

Literature Cited; Intrauterine Devices (IUDs)

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Evaluating the efficacy and safety of silicone O-ring intrauterine devices as a horse contraceptive through a captive breeding trial.

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Abstract : Due to continued on-range population growth there are upwards to 70,000 feral horses against a BLM range ecologist established goal of 27,000. Therefore, the need for an effective means of contraception has significantly increased. A contraceptive that is reversible would allow for the preservation of genetic diversity in targeted populations and alleviate some of the concern over contraception in wild horses. In this study we evaluated the efficacy and safety of an O-ring intrauterine device (IUD) based on earlier studies indicating a greater than 80% retention rate. With this background information we hypothesized that a silicone O-ring IUD with a durometer grade (hardness) of 50 would be retained within the uterus and would be capable of preventing pregnancy in mares under normal pasture breeding conditions. Acceptable rates of loss for the IUDs were predetermined to be not more than 25% during the first month and less than 50% during the three-month trial period. If these parameters were not met the study would then transition to determining "best" fit. Additionally, we also wanted to determine the incidence of adverse reactions to IUDs regarding inflammation, endometrial fibrosis, and cyclicity. The first IUDs inserted had a loss rate of 3/5 over a two month period without stallion exposure. Therefore, five groups of IUDs, of differing sizes and durometers, were manufactured and the second group of O-ring IUDs were inserted into the uterus of 20 young, reproductively healthy mares; the mares were immediately turned out with stallions after IUD insertion. Retention of IUDs was monitored via transrectal ultrasonography at 3-5 day intervals. Biopsy samples were collected at the time of IUD insertion and IUD removal for comparison. After the first 45 days of the pilot study the inserted O-ring IUDs experienced an overall loss rate of 60%, with some IUD configurations having a loss rate of 100%. At that point the IUDs with the lowest rate of loss were selected to be further refined and inserted into a larger group of mares. Three durometer types were inserted in three groups consisting of eight mares each. This step tested the hypothesis that higher durometer IUDs would be less malleable to uterine contractions (during estrus and during the process of breeding) and less likely to be expelled. Over the one-month evaluation period that followed, 11/24 IUDs were lost; many of which were expelled within the first week after insertion. There was no significant increase in endometrial inflammation or fibrosis. Several mares did experience deviations from normal cyclicity. Finally, no pregnancies were established.

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FERTILITY CONTROL USING INTRAUTERINE DEVICES: AN ALTERNATIVE FOR POPULATION CONTROL IN WILD HORSES

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ABSTRACT

The purpose of this study was to develop a contraceptive method for feral horses. The feral horse population has increased significantly in recent years despite attempts to control numbers. As in most wild animal population control programs, contraceptive methods must be easy to apply, cause minimal disruption to the social structure and be fully reversible. In the present study, we tested the effectiveness of an intrauterine device (IUD) for fertility control in mares. Six mares were fitted with a silastic O-ring-shaped IUD on July 1 of Year 1. The IUD-treated mares were turned out with 12 nontreated mares and a fertile stallion in a large pasture until October 20 (112 d). None of the IUD-treated mares and all the nontreated mares became pregnant. The IUD-treated mares were maintained separately from the stallion during the winter. Following removal of the IUD on April 27 of Year 2, the mares were again introduced to the pasture with the stallion together with 6 nontreated mares. For the 6 mares previously treated with an IUD, the mean interval from introduction to the stallion to conception was 17.5 ± 5 d or 1.3 cycles per pregnancy, and all mares produced a normal foal at term. Subsequently, 19 recorded mare breeding seasons resulted in 18 foals. Uterine cytology and histopathology indicate that the IUD causes mild chronic endometritis without permanent changes in the endometrium. We conclude that based on our observations, the O-ring-shaped IUD is an effective, safe and practical contraceptive method for mares.

Key words: intrauterine device, contraception, population control

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INTRODUCTION

Wild horses are a significant public concern and an economic issue facing the government and private citizens in many of the western states. The feral horse population has increased significantly in recent years in spite of attempts to control the numbers. Attempts to control the growth of the wild horse population include adoption programs, confinement in feedlots, vasectomy of the dominant stallion, progesterone releasing implants and remotely-delivered immunization against zona pellucida proteins (12,13,15). Methods such as the adoption program have had little impact on the growing numbers of horses in the wild. Vasectomy of the dominant stallion (harem sire) has been shown to be ineffective as contraceptive because bachelor stallions breed up to one third of the mares of a harem despite efforts of the dominant stallion to guard his harem (14). Hormonal treatments have had limited success due to dosage requirements and ineffective delivery systems. More recently, immunization against porcine zona pellucida proteins (PZP) has been used successfully to control the reproduction of feral horses (13,14). However, this method requires booster immunizations at regular intervals, and thus it may be more applicable for small herds living in confined areas where individual identification and immunization is possible. In several species, including the horse, immunization against PZP or purified zona pellucida (ZP3) was accompanied by disruption of ovarian function and has resulted in anovulation for extended periods of time. In the mare, continued use of PZP-immunocontraception resulted in a gradual increase of ovarian depression and anovulation, 4 anovulatory mares out of 7 immunized mares and 4 out of 5 immunized mares after 3 and 6 yr of booster immunization, respectively (13,14).

As in most wild animal populations, contraceptive methods in feral horses must be effective and fully reversible. Application must be practical, affordable and cause minimal disruption of the social structure of the herd. To be a practical contraceptive method, it must be applied in a single treatment and be effective for years without significant loss of future fertility. This report describes the development of an effective contraceptive method, using an intrauterine device (IUD), which can be administered in a single treatment to selected individual animals and which is fully reversible. Intrauterine devices have been utilized effectively for fertility control in women for many years (1,3,6,8,18,19). With the introduction of new and improved designs, IUDs have received increasing attention as a human contraceptive. Extensive retrospective studies have proven that long-term IUD use provides a safe and effective method of contraception with a low rate of complication (2,7,8,16). In women using IUDs for up to 3 yr, the cumulative pregnancy rate ranged from 2.6 to 7.0% depending on the kind of IUD used (8,19). The complications that may be caused by the use of an IUD include discomfort, infection and perforation of the uterus. However, the percentage of cases associated with problems remains very low. Perforation of the uterus occurred in only 1.2 to 8.7 per 1,000 women in a study involving several thousands of patients (19). Deaths caused directly by the IUD were as low as 1.06 per million (19). In women, the orientation of the uterus is vertical and the major problem involved with the application of an intrauterine device is its uncertain retention in the uterus. Most

of the pregnancies observed in women using an IUD can be attributed to loss of the IUD unbeknown to the patient. The design of human IUDs is the result of a compromise between effective retention and minimal discomfort for the patient. Problems such as discomfort and occasional perforation of the woman's uterus are due to the shape of human intrauterine devices and the method of placement (1). The mare's uterus has a horizontal orientation and loss of the intrauterine device is less likely to be a problem. This led us to believe that safer and more comfortable devices could be used as effective contraceptive devices in mares without encountering the problems occasionally observed in women. Subsequent fertility is an important consideration in the choice of a contraceptive method. In women, the fertility rate upon removal is about 90%, including the patients who have used IUDs as a contraceptive method for more than 5 yr (9,10). The precise mechanism by which IUDs act is still uncertain. Although there are a variety of IUD-related antifertility effects in different animal species, the single factor common to all species appears to be the local inflammatory response in the endometrium to the IUD.

The aim of this study was to develop a practical, economical, safe and reversible method of contraception for mares that would be effective for several years and that would not interfere with future fertility.

MATERIALS AND METHODS

Experimental Design

An IUD was placed in the uterus of 6 mares in the spring of Year 1. The 6 IUD-treated mares, 12 untreated control mares, and a fertile stallion were housed together on 12 acres of irrigated pasture during the summer of Year 1. The mares were examined for pregnancy at regular intervals, and those more than 30 d pregnant were removed from the herd. In the fall of Year 1, IUD-treated mares were separated from the stallion. In the spring of Year 2, the IUD was removed from the uterus and the mares were placed in a large pasture with the stallion and with 6 additional untreated nonpregnant mares. The mares were examined for pregnancy, and 30-d pregnant mares were removed from the herd.

Animals

In Year 1, 18 nonpregnant, nonlactating mares of various light breeds were selected. Mares were selected when judged to be reproductively sound based on palpation per rectum and ultrasonography of the genital tract, vaginoscopy, uterine cytology, uterine culture and an absence of a history of reproductive problems in the past 2 yr. During this examination a uterine biopsy was collected but histopathological examination was performed at the completion of the experiment together with biopsies collected during the experiment (5,17). Eight mares were maiden and 10 mares were multiparous. Three maiden and three multiparous mares (IUD-mares) were randomly

assigned to the IUD-treated group, and the remaining 12 mares (control mares) were assigned to the untreated group. The stallion used for the study was 8 yr old in Year 1, of proven fertility, and had been used successfully for pasture breeding in the previous year. In Year 2, 6 multiparous nonpregnant mares were selected using the same criteria as in Year 1 and were used as control mares.

Placement and Monitoring of Intrauterine Device

On July 1 of Year 1, 12 d after the initial examination, 1 IUD was placed in the uterus of each mare. Following the IUD placement, the 6 IUD-treated, 12 untreated mares and a stallion were introduced to the pasture. Mares were examined at 3-d intervals for adverse reactions (general attitude, vulvar discharge) and reproductive function. Ovarian activity, uterine and cervical tone and pregnancy status, and the presence and location of the IUD was monitored by palpation and ultrasonography per rectum. Pregnancy was diagnosed by ultrasonography on Days 14, 25 and 30. Mares more than 30 d pregnant were removed from the herd. On October 20 of Year 1, the IUD-mares were separated from the stallion. Fourteen days after separation from the stallion, IUD-treated mares were examined again for pregnancy. After having confirmed that none of the IUD-treated mares were pregnant, samples for uterine cytology and culture and an endometrial biopsy were collected. The IUD-treated mares were housed with 34 pregnant mares on pasture and fed grass hay. The IUD-treated mares remained separated from the stallion until May of Year 2. The stallion remained in the same pasture for the remainder of Year 1 and until the end of the experiment in Year 2. On April 27 of Year 2, the ovaries and tubular genitalia of the IUD-treated mares were examined by palpation and ultrasonography per rectum, vaginoscopy was performed and samples for uterine cytology and culture were collected. Subsequently, the IUD was removed manually through the cervix and an endometrial biopsy was taken immediately after removal of the IUD. On May 8, the 6 mares (previously treated with IUD) and 6 new, nonpregnant mares (control group) were re-introduced to the same stallion used in the previous year. The reproductive status of these 12 mares was monitored every other day by palpation and ultrasonography per rectum of the reproductive organs. Mares more than 30 d pregnant were removed from the herd.

Intrauterine Device

A flexible O-ring, with a thickness of 5 mm and an external diameter of 40 mm, was used as an intrauterine device. The ring was made of pure medical grade silastic elastomer (Silastic 382, Dow Corning, Midland MI 48640). The flexibility of the ring was such that the ring could easily be compressed between thumb and index finger. The flexibility allowed easy insertion and removal through the cervix. Spontaneous expulsion through the cervix was prevented by return of the O-ring to its original circular form. Before the placement of the IUD, the perineal area was washed thoroughly as described for artificial insemination (17). The IUD was inserted with a gloved hand using appropriate sterile lubrication. The IUD was inserted in the cervix

and pushed into the uterus with 1 finger. Removal of the IUD was achieved by reaching through the cervix with 1 or 2 fingers. One finger was hooked through the opening of the O-ring and the IUD was then gently removed. No attempt was made to insert or remove the IUD during a specific stage of the estrous cycle.

Interpretation of Uterine Cytology, Culture and Biopsy

Uterine samples for cytological examination were fixed and stained (Diff-Quick Stain, American Scientific Products, McGaw Park, IL; 18). Presence of 1 to 25 polymorphonuclear neutrophils (pmns) per 5 high powered fields (x240) was determined as a few, 25 to 200 as a moderate number and more than 200 as a high number of pmns. Uterine samples were submitted for aerobic and anaerobic cultures. Samples for uterine cytology and culture were evaluated without previous knowledge of treatment status. Uterine biopsies were evaluated in random order after collection of the last sample and were classified on a scale of I to III, with I being essentially a normal endometrium and III indicating severe pathological changes and an expected pregnancy rate of less than 10% (5,11).

RESULTS

Placement, Retention and Removal of Intrauterine Device

Introduction of the collapsed O-ring (Figure 1) was possible in all mares regardless of the stage of the estrous cycle. Placement of the IUD in the uterus was achieved on the first attempt and took less than 1 minute in each mare. In 5 of 6 mares, the IUD remained in the uterus until it was removed 10 mo later (Year 2). In 1 mare the IUD was no longer present at the first examination 3 d after initial placement. A new IUD of the same type was introduced and remained in the uterus until removal in Year 2.

Removal of the IUD was uncomplicated. Three mares were in estrus at the time of removal and their cervix was open and relaxed. Average time needed for removal was less than 1 min. Three mares were in diestrus and the cervix was closed. In the diestrous mares, gentle massage for approximately 1 min was sufficient to dilate the cervix to allow introduction of a finger and the entire removal procedure required less than 3 min per mare. The IUD was removed from all the mares on the first attempt. Age or parity did not have a noticeable effect on the ease of placement or removal.

Pregnancy Rate

In Year 1, all untreated mares were pregnant at the end of the summer. The mean number of estrous cycles per pregnancy rate was 2 ± 0.3 cycles/pregnancy (mean $\pm$ SEM) and ranged between 1 and 4 cycles/pregnancy. None of the IUD-treated

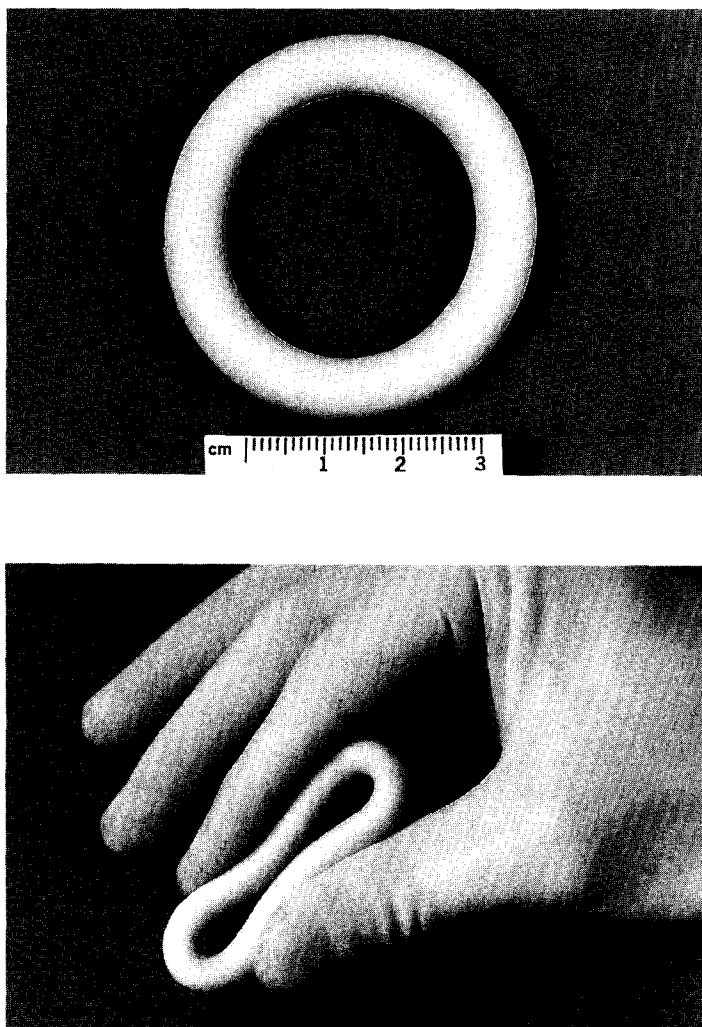


Figure 1. Photograph (top) of the type of IUD used in this study. The IUD could easily be collapsed between thumb and index finger as shown in the bottom photograph.

mares became pregnant as determined by ultrasonography of the uterus per rectum. During the period that the IUD-treated mares were exposed to the stallion (112 d), the estimated number of estrous periods per mare was 5.3 ± 0.3 (range: 4 to 6 cycles). Mares continued to have estrous cycles although estrous cycle length tended to vary more than in the untreated mares. Mean interovulatory interval in IUD-treated mares was 19 ± 1.4 d and intervals ranged between 9 and 35 d based on ovulation observed during estrus.

In Year 2, 5 of 6 untreated control mares were pregnant after the first estrus and one was pregnant after the fourth estrus, resulting in a per cycle pregnancy rate of 1.5 ± 0.5 cycles/pregnancy. For the 6 mares previously treated with IUD, the mean interval from introduction to the stallion to conception was 17.5 ± 5 d (range: 6 to 38 d). Four mares conceived after the first estrus and 2 conceived after the second estrus, resulting in a pregnancy rate of 1.3 ± 0.2 cycles/pregnancy. The pregnancy rate for both groups was not significantly different ($P > 0.05$) based on the Student's t-test. All mares delivered a normal foal at term in the following year (Year 3). The 6 IUD-treated mares were bred during the first postpartum estrus in Year 3. Five mares conceived on the first postpartum estrus (foal heat) and one on the second postpartum estrus. Mares produced a normal foal at term, but not all mares were bred back. For those mares bred again in following years, 13 recorded breeding seasons resulted in 12 successful pregnancies. Information on fertility in subsequent years was not available for control mares used in Year 1 and 2.

Uterine Cytology and Culture

Uterine cytology and culture samples were collected from the six IUD-treated mares before placement of the IUD, 14 d after separation from the stallion and on the day of IUD removal. Before IUD placement, no signs of uterine inflammation (less than 1 pmn per 5 high powered fields) were observed on uterine cytology and culture resulted in no growth in all mares. Fourteen days following separation of the IUD-treated mares from the stallion, few numbers of pmns were observed in 2 mares and moderate numbers of pmns were observed in the 4 remaining mares. Uterine culture in these 4 mares resulted in small numbers of Streptococcus zooepidemicus in 4 mares and very small numbers of Escherichia coli in 1 mare. Immediately before removal of the IUD (spring of Year 2), all mares had either few or moderate numbers of pmns on uterine cytology and 2 mares had very small numbers of Streptococcus zooepidemicus. The inflammatory cells consisted primarily of mature hypersegmented pmns with few lymphocytes scattered among the pmns, indicating a nonseptic but chronic inflammation. Uterine cytology and culture was repeated 1 week after removal of the IUD (May 8), before introduction to pasture with the stallion, and no evidence of uterine inflammation and/or infection was observed at this time.

Uterine Biopsy

The results of the histopathological evaluation of the uterine biopsies are listed in Table 1. At the onset of the experiment (June 18), 5 mares had essentially normal endometrium (Grade I) and one mare had signs of a mild chronic endometritis (Grade II). During the time of the IUD treatment (November 5 and April 27), all mares had mild to severe uterine inflammation. The inflammatory reaction appeared to be severe enough to interfere with fertility. However, histopathological changes did not appear to be of a permanent nature (periglandular fibrosis), suggesting that if the cause of the inflammation was eliminated the mares would return to normal fertility.

Table 1. Histopathological evaluation of uterine biopsy		
Mare ID No	Date	Histopathology of uterine biopsy
298	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, moderate, diffuse, subacute (Grade II)
	Apr 27 (Year 2)	Endometritis, moderate to severe, diffuse, subacute, ulcerative, active (Grade II)
595	Jun 18 (Year 1)	Endometritis, mild, multifocal, chronic (Grade I)
	Nov 5 (Year 1)	Endometritis, mild to moderate, multifocal, chronic, active (Grade II)
	Apr 27 (Year 2)	Endometritis, mild to moderate, multifocal, chronic, active (Grade II)
6556	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, moderate, diffuse, acute, stratum compactum (Grade II)
	Apr 27 (Year 2)	Endometritis, moderate, diffuse, acute (Grade II)
6557	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, subacute, moderate to severe, diffuse, stratum compactum (Grade II)
	Apr 27 (Year 2)	Biopsy not available
6558	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, acute, moderate, diffuse, stratum compactum (Grade II)
	Apr 27 (Year 2)	Endometritis, moderate, diffuse, chronic, active (Grade II)
6559	Jun 18 (Year 1)	Endometritis, mild, focal, chronic (Grade II)
	Nov 5 (Year 1)	Biopsy not available
	Apr 27 (Year 2)	Endometritis, chronic, active, severe, diffuse (Grade III)

Body Condition

The IUD did not appear to interfere with the general health status of the mares. They remained in good body condition and appeared to thrive as well as the control mares during summer and pasture mates during the winter period. Animal health technicians in charge of daily supervision did not observe any loss of weight or appetite, rough hair coat, gastrointestinal problems or vaginal discharge.

DISCUSSION

The results of this experiment indicate that IUDs can be used as a contraceptive in the mare. Mares continued to have estrous cycles and were bred by the stallion but failed to conceive. In our experiment, pregnant mares were removed from the herd to maximize the chances of conception of the remaining nonpregnant mares. Following removal of the IUD all mares returned to normal fertility in a very short interval and the reproductive performance of these mares in the subsequent years suggest that there was no residual effect of the IUD on fertility. At the end of the breeding trial in Year 1, IUD-treated mares were relocated to a different pasture. Upon the return of the mares to the original location, they were not exposed to the stallion until after removal of the IUD. However, the results of uterine cytological and histopathological examination suggested that the condition of the uterus at the time of IUD removal in Year 2 was not different from that observed at the end of the breeding trial in Year 1. In women, the proposed mechanism of contraceptive action of the IUD is a chronic low-grade uterine inflammation. In mares, the same mechanism appears to be present. During IUD treatment, there was cytological and histopathological evidence of endometritis and mares failed to conceive. However, within 1 wk after IUD removal, cytological evidence of endometritis and bacterial growth had subsided and mares had returned to normal fertility.

Of all our large domestic species, the mare lends itself best to the use of IUDs for contraception. Due to its size and the anatomy of the genital tract, placement and removal of the IUD are possible on the standing animal, and instrumentation is not required. Placement was done by someone proficient in reproductive techniques in mares but with no prior training in this specific procedure. The thickness of the IUD ring was such that the ring was readily collapsible when placed between the thumb and index finger but rigid enough to resist the uterine contractions and spontaneous expulsion through the cervix. It is not clear why the IUD was lost by 1 mare within 3 d after placement. Loss may have been the result of improper placement, possibly intracervically. This is supported by the observation that following placement of the same type of IUD, this mare retained the IUD in the uterus for the remainder of the experimental period. In this study, a delay was planned between insertion of the IUD and introduction to the pasture with the stallion. This observations period allowed us to better assess the retention of the IUD and possible adverse reactions (abdominal discomfort). Since no adverse reactions were noted it appears safe to reintroduce

mares to the herd immediately following IUD placement. Following removal of the IUD, a uterine biopsy was collected and mares were kept under close observation for 1 wk. Based on the cytology and culture results obtained 1 wk after removal of the IUD, it appears that mares could be reintroduced to the herd immediately after removal of the IUD.

In our study, we did not collect samples for uterine cytology, culture or biopsy from the nontreated mares because we anticipated that nontreated mares would become pregnant and thus would not be available for subsequent sampling. During IUD treatment, all mares had a mild to moderate number of pmns present in the uterus. Cells appeared nontoxic suggesting that the endometritis was primarily the result of irritation caused by the presence of the IUD. The presence of bacteria at the end of Year 1 and the beginning of Year 2 is difficult to interpret. While bacterial growth at the end of Year 1 could have been the result of exposure to the stallion, it is not clear if the continued presence of bacteria in the spring of Year 2 was related to the IUD. At this time mares had not been exposed to the stallion for 6 mo. It is possible that continued presence of bacteria in the uterus was the result of impaired uterine clearance caused by the IUD, but this hypothesis needs further investigation. Uterine histopathology confirmed that mares had a mild to moderate, chronic endometritis during IUD treatment. The absence of pmns on cytological examination and bacterial growth 1 wk after IUD removal supports the hypothesis that the presence of an IUD may cause chronic bacterial infection in some mares. The design of the IUD, shape and size, may need to be re-evaluated to address this concern. However, it is encouraging that after 1 yr of IUD-treatment there was no histopathological evidence of permanent changes in the uterus, such as periglandular fibrosis, which could impair future fertility of mares following IUD removal. The rapid return to normal on cytological examination and the fertility in the years following IUD treatment support the histopathological observations that long-term potential fertility was not affected by IUD treatment.

The results of this study provide the first evidence that the use of IUDs is a viable alternative for contraception in feral mares. Pregnancy was prevented in all mares during treatment, the mares continued to have normal estrous cycles, side effects were not observed, and the mares returned to normal fertility after IUD removal. While these results are encouraging, larger field trials need to be done to examine the effect of prolonged IUD treatment on subsequent fertility.

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**UNITED STATES ENVIRONMENTAL PROTECTION
AGENCY**

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

October 28, 2020

Dr. Paul Griffin
Research Coordinator
U.S. Department of the Interior
Bureau of Land Management
National Wild Horse and Burro Program
1340 Financial Blvd.
Reno, NV 89502

SUBJECT: M009 Device Determination Review
Product Name: Y-Shaped Silicone IUD for Feral Horses
PRIA Application Number.: 97949PA1
Application Date: 08/18/2020
Decision Number: 565481

BACKGROUND

List of Material(s) Submitted in Application Package:

1. EPA Form 8570-1
2. Cover letter from Bureau of Land Management describing the submission
3. Supplemental Materials- Written description of how the device works and supporting information for an M009 application
4. Label for "Y-Shaped Silicone IUD for Feral Horses"
5. PRIA fee waiver request for federal agencies
6. Safety Data Sheet for material used in the product
7. Cooperative Agreement between Bureau of Land Management and U.S. Geological Survey to develop product
8. Cooperative Agreement with U.S. Geological Survey and Oklahoma State University to conduct efficacy testing
9. **MRID 51243700** Data transmittal sheet
10. **MRID 51243701** Baldrighi, J.M., C.C. Lyman, K. Hornberger, S.S. Germaine, A. Kane, and G.R. Holyoak. 2017. Evaluating the efficacy and safety of silicone O-ring intrauterine devices as a horse contraceptive through a captive breeding trial. *Clinical Theriogenology* 9:471.
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Description of Product:

“The *Y-Shaped Silicone IUD for Feral Horses* is an intrauterine device (IUD) comprised solely of medical-grade, inert, silicone that is suitable for use in female feral horses (free-roaming or “wild” *Equus caballus*). Intended users include government agencies with feral horses in their management purview, Native American tribes that have management authority over feral horses, and authorized feral horse care or rescue sanctuaries that manage feral horses in a free ranging environment.

The *Y-Shaped Silicone IUD for Feral Horses* can mitigate or reduce feral horse population growth rates because these IUDs can provide potentially reversible fertility control for female feral horses. This IUD prevents pregnancy by its physical presence in the mare's uterus as long as the IUD stays in place. In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.” - *Description from supplemental materials*

The description provided by the applicant appears accurate, as evidenced by photographs of the device in the supporting information document provided which details the manufacturing process. A medical grade silicone elastomer is injected into 2-part Y-shaped aluminum molds,

then curing is catalyzed by the presence of platinum and heat to form the product. The IUD is six inches wide, 4 inches high, and 3/8 inches deep.

The Directions for Use on the submitted label state that the product “is to be place in the uterus of feral horse mares by a veterinarian” and “is intended for use in feral mares that are at least approximately 1 year old, where age is determined based on available evidence, such as tooth eruption pattern.” A CAUTION statement on the label warns users that “These IUDs are only to be used in mares that are confirmed to be not pregnant.”

The product is manufactured for U.S. Bureau of Land Management (BLM; EPA Company Number 97949) at EPA Est. No. 97628-MI-1. U.S. BLM has stated in the Supplemental Materials that “the material that will be distributed with the product is limited to the product itself and the label. BLM will not distribute these devices for profit.” Additionally, “no patent has been issued or applied for. BLM has no intention to patent the Y-Shaped Silicone IUD for Feral Horses....the US Government retains the rights for use of the devices.....[and] the Federal Government shall have a non-exclusive, nontransferable, irrevocable, paid-up license to practice or have practiced for or on behalf of the U.S. the subject invention throughout the world.”

Mode of Action and Use Site:

“Intrauterine devices (IUDs) that operate through physical means likely reduce fertility by maintaining the corpus luteum, and perhaps by causing a low-grade irritation of the uterus, triggering hormonal changes in the mare that prevent any fertilized egg implantation (Nie et al. 2003)¹..... IUDs that act through physical means are considered a temporary fertility control method that does not generally cause future sterility, as the devices may be removed manually at a later date (Daels and Hughes 1995)².” -*Description from supplemental materials*

Additionally, while the exact mechanism that causes infertility in wild horses from IUDs is not known, Gradil et al. (2019)³ suggest that IUDs may suppress estrus by extending luteal function through a luminal inflammatory response in the uterus. The hypothesis that an IUD produces a local, sterile, inflammatory reaction was further validated by Ginther et al. (1966)⁴ and Fordyce et al. (2001)⁵. The plastic-coated magnet Upod in Gradil et al. (2019)’s study asserted that no chemical compounds were released but rather the Upod exerted pressure on the uterine walls by contact, perhaps restricting uterine contractions necessary for embryo mobility into the uterus.^{6,7} However, Gradil et al. (2019) postulate that since IUD movement in the uterus is limited, the IUD is most likely impacting only the endometrium.

¹ MRID 51243709

² MRID 51243702

³ MRID 51243704

⁴ Ginther OJ, Woody CO, Janakira K, Casida LE. Effect of an intra-uterine plastic coil on oestrous cycle of heifer. J Reprod Fertil 1966;12:193e8.

⁵ Fordyce G, Jubb TF, Fitzpatrick LA, Whyte TR, Cooper NJ, Bolam MJ, et al. Contraceptive efficacy of an intra-uterine device in brahman cattle. Anim Reprod Sci 2001;65:193e204.

⁶ Leith GS, Ginther OJ. Characterization of intrauterine mobility of the early equine conceptus. Theriogenology 1984;22:401e8.

⁷ McDowell KJ, Sharp DC, Grubaugh W, Thatcher WW, Wilcox CJ. Restricted conceptus mobility results in failure of pregnancy maintenance in mares. Biol Reprod 1988;39:340e8.

IUDs for feral horses have previously been made with plastic-coated magnetic Upods (Gradil et al. 2019), glass marbles or metal ball bearings (Nie et al. 2003), and silastic O-shaped rings (Daels & Hughes 1995), all of which claim to utilize a mechanical mode of action with no chemical substance to prevent pregnancy. The supplemental materials state “As far as BLM knows, no other IUDs are specifically made for use to mitigate feral horse population growth rate, although one product has made related claims”.

The use site is in the uterus of free-roaming or “wild” female feral horses (*Equus caballus*).

Product Claims:

Claims being made by the product on label/labeling materials include⁸:

1. “The *Y-Shaped Silicone IUD for Feral Horses* is an intrauterine device (IUD) comprised solely of medical-grade, inert, silicone that is suitable for use in female feral horses (free-roaming or “wild” *Equus caballus*).” -*Label*
2. “The *Y-Shaped Silicone IUD for Feral Horses* can mitigate or reduce feral horse population growth rates because these IUDs can provide potentially reversible fertility control for female feral horses.” -*Label*
3. “This IUD prevents pregnancy by its physical presence in the mare’s uterus as long as the IUD stays in place. In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.” -*Label*
4. “The *Y-Shaped Silicone IUD for Feral Horses* is made from medical-grade, inert silicone. The IUD prevents pregnancy by its physical presence in the mare’s uterus.” -*Proposed marketing claims in Description from application submission materials*
5. “The *Y-Shaped Silicone IUD for Feral Horses* reduces female wild horse fertility, as long as the IUD stays in place in the mare’s uterus.” -*Proposed marketing claims in Description from application submission materials*
6. “The *Y-Shaped Silicone IUD for Feral Horses* provides potentially reversible fertility control for female wild horses.” -*Proposed marketing claims in Description from application submission materials*
7. “In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.” -*Proposed marketing claims in Description from application submission materials*
8. “Use of the *Y-Shaped Silicone IUD for Feral Horses* is expected to help reduce wild horse population growth rates, and potentially to reduce the total number of wild horses that would need to be removed from public lands.” -*Proposed marketing claims in Description from application submission materials*

⁸ The applicant is claiming to support all of the eight above claims with Holyoak et al. (in press; MRID 51243706). EPA has not reviewed the validity of this data.

DEVICE DETERMINATION

1. Are any substances included or used? If so, how and what is the purpose of the substance in the product?

The product is made solely of medical-implant-grade silicone elastomer, NuSil MED 4860 with a durometer of shore-A 60.

“The silicone is cured by heat, and the silicone curing is catalyzed by addition of platinum. Both the silicone and the platinum are chemically inert. After heat/platinum curing, the components become stable, inert medical grade silicone, such as is used in silicone medical implants.” -

Description from supplemental materials

2. If a substance is generated, is it contained in any way, and if so how?

No substance is generated.

3. How is the resulting output substance used?

No substance is output.

4. Are any of the label/labeling claims false or misleading, as defined in FIFRA?

The purpose of this review is solely to determine whether the product as described is subject to FIFRA as a pesticidal device, so EPA has not evaluated either the potential risks or the possible effectiveness of the product. The only claims that were evaluated were the ones that were submitted as of 08/18/2020 and other claims found in the process of the review. Any additional claims that are added to the labeling/marketing material/website(s) could result in a different determination or later be determined to be false and misleading.

The items listed in the **Product Claims** section above were not found to be false or misleading at this time. No other claims other than those listed in the **Product Claims** section above were evaluated by EPA in this device determination review.

CONCLUSION

After review of the provided materials, the Agency has determined that the *Y-Shaped Silicone IUD for Feral Horses* is mitigating feral horse populations by a mechanical means. The product is not a physical barrier exempt under FIFRA, as the product does not physically block the corpus luteum from entering the fallopian tube, nor does it physically block the fertilized egg from implanting on the endometrium. Rather, the product mechanically causes inflammation of the endometrium from its presence in the uterus, which in turn inhibits successful implantation of the fertilized egg.

This product appears to be a similar mode of action to other feral horse IUDs, such as plastic-coated magnetic Upods, glass marbles or metal ball bearings, and silastic O-shaped rings, which are thought to work by inflammation of the uterus/endometrium. The insertion of a foreign object into the uterus, regardless of the material (e.g., glass, plastic, silicone, etc.), is the mode of action. Hence, this product is not a pesticide requiring registration under FIFRA.

Therefore, the Agency concludes that the silicone material of this product is highly unlikely to be chemically contributing to the pesticidal mode of action (i.e. the product is working solely through a mechanical means) and the ***Y-Shaped Silicone IUD for Feral Horses is a pesticidal device regulated under FIFRA.***

Regulatory Requirements:

Please note that since your product is a pesticide device, it is subject to certain requirements of FIFRA that are set forth in 40 Code of Federal Regulations (CFR) § 152.500. Among other things, FIFRA Section 12(a)(1)(F) makes it unlawful to distribute, sell, or offer to sell pesticide devices that are misbranded. A device may be “misbranded” under several circumstances outlined in FIFRA Section 2 and the applicable regulations, including when its labeling, which includes everything referenced on the label, such as a website, and any other written materials accompanying the device as part of its distribution or sale, is false or misleading in any particular regarding pesticidal or non-pesticidal claims, per 40 C.F.R. § 156.10(a)(5); 7 U.S.C. §§ 136(q)(q)(1)(A). Accordingly, EPA recommends that you have sufficient efficacy data available to substantiate any claims regarding your product, and that you refrain from making any claims that may mislead consumers about the device’s purposes or capabilities, including claims that may mislead consumers to believe that the device is “registered” or has been otherwise evaluated or approved by EPA.

If the product is considered to be a pesticidal device and is subject to regulation under FIFRA Section 2(q)(1) and Section 7, it cannot be misbranded (i.e., make false or misleading claims) and is subject to production, labeling, and child-resistant packaging requirements set forth in:

- labeling as described in 40 CFR 156.10;
- EPA Establishment Number (EPA Est. No.) and reporting for each location where production occurs, as described in 40 CFR 167 (**Note:** production includes facilities where formulation, packaging, repackaging, labeling, and/or relabeling occurs);
- books and record keeping as described in 40 CFR 169.

A pesticidal device is also subject to all FIFRA provisions for pesticide-producing establishment inspections, enforcement, import and export requirements, and child-resistant packaging. Pay particular attention to the product efficacy and safety claims requirements, as these are common problem areas for pesticidal device manufacturers.

Some states require registration of pesticidal devices. Please check with the state where you plan to sell your device to determine their requirements, including requirements for efficacy data.

<https://www.epa.gov/pesticide-advisory-committees-and-regulatory-partners/state-and-territorial-pesticide-agencies>

This letter is not an endorsement of the product and cannot be used in conjunction with the sale, distribution or marketing of the product.

If you have any questions, you may contact Paul Di Salvo at 703-347-0322 or via email at disalvo.paul@epa.gov.

Sincerely,

A handwritten signature in blue ink that reads "Meredith F. Laws". The signature is written in a cursive style with a large, stylized 'M' and 'L'.

Meredith F. Laws, Chief
Invertebrate and Vertebrate Branch 3
Registration Division
Office of Pesticide Programs



United States Department of the Interior
Bureau of Land Management
National Wild Horse and Burro Program
1340 Financial Boulevard, Reno, Nevada 89502



August 18, 2020

TO: U.S. Environmental Protection Agency
Document Processing Desk
Office of Pesticide Programs (7504P)
Registration Division, IVB3
1200 Pennsylvania Avenue, N.W.
Washington DC 20460-0001

RE: Device Determination Request (PRIA code M009); “Y-Shaped Silicone IUD for Feral Horses”

Dear EPA OPP Device Determination panel members,

The U.S. Bureau of Land Management (BLM) is requesting a Device Determination for the product “Y-Shaped Silicone IUD for Feral Horses.” Based on EPA guidelines and early communication with EPA staff, BLM believes that EPA will agree that these intrauterine devices (IUDs) are pest control devices, because they operate through a strictly physical means to mitigate or reduce feral horse population growth by reducing fertility in female feral horses.

Wild horses are a subset of feral horses in the American west, protected by federal law, notably the Wild Free-Roaming Horses and Burros Act of 1971 (as amended). Populations of wild horses, which are feral descendants of domestic horses, typically grow at annual rates of 15-20% or more. Biologically, they are feral horses. As part of its management purview, BLM is charged with keeping the population sizes of these iconic animals at levels that allow for the rest of the ecosystem they live in to be at a ‘thriving natural ecological balance.’ Keeping wild horse populations at managed levels reduces the damage that can be caused by overpopulated wild horse herds. BLM sometimes needs to remove wild horses from public rangelands, but it is preferable in many cases to use fertility control to reduce the rate of their population growth. BLM-funded research that developed and tested the specific design of the *Y-Shaped Silicone IUD for Feral Horses* at issue here. That research showed that this IUD design is safe for the animals, reduces fertility in the horses, and stays in horses long enough to be effective over multiple breeding seasons.

This is an unpatented design, developed as a result of BLM-funded research. BLM funded the U.S. Geological Survey (USGS) to conduct that research. Like BLM, USGS is in the Department of the Interior. USGS formed a financial assistance agreement with Oklahoma State University to develop and test different shapes, sizes, and hardnesses (‘durometer’) of medical-grade silicone IUDs, for use in horses. Along with other supporting documents, I am attaching two agreements, to substantiate that the federal government has rights to use this design. No patent

claim has been made on the design. Also, the financial assistance agreement between USGS (using BLM funds) and Oklahoma State University establishes that, for inventions arising from the USGS - OSU agreement, "...the Federal Government shall have a non-exclusive, nontransferable, irrevocable, paid-up license to practice or have practiced for or on behalf of the U.S. the subject invention throughout the world."

These IUD will be manufactured at a registered pesticide-producing establishment for BLM. BLM would not sell these devices for profit. If another federal, state, or local agency that has management purview over feral horses (such as the US Forest Service, the Department of Defense, or a Native American tribe) requests the IUDs for their own management use in feral horses, BLM would only seek reimbursement for the cost of production.

BLM is submitting EPA Form 8570-1 and additional information as required for Device Determinations on the EPA website. For all of the following, please see the attached "Supplemental Materials" file.

1. EPA issued company number
2. The complete brand names of the product
3. a) Complete copy of the label for the product, and b) a statement of all claims to be made for the product, including all written, printed, or broadcast claims made for the product.
4. Directions for use and warnings or cautionary statements.
5. All material distributed with the product.
6. A detailed written description of how the product works.
7. Starting material inputs or ingredients.
8. Schematic diagram or detailed engineering drawings, diagrams, flow diagrams or patent(s) application information.
9. Photographs of the product from all sides, including digital copies available upon request.
10. Comparisons to current industry products.
11. Provide proof of payment.
12. Additional Information, including copies of agreements and scientific journal articles.

Please contact me if there are any deficiencies in our request for this Device Determination, or if you have any questions. Thank you very much for any guidance and help, and for considering this request.

Sincerely,

A handwritten signature in black ink, appearing to read "Paul Griffin". The signature is stylized with a large, looped "P" and a cursive "Griffin".

Paul Griffin

Y-Shaped Silicone IUD for Feral Horses

The *Y-Shaped Silicone IUD for Feral Horses* is an intrauterine device (IUD) comprised solely of medical-grade, inert, silicone that is suitable for use in female feral horses (free-roaming or “wild” *Equus caballus*). Intended users include government agencies with feral horses in their management purview, Native American tribes that have management authority over feral horses, and authorized feral horse care or rescue sanctuaries that manage feral horses in a free ranging environment.

The *Y-Shaped Silicone IUD for Feral Horses* can mitigate or reduce feral horse population growth rates because these IUDs can provide potentially reversible fertility control for female feral horses. This IUD prevents pregnancy by its physical presence in the mare’s uterus as long as the IUD stays in place. In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.

Directions for Use:

The *Y-Shaped Silicone IUD for Feral Horses* is to be placed in the uterus of feral horse mares by a veterinarian. The *Y-Shaped Silicone IUD for Feral Horses* is intended for use in feral mares that are at least approximately 1 year old, where age is determined based on available evidence, such as tooth eruption pattern.

IUDs must be sterilized before use. The IUD is inserted into the uterus using a thin, tubular applicator, similar to a shielded culture tube commonly used in equine reproductive veterinary medicine, in a manner similar to methods used for uterine culture of domestic mares. Feral mares with IUDs should be individually marked and identified (i.e., with an RFID microchip, or via visible freeze-brand on the hip or neck).

Caution:

These IUDs are only to be used in mares that are confirmed to be not pregnant. Checking pregnancy status can be accomplished by methods such as a transrectal palpation and/or ultrasound performed by a veterinarian. If a *Y-Shaped Silicone IUD for Feral Horses* is inserted in the uterus of a pregnant mare, it may cause the pregnancy to terminate, and the IUD to be expelled.

Manufactured for:

U.S. Bureau of Land Management (97949)

1340 Financial Blvd., Reno, NV 89052

EPA Est.: 97628-MI-1



United States
Environmental Protection Agency
Washington, DC 20460

☐ Registration
☐ Amendment
☐ Other

OPP Identifier Number

Application for Pesticide - Section I

1. Company/Product Number 94979	2. EPA Product Manager (unknown)	3. Proposed Classification ☒ None ☐ Restricted
4. Company/Product (Name) Bureau of Land Management	PM# (unknown)	
5. Name and Address of Applicant (Include ZIP Code) Paul Griffin, 1340 Financial Blvd, Reno, NV 89502 ☐ Check if this is a new address	6. Expedited Review. In accordance with FIFRA Section 3(c)(3) (b)(i), my product is similar or identical in composition and labeling to: EPA Reg. No. _____ Product Name _____	

Section - II

☐ Amendment - Explain below.	☐ Final printed labels in response to Agency letter dated _____
☐ Resubmission in response to Agency letter dated _____	☐ "Me Too" Application.
☐ Notification - Explain below.	☒ Other - Explain below.

Explanation: Use additional page(s) if necessary. (For section I and Section II.)

Requesting a Device Determination (PRIA M009) for "Y-Shaped Silicone IUD for Feral Horses."

Requesting a fee waiver, because the Bureau of Land Management is a federal agency (Department of the Interior)

Y-Shaped Silicone Intrauterine Devices (IUDs) for Feral Horses mitigate these feral animals, by virtue of their physical presence alone.

Section - III

1. Material This Product Will Be Packaged In:				2. Type of Container	
Child-Resistant Packaging ☐ Yes* ☒ No	Unit Packaging ☐ Yes ☒ No	Water Soluble Packaging ☐ Yes ☒ No		☐ Metal	
* Certification must be submitted				☐ Plastic	
	If "Yes" Unit Packaging wgt.	No. per container	If "Yes" Package wgt.	☐ Glass	
				☐ Paper	
				☒ Other (Specify) _____	
3. Location of Net Contents Information ☒ Label ☐ Container		4. Size(s) Retail Container (Not for sale)		5. Location of Label Directions ☒ On Label ☐ On Labeling accompanying product	
6. Manner in Which Label is Affixed to Product Sent in mail with Device		☐ Lithograph ☐ Paper glued ☐ Stenciled		☒ Other _____	

Section - IV

1. Contact Point (Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)			
Name Paul Griffin		Title WHB Research Coordinator	
		Telephone No. (Include Area Code) 970-631-4808	
Certification I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.			6. Date Application Received (Stamped)
2. Signature		3. Title BLM WHB Research Coordinator	
4. Typed Name Paul Griffin		5. Date Aug 18, 2020	

PAPERWORK REDUCTION ACT NOTICE and INSTRUCTIONS

PAPERWORK REDUCTION ACT NOTICE: Public reporting burden for this collection of information is estimated to average 0.85 hour per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to **Director, Collection Strategies Division (2822T) U.S.Environmental Protection Agency,1200 Pennsylvania Ave, NW,Washington, DC20460.**

INSTRUCTIONS: This form is to be used all applications for new registration, amendment, resubmission, to applications for notifications, final printed labeling, reregistration, etc. In order to process an application for a new registration submitted on this form, the following material must accompany the application:

1. Certification with Respect to Citation of Data (EPA Form 8570-34). [If not exempted by 40 CFR 152.81(b)(4)].
2. Confidential Statement of Formula (EPA Form 8570-4);
3. Formulator's Exemption Statement (EPA Form 8570-27);
4. Five copies of draft labeling;
5. Three copies of any data submitted;
6. Authorization letter where applicable;
7. Data Matrix.

Submission of Labeling -Labeling should first be submitted in the form of draft labeling with all applications. Such draft labels may be in the form of typed label text on 8.5 x 11 inch paper for submission or a mockup of the proposed label. If prepared for mockup, it should be constructed in a way as to facilitate storage in an 8.5 x 11 inch file. Mockup labels significantly smaller than 8.5 x 11 inches should be mounted on 8.5 x 11 inch paper for submission.

Submission of Data -Data submitted in support of this application must be submitted in accordance with PR Notice 86-5.

SPECIFIC INSTRUCTIONS: Please read the instructions listed below before completing this application. First determine the type of registration action, listed in Block A, for which you are submitting this application. For applications submitted in connection with new registration actions, Sections I, III, and IV must be completed by the applicant. For applications submitted in connection with amendments actions, resubmissions, notifications, reregistrations, etc., Sections I, II, and IV must be completed by the applicant.

Block A - Check the appropriate action for which you are submitting this form.

Section I - The section must be completed, as applicable, for all registration actions.

1. **Company /Product Number** - Insert your company number, if one has been assigned by EPA. This number may have been assigned to you as a basic registrant, a distributor, or as an establishment. If your product is registered, insert the Product Number.
2. **EPA Product Manager** -If known, fill in the name and PM number of the EPA Product Manager.
3. **Proposed Classification** -Specify the proposed classification of this product. For most products the classification would be "None".
4. **Product Name** -Enter the complete product name of this pesticide as it will appear on the label. The name must be specific to this product only. Duplication of names is not permitted among products of the same company. Do not include any brand name or company line designations.
5. **Name and Address of Applicant** -The name of the firm or person and address shown in your application is the person or firm to whom the registration will be issued. If you are acting on behalf of another party, you must submit authorization from that party to act for them in registration matters. An applicant not residing in the United States must have an authorized agent residing in the United States to act for them in all registration matters. The name and complete mailing address of such an agent must accompany this application.
6. **Expedited Review** -FIFRA section 3 (c) 3 (B) provides for expedited review of applications for registration, or amendments to existing registrations, that are similar or identical to other pesticide products that are currently registered with the EPA. In order for your application to be eligible for expedited review, you must provide us with the EPA Registration Number and product name of the product you believe is similar to or identical to your product. The product must be similar or identical in both formulation and labeled uses.

SECTION II -This section must be completed for all applications submitted to amend the registration only of a currently registered product (Amendment), for a resubmission in response to an Agency letter, for notifications to the Agency, for the submission of final printed labeling, for reregistration and for any other action that pertains to a specific EPA registered product. **The Explanation Section should be used for any additional information regarding Sections I and II.**

1. **Subject of submission** -Check the applicable block and provide the Agency letter date if appropriate. Provide a brief explanation of the purpose(s) for the submission, such as "the addition of a site, pest or crop (specify)"; "amend the Confidential Statement of Formula by..."; "reregistration submission"; "general label revision of direction for use", "notification for...". Attach a separate page if additional space is needed.

SECTION III - This Section must be completed for all applications submitted in connection with new registration or applicable amendments.

1. **Type of Packaging** -Check the appropriate block if your product will be packaged in the indicated packaging types. Indicate the size of the individual packets and number per retail container.
2. **Type of Retail Container** - Indicate type of container in which product will be marketed.
3. **Location of Net Contents** -Indicate the location of the net contents information for your product.
4. **Size(s) of Retail Container** -Specify the net contents of all retail containers for your product.
5. **Location of Use Directions** -Indicate the location of the use directions for your product.
6. **Manner in which label is affixed to product** -Indicate the method product label is attached to retail container.

SECTION IV (Contact Point) -This section must be completed for all applications for Registration actions, i.e., new products registration, resubmission, "me-too," reregistration, etc.

- 1-5. Self-explanatory
6. EPA Use Only

Device Determination supporting materials: “Y-shaped Silicone IUD for Feral Horses”

1. The EPA issued company number

The Bureau of Land Management (BLM) has been issued EPA company number **97949**. The product will be manufactured for BLM by pesticide producing establishment 97628-MI-1.

2. The complete brand names of the product:

For labeling purposes, BLM proposes to refer to these devices as “**Y-shaped Silicone IUD for Feral Horses.**”

3. Complete copy of the label for the product, and a statement of all claims to be made for the product, including all written, printed, or broadcast claims made for the product.

3a. Label

Y-Shaped Silicone IUD for Feral Horses

The *Y-Shaped Silicone IUD for Feral Horses* is an intrauterine device (IUD) comprised solely of medical-grade, inert, silicone that is suitable for use in female feral horses (free-roaming or “wild” *Equus caballus*). Intended users include government agencies with feral horses in their management purview, Native American tribes that have management authority over feral horses, and authorized feral horse care or rescue sanctuaries that manage feral horses in a free ranging environment.

The *Y-Shaped Silicone IUD for Feral Horses* can mitigate or reduce feral horse population growth rates because these IUDs can provide potentially reversible fertility control for female feral horses. This IUD prevents pregnancy by its physical presence in the mare’s uterus as long as the IUD stays in place. In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.

Directions for Use:

The *Y-Shaped Silicone IUD for Feral Horses* is to be placed in the uterus of feral horse mares by a veterinarian. The *Y-Shaped Silicone IUD for Feral Horses* is intended for use in feral mares that are at least approximately 1 year old, where age is determined based on available evidence, such as tooth eruption pattern.

IUDs must be sterilized before use. The IUD is inserted into the uterus using a thin, tubular applicator, similar to a shielded culture tube commonly used in equine reproductive veterinary medicine, in a manner similar to methods used for uterine culture of domestic mares. Feral mares with IUDs should be individually marked and identified (i.e., with an RFID microchip, or via visible freeze-brand on the hip or neck).

Caution:

These IUDs are only to be used in mares that are confirmed to be not pregnant. Checking pregnancy status can be accomplished by methods such as a transrectal palpation and/or ultrasound performed by a veterinarian. If a *Y-Shaped Silicone IUD for Feral Horses* is inserted in the uterus of a pregnant mare, it may cause the pregnancy to terminate, and the IUD to be expelled.

Manufactured for:

U.S. Bureau of Land Management (97949)
1340 Financial Blvd., Reno, NV 89052
EPA Est.: 97628-MI-1

3b. Claims to be made

Note: This product will not be sold for profit by BLM. BLM intends to use the *Y-Shaped Silicone IUD for Feral Horses* as part of BLM wild horse management. BLM may make statements about these IUDs in that context. BLM refers to the feral horses it manages as wild horses, because that is how they are known in the Wild Free-Roaming Horses and Burros Act of 1971 (as amended). But biologically, the animals are the feral descendants of domestic horses (*Equus caballus*). Statements about the composition and effects of these IUDs may include the following.

“The *Y-Shaped Silicone IUD for Feral Horses* is made from medical-grade, inert silicone. The IUD prevents pregnancy by its physical presence in the mare’s uterus.”

“The *Y-Shaped Silicone IUD for Feral Horses* reduces female wild horse fertility, as long as the IUD stays in place in the mare’s uterus.”

“The *Y-Shaped Silicone IUD for Feral Horses* provides potentially reversible fertility control for female wild horses.”

“In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.”

“Use of the *Y-Shaped Silicone IUD for Feral Horses* is expected to help reduce wild horse population growth rates, and potentially to reduce the total number of wild horses that would need to be removed from public lands.”

4. Directions for use and warnings or cautionary statements

Y-Shaped Silicone IUD for Feral Horses is an intrauterine device (IUD) comprised solely of medical-grade, inert, silicone that is suitable for use in female feral horses (free-roaming or “wild” *Equus caballus*). Intended users include government agencies, Native American tribes that have management authority over feral horses, and authorized feral horse care or rescue sanctuaries that manage feral horses in a free ranging environment.

Y-Shaped Silicone IUD for Feral Horses are to be placed in the uterus of feral horse mares by a veterinarian. The *Y-Shaped Silicone IUD for Feral Horses* is intended for use in feral mares that are at least approximately 1 year old, where age is determined based on available evidence, such as tooth eruption pattern. IUDs must be sterilized before use. The IUD is inserted into the uterus using a thin, tubular applicator, similar to a shielded culture tube commonly used in equine reproductive veterinary medicine, in a manner similar to methods used for uterine culture of domestic mares. Feral mares with IUDs should be individually marked and identified (i.e., with an RFID microchip, or via visible freeze-brand on the hip or neck).

Caution: These IUDs are only to be used in mares that are confirmed to be not pregnant.

Checking pregnancy status can be accomplished by methods such as transrectal palpation and/or ultrasound performed by a veterinarian. If a *Y-Shaped Silicone IUD for Feral Horses* is inserted in the uterus of a pregnant mare, it may cause the pregnancy to terminate, and the IUD to be expelled.

5. All material distributed with the product

The material that will be distributed with the product is limited to the product itself and the label. BLM will not distribute these devices for profit. BLM will ensure that all production of *Y-Shaped Silicone IUD for Feral Horses* is by producer(s) that are registered with the EPA as pesticide producing establishments. The label in item #3, above, lists one such manufacturer. In the event that BLM contracts with a different manufacturer, the Establishment number on the label would be changed accordingly, to show that other Establishment number.

6. A detailed written description of how the product works to mitigate a pest

The *Y-Shaped Silicone IUD for Feral Horses* would mitigate feral horse population growth rates, using physical means to reduce the fertility rate for female feral horses that are treated with the device. Where their herds are too large, feral horses can cause ecological damage on public rangelands (Crist et al. 2019).

Intrauterine devices (IUDs) that operate through physical means likely reduce fertility by maintaining the corpus luteum, and perhaps by causing a low-grade irritation of the uterus, triggering hormonal changes in the mare that prevent any fertilized egg implantation (Nie et al. 2003). A 2013 National Academies of Sciences (NAS) report addressing fertility control methods for feral horses considered IUDs, and suggested that research should test whether IUDs cause uterine inflammation, and should also test how well IUDs stay in mares that live and breed with fertile stallions. Since that report, the BLM-funded study performed by the United States Geological Survey (USGS) and Oklahoma State University (OSU) from 2016-2020 (Baldrighi et al. 2017, Holyoak et al. In press) demonstrated that these Y-shaped silicone IUDs prevented pregnancies in all the domestic mares that retained the IUD, even when exposed to fertile stallions. Domestic mares in that study lived in large pastures, mating with fertile stallions. Biweekly ultrasound examinations showed that those IUDs stayed in 75% of treated mares over the course of two breeding seasons. The IUDs in that study were then removed so the researchers could monitor the mares' return to fertility. Uterine health, as measured in terms of inflammation, was not seriously affected by the IUDs, and the majority of IUD-treated mares became pregnant within months after IUD removal (Holyoak et al. In press). The overall results are consistent with results from an earlier study (Daels and Hughes 1995), which used O-shaped silicone IUDs.

Overall, as with other methods of population growth suppression, use of IUDs and other fertility control measures are expected to help reduce population growth rates, extend the time interval between gathers, and reduce the total number of excess animals that will need to be removed from the range (NAS 2013).

IUDs that act through physical means are considered a temporary fertility control method that does not generally cause future sterility, as the devices may be removed manually at a later date (Daels and Hughes 1995). Use of IUDs is an effective fertility control method in women, and IUDs have historically been used in livestock management, including in domestic horses. Insertion of an IUD can be a very rapid procedure, but it does require the mare to be temporarily restrained, such as in a squeeze chute. IUDs in mares may cause, in some cases, physiological effects including discomfort, infection, perforation of the uterus if the IUD is hard

and angular, severe endometritis, uterine edema (Killian et al. 2008), and pyometra (Klabnik-Bradford et al. 2013). However, none of these complications were noted in the study that led to the development of the *Y-Shaped Silicone IUD for Feral Horses*. In women, deaths attributable to IUD use may be as low as 1.06 per million (Daels and Hughes 1995).

The exact mechanism by which IUDs prevent pregnancy is uncertain (Daels and Hughes 1995), but the presence of an IUD in the uterus may prolong the time between estrus in the mare (Turner et al. 2015, Gradil et al. 2019). Some domestic mares did exhibit repeated estrus cycles during the time when they had IUDs (Nie et al. 2003, Killian et al. 2008, Gradil et al. 2019). The main cause for an IUD to not be effective at contraception is its failure to stay in the uterus (Daels and Hughes 1995). As a result, one of the major challenges to using IUDs to control fertility in feral horses is preventing the IUD from being dislodged or otherwise ejected over the course of daily activities, which could include, at times, frequent copulation.

In domestic ponies, Killian et al. (2008) explored the use of three different IUD configurations, including a silastic polymer O-ring with copper clamps, and the “380 Copper T” and “GyneFix” IUDs designed for women. The longest retention time for those three IUD models was seen in the “T” device, which stayed in the uterus of several mares for 3-5 years. Reported contraception rates in that study for IUD-treated mares were 80%, 29%, 14%, and 0% in years 1-4, respectively. The authors surmised that pregnancy resulted after some IUDs fell out of the uterus. Killian et al. (2008) reported high levels of progesterone in non-pregnant, IUD-treated ponies.

At this time, it is thought that any IUD inserted into a pregnant mare could cause the pregnancy to terminate, which may also cause the IUD to be expelled. For that reason, it is expected that the *Y-Shaped Silicone IUD for Feral Horses* would only be inserted in non-pregnant mares. Feral mares receiving IUDs would be checked for pregnancy prior to insertion of an IUD. This can be accomplished by methods such as transrectal palpation and/or ultrasound performed by a veterinarian. Pregnant mares would not receive an IUD. The IUD is inserted into the uterus using a thin, tubular applicator similar to a shielded culture tube, in a manner similar to that routinely used to obtain uterine cultures in domestic mares. If a mare has a zygote or very small, early phase embryo, it is theoretically possible that it will fail to be detected during pregnancy screening, and could develop further without causing the expulsion of the IUD. Feral mares with IUDs should be individually marked and identified, so that they can be monitored occasionally and examined, if necessary, in the future.

A number of studies specifically address the use of other types of IUDs that work in mares exclusively by physical means. Using metallic or glass marbles as IUDs may prevent pregnancy in horses (Nie et al. 2003), but can pose health risks to domestic mares (Turner et al. 2015, Freeman and Lyle 2015). Marbles may break into shards (Turner et al. 2015), and uterine irritation that results from marble IUDs may cause chronic, intermittent colic (Freeman and Lyle 2015). Use of metallic IUDs may lead to severe infection (Klabnik-Bradford et al. 2013). Softer, inert materials such as the medical-grade silicone used in the *Y-Shaped Silicone IUD for Feral Horses* are thought to be far safer, based on experimental studies (Daels and Hughes 1995, Bladighi et al. 2017, Holyoak et al. In press). Daels and Hughes (1995) tested the use of a flexible O-ring IUD, made of silastic, medical-grade polymer, measuring 40 mm in diameter; in five of six breeding domestic mares tested, the IUD was reported to have stayed in the mare for

at least 10 months. In mares with O-ring silicone IUDs, Daels and Hughes (1995) reported some level of uterine irritation, but inferred that the level of irritation was not enough to interfere with a return to fertility after IUD removal.

More recently, several types of soft or flexible IUDs have been tested for use in breeding mares. When researchers attempted to replicate the silicone O-ring IUD study (Daels and Hughes 1995) in a BLM-funded USGS / Oklahoma State University (OSU) with breeding domestic mares, using various configurations of silicone O-ring IUDs, the O-ring design of IUDs fell out at unacceptably high rates over time scales of less than 2 months (Baldrighi et al. 2017). Subsequently, the USGS / OSU researchers tested the *Y-Shaped Silicone IUD for Feral Horses* to determine retention rates and assess effects on uterine health; retention rates were greater than 75% for an 18-month period, and mares returned to good uterine health and reproductive capacity after removal of the IUDs (Holyoak et al. In press). Separately, the University of Massachusetts has developed a plastic-coated IUD that has been effective at prolonging the duration between estrus in non-breeding domestic mares, with over 50 days of diestrus, on average (Gradil 2019). After insertion in the uterus, the three subunits of the University of Massachusetts plastic coated device are held together by magnetic forces as a flexible triangle. A metal detector can be used to determine whether that device is still present in the mare. In an early trial, two sizes of those magnetic IUDs fell out of actively breeding domestic mares at high rates (Holyoak et al., unpublished results). In 2019, the magnetic IUD was used in two trials where mares were exposed to stallions, and in one where mares were artificially inseminated; in all cases, the IUDs were reported to stay in the mares without any pregnancy (Gradil 2019).

Literature Cited

- Baldrighi, J.M., C.C. Lyman, K. Hornberger, S.S. Germaine, A. Kane, and G.R. Holyoak. 2017. Evaluating the efficacy and safety of silicone O-ring intrauterine devices as a horse contraceptive through a captive breeding trial. *Clinical Theriogenology* 9:471.
- Crist, M.R., J.C. Chambers, S.L. Phillips, K.L. Prentice, and L.A. Wiechman, eds. 2019. Science framework for conservation and restoration of the sagebrush biome: Linking the Department of the Interior's Integrated Rangeland Fire Management Strategy to long-term strategic conservation actions. Part 2. Management applications. Gen. Tech. Rep. RMRS-GTR-389. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- Daels, P.F, and J.P. Hughes. 1995. Fertility control using intrauterine devices: an alternative for population control in wild horses. *Theriogenology* 44:629-639.
- Freeman, C.E., and S.K. Lyle. 2015. Chronic intermittent colic in a mare attributed to uterine marbles. *Equine Veterinary Education* 27:469-473.
- Gradil, C. 2019. The Upod IUD: a potential simple, safe solution for long-term, reversible fertility control in feral equids. Oral presentation at the Free Roaming Equids and Ecosystem Sustainability Summit, Reno, Nevada.
- Gradil, C., C.K. Uricchio, and A. Schwartz. 2019. Self-assembling intrauterine device (Upod) modulation of the reproductive cycle in mares. *Journal of Equine Veterinary Science* 83:102690.
- Holyoak, G.R., C.C. Lyman, S.S. Germaine, C.O. Anderson, J.M. Baldrighi, G. Rezabek, S. Wang, and A.J. Kane. In Press. Evaluating the efficacy of a silicone Y-design intrauterine device as a horse contraceptive in a captive breeding trial. *Journal of Wildlife Management*.

- Killian, G., D. Thain, N.K. Diehl, J. Rhyan, and L. Miller. 2008. Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and intrauterine devices. *Wildlife Research* 35:531-539.
- Klabnik-Bradford, J., M.S. Ferrer, C. Blevins, and L. Beard. 2013. Marble-induced pyometra in an Appaloosa mare. *Clinical Theriogenology* 5: 410.
- Nie, G.J., K.E., Johnson, T.D. Braden, and J. G.W. Wenzel. 2003. Use of an intra-uterine glass ball protocol to extend luteal function in mares. *Journal of Equine Veterinary Science* 23:266-273.
- Turner, R.M., D.K. Vanderwall, and R. Stawewski. 2015. Complications associated with the presence of two intrauterine glass balls used for oestrus suppression in a mare. *Equine Veterinary Education* 27:340-343.

7. Starting material inputs or ingredients or specifications used in the operation of the product.

Ingredients in the product must be identified by common name and CAS number if applicable:

The sole ingredients in the IUD manufacture are the components of medical-implant-grade silicone. Specifically, the product used is NuSil MED 4860 with a durometer of shore-A 60. The silicone is cured by heat, and the silicone curing is catalyzed by addition of platinum. Both the silicone and the platinum are chemically inert. After heat / platinum curing, the components become stable, inert medical grade silicone, such as is used in silicone medical implants.

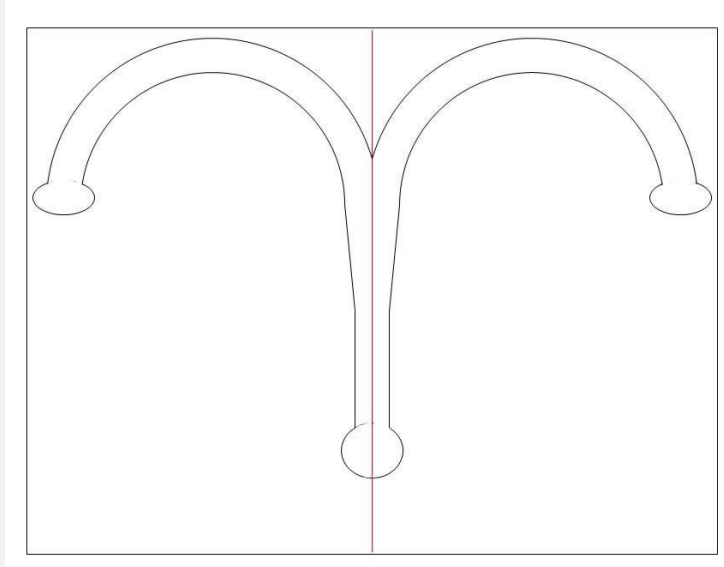
Based on the Safety Data Sheet for NuSil Med 4860, the following table includes the CAS numbers for components of the uncured material.

Name	Product Identifier	%	GHS-US classification
Silamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica	(CAS No) 68909-20-6	20 – 40	Not classified
Siloxanes and Silicones, dimethyl, methyl hydrogen	(CAS No) 68037-59-2	<5	Skin Irrit. 2, H315 Eye Irrit. 2A, H319 STOT SE 3, H335
3-Butyn-2-ol, 2-methyl-	(CAS No) 115-19-5	<1	Flam. Liq. 2, H225 Acute Tox. 4 (Oral), H302 Eye Dam. 1, H318

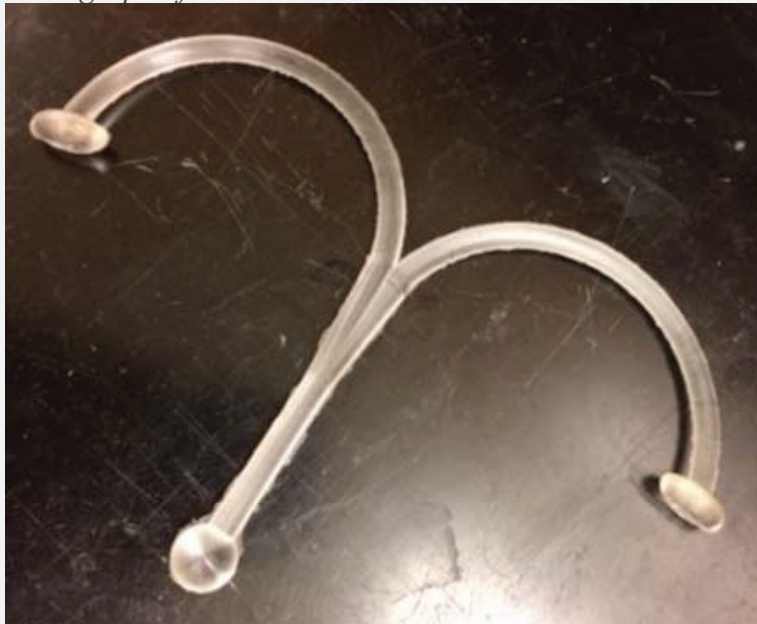
8. Schematic diagram or detailed engineering drawings, diagrams, flow diagrams or patent(s) application information.

The *Y-Shaped Silicone IUD for Feral Horses* is 6 inches wide (the entire width spanning the two curved branches of the “Y”), 4 inches high (from the base of the stem to the maximum height of the curved branches), and 3/8 inches deep (i.e., cylindrical diameter of the stem and branches).

Schematic diagram

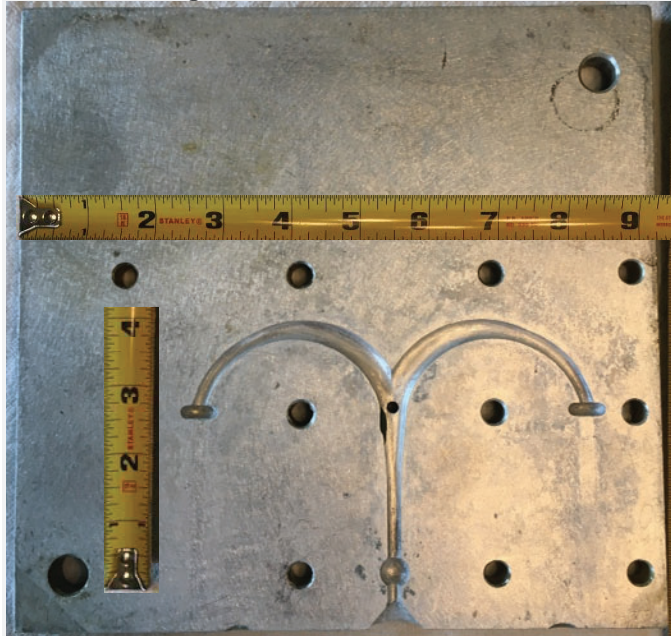


Photograph of IUD



Photograph of aluminum mold used for manufacture (with ruler for scale)

The aluminum mold used for manufacture of the *Y-Shaped Silicone IUD for Feral Horses* is shown below. The picture at left shows half of the 2-part mold, with rulers shown for scale of the width and height. The picture at right shows part of the aluminum molds, with ruler to show scale of the depth.



No patent has been issued or applied for. BLM has no intention to patent the *Y-Shaped Silicone IUD for Feral Horses*. This design of IUD was first developed by BLM-funded researchers at the Oklahoma State University (OSU). The exact funding mechanism was through a BLM-USGS interagency agreement, by which BLM funded the US Geological Survey (USGS) to develop and test silicone IUDs for horses. USGS subsequently used that funding to form a financial assistance agreement with OSU. OSU has submitted no claims for patent rights to the US Patent Office, and has not notified the US Geological Survey or the BLM of any intent to do so, which would be required according to the terms of the USGS-OSU agreement. Furthermore, even if OSU were to pursue patent rights for the *Y-Shaped Silicone IUD for Feral Horses*, the US government retains rights for use of the devices, as specified in the USGS-OSU agreement, section E.3.i.b.1, which states: “With respect to any subject invention in which the recipient retains title, the Federal Government shall have a non-exclusive, nontransferable, irrevocable, paid-up license to practice or have practiced for or on behalf of the U.S. the subject invention throughout the world.” BLM will attempt to attach, as files, the text of the BLM-USGS agreement, and the USGS-OSU agreement.

9. Photographs of the product from all sides, including digital copies available upon request:



10. Comparisons to current industry products:

As far as BLM knows, no other IUDs are specifically made for use to mitigate feral horse population growth rate, although one product has made related claims. It has been common practice on ranches for horse owners to insert foreign objects, such as glass marbles or metal ball bearing, into mare estruses to suppress fertility (i.e., Nie et al. 2003). However, as noted in the literature review above, glass or metallic IUDs can pose health risks to domestic mares (Turner et al. 2015, Freeman and Lyle 2015), so those methods would not be suitable for federally-protected feral horses. The University of Massachusetts has developed a different type of physical IUD for use in horses, which it markets as the iUPOD® (<https://www.iupod.org/iupod>); their web site makes claims about the suitability of that type of IUD to reduce fertility in feral mares. The *Y-Shaped Silicone IUD for Feral Horses* differs from the iUPOD in several ways that BLM is aware of: a) the iUPODs are each a set of three magnetic, ovoid devices, coated in plastic (the exact type of plastic is not known to BLM), while the *Y-Shaped Silicone IUD for Feral Horses* is a single piece of medical-grade silicone; b) no published studies have demonstrated iUPOD effectiveness for multiple breeding seasons in mares that lived and repeatedly copulated with fertile stallions;; and c) BLM has been informed that the University of Massachusetts contracted for the iUPODs to be manufactured in China, while BLM would contract for the manufacture of *Y-Shaped Silicone IUD for Feral Horses* exclusively from American, EPA-registered pesticide device making establishment(s).

11. Provide proof of payment:

The Bureau of Land Management has applied for a fee waiver for processing of this Device Determination request, on the basis of BLM being a federal agency, within the Department of the Interior.

12. Additional Information

The Bureau of Land Management is also attempting to attach a number of additional files with this request. Those include the text of agreements between BLM-USGS, and USGS-Oklahoma State University, to fund the research, as well as the text of a number of scientific papers that are cited in the Supplemental Materials file.

MED-4860 Part A



Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations
Revision date: 09/13/2018

Date of issue: 03/06/2014

Version: 3.0

SECTION 1: Identification

1.1. Product identifier

Product Form Mixture
Product Name MED-4860 Part A
Synonyms Silicone Elastomer

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use of the substance/mixture For professional use only.

1.3. Details of the supplier of the safety data sheet

NuSil Technology LLC
1050 Cindy Lane
Carpinteria, California 93013
USA
(805) 684-8780
ehs@nusal.com
www.nusal.com

1.4. Emergency telephone number

Emergency : 800-424-9300 CHEMTREC (in US); +1 703-527-3887 CHEMTREC (International
number and Maritime)

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

GHS-US classification

Not classified

2.2. Label elements

GHS-US labeling

No labeling applicable

2.3. Other hazards

Other hazards not contributing to the classification None under normal conditions.

2.4. Unknown acute toxicity (GHS US)

No data available

SECTION 3: Composition/Information on ingredients

3.1. Substances

Not applicable

3.2. Mixtures

Name	Product identifier	%	GHS-US classification
Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica	(CAS No) 68909-20-6	20 - 40	Not classified

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Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 4: First aid measures

4.1. Description of first aid measures

First-aid measures general	Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice (show the label where possible).
First-aid measures after inhalation	When symptoms occur: go into open air and ventilate suspected area. Obtain medical attention if breathing difficulty persists.
First-aid measures after skin contact	Remove contaminated clothing. Drench affected area with water for at least 15 minutes. Obtain medical attention if irritation develops or persists.
First-aid measures after eye contact	Rinse cautiously with water for at least 15 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Obtain medical attention.
First-aid measures after ingestion	Rinse mouth. Do NOT induce vomiting. Obtain medical attention.

4.2. Most important symptoms and effects, both acute and delayed

Symptoms/injuries	Not expected to present a significant hazard under anticipated conditions of normal use.
Symptoms/injuries after inhalation	Prolonged exposure may cause irritation.
Symptoms/injuries after skin contact	Prolonged exposure may cause skin irritation.
Symptoms/injuries after eye contact	May cause slight irritation to eyes.
Symptoms/injuries after ingestion	Ingestion may cause adverse effects.
Chronic symptoms	None expected under normal conditions of use.

4.3. Indication of any immediate medical attention and special treatment needed

If exposed or concerned, get medical advice and attention. If medical advice is needed, have product container or label at hand.

SECTION 5: Fire-Fighting measures

5.1. Extinguishing media

Suitable extinguishing media	Water spray, dry chemical, alcohol-resistant foam, carbon dioxide.
Unsuitable extinguishing media	Do not use a heavy water stream. Use of heavy stream of water may spread fire. Application of water stream to hot product may cause frothing and increase fire intensity.

5.2. Special hazards arising from the substance or mixture

Fire hazard	Not considered flammable but may burn at high temperatures.
Explosion hazard	Product is not explosive.
Reactivity	Hazardous reactions will not occur under normal conditions.

5.3. Advice for firefighters

Precautionary measures fire	Exercise caution when fighting any chemical fire. Under fire conditions, hazardous fumes will be present.
Firefighting instructions	Use water spray or fog for cooling exposed containers.

MED-4860 Part A

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

Protection during firefighting

Do not enter fire area without proper protective equipment, including respiratory protection.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

General measures

Avoid prolonged contact with eyes, skin and clothing. Avoid breathing (vapor, mist, spray).

6.1.1. For non-emergency personnel

Protective equipment

Use appropriate personal protective equipment (PPE).

Emergency procedures

Evacuate unnecessary personnel.

6.1.2. For emergency responders

Protective equipment

Equip cleanup crew with proper protection.

Emergency procedures

Upon arrival at the scene, a first responder is expected to recognize the presence of dangerous goods, protect oneself and the public, secure the area, and call for the assistance of trained personnel as soon as conditions permit. Ventilate area.

6.2. Environmental precautions

Prevent entry to sewers and public waters.

6.3. Methods and material for containment and cleaning up

For containment

Contain any spills with dikes or absorbents to prevent migration and entry into sewers or streams.

Methods for cleaning up

Clean up spills immediately and dispose of waste safely. Transfer spilled material to a suitable container for disposal. Contact competent authorities after a spill.

6.4. Reference to other sections

See Section 8 for exposure controls and personal protection and Section 13 for disposal considerations.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Precautions for safe handling

Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Avoid prolonged contact with eyes, skin and clothing. Avoid breathing vapors, mist, spray.

Hygiene measures

Handle in accordance with good industrial hygiene and safety procedures.

7.2. Conditions for safe storage, including any incompatibilities

Technical measures

Comply with applicable regulations.

Storage conditions

Keep container closed when not in use. Store in a dry, cool place. Keep/Store away from direct sunlight, extremely high or low temperatures and incompatible materials.

Incompatible products

Strong acids, strong bases, strong oxidizers.

7.3. Specific end use(s)

For professional use only.

MED-4860 Part A

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

For substances listed in section 3 that are not listed here, there are no established exposure limits from the manufacturer, supplier, importer, or the appropriate advisory agency including: ACGIH (TLV), or OSHA (PEL).

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica (68909-20-6)		
USA OSHA	OSHA PEL (TWA) (mg/m ³)	6 mg/m ³
USA OSHA	OSHA PEL (TWA) (ppm)	20 mppcf (80mg/m ³ /%SiO ₂)

8.2. Exposure controls

Appropriate engineering controls

Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure. Ensure adequate ventilation, especially in confined areas. Ensure all national/local regulations are observed. Gloves. Protective clothing. Protective goggles.

Personal protective equipment



Materials for protective clothing

Chemically resistant materials and fabrics.

Hand protection

Wear protective gloves.

Eye protection

Chemical safety goggles.

Skin and body protection

Wear suitable protective clothing.

Respiratory protection

If exposure limits are exceeded or irritation is experienced, approved respiratory protection should be worn. In case of inadequate ventilation, oxygen deficient atmosphere, or where exposure levels are not known wear approved respiratory protection.

Other information

When using, do not eat, drink or smoke.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	: Liquid
Appearance	: Colorless
Odor	: Odorless
Odor threshold	: No data available
pH	: No data available
Evaporation Rate	: No data available
Melting point	: No data available
Freezing point	: No data available
Boiling point	: No data available
Flash point	: > 135 °C (> 275 °F)
Auto-ignition Temperature	: No data available
Decomposition temperature	: No data available
Flammability (solid, gas)	: No data available
Vapor pressure	: No data available
Relative vapor density at 20 °C	: No data available

MED-4860 Part A

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

Specific gravity	: > 1
Solubility	: No data available
Partition coefficient: n-octanol/water	: No data available
Viscosity	: No data available

9.2. Other information

VOC content < 1 %

SECTION 10: Stability and reactivity

10.1. Reactivity

Hazardous reactions will not occur under normal conditions.

10.2. Chemical stability

Stable under recommended handling and storage conditions (see section 7).

10.3. Possibility of hazardous reactions

Hazardous polymerization will not occur.

10.4. Conditions to avoid

Direct sunlight, extremely high or low temperatures, and incompatible materials.

10.5. Incompatible materials

Strong acids, strong bases, strong oxidizers.

10.6. Hazardous decomposition products

Silicon oxides. Carbon oxides (CO, CO₂). Will decompose above 150 °C (> 300 °F) releasing formaldehyde vapors. Formaldehyde is a potential carcinogen and can act as a potential skin and respiratory sensitizer. Formaldehyde can also cause respiratory and eye irritation.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Acute toxicity	Not classified
Skin corrosion/irritation	Not classified
Serious eye damage/irritation	Not classified
Respiratory or skin sensitization	Not classified
Germ cell mutagenicity	Not classified
Carcinogenicity	Not classified
Reproductive toxicity	: Not classified
STOT-single exposure	: Not classified
STOT-repeated exposure	: Not classified
Aspiration hazard	Not classified
Symptoms/injuries after inhalation	Prolonged exposure may cause irritation.
Symptoms/injuries after skin contact	Prolonged exposure may cause skin irritation.
Symptoms/injuries after eye contact	May cause slight irritation to eyes.
Symptoms/injuries after ingestion	Ingestion may cause adverse effects.
Chronic symptoms	None expected under normal conditions of use.

MED-4860 Part A

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 12: Ecological information

12.1. Toxicity

No additional information available

12.2. Persistence and degradability

MED-4860 Part A

Persistence and degradability	Not established.
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12.3. Bioaccumulative potential

MED-4860 Part A

Bioaccumulative potential	Not established.
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12.4. Mobility in soil

No additional information available

12.5. Other adverse effects

Other information	Avoid release to the environment.
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SECTION 13: Disposal considerations

13.1. Waste treatment methods

Product/Packaging disposal recommendations	Dispose of contents/container in accordance with local, regional, national, and international regulations.
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Ecology - waste materials	Avoid release to the environment.
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SECTION 14: Transport information

In accordance with DOT / IMDG / IATA

14.1. UN number

Not applicable

14.2. UN proper shipping name

Not applicable

14.3. Additional information

Other information	No supplementary information available.
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Transport by sea

No additional information available

Air transport

No additional information available

SECTION 15: Regulatory information

15.1. US Federal regulations

All components of this product are listed or exempted from being listed on the United States Environmental Protection Agency Toxic Substances Control Act (TSCA) inventory.

15.2. US State regulations

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica (68909-20-6)

U.S. - Texas - Effects Screening Levels - Long Term

U.S. - Texas - Effects Screening Levels - Short Term
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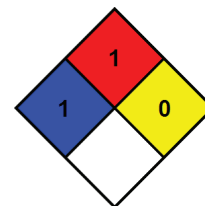
MED-4860 Part A

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 16: Other information, including date of preparation or last revision

Revision date	09/13/2018
Other information	This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200.
NFPA health hazard	1 - Exposure could cause irritation but only minor residual injury even if no treatment is given.
NFPA fire hazard	1 - Must be preheated before ignition can occur.
NFPA reactivity	0 - Normally stable, even under fire exposure conditions, and are not reactive with water.
HMIS III Rating	
Health	1 Slight Hazard - Irritation or minor reversible injury possible
Flammability	1 Slight Hazard
Physical	0 Minimal Hazard



The information provided in this Safety Data Sheet (SDS) was prepared based on data believed to be accurate as of the date of this SDS. TO THE GREATEST EXTENT PERMITTED BY LAW, NUSIL TECHNOLOGY LLC AND ITS AFFILIATED COMPANIES ("NUSIL") EXPRESSLY DISCLAIMS ANY AND ALL REPRESENTATIONS AND WARRANTIES REGARDING THE INFORMATION CONTAINED HEREIN INCLUDING, WITHOUT LIMITATION, AS TO ACCURACY, COMPLETENESS, FITNESS FOR PURPOSE OR USE, MERCHANTABILITY, NON-INFRINGEMENT, PERFORMANCE, SAFETY, SUITABILITY AND STABILITY. This SDS is intended as a guide to the appropriate use, handling, storage and disposal of the product to which it relates by properly trained personnel, and is not intended to be comprehensive. Users of NuSil's products are advised to perform their own tests and to exercise their own judgment to determine the safety, suitability and appropriate use, handling, storage and disposal of each product and product combination for their own purposes and uses. TO THE GREATEST EXTENT PERMITTED BY LAW, NUSIL DISCLAIMS LIABILITY FOR, AND BY USING NUSIL'S PRODUCTS PURCHASER AGREES THAT UNDER NO CIRCUMSTANCES SHALL NUSIL BE LIABLE FOR, SPECIAL, INDIRECT, INCIDENTAL, PUNITIVE OR CONSEQUENTIAL DAMAGES OF ANY TYPE OR KIND, INCLUDING WITHOUT LIMITATION, FOR LOSS OF PROFITS, REPUTATIONAL DAMAGE, PRODUCT RECALL OR BUSINESS INTERRUPTION.

Nusil US GHS SDS

MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations
Revision date: 09/19/2018

Date of issue: 12/20/2013

Version: 3.0

SECTION 1: Identification

1.1. Product identifier

Product Form Mixture
Product Name MED-4860 Part B
Synonyms Silicone Elastomer

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use of the substance/mixture For professional use only.

1.3. Details of the supplier of the safety data sheet

NuSil Technology LLC
1050 Cindy Lane
Carpinteria, California 93013
USA
(805) 684-8780
ehs@nusil.com
www.nusil.com

1.4. Emergency telephone number

Emergency number : 800-424-9300 CHEMTREC (in US); +1 703-527-3887 CHEMTREC (International and Maritime)

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

GHS-US classification

Not classified

2.2. Label elements

GHS-US labeling

No labeling applicable

2.3. Other hazards

Other hazards not contributing to the classification None under normal conditions.

2.4. Unknown acute toxicity (GHS US)

No data available

SECTION 3: Composition/Information on ingredients

3.1. Substances

Not applicable

3.2. Mixtures

Name	Product identifier	%	GHS-US classification
Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica	(CAS No) 68909-20-6	20 – 40	Not classified
Siloxanes and Silicones, dimethyl, methyl hydrogen	(CAS No) 68037-59-2	<5	Skin Irrit. 2, H315 Eye Irrit. 2A, H319 STOT SE 3, H335

MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

3-Butyn-2-ol, 2-methyl-	(CAS No) 115-19-5	< 1	Flam. Liq. 2, H225 Acute Tox. 4 (Oral), H302 Eye Dam. 1, H318
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SECTION 4: First aid measures

4.1. Description of first aid measures

First-aid measures general	Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice (show the label where possible).
First-aid measures after inhalation	When symptoms occur: go into open air and ventilate suspected area. Obtain medical attention if breathing difficulty persists.
First-aid measures after skin contact	Remove contaminated clothing. Drench affected area with water for at least 15 minutes. Obtain medical attention if irritation develops or persists.
First-aid measures after eye contact	Rinse cautiously with water for at least 15 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Obtain medical attention.
First-aid measures after ingestion	Rinse mouth. Do NOT induce vomiting. Obtain medical attention.

4.2. Most important symptoms and effects, both acute and delayed

Symptoms/injuries	Not expected to present a significant hazard under anticipated conditions of normal use.
Symptoms/injuries after inhalation	Prolonged exposure may cause irritation.
Symptoms/injuries after skin contact	Prolonged exposure may cause skin irritation.
Symptoms/injuries after eye contact	May cause slight irritation to eyes.
Symptoms/injuries after ingestion	Ingestion may cause adverse effects.
Chronic symptoms	None expected under normal conditions of use.

4.3. Indication of any immediate medical attention and special treatment needed

If exposed or concerned, get medical advice and attention. If medical advice is needed, have product container or label at hand.

SECTION 5: Fire-Fighting measures

5.1. Extinguishing media

Suitable extinguishing media	Water spray, dry chemical, alcohol-resistant foam, carbon dioxide.
Unsuitable extinguishing media	Do not use a heavy water stream. Use of heavy stream of water may spread fire. Application of water stream to hot product may cause frothing and increase fire intensity.

5.2. Special hazards arising from the substance or mixture

Fire hazard	Not considered flammable but may burn at high temperatures.
Explosion hazard	Product is not explosive.
Reactivity	Hazardous reactions will not occur under normal conditions.

MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

5.3. Advice for firefighters

Precautionary measures fire

Exercise caution when fighting any chemical fire. Under fire conditions, hazardous fumes will be present.

Firefighting instructions

Use water spray or fog for cooling exposed containers.

Protection during firefighting

Do not enter fire area without proper protective equipment, including respiratory protection.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

General measures

Avoid prolonged contact with eyes, skin and clothing. Avoid breathing (vapor, mist, spray).

6.1.1. For non-emergency personnel

Protective equipment

Use appropriate personal protective equipment (PPE).

Emergency procedures

Evacuate unnecessary personnel.

6.1.2. For emergency responders

Protective equipment

Equip cleanup crew with proper protection.

Emergency procedures

Upon arrival at the scene, a first responder is expected to recognize the presence of dangerous goods, protect oneself and the public, secure the area, and call for the assistance of trained personnel as soon as conditions permit. Ventilate area.

6.2. Environmental precautions

Prevent entry to sewers and public waters.

6.3. Methods and material for containment and cleaning up

For containment

Contain any spills with dikes or absorbents to prevent migration and entry into sewers or streams.

Methods for cleaning up

Clean up spills immediately and dispose of waste safely. Transfer spilled material to a suitable container for disposal. Contact competent authorities after a spill.

6.4. Reference to other sections

See Section 8 for exposure controls and personal protection and Section 13 for disposal considerations.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Precautions for safe handling

Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Avoid prolonged contact with eyes, skin and clothing. Avoid breathing vapors, mist, spray.

Hygiene measures

Handle in accordance with good industrial hygiene and safety procedures.

7.2. Conditions for safe storage, including any incompatibilities

Technical measures

Comply with applicable regulations.

Storage conditions

Keep container closed when not in use. Store in a dry, cool place. Keep/Store away from direct sunlight, extremely high or low temperatures and incompatible materials.

Incompatible products

Strong acids, strong bases, strong oxidizers.

7.3. Specific end use(s)

For professional use only.

MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

For substances listed in section 3 that are not listed here, there are no established exposure limits from the manufacturer, supplier, importer, or the appropriate advisory agency including: ACGIH (TLV), or OSHA (PEL).

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica (68909-20-6)		
USA OSHA	OSHA PEL (TWA) (mg/m ³)	6 mg/m ³
USA OSHA	OSHA PEL (TWA) (ppm)	20 mppcf (80mg/m ³ /%SiO ₂)

8.2. Exposure controls

Appropriate engineering controls

Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure. Ensure adequate ventilation, especially in confined areas. Ensure all national/local regulations are observed.

Personal protective equipment

Gloves. Protective clothing. Protective goggles.



Materials for protective clothing

Chemically resistant materials and fabrics.

Hand protection

Wear protective gloves.

Eye protection

Chemical safety goggles.

Skin and body protection

Wear suitable protective clothing.

Respiratory protection

If exposure limits are exceeded or irritation is experienced, approved respiratory protection should be worn. In case of inadequate ventilation, oxygen deficient atmosphere, or where exposure levels are not known wear approved respiratory protection.

Other information

When using, do not eat, drink or smoke.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	: Liquid
Appearance	: Colorless
Odor	: Odorless
Odor threshold	: No data available
pH	: No data available
Evaporation Rate	: No data available
Melting point	: No data available
Freezing point	: No data available
Boiling point	: No data available
Flash point	: > 135 °C (> 275 °F)
Auto-ignition Temperature	: No data available
Decomposition temperature	: No data available
Flammability (solid, gas)	: No data available
Vapor pressure	: No data available
Relative vapor density at 20 °C	: No data available

MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

Specific gravity	: > 1
Solubility	: No data available
Partition coefficient: n-octanol/water	: No data available
Viscosity	: No data available

9.2. Other information

VOC content < 1 %

SECTION 10: Stability and reactivity

10.1. Reactivity

Hazardous reactions will not occur under normal conditions.

10.2. Chemical stability

Stable under recommended handling and storage conditions (see section 7).

10.3. Possibility of hazardous reactions

Hazardous polymerization will not occur.

10.4. Conditions to avoid

Direct sunlight, extremely high or low temperatures, and incompatible materials.

10.5. Incompatible materials

Strong acids, strong bases, strong oxidizers.

10.6. Hazardous decomposition products

Silicon oxides. Carbon oxides (CO, CO₂). Will decompose above 150 °C (> 300 °F) releasing formaldehyde vapors. Formaldehyde is a potential carcinogen and can act as a potential skin and respiratory sensitizer. Formaldehyde can also cause respiratory and eye irritation.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Acute toxicity	Not classified
Skin corrosion/irritation	Not classified
Serious eye damage/irritation	Not classified
Respiratory or skin sensitization	Not classified
Germ cell mutagenicity	Not classified
Carcinogenicity	Not classified
Reproductive toxicity	: Not classified
STOT-single exposure	: Not classified
STOT-repeated exposure	: Not classified
Aspiration hazard	Not classified
Symptoms/injuries after inhalation	Prolonged exposure may cause irritation.
Symptoms/injuries after skin contact	Prolonged exposure may cause skin irritation.
Symptoms/injuries after eye contact	May cause slight irritation to eyes.
Symptoms/injuries after ingestion	Ingestion may cause adverse effects.
Chronic symptoms	None expected under normal conditions of use.

MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 12: Ecological information

12.1. Toxicity

Ecology – general Not classified

12.2. Persistence and degradability

MED-4860 Part B

Persistence and degradability	Not established.
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12.3. Bioaccumulative potential

MED-4860 Part B

Bioaccumulative potential	Not established.
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12.4. Mobility in soil

No additional information available

12.5. Other adverse effects

Other information Avoid release to the environment.

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Product/Packaging disposal Dispose of contents/container in accordance with local, regional, national, and international regulations.

Ecology - waste materials Avoid release to the environment.

SECTION 14: Transport information

In accordance with DOT / IMDG / IATA

14.1. UN number

Not applicable

14.2. UN proper shipping name

Not applicable

14.3. Additional information

Other information No supplementary information available.

Transport by sea

No additional information available

Air transport

No additional information available

SECTION 15: Regulatory information

15.1. US Federal regulations

All components of this product are listed or exempted from being listed on the United States Environmental Protection Agency Toxic Substances Control Act (TSCA) inventory.

15.2. US State regulations

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica (68909-20-6)
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U.S. - Texas - Effects Screening Levels - Long Term

U.S. - Texas - Effects Screening Levels - Short Term
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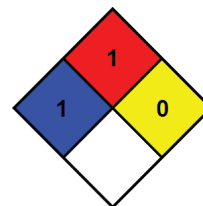
MED-4860 Part B

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

SECTION 16: Other information, including date of preparation or last revision

Revision date	09/13/2018
Other information	This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200.
NFPA health hazard	1 - Exposure could cause irritation but only minor residual injury even if no treatment is given.
NFPA fire hazard	1 - Must be preheated before ignition can occur.
NFPA reactivity	0 - Normally stable, even under fire exposure conditions, and are not reactive with water.
HMIS III Rating	
Health	1 Slight Hazard - Irritation or minor reversible injury possible
Flammability	1 Slight Hazard
Physical	0 Minimal Hazard



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Nusil US GHS SDS



United States Department of the Interior
Bureau of Land Management
National Wild Horse and Burro Program
1340 Financial Boulevard, Reno, Nevada 89502



August 18, 2020

TO: U.S. Environmental Protection Agency
Document Processing Desk
Office of Pesticide Programs (7504P)
Registration Division, IVB3
1200 Pennsylvania Avenue, N.W.
Washington DC 20460-0001

RE: Request for Federal Exemption from PRIA Registration Service Fees, for Device Determination Request (PRIA code M009); “Y-Shaped Silicone IUD for Feral Horses”

Dear EPA Office of Pesticide Programs Staff,

I request an exemption from PRIA registration service fees, for the Bureau of Land Management (BLM) Device Determination request for “Y-Shaped Silicone IUD for Feral Horses.”

The BLM (www.blm.gov) is a federal land management agency within the U.S. Department of the Interior. BLM recently registered with the EPA under company number 94979.

The physical address associated with BLM’s company number (94979) is 1340 Financial Boulevard, Reno, Nevada, 89502. That address is the location of the headquarters of the BLM’s Wild Horse and Burro (WHB) Program (www.blm.gov/programs/wild-horse-and-burro). BLM’s WHB program is responsible for management of the majority of wild horses and burros in the western USA, as directed by federal law (Wild Free-Roaming Horses and Burros Act of 1971, as amended). I (Paul Griffin) am the agency contact with EPA for the WHB program within BLM, and for the Device Determination request. My title is Research Coordinator for the WHB program. My email is pgriffin@blm.gov, and my phone number is 970-631-4808. An alternate agency contact is my supervisor, Alan Shepherd, On-Range Branch Chief for the WHB program; his email is ashepher@blm.gov.

Please contact me if you have any questions about this fee exemption request. Thank you very much for all your guidance and help.

Sincerely,

Paul Griffin



United States Department of the Interior
United States Geological Survey
Biological Resources Division

Fort Collins Science Center
2150 Centre Avenue, Building C
Fort Collins, CO 80526-8118

June 18, 2014

To: Holle' Hooks
Bureau of Land Management
20 M. Street SE
Washington, DC 20003

From: David B. Hamilton, Director
USGS, BRD, Fort Collins Science Center
Fort Collins, Colorado 80526-8118

Subject: Agreement Number 4500065781, Science Assistance to the Bureau of Land Management
Wild Horse and Burro Program

On behalf of the U.S. Geological Survey, I am pleased to accept Reimbursable Agreement Number 4500065781, in the amount of \$3,264,000.00. We understand the performance period for this agreement is from June 16, 2014 through June 15, 2019. The Principal Investigator is Zack Bowen . His contact information is: USGS, FORT, 2150 Centre Avenue, Building C, Fort Collins, Colorado 80526, Phone: 970-226-9218, Email: bowenz@usgs.gov

Attached is one copy of the fully executed agreement. The administrative contact for this agreement is Andrea Pegg, USGS, FORT, 2150 Centre Avenue, Building C, Fort Collins, CO 80526-8118, Phone: (970) 226-9171, e-mail: pegga@usgs.gov .

Sincerely,

David B. Hamilton
Center Director

Enclosure

cc: Zack Bowen, Branch Chief
Andrea Pegg



UNITED STATES DEPARTMENT OF THE INTERIOR

INTER/INTRA-AGENCY AGREEMENT (IAA)

I. Period of Performance



START	END
06/16/2014	06/15/2019

Buyer has work performed for them by the Seller named in item 6b.

Seller to perform work as described herein for the agency named in item 6a.

SEE INSTRUCTIONS ON PAGE 2

2. Common Document Number (Agreement Number)

4500065781

3. Check appropriate box

☒ Original

☐ Modification No. 001

4. Under the authority of (Cite authorities):

☐ 43 U.S.C. 1701 et seq., (FLPMA)

☒ Department of the Interior Appropriation Act for FY

☐ 31 U.S.C. 1535 (the Economy Act)

☐ Working Capital Fund (WCF)

☐ Southern Nevada Public Land Management Act

5. Description of Work: See attached IGO Articles

PROJECT TITLE: Science Assistance to the Bureau of Land Management
Wild Horse and Burro Program

Buyer	Seller
6a. Bureau of Land Management Agency: Address: 20 M. Street SE, Washington DC 20003 Finance POC: Holle' Hooks, Budget Officer Email: hhooks@blm.gov Phone: (405) 791-1066 Fax: (405) 790-1056 Technical Point of Contact: Dean Bolstad, Senior WHB Advisor Email: dbolstad@blm.gov Phone: (202) 912-7297 Fax: (202) 912-7182	6b. Agency: United States Geological Survey Address: 2150 Centre Ave. Building C Fort Collins, CO 80526-8118 Finance POC: Sheri Bishop-Cotner Email: bishopcotners@usgs.gov Phone: (970) 226-9293 Fax: (970) 226-9230 Technical Point of Contact: Zachary H. Bowen Email: bowenz@usgs.gov Phone: (970) 226-9218 Fax: (970) 226-9230

ACCOUNT DATA

BUYER

SELLER

7. Agency Location Code	7a. 14-11-0008	7b. 14-08-001
8. BPN Number (DUNS #) FSN	8a. 926038563	8b. 130900488
9. Treasury Account Symbol (TAFS)	9a. 14X1109	9b. 144/50804
10. Standard General Ledger	10a.	10b.
11. Cost Structure/Account	11a. 14XL1109AF LLWO260000 L10600000 PC0000 LXSINASR0000	11b.
12. Business Event Type Code	12a. DISB	12b. COLL
13. Requisition Number for Buyer/Project Account for Seller	13a. 4500065781	13b.
14. Contract Line Number for Buyer/Proposal Number or other data for Seller	14a.	14b.
15. Buyer provide Expiration of Funding Source (Date or indefinite)	15a.	15b. NOTE: Seller, ensure project completion by this date (Seller must not incur additional costs) See Block 15a

16. Amount Obligated by Buyer

a. Initial or current obligation: \$3,264,000

b. Modification Amount (check one)

☐ Increase

☐ Decrease

c. Total obligation:

17. Bill To (Name and Address, including zip code of **Finance Office**):

Name: Bureau of Land Management

Address: Bldg 50, PO Box 25047, Denver Federal Center
Denver, CO 80225

18. Billing for Federal Agencies and DOD will be processed via IPAC. (billing will be done ☐ bi-weekly ☒ monthly ☐ quarterly ☐ in advance)

Upon Approval, this agreement constitutes an obligation against Buyer requesting the work; or authority to proceed with work by Seller for the herein named agency in anticipation of reimbursement.

19. Approved for Buyer:

(Contracting Officer or other Authorized Signature) *other only for WCF

20. Approved by Seller:

(Seller's Authorizing Signature)

19a. Name (Type): Holle' Hooks

20a. Name (Type): David B. Hamilton

19b. Title: Wild Horse and Burro Specialist/ Budget

19c. Date: 6/17/14

20b. Title: Center Director

20c. Date: 6/18/14

INTRA-GOVERNMENTAL ORDER (IGO) ARTICLES

I. PROJECT TITLE:

Science Assistance to the Bureau of Land Management (BLM) Wild Horse and Burro Program

II. OBJECTIVE:

The purpose of this IGO is to effectively and conveniently acquire a broad range of scientific assistance to augment BLM's efficiency in responding to management issues associated with wild horses and burros. The abilities and skills acquired from the United States Geological Survey (USGS) in conducting environmental and ecological research and technical assistance will assist the BLM in providing timely resolution of technical requests, promote prompt management decisions and effective progression of project schedules, and support the overall mission of the BLM.

III. STATEMENT OF WORK:

The BLM needs technical assistance to complete scientific studies to support wild horse and burro management. Potential topics include population estimation, population growth suppression, population demography, genetics, habitat use, estimation of carrying capacity, assessment of environmental effects of wild horses and burros and other grazers, ecological assessments, socioeconomic analyses, population models, and data resources for planning. Because of the complexity and diversity of problems encountered in these areas, this IGO requests services from the USGS in a broad range of possible disciplines. These services may include, but are not limited to: environmental sampling; ecological assessments; modeling; population demography assessments; population surveys; monitoring design; biological surveys; laboratory analyses; office assistance for collating and interpretation of data and technical advisory roles; coordination and facilitation of scientific reviews; Global Positioning Systems (GPS) and Geographic Information Systems (GIS) support; and photogrammetry and photo interpretation.

Specific tasks envisioned at the time of this agreement may include but will not be limited to the following:

1. SpayVac pasture study
2. SpayVac study in free-ranging herd(s)
3. Sentinel population demographic studies (3 to 5 Herd Management Areas)
4. Fecal analysis study to estimate population size, genetic diversity, and the degree to which wild horses and burros disperse invasive weed seeds
5. Radio marking for tracking individual animals
6. Ovariectomy pen study
7. Ovariectomy study in free-ranging herd(s)
8. Wild burro population estimation
9. Develop a model to project costs, benefits, and population growth for various management alternatives
10. Methods and protocols for differentiating wild horse and burro environmental effects from those of livestock and wildlife
11. Modeling for wild horse carrying capacity

Other tasks that are not specifically identified above but that are supportive of the BLM Wild Horse and Burro Program, including recommendations contained in the June 2013 National Academy of Sciences Report, may be ordered under this agreement.

IGO ARTICLES

For research studies, the USGS will provide the BLM with individual, project-specific proposals utilizing the BLM Research Proposal Format (Attachment 1). For projects that are not research studies, the USGS will provide the BLM with a Statement of Work. The USGS and the BLM will collaborate to develop these documents. Research proposals and statements of work will provide detailed descriptions, performance periods, and associated costs for each task that is ordered under this IGO as the work is identified and before it is started. No work will proceed nor will charges be made against this agreement until the research proposal or a statement of work is approved in writing by BLM.

A. The USGS agrees to:

1. Provide the requested experienced personnel, at the appropriate skill level capable of effectively achieving a BLM work request or technical task.
2. Provide deliverables, including summary reports or memoranda outlining the progress of specific tasks and projects. These progress reports will describe project progress, transfer data files, outline upcoming efforts, defined schedules, describe problems encountered, and detail labor hours used and other project-related costs. Specific deliverables will be described in individual statements of work or research proposals.
3. Supply any additional equipment as necessary in order to complete specific tasks outlined in this Inter-Governmental Order (IGO).
4. Submit results of scientific studies to peer-reviewed journals or other appropriate outlets for publication.

Note: Responsible official for the USGS is to ensure all personnel who work on this project and any expenses incurred are charged to the account classification number identified in the IGO.

B. The BLM agrees to:

1. Work with the USGS on a case-by-case basis to agree to a reasonable schedule for the implementation and completion of a specific work request.
2. Make specific and unique surveying and geophysical instruments (e.g., mapping and survey GPS systems) available to the USGS when required and as available.
3. Provide, upon request, the in-house expertise to assist USGS in identifying and study areas, data collection, coordination with local BLM offices, and general support.

IV. REPORTS:

Annual progress reports will outline progress on each project, results, transfer data files, outline upcoming efforts, schedules or problems encountered as well as labor hours used and other project-related costs. These reports will be submitted to the BLM Technical Contact in the form of two hard copies and one electronic copy by 31 December of each year.

V. AVAILABILITY OF APPROPRIATED FUNDS

The ability of the parties to carry out their responsibilities under this IGO is subject to their respective funding procedures and the availability of appropriated funds. Should either party encounter budgetary problems in the course of its respective internal procedures which may affect the activities to be carried out under this IGO, that party will notify the other party in writing in a timely manner.

Available funding provided by the BLM under this IGO is no year funding.

IGO ARTICLES

VI. ADVANCE FUNDING (ADVANCE PAYMENT)

There is no provision for advanced funding within this IGO.

VII. SETTLEMENT OF DISPUTES

The parties under this IGO are responsible for resolving any disputes that may arise within 30 business days of the billing date. If the dispute cannot be resolved within this period, then the matter will be referred the following business day to the Department of Interior Financial Management Office (PFM).

VIII. FINANCIAL ARRANGEMENTS (PAYMENT):

This IGO is not to exceed the amount as stated herein [\$7,000,000.00] inclusive of all Modifications. The charges for goods/services will include both direct and indirect costs applicable to this IGO. The USGS will submit their billing through the Intra-governmental Payment and Collection (IPAC) system or the Intra-governmental Transaction Portal – whichever is applicable. The bill will reference the BLM's Dun & Bradstreet Number, the Requesting Agency Location Code (ALC), the Treasury Account Symbol, the Accounting Classification Reference Code(s), the Obligating Document Number, a brief description of the service performed, the Accounts Payable POC name and phone number, and identify the amount of money being billed for each project on every invoice.

A copy of the supporting documentation will be forwarded to the BLM initiating office when the bill is prepared. Payments will be made monthly.

The BLM shall not be obligated to pay for, nor will the USGS be obligated to perform any effort that will require the expenditure of Federal funds above the amount obligated.

The USGS agrees to bill prior to the 25th day of each month. The USGS will not issue any bills during the last five days of the month.

IX. TERMS OF IGO:

This IGO shall become effective upon signature by both parties (BLM and USGS) and shall remain in effect until five years after initiation unless terminated in accordance with paragraph VII. The effective date will be determined based upon the last party who signed and dated the IGO.

X. TERMINATION:

This IGO may be terminated by either party upon 30 days written notice. If the IGO is cancelled by the BLM, the USGS will be reimbursed for costs incurred prior to cancellation, plus any termination costs. All costs claimed by the USGS must be itemized and furnished to the BLM.

XI. MODIFYING THE IGO:

Either party under this IGO may propose to make changes under this IGO by notifying the other party in writing. All changes under this IGO must be modified and agreed upon by both parties in writing.

IGO ARTICLES

XII. POINTS OF CONTACT:

Changes to the Points of Contact identified below may be made by written notification to each of the parties under this IGO.

REQUESTING AGENCY (BLM)

IGO Technical Contact:

Name: Dean Bolstad
Title: Natural Resources P.M.
Address: 20 M Street, SE, Room 2134LM
Washington, DC 20003
Phone: (202) 912-7297
Fax: (202) 912-7182
Email: dbolstad@blm.gov

Budget Contact:

Name: Holle' Hooks
Title: Budget Officer
Address: BLM – Moore, OK
221 North Service Rd
Moore, OK 73160-4946
Phone: (405) 791-1066
Fax: (405) 790-1056
Email: hhooks@blm.gov

Payment Contact:

Name: Ashley Whitham
Title: Accounting Technician
Address: BLM, DFC, Bldg. 50
PO Box 25047, Lakewood, CO 80225
Phone: (303) 236-6614
Fax: (303) 236-6412
Email: hudson@blm.gov

SERVICING AGENCY (USGS)

IGO Technical Contact:

Name: Zachary H. Bowen
Title: Chief, Ecosystem Dynamics
Address: USGS- FORT
Phone: 970-226-9218
Fax: 970-226-9230
Email: bowenz@usgs.gov

Budget Contact:

Name: Sheri Bishop-Cotner
Title: Budget Analyst
Address: USGS-FORT
Phone: 970-226-9293
Fax: 970-226-9230
Email: bishop-cotners@usgs.gov

Billing Contact:

Name: Andrea Pegg
Title: Finance Specialist
Address: USGS-FORT
Phone: 970-226-9171
Fax: 970-226-9230
Email: pegga@usgs.gov

COOPERATIVE AGREEMENT NUMBER G16AC00050 BETWEEN

THE UNITED STATES GEOLOGICAL SURVEY

AND

OKLAHOMA STATE UNIVERSITY

OFFER AND ACCEPTANCE:

The United States of America, acting by and through the USGS, hereby offers a Cooperative Agreement to Oklahoma State University for all approved costs up to and not exceeding \$121,269.20 (See B.1) for support described herein. Acceptance of a Federal Financial Assistance award from the Department of the Interior (DOI) carries with it the responsibility to be aware of and comply with the terms and conditions of award. Acceptance is defined as the start of work, drawing down funds, or accepting the award by signature or electronic means. Awards are based on the application submitted to and approved by DOI and are subject to the terms and conditions incorporated either directly or by reference below.

SECTION A – PROJECT DESCRIPTION

A.1 Project Description

The goal of this research is to evaluate the effectiveness of silicone intrauterine contraceptive devices (hereafter IUDs) in horses through a captive breeding trial.

Specific study objectives are as follows.

- 1) Quantify the effectiveness of IUDs in preventing pregnancy over 3 breeding seasons post-insertion, with mares exposed to fertile stallions in a pasture setting throughout each breeding season.
- 2) Estimate the incidence of adverse reactions to IUDs over 3 breeding seasons post insertion in mares. Will compare reactions (ovarian function and endometrial inflammation, infection, and fibrosis) between treated animals and untreated controls.
- 3) Document the relationship between fibrosis severity and pregnancy rate following removal of IUDs in a 4th breeding season.

A.2 USGS Involvement

Substantial involvement is anticipated through the terms of this Agreement between the USGS and the Recipient. A summary of the involvement is as follows: Given the leadership of USGS scientists in the field of ungulate population ecology, specifically feral horse and burro ecology, USGS researchers will be directly involved in all aspects of hypothesis generation, data gathering, statistical evaluation, interpretation and presentation/publication of results. USGS scientists provide complimentary expertise to that of outside collaborators, including long-standing relations with stakeholders such as the Bureau of Land Management and the U.S. Forest Service, who will use these results.

The recipient, USGS researchers, and other collaborators may alternately lead analyses focused on different parts of this work, but we anticipate that in all cases those analyses will be collaborative in nature. The USGS will also institutionally provide sample storage space at the Fort Collins Science Center, some administrative support, and genetic and other lab analyses if needed. All final products will involve the recipient, USGS researchers, and others collaborators as authors.

SECTION B – FUNDING AND AWARD PERIODS

B.1 Funding

- a) The total estimated cost of the USGS share for the performance of this Agreement is **\$486,939** inclusive of any renewal years.
- b) The amount of federal funds obligated under this Agreement, presently the sum of **\$121,269.20**, shall be available for payment of costs incurred by the Recipient in performance of this Agreement from the effective **March 15, 2016**, through **January 15, 2017**. In no event shall costs be incurred in performance of this Agreement in excess of the funds currently obligated.

B.2 Award Periods

The initial budget period is from **March 15, 2016**, through **January 15, 2017**. The total project period is from **March 15, 2016**, through **March 14, 2021**.

The USGS will consider continued funding for this project upon (1) availability of funds, (2) the Recipient showing satisfactory progress and the USGS' determination that continuation of this project would be in the best interests of the Government, and (3) strict adherence to the terms and conditions of this Agreement. Any funding that may be provided in excess of the amount currently obligated is subject to the availability of funds and their obligation in accordance with applicable law.

B.3 Pre-Agreement Costs

The Recipient is not authorized to incur costs prior to the award of this Agreement. Costs incurred prior to the award of this agreement are not allowable.

SECTION C - DELIVERABLES

C.1 Progress Reports

- a) The Recipient shall submit two copies of each Progress Report to the USGS Project Officer and one copy of the transmittal letter to the USGS Contracting Officer. Unless otherwise specified in this Agreement, annual progress reports should be submitted at least sixty (60) days prior to the end of the current budget period to allow adequate time for the designated office to review the report. In the case of multi-year Agreements, failure to submit timely reports may delay processing of funding increments. For Agreements with a total anticipated performance period of twelve months (12) months or less, only a Final Technical Report will be required. A progress report is not required in the final budget year, unless the Recipient requests an extension to the project period.
- b) The progress reports shall include the following information:
 - (i) A comparison of actual accomplishments to the objectives of the Agreement established for the budget period and overall progress in response to the performance metrics.
 - (ii) The reasons why established goals were not met, if appropriate.
 - (iii) Additional pertinent information including, when appropriate, analysis and explanation of cost overruns or high unit costs.
 - (iv) An outline of anticipated activities and adjustments to the program during the next budget period.
- c) Between the required reporting dates, events may occur which have significant impact upon the project or program. In such cases, the Recipient shall inform the USGS as soon as the following types of conditions become known:
 - (i) Problems, delays, or adverse conditions which will materially impair the ability to meet the objective of the Agreement. This disclosure must include a statement of the action taken, or contemplated, and any assistance needed to resolve the situation.
 - (ii) Favorable developments which enable meeting time schedules and objectives sooner or at less cost than anticipated or producing more or different beneficial results than originally planned.

C.2 Final Technical Report

- a) The Recipient shall submit two copies of the final technical report to the USGS Project Officer and one copy of the transmittal letter to the USGS Contracting Officer. The final performance report will be due 90 calendar days after the period of performance end date.

-
- b) The final technical report shall document and summarize the results of Recipient's work. The report shall include a quantitative description of activities and overall progress in response to the performance metrics which documents and summarizes the results of the entire Agreement. The final report shall include tables, graphs, diagrams, sketches, etc., as required to explain the results achieved under the Agreement. The report shall also include recommendations and conclusions based upon both the experience and the results obtained.

C.3 Annual Financial Reports

- a) The Recipient will submit an annual SF 425, Federal Financial Report, for each individual USGS award. The SF 425 is available at http://www.whitehouse.gov/omb/grants_forms. The SF 425 will be due ninety (90) calendar days after the grant year (i.e., 12 months after the approved effective date of the Agreement and every 12 months thereafter until the expiration date of the Agreement). USGS acknowledges that this annual reporting schedule may not always correspond with a specific budget period.
- b) The SF 425 must be submitted electronically through the FedConnect Message Center (www.fedconnect.net) or, if FedConnect is not available, by e-mail to SF425@usgs.gov with a cc to the Contracting Officer. Recipient must include the USGS award number in the subject line of all correspondence. If, after 90 days, Recipient has not submitted a report, the Recipient's account in ASAP will be placed in a manual review status until the report is submitted.

C.4 Final Financial Report

- a) The Recipient will liquidate all obligations incurred under the award and submit a final SF 425, Federal Financial Report in accordance with C.3.b. no later than 90 calendar days after the Agreement completion date.
- b) Recipient will promptly return any unexpended federal cash advances or will complete a final draw from ASAP to obtain any remaining amounts due. Once 120 days has passed since the Agreement completion date, USGS shall unilaterally deobligate federal funds as reflected in the Final SF425.
- c) Subsequent revision to the final SF 425 will be considered only as follows:
- i. When the revision results in a balance due to the Government, the Recipient must submit a revised final SF 425, Federal Financial Report, and refund the excess payment whenever the overcharge is discovered, no matter how long the lapse of time since the original due date of the report.

-
- ii. When the revision represents additional reimbursable costs claimed by the Recipient, a revised final SF 425 may be submitted to the USGS Contracting Officer with an explanation. If approved, the USGS will either request and pay a final invoice or reestablish the ASAP subaccount to permit the Recipient to make a revised final draw. Any revised final report representing additional reimbursable amounts must be submitted no later than 1 year from the due date of the original report, i.e., 15 months following the Agreement completion date. USGS will not accept any revised SF 425 covering additional expenditures after that date and will return any late request for additional payment to the Recipient.

C.5 Publications

a) Acknowledgment of Support

Recipient is responsible for assuring that an acknowledgment of USGS support:

1. is made in any publication (including World Wide Web pages) of any material based on or developed under this Agreement, in the following terms:

This material is based upon work supported by the U.S. Geological Survey under Grant/Cooperative Agreement No. (*insert agreement number*).

2. is orally acknowledged during all news media interviews, including popular media such as radio, television and news magazines.

b) Disclaimer

Recipient is responsible for assuring that every publication of material (including World Wide Web pages) based on or developed under this Agreement, contains the following disclaimer:

The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey. Mention of trade names or commercial products does not constitute their endorsement by the U.S. Geological Survey.

c) Publication

Publication of the results of any project carried out under this assistance award is authorized in professional journals, trade magazines, or may be made by the USGS. Such manuscripts or publications submitted to journals or professional publications for publication shall be accompanied by the following notation:

This manuscript is submitted for publication with the understanding that the United States Government is authorized to reproduce and distribute reprints for Governmental purposes.

d) Copies for USGS

Recipient is responsible for assuring that the USGS Project Office is provided access to, either electronically or in paper form, a copy of every publication planned for publication simultaneously with its submission for publication. One reprint of each published article shall be submitted to the USGS Project Office immediately following publication.

e) Department of the Interior Requirements

Two copies of each publication produced under a Grant or Cooperative Agreement shall be sent to the Natural Resources Library with a transmittal that identifies the sender and the publication. The address of the library is:

U.S. Department of the Interior
Natural Resources Library
Division of Information and Library Services
Gifts and Exchange Section
18th and C Streets, NW
Washington, DC 20240

SECTION D – ASSISTANCE ADMINISTRATIVE DATA

D.1 Assistance Administration

This Agreement will be administered by the USGS Contracting Officer indicated on the award cover page. Written communications shall make reference to the Assistance Award number and shall be mailed (or emailed) to the Contracting Officer.

This Agreement will be administered by:

U.S. Geological Survey
Office of Acquisition and Grants
12201 Sunrise Valley Drive, MS 205
Reston, VA 20192
Attn: Faith Graves
Telephone: 703-648-7356
Fax: 703-648-7901
Email: fgraves@usgs.gov

Written communications shall make reference to the Assistance Award number and shall be mailed (or emailed) to the above address.

D.2 Payment

Payments under financial assistance awards must be made using the Department of the Treasury Automated Standard Application for Payments (ASAP) system (www.asap.gov).

- a) The Recipient agrees that it has established or will establish an account with ASAP. USGS will initiate enrollment in ASAP. If the Recipient does not currently have an ASAP account, they must designate an individual (name, title, address, phone and e-mail) who will serve as the Point of Contact (POC).
- b) With the award of each grant/cooperative agreement, a sub-account will be set up from which the Recipient can draw down funds. After Recipients complete enrollment in ASAP and link their banking information to the USGS ALC (14080001), it may take up to 10 days for sub-accounts to be activated and for funds to be authorized for drawdown in ASAP.
- c) Inquiries regarding payment should be directed to ASAP at 855-868-0151.
- d) Payments may be drawn in advance only as needed to meet immediate cash disbursement needs.

D.3 Revisions and Prior Approvals

Modifications to this Agreement shall generally be executed by mutual written consent of the parties, with the exception of certain purely administrative changes that may be executed unilaterally by the USGS. Recipients may make certain limited budgetary and programmatic changes without prior USGS approval as outlined in 2 CFR 200.308 and 200.407. Any proposed change which requires prior written approval of the USGS shall be submitted in writing to the address at D.1 at least thirty (30) days prior to the requested effective date of the proposed change. The USGS will respond to the change request within thirty (30) days of receipt.

- a) Extensions. Recipients are specifically advised that requests for extension or other change to the budget or project period(s) require prior written approval. Such requests must be submitted as outlined above and be accompanied by a statement supporting the extension and a revised budget indicating the planned use of all unexpended funds during the proposed extension period.
- b) Transfer of Funds. Recipients are specifically advised that prior written approval of the USGS Contracting Officer is not required for transfer of funds between direct cost categories when the cumulative amount of the transfer during the performance period does not exceed ten percent (10%) of the total USGS award. Prior written approval is required from the USGS Contracting Officer for transfers of funds in excess of the ten percent limitation.

-
- c) Carry Forward of Funds. Recipients are specifically advised that prior written approval by the USGS Contracting Officer is required to carry forward unobligated balances to subsequent budget periods. It is expected that funds be expended during the budget period for which they are obligated. The request must include the amount of funds to be carried over, why the carry-over of funds is necessary, and for how long the funds should be carried over.

SECTION E - GENERAL PROVISIONS

E.1 Cost Principles, Audit, And Administrative Requirements

The Recipient shall be subject to the following regulations, which are incorporated herein by reference. Copies of these regulations can be obtained from the Internet at:

http://www.whitehouse.gov/omb/grants_docs

☒ Educational Institutions / State and Local Governments / Non-Profit Organizations

2 CFR Part 200, *Uniform Administrative Requirements, Cost Principles and Audit Requirements for Federal Awards*, as implemented by the Department of the Interior in 2 CFR Part 1402 and 43 CFR Part 12.

☐ Foreign Entities

• **Administrative Requirements**

Foreign entities are subject to the requirements applicable to non-Federal entities in 2 CFR Part 200, Subparts A through D and:

Foreign public entities are also subject to the requirements specific to States, with the following exceptions:

- The State payment procedures in 200.305(a) do not apply. Foreign public entities must follow the payment procedures in 200.305(b).
- The requirements in 200.321 “Contracting with small and minority businesses, women’s business enterprises, and labor surplus area firms” do not apply.
- The requirements in 200.322 “Procurement of recovered materials” do not apply.

Foreign non-profit organizations (see definition in 2 CFR 200.70) are also subject to the requirements specific to non-profit organizations.

Foreign Institutions of Higher Education (IHEs) (i.e., institutions located outside the United States that meet the definition in 20 U.S.C. 1001) are also subject to the requirements specific to IHEs.

- **Cost Principles**

Foreign for-profit entities are subject to the cost principles in 48 CFR 1, Subpart 31.2.

Foreign hospitals (i.e., a facility licensed as a hospital under the law of any foreign governmental entity or a facility operated as a hospital by a foreign public entity) are subject to the cost principles in 45 CFR Part 74, Appendix E.

All other foreign entities are subject to the requirements applicable to non-Federal entities in 2 CFR Part 200, Subpart E.

Foreign public entities are also subject to the requirements specific to States.

- **Indirect Cost Rate Negotiations**

Foreign IHEs: Appendix III to Part 200—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for IHEs. The U.S. Department of Health and Human Services (HHS) is the cognizant agency for indirect costs for foreign IHEs. Visit HHS' Cost Allocation Services website at <https://rates.psc.gov/> for more information.

Foreign non-profit organizations: Appendix IV to Part 200—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Nonprofit Organizations.

Foreign public entities: Appendix VII to Part 200—States and Local Government and Indian Tribe Indirect Cost Proposals.

Foreign for-profit entities: Contact the National Interior Business Center (IBC), Indirect Cost Services by telephone at (916) 566-7111 or by e-mail at: ics@ibc.doi.gov. Visit the IBC's Indirect Cost Services website at http://www.doi.gov/ibc/services/Indirect_Cost_Services/index.cfm for more information.

Foreign hospitals: 45 CFR Part 74, Appendix E—Principles for Determining Cost Applicable to Research and Development Under Grants and Contracts with Hospitals. HHS is the cognizant agency for indirect costs for foreign hospitals. Visit HHS' Cost Allocation Services website at <https://rates.psc.gov/> for more information.

☐ For-Profit Entities, Individuals, and Others Not Covered Above

- **Administrative Requirements**

2 CFR Part 200, Subparts A through D, *Uniform Administrative Requirements, Cost Principles and Audit Requirements for Federal Awards*

- **Cost Principles**

48 CFR 1, Subpart 31.2, *Contracts with Commercial Organizations*

- **Indirect Cost Rate Negotiations**

For information on indirect cost rate negotiations, contact the Interior Business Center (IBC) Indirect Cost Services Division by telephone at (916) 566-7111 or by e-mail at: ics@ibc.doi.gov. Visit the IBC Indirect Cost Services Division website at

http://www.doi.gov/ibc/services/Indirect_Cost_Services/index.cfm for more information.

E.2 Additional Regulations

This award is subject to the following additional Governmentwide regulations:

- 2 CFR 180, Governmentwide Debarment and Suspension (Nonprocurement)
- 2 CFR 182, Governmentwide Requirements for Drug-Free Workplace (Financial Assistance)

This award is subject to the following additional regulations of the U.S. Department of the Interior:

- 2 CFR Part 1400, Nonprocurement Debarment and Suspension
- 2 CFR Part 1401, Requirements for a Drug Free Workplace (Financial Assistance)
- 43 CFR Part 17, Nondiscrimination in Federally Assisted Programs of the Department of the Interior
- 43 CFR Part 18, New Restrictions on Lobbying
 - Submission of an application also represents the applicant's certification of the statements in 43 CFR Part 18, Appendix A, Certification Regarding Lobbying
- 43 CFR Part 41, Nondiscrimination on the Basis of Sex in Education Programs or Activities Receiving Federal Financial Assistance [*Applies only if this award provides assistance to an education program or student(s)*]

E.3 Additional Articles Required For Compliance with Statute or Regulation

a) The Seat Belt Provision (Executive Order 13043)

Recipients of grants/cooperative agreements and/or sub-awards are encouraged to adopt and enforce on-the-job seat belt use policies and programs for their employees when operating company owned, rented, or personally owned vehicles. These measures include, but are not limited to, conducting education, awareness, and other appropriate programs for their employees about the importance of wearing seatbelts and the consequences of not wearing them.

b) Federal Leadership on Reducing Text Messaging while Driving (Executive Order 13513)

Recipients are encouraged to adopt and enforce policies that ban text messaging while driving, including conducting initiatives of the type described in section 3(a) of the order.

(http://www.whitehouse.gov/the_press_office/Executive-Order-Federal-Leadership-on-Reducing-Text-Messaging-while-Driving/)

c) Use of U.S. Flag Air Carriers (49 USC Section 40118)

Any air transportation to, from, between or within a country other than the U.S. of persons or property, the expense of which will be paid in whole or in part by U.S. Government funding, must be performed by, or under a code-sharing arrangement with, a U.S. flag air carrier if service provided by such a carrier is "available" (49 U.S.C. 40118, commonly referred to as the Fly America Act). Tickets (or documentation for electronic tickets) must identify the U.S. flag air carrier's designator code and flight number. See the Federal Travel Regulation §301-10.131 - §301-10.143 for definitions, exceptions, and documentation requirements. (See also Comp. Gen. Decision B-240956, dated September 25, 1991.)

d) Trafficking in Persons (2 CFR Part 175)

a. *Provisions applicable to a recipient that is a private entity.*

1. You as the recipient, your employees, subrecipients under this award, and subrecipients' employees may not—
 - i. Engage in severe forms of trafficking in persons during the period of time that the award is in effect;
 - ii. Procure a commercial sex act during the period of time that the award is in effect; or
 - iii. Use forced labor in the performance of the award or subawards under the award.
2. We as the Federal awarding agency may unilaterally terminate this award, without penalty, if you or a subrecipient that is a private entity —
 - i. Is determined to have violated a prohibition in paragraph a.1 of this award term; or
 - ii. Has an employee who is determined by the agency official authorized to terminate the award to have violated a prohibition in paragraph a.1 of this award term through conduct that is either—
 - A. Associated with performance under this award; or
 - B. Imputed to you or the subrecipient using the standards and due process for imputing the conduct of an individual to an organization that are provided in 2 CFR part 180, "OMB Guidelines to Agencies on Governmentwide Debarment and Suspension (Nonprocurement)," as implemented by our agency at 2 CFR part 1400.

b. *Provision applicable to a recipient other than a private entity.*

We as the Federal awarding agency may unilaterally terminate this award, without penalty, if a subrecipient that is a private entity—

1. Is determined to have violated an applicable prohibition in paragraph a.1 of this award term; or
2. Has an employee who is determined by the agency official authorized to terminate the award to have violated an applicable prohibition in paragraph a.1 of this award term through conduct that is either—
 - i. Associated with performance under this award; or

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- ii. Imputed to the subrecipient using the standards and due process for imputing the conduct of an individual to an organization that are provided in 2 CFR part 180, “OMB Guidelines to Agencies on Governmentwide Debarment and Suspension (Nonprocurement),” as implemented by our agency at 2 CFR part 1400.

c. Provisions applicable to any recipient.

1. You must inform us immediately of any information you receive from any source alleging a violation of a prohibition in paragraph a.1 of this award term.
2. Our right to terminate unilaterally that is described in paragraph a.2 or b of this section:
 - i. Implements section 106(g) of the Trafficking Victims Protection Act of 2000 (TVPA), as amended (22 U.S.C. 7104(g)), and
 - ii. Is in addition to all other remedies for noncompliance that are available to us under this award.
3. You must include the requirements of paragraph a.1 of this award term in any subaward you make to a private entity.

d. Definitions.

For purposes of this award term:

1. “Employee” means either:
 - i. An individual employed by you or a subrecipient who is engaged in the performance of the project or program under this award; or
 - ii. Another person engaged in the performance of the project or program under this award and not compensated by you including, but not limited to, a volunteer or individual whose services are contributed by a third party as an in-kind contribution toward cost sharing or matching requirements.
2. “Forced labor” means labor obtained by any of the following methods: the recruitment, harboring, transportation, provision, or obtaining of a person for labor or services, through the use of force, fraud, or coercion for the purpose of subjection to involuntary servitude, peonage, debt bondage, or slavery.
3. “Private entity”:
 - i. Means any entity other than a State, local government, Indian tribe, or foreign public entity, as those terms are defined in 2 CFR 175.25.
 - ii. Includes:
 - A. A nonprofit organization, including any nonprofit institution of higher education, hospital, or tribal organization other than one included in the definition of Indian tribe at 2 CFR 175.25(b).
 - B. A for-profit organization.
4. “Severe forms of trafficking in persons,” “commercial sex act,” and “coercion” have the meanings given at section 103 of the TVPA, as amended (22 U.S.C. 7102).

e) Reporting Subawards and Executive Compensation Information (2 CFR Part 170).

a. *Reporting of first-tier subawards.*

1. *Applicability.* Unless you are exempt as provided in paragraph d. of this award term, you must report each action that obligates \$25,000 or more in Federal funds that does not include Recovery funds (as defined in section 1512(a)(2) of the American Recovery and Reinvestment Act of 2009, Pub. L. 111-5) for a subaward to an entity (see definitions in paragraph e. of this award term).

2. *Where and when to report.*

i. You must report each obligating action described in paragraph a.1. of this award term to <http://www.fsrs.gov>.

ii. For subaward information, report no later than the end of the month following the month in which the obligation was made. (For example, if the obligation was made on November 7, 2010, the obligation must be reported by no later than December 31, 2010.)

3. *What to report.* You must report the information about each obligating action that the submission instructions posted at <http://www.fsrs.gov> specify.

b. *Reporting Total Compensation of Recipient Executives.*

1. *Applicability and what to report.* You must report total compensation for each of your five most highly compensated executives for the preceding completed fiscal year, if—

i. the total Federal funding authorized to date under this award is \$25,000 or more;

ii. in the preceding fiscal year, you received—

(A) 80 percent or more of your annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

(B) \$25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

iii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at <http://www.sec.gov/answers/execomp.htm>.)

2. *Where and when to report.* You must report executive total compensation described in paragraph b.1. of this award term:

i. As part of your registration profile at <https://www.sam.gov>.

ii. By the end of the month following the month in which this award is made, and annually thereafter.

c. *Reporting of Total Compensation of Subrecipient Executives.*

1. *Applicability and what to report.* Unless you are exempt as provided in paragraph d. of this award term, for each first-tier subrecipient under this award, you shall report the names and total compensation of each of the subrecipient's five most highly compensated executives for the subrecipient's preceding completed fiscal year, if—

- i. in the subrecipient's preceding fiscal year, the subrecipient received—
 - (A) 80 percent or more of its annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and
 - (B) \$25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts), and Federal financial assistance subject to the Transparency Act (and subawards); and
- ii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at <http://www.sec.gov/answers/execomp.htm>.)

2. *Where and when to report.* You must report subrecipient executive total compensation described in paragraph c.1. of this award term:

- i. To the recipient.
- ii. By the end of the month following the month during which you make the subaward. For example, if a subaward is obligated on any date during the month of October of a given year (*i.e.*, between October 1 and 31), you must report any required compensation information of the subrecipient by November 30 of that year.

d. *Exemptions*

If, in the previous tax year, you had gross income, from all sources, under \$300,000, you are exempt from the requirements to report:

- i. Subawards,
and
- ii. The total compensation of the five most highly compensated executives of any subrecipient.

e. *Definitions.* For purposes of this award term:

1. *Entity* means all of the following, as defined in 2 CFR part 25:

- i. A Governmental organization, which is a State, local government, or Indian tribe;
- ii. A foreign public entity;
- iii. A domestic or foreign nonprofit organization;
- iv. A domestic or foreign for-profit organization;
- v. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.

2. *Executive* means officers, managing partners, or any other employees in management positions.

3. *Subaward*:

- i. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.
- ii. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. __ .210 of the attachment to OMB Circular A-133, “Audits of States, Local Governments, and Non-Profit Organizations”).
- iii. A subaward may be provided through any legal agreement, including an agreement that you or a subrecipient considers a contract.

4. *Subrecipient* means an entity that:

- i. Receives a subaward from you (the recipient) under this award; and
- ii. Is accountable to you for the use of the Federal funds provided by the subaward.

5. *Total compensation* means the cash and noncash dollar value earned by the executive during the recipient's or subrecipient's preceding fiscal year and includes the following (for more information see 17 CFR 229.402(c)(2)):

- i. *Salary and bonus*.
- ii. *Awards of stock, stock options, and stock appreciation rights*. Use the dollar amount recognized for financial statement reporting purposes with respect to the fiscal year in accordance with the Statement of Financial Accounting Standards No. 123 (Revised 2004) (FAS 123R), Shared Based Payments.
- iii. *Earnings for services under non-equity incentive plans*. This does not include group life, health, hospitalization or medical reimbursement plans that do not discriminate in favor of executives, and are available generally to all salaried employees.
- iv. *Change in pension value*. This is the change in present value of defined benefit and actuarial pension plans.
- v. *Above-market earnings on deferred compensation which is not tax-qualified*.
- vi. Other compensation, if the aggregate value of all such other compensation (e.g. severance, termination payments, value of life insurance paid on behalf of the employee, perquisites or property) for the executive exceeds \$10,000.

f) System of Award Management and Universal Identifier Requirements (2 CFR Part 25)

a. *Requirement for System of Award Management*

Unless you are exempted from this requirement under 2 CFR 25.110, you as the recipient must maintain the currency of your information in the SAM until you submit the final financial report required under this award or receive the final payment, whichever is later. This requires that you review and update the information at least annually after the initial registration, and more frequently if required by changes in your information or another award term.

b. *Requirement for Unique Entity identifier Numbers*

If you are authorized to make subawards under this award, you:

1. Must notify potential subrecipients that no entity (*see* definition in paragraph C of this award term) may receive a subaward from you unless the entity has provided its unique entity identifier number to you.
2. May not make a subaward to an entity unless the entity has provided its DUNS number to you.

c. *Definitions*

For purposes of this award term:

1. *System of Award Management (SAM)* means the Federal repository into which an entity must provide information required for the conduct of business as a recipient. Additional information about registration procedures may be found at the SAM Internet site (currently at <http://www.sam.gov>).
2. *Unique entity identifier* means the identifier required for SAM registration to uniquely identify business entities.
3. *Entity*, as it is used in this award term, means all of the following, as defined at 2 CFR part 25, subpart C:
 - i. A Governmental organization, which is a State, local government, or Indian Tribe;
 - ii. A foreign public entity;
 - iii. A domestic or foreign nonprofit organization;
 - iv. A domestic or foreign for-profit organization; and
 - v. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.
4. *Subaward*:
 - i. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.
 - ii. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see 2 CFR 200.330).
 - iii. A subaward may be provided through any legal agreement, including an agreement that you consider a contract.
5. *Subrecipient* means an entity that:
 - i. Receives a subaward from you under this award; and
 - ii. Is accountable to you for the use of the Federal funds provided by the subaward.

g) Prohibition on Members of Congress Making Contracts with Federal Government (41 USC Section 6306)

No member of or delegate to the United States Congress or Resident Commissioner shall be admitted to any share or part of this award, or to any benefit that may arise therefrom; this provision shall not be construed to extend to an award made to a corporation for the public's general benefit.

h) Pilot Program for Enhancement of Recipient and Subrecipient Employee Whistleblower Protection (41 USC Section 4712)

This requirement applies to all awards issued after July 1, 2013 and shall be in effect until January 1, 2017.

- a. This award and related subawards and contracts over the simplified acquisition threshold and all employees working on this award and related subawards and contracts over the simplified acquisition threshold are subject to the whistleblower rights and remedies in the pilot program on award recipient employee whistleblower protections established at 41 U.S.C. 4712 by section 828 of the *National Defense Authorization Act for Fiscal Year 2013* (P.L. 112-239).
- b. Recipients, and their subrecipients and contractors awarded contracts over the simplified acquisition threshold related to this award, shall inform their employees in writing, in the predominant language of the workforce, of the employee whistleblower rights and protections under 41 U.S.C. 4712.
- c. The recipient shall insert this clause, including this paragraph (c), in all subawards and contracts over the simplified acquisition threshold related to this award.

i) Patent Rights (37 CFR § 401.14)

Unless otherwise provided in the Agreement, if this Agreement is for experimental, developmental, or research work, the following clause (implementing the Bayh-Dole Act, [35 U.S.C. § 200 et seq.]) shall apply. The recipient shall include this clause in all subawards for experimental, developmental, or research activities.

a. *Definitions*

- 1. INVENTION means any invention or discovery which is or may be patentable or otherwise protectable under Title 35 of the USC, to any novel variety of plant which is or may be protected under the Plant Variety Protection Act (7 U.S.C. § 2321 et seq.).
- 2. SUBJECT INVENTION means any invention of the recipient conceived or first actually reduced to practice in the performance of work under this Agreement, provided that in the case of a variety of plant, the date of determination (as defined in section 41(d)) must also occur during the period of performance.
- 3. PRACTICAL APPLICATION means to manufacture in the case of a composition or product, to practice in the case of a process or method, or to operate in the case of a machine or system; and, in each case, under such conditions as to establish that the invention is being utilized and that its benefits are to the extent permitted by law or Government regulations available to the public on reasonable terms.
- 4. MADE when used in relation to any invention means the conception or first actual reduction to practice of such invention.

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5. SMALL BUSINESS FIRM means a small business concern as defined at section 2 of Pub. L. 85-536 (15 U.S.C. 632) and implementing regulations of the Administrator of the Small Business Administration. For the purpose of this clause, the size standards for small business concerns involved in government procurement and subcontracting at 13 CFR 121.3-8 and 13 CFR 121.3-12, respectively, will be used.
 6. NON-PROFIT ORGANIZATION means a domestic university or other institution of higher education or an organization of the type described in Section 501(c)(3) of the Internal Revenue Code of 1954 (26 U.S.C. § 501(c)) and exempt from taxation under Section 501(a) of the Internal Revenue Code (26 U.S.C. § 501(a)) or any domestic non-profit scientific or educational organization qualified under a State non-profit organization statute.
 - b. Allocation of Principal Rights The recipient may retain the entire right, title, and interest throughout the world to each subject invention subject to the provisions of this Patent Rights clause and 35 U.S.C. § 203. With respect to any subject invention in which the recipient retains title, the Federal Government shall have a non-exclusive, nontransferable, irrevocable, paid-up license to practice or have practiced for or on behalf of the U.S. the subject invention throughout the world. If the Agreement indicates it is subject to an identified international agreement or treaty, the U.S. Geological Survey (USGS) also has the right to direct the recipient to convey to any foreign participant such patent rights to subject inventions as are required to comply with that agreement or treaty.

b. *Allocation of Principal Rights*

1. The recipient may retain the entire right, title, and interest throughout the world to each subject invention subject to the provisions of this Patent Rights clause, including (2) below, and 35 U.S.C. § 203. With respect to any subject invention in which the recipient retains title, the Federal Government shall have a non-exclusive, nontransferable, irrevocable, paid-up license to practice or have practiced for or on behalf of the U.S. the subject invention throughout the world. If the Agreement indicates it is subject to an identified international agreement or treaty, the U.S. Geological Survey (USGS) also has the right to direct the recipient to convey to any foreign participant such patent rights to subject inventions as are required to comply with that agreement or treaty.
2. If the recipient performs services at a Government owned and operated laboratory or at a Government owned and recipient operated laboratory directed by the Government to fulfill the Government's obligations under a Cooperative Research and Development Agreement (CRADA) authorized by 15 U.S.C. 3710a, the Government may require the recipient to negotiate an agreement with the CRADA collaborating party or parties regarding the allocation of rights to any subject invention the recipient makes, solely or jointly, under the CRADA. The agreement shall be negotiated prior to the recipient undertaking the CRADA work or, with the permission of the Government, upon the identification of a subject invention. In the absence of such an agreement, the recipient agrees to grant the collaborating party or parties an option for a license in its inventions of the same scope and terms set forth in the CRADA for inventions made by the Government.

c. *Invention Disclosure, Election of Title and Filing of Patent Applications by Recipient*

1. The recipient will disclose each subject invention to USGS within two months after the inventor discloses it in writing to recipient personnel responsible for the administration of patent matters. The disclosure to USGS shall be in the form of a written report and shall identify the Agreement under which the invention was made and the inventor(s). It shall be sufficiently complete in technical detail to convey a clear understanding of the nature, purpose, operation, and, to the extent known, the physical, chemical, biological or electrical characteristics of the invention. The disclosure shall also identify any publication, on sale or public use of the invention, whether a manuscript describing the invention has been submitted for publication and, if so, whether it has been accepted for publication, at the time of disclosure. In addition, after disclosure to USGS, the recipient will promptly notify USGS of the acceptance of any manuscript describing the invention for publication, or of any on sale or public use planned by the recipient.
2. The recipient will elect in writing whether or not to retain title to any such invention by notifying USGS within two years of disclosure to USGS. However, in any case where publication, on sale, or public use has initiated the one-year statutory period wherein valid patent protection can still be obtained in the U.S., the period for election of title may be shortened by USGS to a date that is no more than 60 days prior to the end of the statutory period.
3. The recipient will file its initial patent application on an invention to which it elects to retain title within one year after election of title or, if earlier, prior to the end of any statutory period wherein valid patent protection can be obtained in the U.S. after a publication, on sale, or public use. The recipient will file patent applications in additional countries or international patent offices within either ten months of the corresponding initial patent application, or six months from the date when permission is granted by the Commissioner of Patents and Trademarks to file foreign patent applications when such filing has been prohibited by a Secrecy Order.
4. Requests for extension of the time for disclosure to USGS, election, and filing under subparagraphs 1., 2., and 3. may, at the discretion of USGS, be granted.

d. *Conditions When the Government May Obtain Title*

The recipient will convey to USGS, upon written request, title to any subject invention:

1. if the recipient fails to disclose or elect the subject invention within the times specified in paragraph c. above, or elects not to retain title, provided that USGS may only request title within 60 days after learning of the failure of the recipient to disclose or elect within the specified times;
2. in those countries in which the recipient fails to file patent applications within the times specified in paragraph c. above, but prior to its receipt of the written request of USGS, the recipient shall continue to retain title in that country; or in any country in which the recipient decides not to continue the prosecution of any application for, to pay the maintenance fees on, or defend in a reexamination or opposition proceeding on, a patent on a subject invention.

e. *Minimum Rights to Recipient*

1. The recipient will retain a non-exclusive royalty-free license throughout the world in each subject invention to which the Government obtains title, except if the recipient fails to disclose the subject invention within the times specified in paragraph c. above. The recipient's license extends to its domestic subsidiaries and affiliates, if any, within the corporate structure of which the recipient is a party and includes the right to grant sublicenses of the same scope to the extent the recipient was legally obligated to do so at the time the Agreement was made. The license is transferable only with the approval of USGS except when transferred to the successor of that part of the recipient's business to which the invention pertains.
2. The recipient's domestic license may be revoked or modified by USGS to the extent necessary to achieve expeditious practical application of the subject invention pursuant to an application for an exclusive license submitted in accordance with applicable provisions at 37 CFR Part 404. This license will not be revoked in that field of use or the geographical areas in which the recipient has achieved practical application and continues to make the benefits of the invention reasonably accessible to the public. The license in any foreign country may be revoked or modified at discretion of USGS to the extent the recipient, its licensees, or its domestic subsidiaries or affiliates have failed to achieve practical application in that foreign country.
3. Before revocation or modification of the license, USGS will furnish the recipient a written notice of its intention to revoke or modify the license, and the recipient will be allowed thirty days (or such other time as may be authorized by USGS for good cause shown by the recipient) after the notice to show cause why the license should not be revoked or modified. The recipient has the right to appeal, in accordance with applicable regulations in 37 CFR Part 404 concerning the licensing of Government-owned inventions, any decision concerning the revocation or modification of its license.

f. *Recipient Action to Protect Government's Interest*

1. The recipient agrees to execute or to have executed and promptly deliver to USGS all instruments necessary to: (i) establish or confirm the rights the Government has throughout the world in those subject inventions for which the recipient retains title; and (ii) convey title to USGS when requested under paragraph d. above, and to enable the Government to obtain patent protection throughout the world in that subject invention.

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2. The recipient agrees to require, by written agreement, its employees, other than clerical and non-technical employees, to disclose promptly in writing to personnel identified as responsible for the administration of patent matters and in a format suggested by the recipient each subject invention made under this Agreement in order that the recipient can comply with the disclosure provisions of paragraph c. above, and to execute all papers necessary to file patent applications on subject inventions and to establish the Government's rights in the subject inventions. The disclosure format should require, as a minimum, the information requested by paragraph c.1 above. The recipient shall instruct such employees through the employee agreements or other suitable educational programs on the importance of reporting inventions in sufficient time to permit the filing of patent applications prior to U.S. or foreign statutory bars.
 3. The recipient will notify USGS of any decision not to continue prosecution of a patent application, pay maintenance fees, or defend in a reexamination or opposition proceeding on a patent, in any country, not less than 30 days before the expiration of the response period required by the relevant patent office.
 4. The recipient agrees to include, within the specification of any U.S. patent application and any patent issuing thereon covering a subject invention, the following statement: "This invention was made with Government support under (identify the Agreement) awarded by the U.S. Geological Survey. The Government has certain rights in this invention."
 5. The recipient or its representative will complete, execute and forward to USGS a confirmation of a License to the U.S. Government and the page of a United States patent application that contains the Federal support clause within two months of filing any domestic or foreign patent application.

g. *Subcontracts*

1. The recipient will include this Patent Rights clause, suitably modified to identify the parties, in all subcontracts, regardless of tier, for experimental, developmental or research work. The subcontractor will retain all rights provided for the recipient in this Patent Rights clause, and the recipient will not, as part of the consideration for awarding the subcontract, obtain rights in the subcontractors' subject inventions.
2. In the case of subcontracts, at any tier, when the prime award by USGS was a contract (but not a cooperative agreement), USGS, subcontractor, and contractor agree that the mutual obligations of the parties created by this Patent Rights clause constitute a contract between the subcontractor and the Foundation with respect to those matters covered by this Patent Rights clause.

h. *Reporting on Utilization of Subject Inventions*

The recipient agrees to submit on request periodic reports no more frequently than annually on the utilization of a subject invention or on efforts at obtaining such utilization that are being made by the recipient or its licensees or assignees. Such reports shall include information regarding the status of development, date of first commercial sale or use, gross royalties received by the recipient and such other data and information as USGS may reasonably specify. The recipient also agrees to provide additional reports in connection with any march-in proceeding undertaken by USGS in accordance with paragraph j. of this Patent Rights clause. As required by 35 U.S.C. § 202(c)(5), USGS agrees it will not disclose such information to persons outside the Government without the permission of the recipient.

i. *Preference for United States Industry*

Notwithstanding any other provision of this Patent Rights clause, the recipient agrees that neither it nor any assignee will grant to any person the exclusive right to use or sell any subject invention in the U.S. unless such person agrees that any products embodying the subject invention or produced through the use of the subject invention will be manufactured substantially in the U.S. However, in individual cases, the requirement for such an agreement may be waived by USGS upon a showing by the recipient or its assignee that reasonable but unsuccessful efforts have been made to award licenses on similar terms to potential licensees that would be likely to manufacture substantially in the U.S. or that under the circumstances domestic manufacture is not commercially feasible.

j. *March-in Rights*

The recipient agrees that with respect to any subject invention in which it has acquired title, USGS has the right in accordance with procedures at 37 CFR § 401.6 and USGS regulations at 45 CFR § 650.13 to require the recipient, an assignee or exclusive licensee of a subject invention to grant a non-exclusive, partially exclusive, or exclusive license in any field of use to a responsible applicant or applicants, upon terms that are reasonable under the circumstances and if the recipient, assignee, or exclusive licensee refuses such a request, USGS has the right to grant such a license itself if USGS determines that:

1. such action is necessary because the recipient or assignee has not taken or is not expected to take within a reasonable time, effective steps to achieve practical application of the subject invention in such field of use;
2. such action is necessary to alleviate health or safety needs which are not reasonably satisfied by the recipient, assignee, or their licensees;
3. such action is necessary to meet requirements for public use specified by Federal regulations and such requirements are not reasonably satisfied by the recipient, assignee, or licensee; or
4. such action is necessary because the agreement required by paragraph i. of this Patent Rights clause has not been obtained or waived or because a licensee of the exclusive right to use or sell any subject invention in the U.S. is in breach of such agreement.

k. *Special Provisions for Agreements with Non-profit Organizations*

If the recipient is a nonprofit organization, it agrees that:

-
1. rights to a subject invention in the U.S. may not be assigned without the approval of USGS, except where such assignment is made to an organization which has as one of its primary functions the management of inventions, provided that such assignee will be subject to the same provisions as the recipient;
 2. the recipient will share royalties collected on a subject invention with the inventor, including Federal employee co-inventors (when USGS deems it appropriate) when the subject invention is assigned in accordance with 35 U.S.C. § 202(e) and 37 CFR § 401.10;
 3. the balance of any royalties or income earned by the recipient with respect to subject inventions, after payment of expenses (including payments to inventors) incidental to the administration of subject inventions, will be utilized for the support of scientific or engineering research or education; and
 4. it will make efforts that are reasonable under the circumstances to attract licensees of subject inventions that are small business firms and that it will give preference to a small business firm if the recipient determines that the small business firm has a plan or proposal for marketing the invention which, if executed, is equally likely to bring the invention to practical application as any plans or proposals from applicants that are not small business firms; provided that the recipient is also satisfied that the small business firm has the capability and resources to carry out its plan or proposal. The decision whether to give a preference in any specific case will be at the discretion of the recipient. However, the recipient agrees that the Secretary of Commerce may review the recipient's licensing program and decisions regarding small business applicants, and the recipient will negotiate changes to its licensing policies, procedures or practices with the Secretary when the Secretary's review discloses that the recipient could take reasonable steps to implement more effectively the requirements of this paragraph k.4.

1. *Communications*

All communications required by this Patent Rights clause must be submitted through Benjamin Henry, Technology Transfer Specialist, Office of Policy and Analysis (OPA), U.S. Geological Survey, Reston, VA 20192, (703) 648-4344, bhenry@usgs.gov.

E.4 Additional General Terms and Conditions

a) Research Integrity

- 1) USGS requires that all grant or cooperative agreement Recipient organizations adhere to the Federal Policy on Research Misconduct, Office of Science and Technology Policy, December 6, 2000, 65 Federal Register (FR) 76260. The Federal Policy on Research Misconduct outlines requirements for addressing allegations of research misconduct, including the investigation, adjudication, and appeal of allegations of research misconduct and the implementation of appropriate administrative actions.
- 2) The Recipient must promptly notify the USGS Project Office when research misconduct that warrants an investigation pursuant to the Federal Policy on Research Misconduct is alleged.

b) Access To Research Data

- 1) Recipients that are institutions of higher education, hospitals, or non-profit organizations are required to release research data first produced in a project supported in whole or in part with Federal funds that are cited publicly and officially by a Federal agency in support of an action that has the force and effect of law (e.g., regulations and administrative orders). “Research data” is defined as the recorded factual material commonly accepted in the scientific community as necessary to validate research findings. It does not include preliminary analyses; drafts of scientific papers; plans for future research; peer reviews; communications with colleagues; physical objects (e.g., laboratory samples, audio or video tapes); trade secrets; commercial information; materials necessary to be held confidential by a researcher until publication in a peer-reviewed journal; information that is protected under the law (e.g., intellectual property); personnel and medical files and similar files, the disclosure of which would constitute an unwarranted invasion of personal privacy; or information that could be used to identify a particular person in a research study.
- 2) These requirements do not apply to commercial organizations or to research data produced by State or local governments. However, if a State or local governmental grantee contracts with an educational institution, hospital, or non-profit organization, and the contract results in covered research data, those data are subject to these disclosure requirements.
- 3) Requests for the release of research data subject to this policy are required to be made to USGS, which will handle them as FOIA requests under 43 CFR 2.25. If the data are publicly available, the requestor will be directed to the public source. Otherwise, the USGS Contract Officer, in consultation with the affected Recipient and the PI, will handle the request. This policy also provides for assessment of a reasonable fee to cover Recipient costs as well as (separately) the USGS costs of responding.

c) Conflict of Interest

The Recipient must establish safeguards to prohibit its employees and Subrecipients from using their positions for purposes that constitute or present the appearance of a personal or organizational conflict of interest. The Recipient is responsible for notifying the USGS Contracting Officer in writing of any actual or potential conflicts of interest that may arise during the life of this award. Conflicts of interest include any relationship or matter which might place the Recipient or its employees in a position of conflict, real or apparent, between their responsibilities under the agreement and any other outside interests. Conflicts of interest may also include, but are not limited to, direct or indirect financial interests, close personal relationships, positions of trust in outside organizations, consideration of future employment arrangements with a different organization, or decision-making affecting the award that would cause a reasonable person with knowledge of the relevant facts to question the impartiality of the Recipient and/or Recipient's employees and Sub-recipients in the matter.

The USGS Contracting Officer and the servicing Ethics Counselor will determine if a conflict of interest exists. If a conflict of interest exists, the USGS Contracting Officer will determine whether a mitigation plan is feasible. Mitigation plans must be approved by the USGS Contracting Officer in writing. Failure to resolve conflicts of interest in a manner that satisfies the government may be cause for termination of the award.

Failure to make required disclosures may result in any of the remedies described in 2 CFR § 200.338, Remedies for Noncompliance, including suspension or debarment (see also 2 CFR Part 180).

d) Program Income

- 1) If the Recipient is an educational institution or nonprofit research organization, any other program income will be added to funds committed to the project by the Federal awarding agency and Recipient and be used to further eligible project or program objectives, as described in 2 CFR 200.307(e)(2).
- 2) For all other types of Recipients, any other program income will be deducted from total allowable costs to determine the net allowable costs before calculating the Government's share of reimbursable costs, as provided in 2 CFR 200.307(e)(1).

e) Government Furnished Equipment or Equipment Authorized for Purchase

Title to equipment acquired wholly or in part with Federal funds shall be vested in the Recipient unless otherwise specified in the award document. The Recipient shall retain control and maintain an inventory of such equipment as long as there is a need for such equipment to accomplish the purpose of the project, whether or not the project continues to be supported by Federal funds. When there is no longer a need for such equipment to accomplish the purpose of the project, the Recipient shall use the equipment in connection with other Federal awards the Recipient has received. Disposal of equipment shall be in accordance with 2 CFR 200.313.

No equipment is provided or authorized for purchase on this grant/cooperative agreement.

SECTION F – SPECIAL PROVISIONS

NONE.

SECTION G – DOCUMENTS INCORPORATED BY REFERENCE AND ORDER OF PRECEDENCE

G.1 Documents Incorporated By Reference

The following documents are hereby incorporated into this Agreement by reference:

- 1) The Recipient’s proposal titled: “The Efficacy and Safety of Silicone O-Ring Intrauterine Devices as a Horse Contraceptive through a Captive Breeding Trial”, dated, January 19, 2016.
- 2) The Recipient’s application for financial assistance (SF424, SF424A, SF424B), dated January 19, 2016.

G.2 Order of Precedence

In the event of any inconsistency within this Agreement, the following order of precedence shall be followed:

- 1) The cover page.
- 2) Sections A through F of this Agreement.
- 3) Documents incorporated by reference (see G.1) in the order in which they are incorporated.

– END OF ASSISTANCE AWARD DOCUMENT –

Literature Cited in Supplemental Materials

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- MRID 51243702** Daels, P.F, and J.P. Hughes. 1995. Fertility control using intrauterine devices: an alternative for population control in wild horses. *Theriogenology* 44:629-639.
- MRID 51243703** Freeman, C.E., and S.K. Lyle. 2015. Chronic intermittent colic in a mare attributed to uterine marbles. *Equine Veterinary Education* 27:469-473.
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- MRID 51243709** Nie, G.J., K.E., Johnson, T.D. Braden, and J. G.W. Wenzel. 2003. Use of an intra-uterine glass ball protocol to extend luteal function in mares. *Journal of Equine Veterinary Science* 23:266-273.
- MRID 51243710** Turner, R.M., D.K. Vanderwall, and R. Stawiecki. 2015. Complications associated with the presence of two intrauterine glass balls used for oestrus suppression in a mare. *Equine Veterinary Education* 27:340-343.



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Evaluating the efficacy and safety of silicone O-ring intrauterine devices as a horse contraceptive through a captive breeding trial.

Author(s) : [Baldrigi, J. M. \(/cabdirect/search/?q=au%3a%22Baldrigi%2c+J.+M.%22\)](#); [Lyman, C. C. \(/cabdirect/search/?q=au%3a%22Lyman%2c+C.+C.%22\)](#); [Hornberger, K. \(/cabdirect/search/?q=au%3a%22Hornberger%2c+K.%22\)](#); [Germaine, S. S. \(/cabdirect/search/?q=au%3a%22Germaine%2c+S.+S.%22\)](#); [Kane, A. \(/cabdirect/search/?q=au%3a%22Kane%2c+A.%22\)](#); [Holyoak, G. R. \(/cabdirect/search/?q=au%3a%22Holyoak%2c+G.+R.%22\)](#)

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Editors : [Shipley, C. F. \(/cabdirect/search/?q=ed%3a%22Shipley%2c+C.+F.%22\)](#)

Journal article : [Clinical Theriogenology \(/cabdirect/search/?q=do%3a%22Clinical+Theriogenology%22\)](#) 2017 Vol.9 No.3 pp.471

Abstract : Due to continued on-range population growth there are upwards to 70,000 feral horses against a BLM range ecologist established goal of 27,000. Therefore, the need for an effective means of contraception has significantly increased. A contraceptive that is reversible would allow for the preservation of genetic diversity in targeted populations and alleviate some of the concern over contraception in wild horses. In this study we evaluated the efficacy and safety of an O-ring intrauterine device (IUD) based on earlier studies indicating a greater than 80% retention rate. With this background information we hypothesized that a silicone O-ring IUD with a durometer grade (hardness) of 50 would be retained within the uterus and would be capable of preventing pregnancy in mares under normal pasture breeding conditions. Acceptable rates of loss for the IUDs were predetermined to be not more than 25% during the first month and less than 50% during the three-month trial period. If these parameters were not met the study would then transition to determining "best" fit. Additionally, we also wanted to determine the incidence of adverse reactions to IUDs regarding inflammation, endometrial fibrosis, and cyclicity. The first IUDs inserted had a loss rate of 3/5 over a two month period without stallion exposure. Therefore, five groups of IUDs, of differing sizes and durometers, were manufactured and the second group of O-ring IUDs were inserted into the uterus of 20 young, reproductively healthy mares; the mares were immediately turned out with stallions after IUD insertion. Retention of IUDs was monitored via transrectal ultrasonography at 3-5 day intervals. Biopsy samples were collected at the time of IUD insertion and IUD removal for comparison. After the first 45 days of the pilot study the inserted O-ring IUDs experienced an overall loss rate of 60%, with some IUD configurations having a loss rate of 100%. At that point the IUDs with the lowest rate of loss were selected to be further refined and inserted into a larger group of mares. Three durometer types were inserted in three groups consisting of eight mares each. This step tested the hypothesis that higher durometer IUDs would be less malleable to uterine contractions (during estrus and during the process of breeding) and less likely to be expelled. Over the one-month evaluation period that followed, 11/24 IUDs were lost; many of which were expelled within the first week after insertion. There was no significant increase in endometrial inflammation or fibrosis. Several mares did experience deviations from normal cyclicity. Finally, no pregnancies were established.

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Organism descriptor(s) : Equus, horses

Descriptor(s) : adverse effects, contraception, contraceptive properties, efficacy, feral animals, feral horses, intrauterine devices, mares, population control, potency, silicon

Identifier(s) : adverse reactions, birth control, mare

Broader term(s) : Equus, Equidae, Perissodactyla, mammals, vertebrates, Chordata, animals, eukaryotes

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FERTILITY CONTROL USING INTRAUTERINE DEVICES: AN ALTERNATIVE FOR POPULATION CONTROL IN WILD HORSES

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ABSTRACT

The purpose of this study was to develop a contraceptive method for feral horses. The feral horse population has increased significantly in recent years despite attempts to control numbers. As in most wild animal population control programs, contraceptive methods must be easy to apply, cause minimal disruption to the social structure and be fully reversible. In the present study, we tested the effectiveness of an intrauterine device (IUD) for fertility control in mares. Six mares were fitted with a silastic O-ring-shaped IUD on July 1 of Year 1. The IUD-treated mares were turned out with 12 nontreated mares and a fertile stallion in a large pasture until October 20 (112 d). None of the IUD-treated mares and all the nontreated mares became pregnant. The IUD-treated mares were maintained separately from the stallion during the winter. Following removal of the IUD on April 27 of Year 2, the mares were again introduced to the pasture with the stallion together with 6 nontreated mares. For the 6 mares previously treated with an IUD, the mean interval from introduction to the stallion to conception was 17.5 ± 5 d or 1.3 cycles per pregnancy, and all mares produced a normal foal at term. Subsequently, 19 recorded mare breeding seasons resulted in 18 foals. Uterine cytology and histopathology indicate that the IUD causes mild chronic endometritis without permanent changes in the endometrium. We conclude that based on our observations, the O-ring-shaped IUD is an effective, safe and practical contraceptive method for mares.

Key words: intrauterine device, contraception, population control

Acknowledgements

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INTRODUCTION

Wild horses are a significant public concern and an economic issue facing the government and private citizens in many of the western states. The feral horse population has increased significantly in recent years in spite of attempts to control the numbers. Attempts to control the growth of the wild horse population include adoption programs, confinement in feedlots, vasectomy of the dominant stallion, progesterone releasing implants and remotely-delivered immunization against zona pellucida proteins (12,13,15). Methods such as the adoption program have had little impact on the growing numbers of horses in the wild. Vasectomy of the dominant stallion (harem sire) has been shown to be ineffective as contraceptive because bachelor stallions breed up to one third of the mares of a harem despite efforts of the dominant stallion to guard his harem (14). Hormonal treatments have had limited success due to dosage requirements and ineffective delivery systems. More recently, immunization against porcine zona pellucida proteins (PZP) has been used successfully to control the reproduction of feral horses (13,14). However, this method requires booster immunizations at regular intervals, and thus it may be more applicable for small herds living in confined areas where individual identification and immunization is possible. In several species, including the horse, immunization against PZP or purified zona pellucida (ZP3) was accompanied by disruption of ovarian function and has resulted in anovulation for extended periods of time. In the mare, continued use of PZP-immunocontraception resulted in a gradual increase of ovarian depression and anovulation, 4 anovulatory mares out of 7 immunized mares and 4 out of 5 immunized mares after 3 and 6 yr of booster immunization, respectively (13,14).

As in most wild animal populations, contraceptive methods in feral horses must be effective and fully reversible. Application must be practical, affordable and cause minimal disruption of the social structure of the herd. To be a practical contraceptive method, it must be applied in a single treatment and be effective for years without significant loss of future fertility. This report describes the development of an effective contraceptive method, using an intrauterine device (IUD), which can be administered in a single treatment to selected individual animals and which is fully reversible. Intrauterine devices have been utilized effectively for fertility control in women for many years (1,3,6,8,18,19). With the introduction of new and improved designs, IUDs have received increasing attention as a human contraceptive. Extensive retrospective studies have proven that long-term IUD use provides a safe and effective method of contraception with a low rate of complication (2,7,8,16). In women using IUDs for up to 3 yr, the cumulative pregnancy rate ranged from 2.6 to 7.0% depending on the kind of IUD used (8,19). The complications that may be caused by the use of an IUD include discomfort, infection and perforation of the uterus. However, the percentage of cases associated with problems remains very low. Perforation of the uterus occurred in only 1.2 to 8.7 per 1,000 women in a study involving several thousands of patients (19). Deaths caused directly by the IUD were as low as 1.06 per million (19). In women, the orientation of the uterus is vertical and the major problem involved with the application of an intrauterine device is its uncertain retention in the uterus. Most

of the pregnancies observed in women using an IUD can be attributed to loss of the IUD unbeknown to the patient. The design of human IUDs is the result of a compromise between effective retention and minimal discomfort for the patient. Problems such as discomfort and occasional perforation of the woman's uterus are due to the shape of human intrauterine devices and the method of placement (1). The mare's uterus has a horizontal orientation and loss of the intrauterine device is less likely to be a problem. This led us to believe that safer and more comfortable devices could be used as effective contraceptive devices in mares without encountering the problems occasionally observed in women. Subsequent fertility is an important consideration in the choice of a contraceptive method. In women, the fertility rate upon removal is about 90%, including the patients who have used IUDs as a contraceptive method for more than 5 yr (9,10). The precise mechanism by which IUDs act is still uncertain. Although there are a variety of IUD-related antifertility effects in different animal species, the single factor common to all species appears to be the local inflammatory response in the endometrium to the IUD.

The aim of this study was to develop a practical, economical, safe and reversible method of contraception for mares that would be effective for several years and that would not interfere with future fertility.

MATERIALS AND METHODS

Experimental Design

An IUD was placed in the uterus of 6 mares in the spring of Year 1. The 6 IUD-treated mares, 12 untreated control mares, and a fertile stallion were housed together on 12 acres of irrigated pasture during the summer of Year 1. The mares were examined for pregnancy at regular intervals, and those more than 30 d pregnant were removed from the herd. In the fall of Year 1, IUD-treated mares were separated from the stallion. In the spring of Year 2, the IUD was removed from the uterus and the mares were placed in a large pasture with the stallion and with 6 additional untreated nonpregnant mares. The mares were examined for pregnancy, and 30-d pregnant mares were removed from the herd.

Animals

In Year 1, 18 nonpregnant, nonlactating mares of various light breeds were selected. Mares were selected when judged to be reproductively sound based on palpation per rectum and ultrasonography of the genital tract, vaginoscopy, uterine cytology, uterine culture and an absence of a history of reproductive problems in the past 2 yr. During this examination a uterine biopsy was collected but histopathological examination was performed at the completion of the experiment together with biopsies collected during the experiment (5,17). Eight mares were maiden and 10 mares were multiparous. Three maiden and three multiparous mares (IUD-mares) were randomly

assigned to the IUD-treated group, and the remaining 12 mares (control mares) were assigned to the untreated group. The stallion used for the study was 8 yr old in Year 1, of proven fertility, and had been used successfully for pasture breeding in the previous year. In Year 2, 6 multiparous nonpregnant mares were selected using the same criteria as in Year 1 and were used as control mares.

Placement and Monitoring of Intrauterine Device

On July 1 of Year 1, 12 d after the initial examination, 1 IUD was placed in the uterus of each mare. Following the IUD placement, the 6 IUD-treated, 12 untreated mares and a stallion were introduced to the pasture. Mares were examined at 3-d intervals for adverse reactions (general attitude, vulvar discharge) and reproductive function. Ovarian activity, uterine and cervical tone and pregnancy status, and the presence and location of the IUD was monitored by palpation and ultrasonography per rectum. Pregnancy was diagnosed by ultrasonography on Days 14, 25 and 30. Mares more than 30 d pregnant were removed from the herd. On October 20 of Year 1, the IUD-mares were separated from the stallion. Fourteen days after separation from the stallion, IUD-treated mares were examined again for pregnancy. After having confirmed that none of the IUD-treated mares were pregnant, samples for uterine cytology and culture and an endometrial biopsy were collected. The IUD-treated mares were housed with 34 pregnant mares on pasture and fed grass hay. The IUD-treated mares remained separated from the stallion until May of Year 2. The stallion remained in the same pasture for the remainder of Year 1 and until the end of the experiment in Year 2. On April 27 of Year 2, the ovaries and tubular genitalia of the IUD-treated mares were examined by palpation and ultrasonography per rectum, vaginoscopy was performed and samples for uterine cytology and culture were collected. Subsequently, the IUD was removed manually through the cervix and an endometrial biopsy was taken immediately after removal of the IUD. On May 8, the 6 mares (previously treated with IUD) and 6 new, nonpregnant mares (control group) were re-introduced to the same stallion used in the previous year. The reproductive status of these 12 mares was monitored every other day by palpation and ultrasonography per rectum of the reproductive organs. Mares more than 30 d pregnant were removed from the herd.

Intrauterine Device

A flexible O-ring, with a thickness of 5 mm and an external diameter of 40 mm, was used as an intrauterine device. The ring was made of pure medical grade silastic elastomer (Silastic 382, Dow Corning, Midland MI 48640). The flexibility of the ring was such that the ring could easily be compressed between thumb and index finger. The flexibility allowed easy insertion and removal through the cervix. Spontaneous expulsion through the cervix was prevented by return of the O-ring to its original circular form. Before the placement of the IUD, the perineal area was washed thoroughly as described for artificial insemination (17). The IUD was inserted with a gloved hand using appropriate sterile lubrication. The IUD was inserted in the cervix

and pushed into the uterus with 1 finger. Removal of the IUD was achieved by reaching through the cervix with 1 or 2 fingers. One finger was hooked through the opening of the O-ring and the IUD was then gently removed. No attempt was made to insert or remove the IUD during a specific stage of the estrous cycle.

Interpretation of Uterine Cytology, Culture and Biopsy

Uterine samples for cytological examination were fixed and stained (Diff-Quick Stain, American Scientific Products, McGaw Park, IL; 18). Presence of 1 to 25 polymorphonuclear neutrophils (pmns) per 5 high powered fields (x240) was determined as a few, 25 to 200 as a moderate number and more than 200 as a high number of pmns. Uterine samples were submitted for aerobic and anaerobic cultures. Samples for uterine cytology and culture were evaluated without previous knowledge of treatment status. Uterine biopsies were evaluated in random order after collection of the last sample and were classified on a scale of I to III, with I being essentially a normal endometrium and III indicating severe pathological changes and an expected pregnancy rate of less than 10% (5,11).

RESULTS

Placement, Retention and Removal of Intrauterine Device

Introduction of the collapsed O-ring (Figure 1) was possible in all mares regardless of the stage of the estrous cycle. Placement of the IUD in the uterus was achieved on the first attempt and took less than 1 minute in each mare. In 5 of 6 mares, the IUD remained in the uterus until it was removed 10 mo later (Year 2). In 1 mare the IUD was no longer present at the first examination 3 d after initial placement. A new IUD of the same type was introduced and remained in the uterus until removal in Year 2.

Removal of the IUD was uncomplicated. Three mares were in estrus at the time of removal and their cervix was open and relaxed. Average time needed for removal was less than 1 min. Three mares were in diestrus and the cervix was closed. In the diestrous mares, gentle massage for approximately 1 min was sufficient to dilate the cervix to allow introduction of a finger and the entire removal procedure required less than 3 min per mare. The IUD was removed from all the mares on the first attempt. Age or parity did not have a noticeable effect on the ease of placement or removal.

Pregnancy Rate

In Year 1, all untreated mares were pregnant at the end of the summer. The mean number of estrous cycles per pregnancy rate was 2 ± 0.3 cycles/pregnancy (mean $\pm$ SEM) and ranged between 1 and 4 cycles/pregnancy. None of the IUD-treated

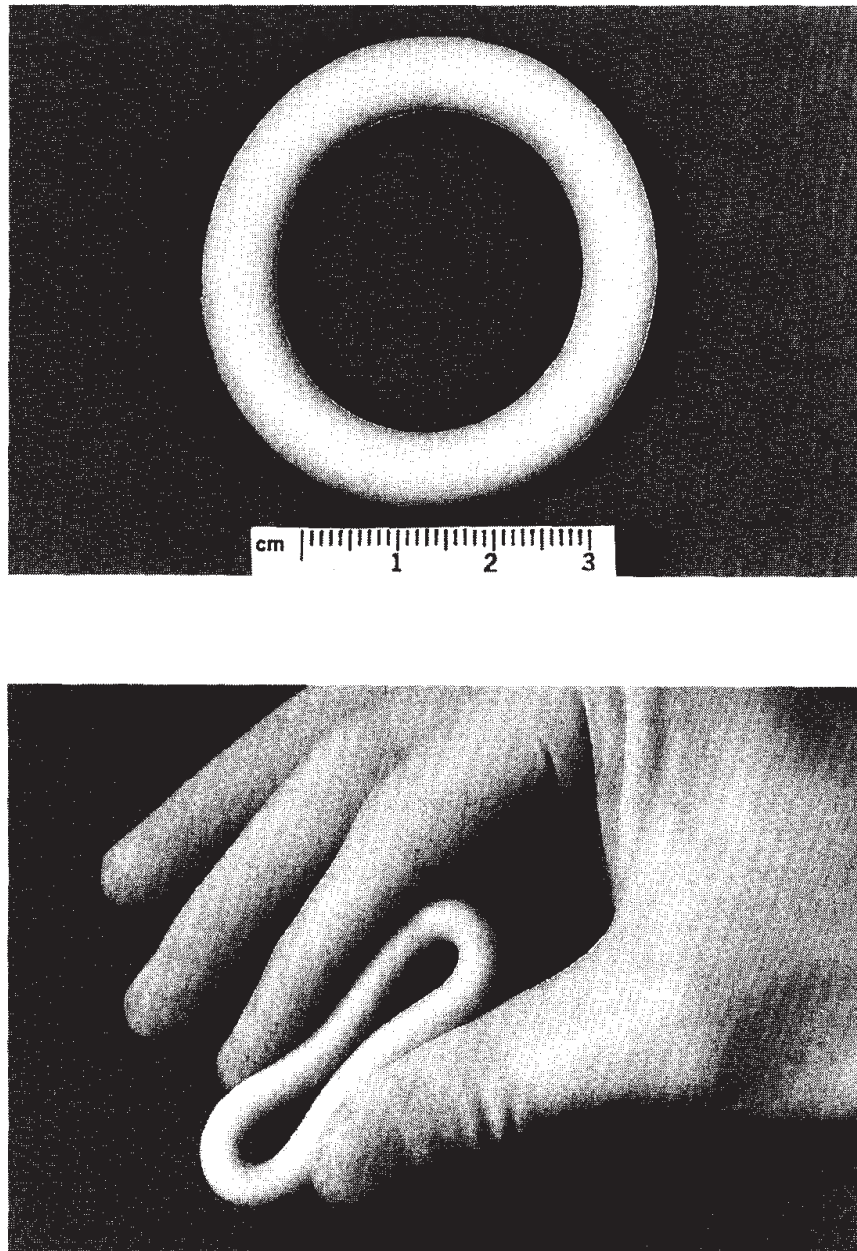


Figure 1. Photograph (top) of the type of IUD used in this study. The IUD could easily be collapsed between thumb and index finger as shown in the bottom photograph.

mares became pregnant as determined by ultrasonography of the uterus per rectum. During the period that the IUD-treated mares were exposed to the stallion (112 d), the estimated number of estrous periods per mare was 5.3 ± 0.3 (range: 4 to 6 cycles). Mares continued to have estrous cycles although estrous cycle length tended to vary more than in the untreated mares. Mean interovulatory interval in IUD-treated mares was 19 ± 1.4 d and intervals ranged between 9 and 35 d based on ovulation observed during estrus.

In Year 2, 5 of 6 untreated control mares were pregnant after the first estrus and one was pregnant after the fourth estrus, resulting in a per cycle pregnancy rate of 1.5 ± 0.5 cycles/pregnancy. For the 6 mares previously treated with IUD, the mean interval from introduction to the stallion to conception was 17.5 ± 5 d (range: 6 to 38 d). Four mares conceived after the first estrus and 2 conceived after the second estrus, resulting in a pregnancy rate of 1.3 ± 0.2 cycles/pregnancy. The pregnancy rate for both groups was not significantly different ($P > 0.05$) based on the Student's t-test. All mares delivered a normal foal at term in the following year (Year 3). The 6 IUD-treated mares were bred during the first postpartum estrus in Year 3. Five mares conceived on the first postpartum estrus (foal heat) and one on the second postpartum estrus. Mares produced a normal foal at term, but not all mares were bred back. For those mares bred again in following years, 13 recorded breeding seasons resulted in 12 successful pregnancies. Information on fertility in subsequent years was not available for control mares used in Year 1 and 2.

Uterine Cytology and Culture

Uterine cytology and culture samples were collected from the six IUD-treated mares before placement of the IUD, 14 d after separation from the stallion and on the day of IUD removal. Before IUD placement, no signs of uterine inflammation (less than 1 pmn per 5 high powered fields) were observed on uterine cytology and culture resulted in no growth in all mares. Fourteen days following separation of the IUD-treated mares from the stallion, few numbers of pmns were observed in 2 mares and moderate numbers of pmns were observed in the 4 remaining mares. Uterine culture in these 4 mares resulted in small numbers of Streptococcus zooepidemicus in 4 mares and very small numbers of Escherichia coli in 1 mare. Immediately before removal of the IUD (spring of Year 2), all mares had either few or moderate numbers of pmns on uterine cytology and 2 mares had very small numbers of Streptococcus zooepidemicus. The inflammatory cells consisted primarily of mature hypersegmented pmns with few lymphocytes scattered among the pmns, indicating a nonseptic but chronic inflammation. Uterine cytology and culture was repeated 1 week after removal of the IUD (May 8), before introduction to pasture with the stallion, and no evidence of uterine inflammation and/or infection was observed at this time.

Uterine Biopsy

The results of the histopathological evaluation of the uterine biopsies are listed in Table 1. At the onset of the experiment (June 18), 5 mares had essentially normal endometrium (Grade I) and one mare had signs of a mild chronic endometritis (Grade II). During the time of the IUD treatment (November 5 and April 27), all mares had mild to severe uterine inflammation. The inflammatory reaction appeared to be severe enough to interfere with fertility. However, histopathological changes did not appear to be of a permanent nature (periglandular fibrosis), suggesting that if the cause of the inflammation was eliminated the mares would return to normal fertility.

Table 1. Histopathological evaluation of uterine biopsy

Mare ID No	Date	Histopathology of uterine biopsy
298	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, moderate, diffuse, subacute (Grade II)
	Apr 27 (Year 2)	Endometritis, moderate to severe, diffuse, subacute, ulcerative, active (Grade II)
595	Jun 18 (Year 1)	Endometritis, mild, multifocal, chronic (Grade I)
	Nov 5 (Year 1)	Endometritis, mild to moderate, multifocal, chronic, active (Grade II)
	Apr 27 (Year 2)	Endometritis, mild to moderate, multifocal, chronic, active (Grade II)
6556	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, moderate, diffuse, acute, stratum compactum (Grade II)
	Apr 27 (Year 2)	Endometritis, moderate, diffuse, acute (Grade II)
6557	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, subacute, moderate to severe, diffuse, stratum compactum (Grade II)
	Apr 27 (Year 2)	Biopsy not available
6558	Jun 18 (Year 1)	Essentially normal endometrium (Grade I)
	Nov 5 (Year 1)	Endometritis, acute, moderate, diffuse, stratum compactum (Grade II)
	Apr 27 (Year 2)	Endometritis, moderate, diffuse, chronic, active (Grade II)
6559	Jun 18 (Year 1)	Endometritis, mild, focal, chronic (Grade II)
	Nov 5 (Year 1)	Biopsy not available
	Apr 27 (Year 2)	Endometritis, chronic, active, severe, diffuse (Grade III)

Body Condition

The IUD did not appear to interfere with the general health status of the mares. They remained in good body condition and appeared to thrive as well as the control mares during summer and pasture mates during the winter period. Animal health technicians in charge of daily supervision did not observe any loss of weight or appetite, rough hair coat, gastrointestinal problems or vaginal discharge.

DISCUSSION

The results of this experiment indicate that IUDs can be used as a contraceptive in the mare. Mares continued to have estrous cycles and were bred by the stallion but failed to conceive. In our experiment, pregnant mares were removed from the herd to maximize the chances of conception of the remaining nonpregnant mares. Following removal of the IUD all mares returned to normal fertility in a very short interval and the reproductive performance of these mares in the subsequent years suggest that there was no residual effect of the IUD on fertility. At the end of the breeding trial in Year 1, IUD-treated mares were relocated to a different pasture. Upon the return of the mares to the original location, they were not exposed to the stallion until after removal of the IUD. However, the results of uterine cytological and histopathological examination suggested that the condition of the uterus at the time of IUD removal in Year 2 was not different from that observed at the end of the breeding trial in Year 1. In women, the proposed mechanism of contraceptive action of the IUD is a chronic low-grade uterine inflammation. In mares, the same mechanism appears to be present. During IUD treatment, there was cytological and histopathological evidence of endometritis and mares failed to conceive. However, within 1 wk after IUD removal, cytological evidence of endometritis and bacterial growth had subsided and mares had returned to normal fertility.

Of all our large domestic species, the mare lends itself best to the use of IUDs for contraception. Due to its size and the anatomy of the genital tract, placement and removal of the IUD are possible on the standing animal, and instrumentation is not required. Placement was done by someone proficient in reproductive techniques in mares but with no prior training in this specific procedure. The thickness of the IUD ring was such that the ring was readily collapsible when placed between the thumb and index finger but rigid enough to resist the uterine contractions and spontaneous expulsion through the cervix. It is not clear why the IUD was lost by 1 mare within 3 d after placement. Loss may have been the result of improper placement, possibly intracervically. This is supported by the observation that following placement of the same type of IUD, this mare retained the IUD in the uterus for the remainder of the experimental period. In this study, a delay was planned between insertion of the IUD and introduction to the pasture with the stallion. This observations period allowed us to better assess the retention of the IUD and possible adverse reactions (abdominal discomfort). Since no adverse reactions were noted it appears safe to reintroduce

mares to the herd immediately following IUD placement. Following removal of the IUD, a uterine biopsy was collected and mares were kept under close observation for 1 wk. Based on the cytology and culture results obtained 1 wk after removal of the IUD, it appears that mares could be reintroduced to the herd immediately after removal of the IUD.

In our study, we did not collect samples for uterine cytology, culture or biopsy from the nontreated mares because we anticipated that nontreated mares would become pregnant and thus would not be available for subsequent sampling. During IUD treatment, all mares had a mild to moderate number of pmns present in the uterus. Cells appeared nontoxic suggesting that the endometritis was primarily the result of irritation caused by the presence of the IUD. The presence of bacteria at the end of Year 1 and the beginning of Year 2 is difficult to interpret. While bacterial growth at the end of Year 1 could have been the result of exposure to the stallion, it is not clear if the continued presence of bacteria in the spring of Year 2 was related to the IUD. At this time mares had not been exposed to the stallion for 6 mo. It is possible that continued presence of bacteria in the uterus was the result of impaired uterine clearance caused by the IUD, but this hypothesis needs further investigation. Uterine histopathology confirmed that mares had a mild to moderate, chronic endometritis during IUD treatment. The absence of pmns on cytological examination and bacterial growth 1 wk after IUD removal supports the hypothesis that the presence of an IUD may cause chronic bacterial infection in some mares. The design of the IUD, shape and size, may need to be re-evaluated to address this concern. However, it is encouraging that after 1 yr of IUD-treatment there was no histopathological evidence of permanent changes in the uterus, such as periglandular fibrosis, which could impair future fertility of mares following IUD removal. The rapid return to normal on cytological examination and the fertility in the years following IUD treatment support the histopathological observations that long-term potential fertility was not affected by IUD treatment.

The results of this study provide the first evidence that the use of IUDs is a viable alternative for contraception in feral mares. Pregnancy was prevented in all mares during treatment, the mares continued to have normal estrous cycles, side effects were not observed, and the mares returned to normal fertility after IUD removal. While these results are encouraging, larger field trials need to be done to examine the effect of prolonged IUD treatment on subsequent fertility.

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Chronic intermittent colic in a mare attributed to uterine marbles

By: [Freeman, CE](#) (Freeman, C. E.)^[1]; [Lyle, SK](#) (Lyle, S. K.)^[1]

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Abstract

A 13-year-old Quarter Horse mare presented for evaluation of chronic intermittent colic. Following extensive diagnostics, abdominal radiographs revealed two round, radiopaque objects in the caudal abdomen. Palpation per rectum and transrectal ultrasonography of the reproductive tract confirmed that the round objects were uterine marbles. Dinoprost tromethamine (Lutalyse, 5mg i.m. q. 24h for 2days) was administered to bring the mare into oestrus, and both uterine marbles were manually removed from the uterus following digital dilation of the relaxed cervix. Follow-up with the owner 12months after discharge revealed that the mare had shown no further signs of abdominal discomfort since having the uterine marbles removed. To the authors' knowledge, this is the first published report of chronic intermittent colic attributed to uterine marbles in a mare.

Keywords

Author Keywords: [horse](#); [intermittent colic](#); [uterine marble](#); [oestrus suppression](#); [abdominal radiograph](#)KeyWords Plus: [INTRAUTERINE-DEVICE](#); [ESTROUS-CYCLE](#); [PONY MARES](#); [GNRH](#); [SUPPRESSION](#); [OXYTOCIN](#); [DIESTRUS](#); [ESTRUS](#); [BEHAVIOR](#); [HORSES](#)

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Original Research

Self-Assembling Intrauterine Device (Upod) Modulation of the Reproductive Cycle in Mares

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ABSTRACT

The present study evaluated a novel intrauterine device for its effect on estrus suppression. The self-assembling intrauterine device (Upod) consists of three 12 mm × 26 mm elliptically shaped units each with a magnetic core and coated with a polymer (total weight 22.5 g). Each magnetic unit is inserted independently from the other. Once inside the uterus, these magnetic elliptical units self-assemble and adapt the lower energy “ring” conformation. In mares, the devices can be inserted at any stage of the estrous cycle without the need for multiple exams. Shatter-proof grade material is used to assure safety for long-term use. The Upod is inserted and retrieved using an applicator and magnetic retriever. In experiment 1, Upods were inserted postovulation in 15 mares during estrus. The mean duration of diestrus was 73.4 ± 36.6 days (mean $\pm$ S.D.). Endometrial biopsies showed no significant differences before and after Upod placement. In experiment 2, Upods were inserted regardless of the stage of the estrous cycle in 14 mares. The mean duration of diestrus was 51.3 ± 22.7 days (mean $\pm$ S.D.). The presence of the Upod was monitored by transrectal ultrasound and an external handheld metal detector. Progesterone was assayed to monitor corpus luteum lifespan, and the mean duration of diestrus was significantly extended in both experiments compared to the control group. Eight mares were randomly selected to be bred the following year, with 100% conception rate post-Upod retrieval.

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1. Introduction

Mares in estrus can behave in ways that can interfere with handling and competition. Current methods to suppress estrus behavior include pharmacological treatments, glass marbles, and negative reinforcement strategies.

The use of pharmacological therapies, a common strategy, is to administer synthetic progesterone compounds to horses on a daily basis [1]. Different methods of application include intramuscular (IM) administration of exogenous progesterone [2] or oral administration of the synthetic progestin altrenogest (Merck, Madison, NJ, 07940) [3]. The U.S. Food and Drug Administration recently

highlighted the potential health risks, including reproductive system disorders and other adverse effects, in people who are exposed to altrenogest through contact with skin or product residue on barn surfaces, equipment, or treated animals [4]. The administration of exogenous oxytocin during diestrus can disrupt luteolysis; however, it requires IM administration every 12 hours [5]. GnRH vaccines, antagonists, or agonists with highly variable individual responses and irreversibility have also been used [6].

Another pharmacological therapy includes injections of medroxyprogesterone acetate [7]; however, this drug is considered a controlled medication and has been prohibited in Federation Equestre Internationale competition since 2011.

All these treatments require frequent administration and generally only result in a short-term effect. Therefore, the use of these pharmacological therapies for behavioral control is limited.

Another strategy is the use of 25 mm or 35 mm glass marbles placed in the uterus of mares to extend the functional lifespan of the corpus luteum [8]. The 25-mm glass ball was spontaneously expelled from the uterus of 6 of 12 mares (50%), whereas the 35-mm glass ball had 100% retention rate. However, an extended mean luteal function of 87 days (range 76–109 days) occurred in

Animal welfare/ethical statement: All experimental procedures were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee.

Conflict of interest statement: Partial costs defrayed by a Technology Development Fund, UMass. (UMass has applied for a patent).

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only 7 of 62 diestrus periods (11%) among mares following glass ball placement. When mares were bred in the season subsequent to glass ball removal, 17 of 23 (74%) conceived. Complications have been reported with the use of glass marbles, including chronic endometritis, pyometra, and glass shards becoming adhered to the endometrium [9]. Morris et al. described several cases involving fragmentation of marbles in the uterine lumen [10].

This raises the question: Do the control mechanisms of an embryo and a self-assembling intrauterine device (Upod) differ on luteal function? Estrus suppression using Upods extend luteal function, but the biological mechanism is unknown. A luminal inflammatory response in the uterus without observable changes in the endometrium could somehow be involved. It is generally accepted that intrauterine devices (IUDs) produce a local, sterile, inflammatory reaction [11,12]. The Upod releases no chemical compounds but may exert pressure on the uterine walls by physical contact. Embryo mobility is due to uterine contractions that move them in the uterus [13], and unrestricted motility is a prerequisite for pregnancy maintenance [14]. However, IUD movement in the uterus is limited [15], and thus local influence directly on the endometrium is most likely. For example, after mechanical stress, uteroferrin [16] may be involved in luteostasis in the mare and be reflective of chronic inflammation.

Important factors considered in the development of the Upod were reversibility and absence of any lasting detrimental effects on fertility as any mare currently engaged in a sporting career may be required to breed. The parameters set were as follows: (1) efficacy; (2) safe to be used in mares; (3) minimal distortion of the uterine lumen; (4) high retention rate in the uterus; (5) easy for the veterinarian to insert through the cervix with or without an applicator; (6) horse owner ability to monitor if the device is present by noninvasive means (readily available handheld metal detectors); (7) easily visualized by ultrasound; (8) and easy to retrieve.

The purpose of this study was to develop and test an Upod that would suppress the expression of estrus in mares by extending the lifespan of corpora lutea. Specifically, the study's objectives were to evaluate (1) Upod retention rates, (2) the effect of Upods in suppressing the frequency of estrus, (3) uterine integrity following Upod removal, and (4) fertility post-Upod retrieval.

2. Materials and Methods

All experimental procedures were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee.

2.1. Animals

Thirty-eight mares (Upod treatment $n = 38$) of mixed light horse breeds, aged 3–20 years, with no history of reproductive failure, clinically normal and nonpregnant, were used over a five-year period (2013–2018) for the development and testing of the novel Upod device. The mean $\pm$ standard deviation (SD) of age at time of Upod insertion was 11.5 ± 7.1 years. Seven mares (control $n = 7$) were also randomly chosen to serve as controls for the study. The mean $\pm$ SD of age for the control mares was 8.6 ± 5.9 years (range 3–17 years old). Eight mares (fertility $n = 8$) were randomly selected to be bred post-Upod retrieval. The mean $\pm$ SD of age at time of breeding was 11.1 ± 6.2 years (range 4–20 year old).

All mares were housed and cared for at the University of Massachusetts Hadley Farm. Mares were fed free-choice forage (mixed grass hay and pasture), a ration balancer, and had 24/7 access to water and a plain white salt block. The majority of mares lived outside in small herds with access to a three-sided shelter; five of

the mares were individually stalled overnight and received a minimum of 6 hours of daily turnout.

2.2. Intrauterine Device

The intrauterine device used was a frameless, self-assembling, sterilized device (Upod; 12 mm $\times$ 26 mm) made of shatter-proof inert FDA grade material with a mean total weight of 22.5 g (Fig. 1). For comparison, a 35-mm glass marble weighs 53 g.

Three dimensional printing was used to design an ergonomic, magnetic-based Upod that can be adapted to several mammalian species. The Upod consists of three magnetic elliptically shaped units each with a magnetic core coated with a polymer, inserted independently from each other. In mares, the device can be inserted at any stage of the estrous cycle without the need for multiple exams or repeated darting in the case of wild equids. Once inside the uterus, these magnetic elliptical units will self-assemble and adapt the lower energy triad conformation.

The ability of the device to self-assemble after insertion remain in a stable configuration and then disassemble for removal is governed by magnetic forces and required a few physical design considerations that were tested and optimized (Figs. 1 and 2). The “ring” shape formed by the three magnets is a singularly unique configuration that minimizes the magnetic energy of the system. In physics terminology, the system does whatever necessary to achieve a complete “circuit” of magnetic flux closure, ultimately leading to the “ring” shape. The shape of the package material surrounding the magnet provides an additional constraint that modifies this stability. Minimizing uterine lumen (cavity) deformity may be important to reduce device expulsion/rejection.

The field stability is aided by the ring geometry, in that, even if one has a somewhat lower coercivity material, there is a strong energetic tendency to stay in a ring formation. Thus, the magnetic field strength is stable, especially once it is in the ring configuration.

Stability and on-demand disassembly were investigated and optimized using computer modeling and experimental prototypes. Package prototypes of different shapes were made by 3D printing. Package shape was found to also impact biocompatibility, as rounded surfaces that allow for small fluctuations in ring configuration helped to keep the surfaces free of biofilm formation (ongoing research). Because of its biocompatibility, Teflon (PTFE) was identified as a preferred package material, and next generation designs will include either gold-copper 2^+ bands or progesterin eluting polymers.

2.3. Insertion and Retrieval of Upod

Upods for mares in estrus (or within 48 hours postovulation) can be inserted using an Upod applicator with the 3 units loaded in tandem or alternatively can be manually inserted. The 3 units are pushed through the cervix until just clear of the internal cervical os.

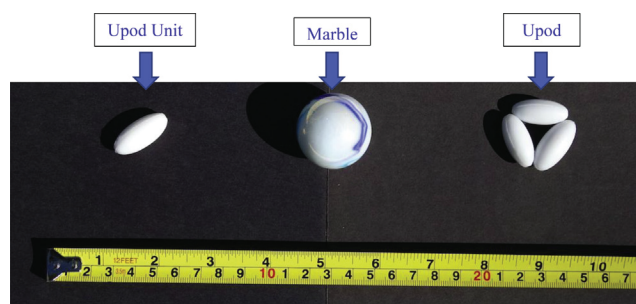


Fig. 1. Upod (7.5 g each = 22.5 g total); 35 mm glass marble (53 g).

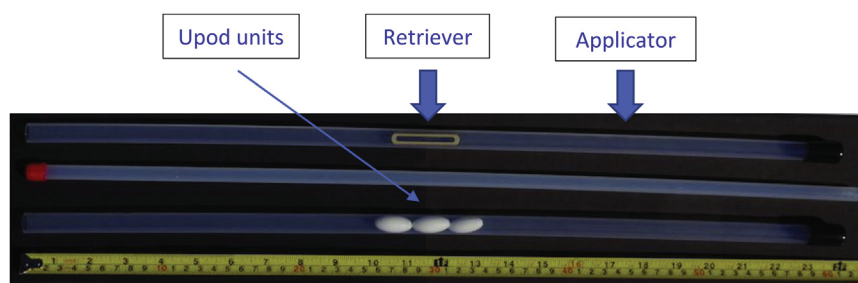


Fig. 2. Upod magnetic retriever and applicator.

The location of the devices should be checked with ultrasound. The Upod will self-aggregate in the uterus in a “ring” configuration (please see [video 1. Upod Insertion Horse Model - Supplementary material](#)). For mares with a closed cervix (e.g., early estrus, diestrus, maiden mares), insert individual units by hand. Place the 3 units of the Upod in one's hand and push them through the cervix one by one.

To retrieve the Upods, induce estrus with 250 µg cloprostenol (Estrumate; Merck Animal Health, Millsboro, DE, 19966-4217) and retrieve the Upod in mid-estrus using the specially designed magnetic retriever with the applicator. The retriever is attached to a fluorocarbon line. Insert the applicator loaded with the retriever through the cervix and push the retriever into the uterus, making sure the retriever drops into the uterus, or sweep the uterus with the applicator and retriever until contact with the Upod. One can either (1) keep the applicator in the cervix as one pulls the tag line and feels the device with the index finger at the end of the applicator, or (2) withdraw the applicator, leaving and holding on to the tag line, before pulling and feeling the weight of the Upod attached to the retriever (please see [video 2. Upod Retriever Horse Model - Supplementary material](#)).

2.4. Experimental Design

Mares were examined at the time of Upod insertion by transrectal palpation and ultrasonography to rule out pregnancy, presence of uterine fluid or other pathologies (e.g., urine pooling, granuloma cell tumor). At the time of Upod insertion, the perineal area was scrubbed with 7.5% povidone-iodine, and mares were sedated with 0.02–0.04 mg/kg IV detomidine hydrochloride (Dormosedan HCL; Zoetis US, Parsippany, NJ, 07057) (please see [video 3. Upod Placement - Supplementary material](#)). After insertion of the Upod, 3.1 g of disodium/clavulanate potassium (Absolute Veterinary Compounding Pharmacy Weatherford, TX, 76086-4779) diluted in 10 mL of sterile water was introduced into the uterus (please see [video 4. Intrauterine Infusion - Supplementary material](#)). Mares were examined by transrectal ultrasonography within 5 minutes postinsertion (please see [video 5. Upod Ultrasound Detection - Supplementary material](#)) and then weekly (Fig. 3). The number and size of follicles, the corpora lutea, and the presence of intrauterine fluid were assessed with ultrasonography and recorded. Transabdominal detection of the Upod, using a handheld magnetic detector wand, was also performed weekly for the first month and then biweekly.

2.4.1. Pilot Study

Phase one of the study consisted of testing several prototypes of the novel intrauterine device on nine mares ($n = 9$) over a two-year period (2013–2015). Specifically, the techniques for insertion, visualization, transabdominal detection, and retrieval of the device were tested. Estrus was induced with an IM injection of 250 µg

cloprostenol (Estrumate), and the Upod was pushed through the cervix into the uterus 48–72 hours postovulation, using the double-glove technique [17]. Mares also received an IM injection of 250 µg cloprostenol (Estrumate) to induce estrus to facilitate the removal of the Upod.

2.4.2. Experiment 1

Phase two of the study consisted of testing the refined Upod design on 15 mares ($n = 15$) during the 2016 breeding season. Specifically, retention of the Upod and effect on uterine health were investigated. Upods were inserted and retrieved as described previously in the pilot study. The device was retrieved after 3 months (4 mares), 5 months (2 mares), 13 months (3 mares), 14 months (4 mares), 17 months (1 mare), or 18 months (1 mare). Serum samples were collected for progesterone assay at the time of Upod insertion to confirm the mares were in estrus and then assayed every 2 weeks to monitor corpus luteum function. Transabdominal detection was also performed at each blood draw. Samples for uterine biopsies were taken before Upod insertion and at the time of Upod retrieval. Samples were taken during estrus based on edema and vascular congestion. A postinsertion uterine biopsy specimen was obtained from the ventral wall at the base of either horn, ipsilateral to last observed Upod location; if the Upod was located in the uterine body, the biopsy was taken from the base of the right horn.

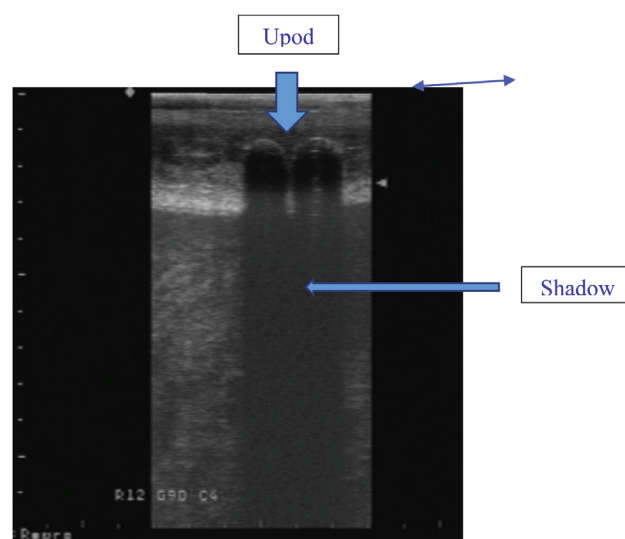


Fig. 3. Ultrasound image of a Upod (down thick arrow) in the uterus; Uterus on top; Bladder at the bottom. Note the absence of fluid in the lumen (cavity) of the uterus, and particularly in the vicinity of the Upod, indicating absence of clinical signs of inflammation. Please notice the shadowing artifact produced by the device (horizontal thin arrow).

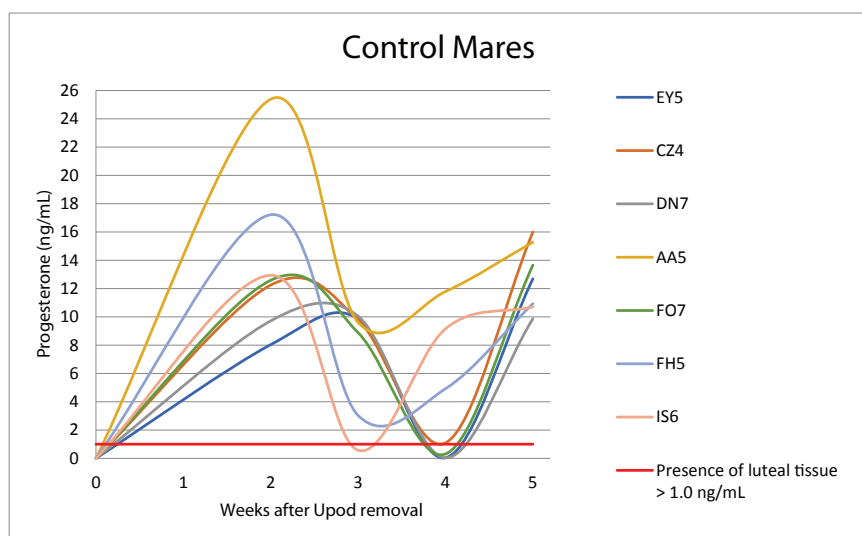


Fig. 4. Progesterone profiles of control mares post-Upod retrieval.

2.4.3. Experiment 2

Phase three of the study consisted of testing the Upod on 14 mares ($n = 14$) during the 2017 breeding season. Mare progesterone profiles were collected on mares with an intrauterine device ($n = 14$) and after the intrauterine device was removed as control data ($n = 7$). Specifically, progesterone was assayed at the time of Upod insertion, then weekly in the first month postinsertion, and then every 2 weeks. Progesterone was assayed weekly from the control mares for an additional five weeks after Upod retrieval. Transabdominal detection was also performed at each blood draw. In addition, the insertion of the device during diestrus was tested. In this phase, mares were not induced into estrus. Upods were inserted late in the breeding season into the uterus of 14 mares, regardless of the stage of the estrous cycle. Four of the mares in this study had nursing foals by their side for the duration of the study (Upods were inserted 3–10 weeks postpartum). The devices were retrieved at 3 months postinsertion (end of September) for 10 mares and at 5 months postinsertion (end of November at time of weaning) for the 4 postpartum mares.

2.4.4. Experiment 3

Phase four of the study consisted of post-Upod fertility testing during the 2018 breeding season. Eight mares ($n = 8$) were randomly assigned to be inseminated with fresh cooled or frozen semen on the subsequent season following Upod retrieval. These mares had carried the devices for at least 3 months before being inseminated, post-Upod removal. Mares were ultrasounded transrectally 14 days postovulation to check for pregnancy.

2.5. Analysis of Blood and Tissue Samples

Serum for progesterone analysis was performed at a reference laboratory using MP Biomedicals Progesterone RIA (Animal Health Diagnostic Center, Ithaca, NY, 14853), without previous knowledge of treatment status. Reference progesterone values were as follows: Absence of active luteal tissue: 0.1–0.5 ng/mL; borderline for the presence of luteal tissue: 0.5–1.0 ng/mL; presence of luteal tissue: > 1.0 ng/mL.

Endometrial biopsies were fixed in 10% neutral-buffered formalin, routinely processed, and stained with hematoxylin and eosin (H&E). Tissue sections were evaluated by a board-certified

veterinary pathologist blinded to treatment history and graded as described by Kenney and Doig [18].

2.6. Statistical Analysis

For analysis of the results, independent samples T-tests were used to detect significant ($P < .05$) differences in mean diestrus durations and interestrus intervals (IEI) between control mares and Upod mares with sustained progesterone levels and persistent diestrus (Upod-P) as estimated by progesterone levels (SPSS; StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX, 77845). The W/S test for normality ($P > .10$, accepts the null hypothesis that the data were normally distributed) was used to determine normal distribution of the data before statistical analysis; the Kolmogorov–Smirnov test confirmed normal distribution in SPSS.

3. Results

3.1. Control Mares—Duration of Diestrus

Seven mares ($n = 7$) were randomly chosen to serve as controls for the study (Fig. 4). Mean duration of diestrus for control mares was 16.6 ± 7.4 days (mean $\pm$ S.D.).

3.2. Pilot Study

Nine mares ($n = 9$) were used in the pilot study. The techniques for insertion, visualization, transabdominal detection, and retrieval of the device were tested and refined. Stability and on-demand disassembly were investigated and optimized.

Table 1
Average diestrus length and interestrus interval (IEI) in days in study 1 and 2.

Group	Duration of Diestrus (d)	IEI (d)
Control mares ($n = 7$)	16.6 ± 7.4 (7–28)	23.6 ± 7.4 (14–35)
Study 1 ($n = 15$)	73.4 ± 36.6 (20–155) ^a	75.7 ± 36.0 (27–162) ^a
Study 2 ($n = 14$)	51.3 ± 22.7 (21–92) ^a	57.5 ± 23.8 (28–99) ^a

Average diestrus length and interestrus interval (IEI) in days represented as mean $\pm$ SD (range).

Based on serial progesterone concentration value decline to <1 ng/mL indicating absence of luteal tissue. Values in brackets are minimum and maximum values.

^a $P < .05$.

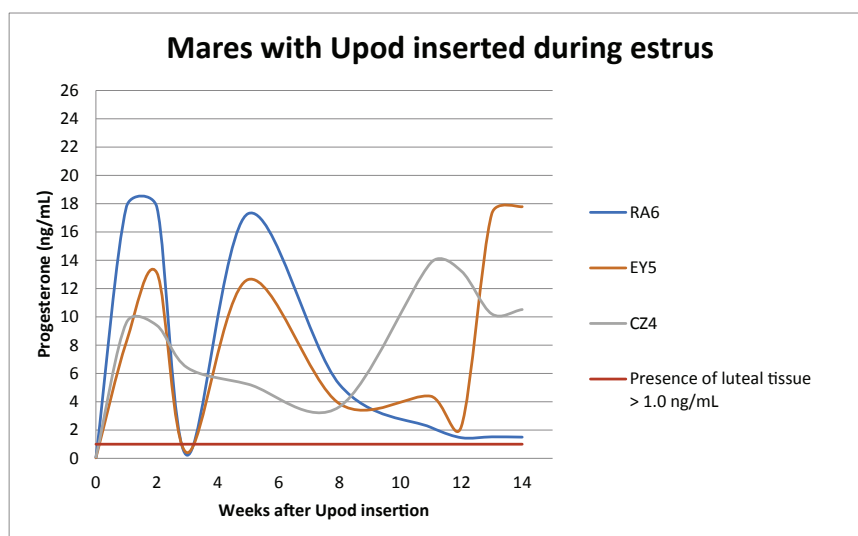


Fig. 5. Progesterone profiles of mares with Upod inserted during estrus.

3.3. Experiment 1—Duration of Diestrus, Retention, and Uterine Biopsies

Mares ($n = 15$) were induced into estrus and had a mean progesterone concentration of 1.7 ± 3.8 ng/mL at time of Upod insertion. Mean duration of extended diestrus for Upod mares was 73.4 ± 36.6 days (mean $\pm$ S.D.), a significant ($P < .05$) difference compared to control group (Table 1). Two mares underwent luteolysis 3 weeks following device insertion (Fig. 5).

Upod retention was 100% and the devices were purposely removed at the end of the breeding season (75–227 days post-insertion). Total Upod retention in this experiment was 2,377 days (equivalent to 113 mare-21 day average cycles).

The results of the histopathological evaluation of the uterine biopsies are shown in Table 2. Before Upod insertion (time 0), 7/15 mares had normal endometrium (Grade I) and 8/15 mares had signs of a mild chronic endometritis (Grade II). After Upod removal (time 1), there were no significant histopathological changes in the grade of the uterine biopsy for all the mares. Changes in inflammation scores in endometrial biopsy specimens obtained at time 0 and time of retrieval were i) increase 4/15 (26.7%); decrease 3/15 (20.0%); no change 8/15 (53.3%). None of the mares showed fluid accumulations in the uterus during diestrus in the cycle preceding Upod insertion. In 2 Upod mares, small accumulation of non-echogenic intrauterine fluid ($\leq 5 \times 5$ mm) that dispersed over time was seen during estrus but not during the extended luteal phase. Uterine edema was observed in Upod mares in estrus in the presence of a preovulatory follicle. No vaginal discharge was observed in any mare carrying Upods or at time of removal.

3.4. Experiment 2—Duration of Diestrus, Retention, and Progesterone Profiles

Mares ($n = 14$) were not induced into estrus and had a mean progesterone concentration of 5.6 ± 5.6 ng/mL at time of Upod insertion. Mean duration of diestrus for Upod mares was 54.6 ± 24.1 days in mares that were in diestrus ($n = 10$) and 42.9 ± 18.9 days for mares in estrus ($n = 2$) or without ovarian activity at time of insertion ($n = 2$). Mean duration of diestrus for all 14 mares was 51.3 ± 22.7 days. A significant ($P < .05$) difference was found compared to control group (Table 1). Two mares underwent luteolysis 1 week following device insertion (Fig. 6).

Upod retention was 100% for the duration of the experiment (98–161 days). Total Upod retention in this experiment was 1,491 days (equivalent to 71 mare-21 day average cycles). Progesterone profiles are shown in Figs. 4–6.

Twelve of the mares (85.7%) experienced extended luteal function, and the average duration of diestrus was 74.1 ± 11.1 days (range 64–92). Nineteen estrous cycles were observed over the 98-day study; 63.2% (12/19) of those cycles had prolonged luteal function of more than 35 days (Table 3).

We observed that Upod-treated mares ≤ 15 years of age had a slightly longer extended luteal function (56.3 ± 15.9 days) than mares > 15 years of age (44.5 ± 29.8 days); however, this difference was not statistically significant ($P = .35$).

3.5. Experiment 3—Fertility

Eight mares (3 maiden and 5 multiparous) were randomly selected to test post-Upod fertility. Eight of the eight mares conceived using artificial insemination with fresh cooled or frozen semen for 100% conception rate. The oldest mare in the group successfully conceived post-Upod retrieval using frozen semen at the age of 20 years.

Table 2
Histopathology category^a of endometrial biopsy specimens in study 1.

Mare ID	Time 0 (Upod Insertion)	Time 1 (Upod Retrieval)
AA5	IIA	I
CZ4	IIA	IIA
EY5	I	IIA
FH5	I	I
IL6	I	IIA
IS6	I	I
JR6	I	I
LI6	I	I
LY5	I	IIA
MA4	IIA	IIA
NE4	IIA	I
NS5	IIA	I
RA6	IIA	IIB
SL5	IIA	IIA
TK6	IIA	IIA

Grade I (normal) or IIA (mild lesions).

^a Kenney/Doig grading. Samples taken at estrus based on edema and vascular congestion.

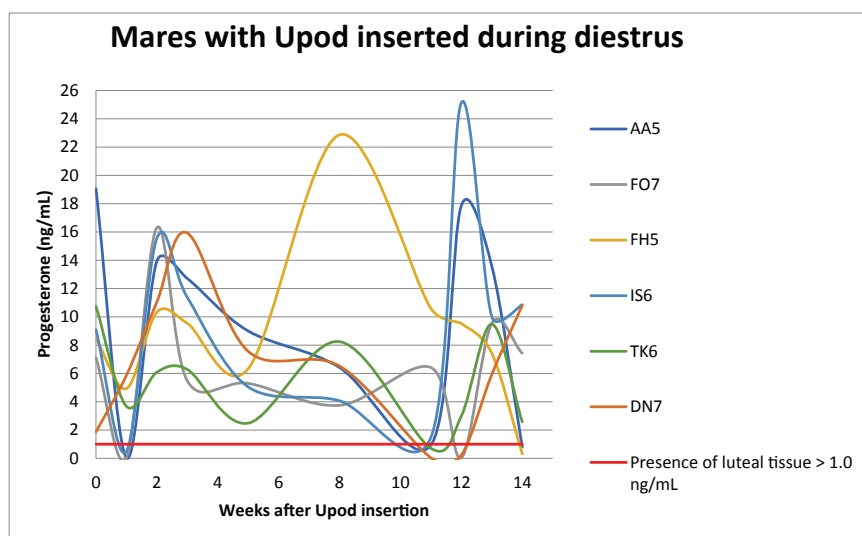


Fig. 6. Progesterone profiles of mares with Upod inserted during diestrus.

4. Discussion

This is a novel device manufactured with inert FDA shatter-proof grade material. The Upod's long-term safety and minimal distortion of the uterine lumen (cavity) decrease the risk for discomfort/pain/inflammation resulting in a high retention rate. Because this device has elastic potential energy, the three vertices will dissipate forces against the uterine walls. This decreases potential trauma as they deform ergonomically and adapt to the uterine cavity's ever-changing conformation. The core of this Upod contains a magnet of high coercivity material. The magnetic field strength will not degrade over time, provided it is made with a sufficiently "hard" (i.e., high coercivity) ferromagnetic material. The detection of the device in the uterus by ultrasound is very easy, particularly in diestrus mares. Another consideration of the design was that a layperson can monitor, by noninvasive means (common handheld metal detectors), if the device is present (please see [video 6. Upod Metal Detection - Supplementary material](#)). Insertion and retrieval of the device can be accomplished quickly, with minimal effort. Disassembly is initiated by introducing a magnetic probe, which disrupts the stability of the ring (Fig. 2). The initial retrieval prototype included a stainless-steel metal stylet with a magnetic tip with an inverted north pole. In mares with a pendulous uterus, it required bending the stylet over the pubic brim.

Time of insertion, Upod properties, and individual animal differences all seem to affect the outcome. Mare age may play a role. Nie et al. [8] showed that intrauterine glass ball-treated mares with extended luteal function were younger than those that did not exhibit prolonged luteal lifespan. Klein et al. [16] also observed that the IUD (water-filled, 3.6 g, 20 mm plastic ball) in mares with extended luteal function were younger than IUD mares with normal luteal function. Luteolysis seemed more likely

in Upod mares that exhibited more severe endometrial angio-pathies. Young age and normality of the endometrial blood vessels may improve the efficacy of Upods, suggesting that adequate perfusion and drainage of the endometrium may be important requirements for Upod effectiveness. It is possible that the "signal" from the Upod to prevent luteolysis is mediated through endometrial-ovarian vascular system. Two mares underwent luteolysis 3 and 1 week, respectively, following device insertion (Figs. 5 and 6). The devices were not able to block luteolysis in the first cycle subsequent to device insertion (experiment 1); however, diestrus periods were extended afterward. In the two mares with the Upod inserted during diestrus (experiment 2), the devices were not able to block luteolysis. The insertion procedure could have triggered an endogenous peak of prostaglandin and/or enable to block it. Timing of insertion, relative to endogenous spike in prostaglandin release on day 13–14 of the estrous cycle, has to be considered.

Upod mares exhibited subdued estrous-related behaviors and improved rideability. There was a general demeanor shift, even when in estrus. This was especially apparent when the Upod was retrieved. However, judging behavior is subjective, and to avoid prejudice, further studies with blinded evaluators and objective scoring system are needed to assess changes in behavior.

In experiment 2, Upods were inserted into the uterus of mares, (1) regardless of the stage of the estrous cycle and, (2) included postpartum mares. The rationale for was (1) to be able to insert the device as one visit outpatient procedure, without the need for repeat exams to determine stage of the estrous cycle; and (2) to envision future application in feral horses, where the device would be inserted in mares first seen with a young foal, within 30 days postpartum, without the need for additional exams to rule out pregnancy and disturb harem social fabric.

Table 3

Extended luteal function in mares with Upod versus control mares.

Group	Length of Study	Cycles Per Mare	Extended Diestrus ^a		
			Mares	Cycles	Length
Control mares (n = 7)	35 d	1.0 ± 0.0	0/7 (0%)	0/7 (0%)	n/a
Upod mares (n = 14)	98 d	1.4 ± 0.5	12/14 (85.7%)	12/19 (63.2%)	74.1 ± 11.1 (64–92)

^a Prolonged luteal function >35 days (Nie, 2003).

The potential use of this device is not restricted to horses. Inert nonmedicated, medicated (levonorgestrel-eluting), or copper Upods could potentially be used in other mammalian species as a reversible contraceptive device. Additional applications include control of feral populations of equids and wildlife. This would alleviate the need for repeated administration of ovotoxic industrial chemicals [19], immunogens to disrupt folliculogenesis [20], or GnRH vaccines [21] with highly variable individual responses and irreversibility. Field trials are ongoing to test retention and contraception in feral horse populations with a stallion harem. Ongoing research is being conducted on time-releasing progesterone-eluting Upods that may have application in transition and embryo transfer mares. We recommend that these devices are used by a licensed veterinarian only.

5. Conclusions

The inert, frameless, self-assembling Upods used in this study show a high retention rate. Endometrial integrity was not significantly affected by the device. Fertility post-Upod retrieval was not affected by the device and 100% of mares bred in the following breeding season successfully conceived. Hormone-free estrous behavior regulators have significant advantages over other methods, including (1) high efficacy, (2) 100% retention rate, (3) safe for health of the uterus, (4) easy to insert and retrieve, and (5) easy to detect with external detector and visualize with ultrasound. The Upod design decreased the risk of damage to the cervix with a streamline magnetic retrieval technique. Even with a high retention rate, the mares do eventually recycle without Upod expulsion, possibly decreasing the risk of infection from long-term use.

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Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jevs.2019.02.009>.

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2016 UMass Amherst Start-up Company

PearlPod LLC

PearlPod LLC is based on technology developed by Veterinarian Carlos Gradil, who currently serves as PearlPod's interim CEO. His initial goal was to improve control of female horse behavior. Female horses can behave erratically during their mating season, which can be disturbing or even dangerous to riders. The traditional remedy has been to insert a glass marble into the uterus, which stimulates progesterone secretion and calms behavior. But marbles are known to fall out easily, and their presence or absence can't be easily detected.

Dr. Gradil has developed an elegant solution to this problem. He developed an IUD made up of plastic-coated magnets, which self-assemble into a shape that keeps them from falling out, while at the same time their individually small size makes insertion and removal very easy. Their presence can be confirmed by ultrasound, or easier yet, they can be detected by simply holding a hand-held metal detector to the mare's abdomen. At the end of the mating season the magnets can easily be retrieved from the uterus using a magnetic wand. The PearlPod IUDs should be useful not only for behavior control, but also to allow horse breeders to synchronize horse ovulation for same-day artificial insemination.



Other Products

PearlPod plans to develop modified devices that include traditional copper or progesterone-eluting coatings to make these IUDs capable of providing reliable contraception. Because of their simple design, ease of insertion and ease of removal, these devices could be used for controlling animal populations in the wild, and to provide a non-surgical means of controlling fertility in pets. And these IUDs would offer considerable advantages over currently-available human IUDs, which can be challenging to remove, sometimes requiring the device be torn from the uterus wall. In addition, over 100,000 IUDs in the U.S. fall out each year; current IUDs have a string coming out of the cervix to assure they are still there. The PearlPod IUD would need no string, because PearlPod can provide a specially developed metal detector app to allow a woman to easily confirm the IUD is still in place with her cell phone.

Evaluating the efficacy of a silicone Y-design intrauterine device as a horse contraceptive in a captive breeding trial

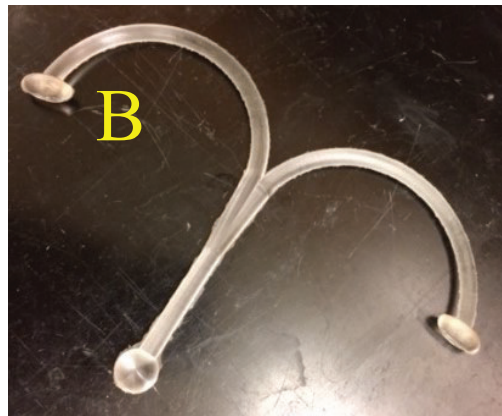
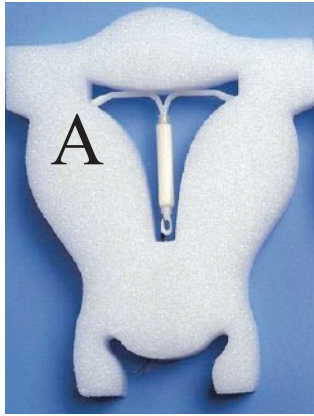
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Due to continued on-range population growth on western rangelands in the United States there are now populations of feral horses administered by the Bureau for Land Management (BLM) which far exceed the desired population size established by range ecologists for their grazing habitat. Therefore, an effective, long-lasting contraceptive for feral horses on the western range would be useful. The main goals of this study were to develop a long-lasting intrauterine device (IUD) that could reduce pregnancies within the US feral horse population, and which allowed for the potential return to fertility after IUD removal.

Our group progressed through a series of studies examining the efficacy and safety of several sizes and degrees of hardness (durometer) of a silicone elastomer polymer O-ring intrauterine device (IUD); these were based on earlier studies indicating a greater than 80% retention rate of an O-ring IUD¹. In our early trials we observed that a majority of the O-ring IUDs were expelled within 14 days of insertion. Most likely a result of either uterine expulsion forces or by causing the mares to have an abbreviated diestrus and inducing estrus with subsequent frequent breeding behavior soon after placement of the IUD². We explored multiple variations of the O-ring design which all failed to resist uterine expulsion in the face of immediate, subsequent breeding behavior.²

Manufacturers of IUDs for humans have described their devices as having a “fundus seeking design” (see image A, below; Flexi-T (Bayer)). The Flexi-T® IUD is a flexible T or Y type designed IUD that is intended to match the luminal space in the fundus and upper uterine body between the promontory bulges of the myometrium. The intent is that the closer the placement of this IUD to the fundus where the arms sit snugly above the myometrial promontory, the more likely the device would remain in place. This same fundus seeking idea was what caused us to reject the O-ring design upon seeing the unexpectedly high expulsion rates, even with the higher durometer grades. We therefore developed a Y-design silastic prototype based on the luminal dimensions of most mares. While we understood that the studies we used in our developmental processes were performed in the human possessing a unicornuate uterus with a menstrual cycle very different than that of the equine (which possesses a bicornual uterus with a seasonally associated estrous cycle), the human based findings did shed light on IUD movement throughout the equine uterus. One major factor that is unique to the equine is the fact that the stallion ejaculates through a soft, dilated cervix directly into the uterus. Thus the washing and expulsion effects of ejaculation and post-breeding uterine clearance on an IUD are not seen in the human and most of our other domestic animal species. Therefore, an IUD design that withstands both the endogenous and exogenous forces of equine breeding is essential for long-term retention. Therefore, we felt the most effective and tolerated IUD design should cause a minimum of distortion of the uterine lumen during the most active phases of contraction. Based on the fundus seeking design (A below) utilized for a human IUD, Flexi-T (Bayer) which ameliorates normal uterine expulsion forces, we extrapolated from those data to formulate a Y-design specific for a mare’s uterine shape. We consequently designed a ‘Y’ shaped silastic IUD (B below).



For the initial study evaluating the Y-design, we compared 50 to 60 durometer IUDs with each mare received either a 50 or 60 durometer Y-design IUD. Additionally, every other mare received an intramuscular injection of either saline, or long-acting progesterone; the co-administration of a long-acting progesterone at the time of IUD insertion was to reduce myometrial contractility in response to a luminal “foreign body” and consequently increase retention by hormonally reducing expulsory forces. It would also prevent estrus and/or lengthen the diestrus phase of these mares by reducing the IUD insertion induced prostaglandin production. This process allowed for an equally randomized, split plot design of equal numbers of mares (10 mares) in each treatment for IUD hardness, and co-administration of progesterone. The desired predetermined loss rate was no more than 25% overall, giving a $\geq 75\%$ retention rate.

For the long-term study, once we saw that the 60 durometer Y IUD had a higher retention rate over the first 60 days, mares still retaining a 50 durometer IUD had their IUDs replaced with a 60 durometer Y-design IUD. All of the mares in both breeding pods were then monitored once every two weeks to determine IUD retention and pregnancy rates for the next two years.

Materials and Methods:

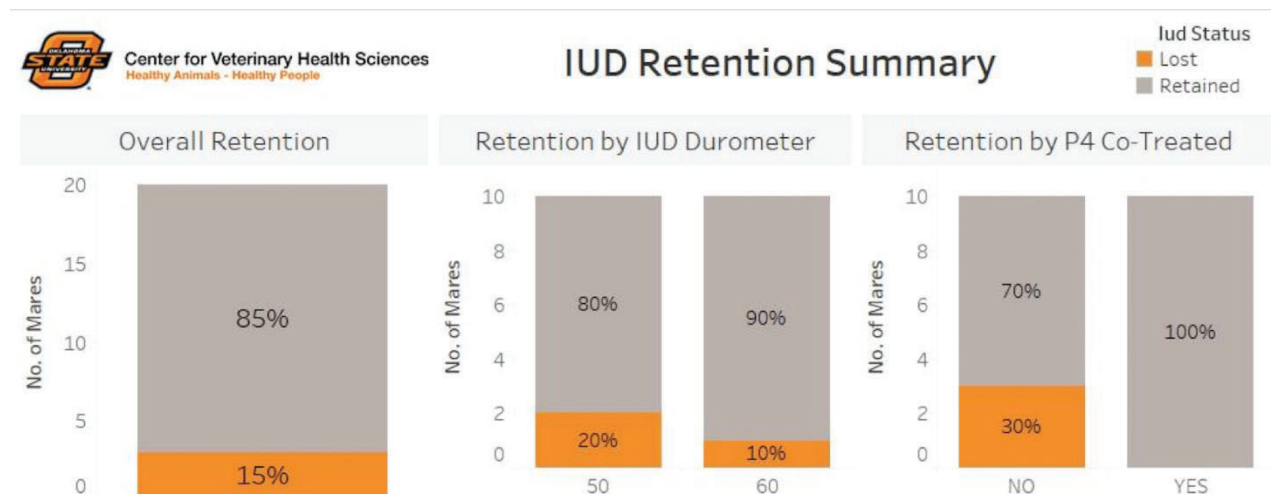
Twenty light breed mares between the age of 4 and 10 years were used in this study. The mares were randomly divided into two groups with one fertility tested stallion placed in each group as breeding pods. An initial comparison trial starting on (dates), 2017 was designed with each pod equally divided with 5 mares receiving a 50 durometer IUD and the other receiving a 60 durometer IUD. Additionally, every other mare received an IM injection of saline or long-acting progesterone (LA P4) (1,500 mg, BET Pharmaceutical, Lexington, KY, USA) in a 4x4 plot design. The acceptable retention rate was set at 70-75% over a 60-day trial period.

The initial 60-day trial period strongly suggested that the best performance was with the 60 durometer IUDs and those mares that received the LA P4 (see Table 1, below). With that then we removed the 50 durometer IUDs and replaced them with 60 durometer IUDs, with all mares receiving 1,500 mg co-administered of LA P4 via IM injection. The mares remained in their original breeding pods. After two breeding seasons, all of the IUDs were removed on (dates), 2019 and the mares entered a final phase where they were allowed to breed naturally to assess the return to fertility post-IUD removal. At the initiation of this phase all mares had endometrial biopsies taken to assess the histopathology of the IUDs, then returned to their original breeding pod (10 mares /1 stallion). This phase lasted from April 15 to October 15, giving the mares 6 months of non-contracepted stallion exposure.

Results:

5 mares receiving a 60 durometer IUD.

Seventeen of the 20 mares retained the Y-designed IUD, giving an 85% retention over the 60 day initial trial. For the 50 durometer vs 60 durometer comparison resulted in 8 of 10 vs 9 of 10 respectively. The co-administration of LA P4 cohort had 10 of 10 retention over the 60-day trial period. These results are presented in Table 1 below.



After all the mares received a 60 durometer IUD over the following two breeding seasons, with the mares continually in their respective breeding pods of 10 mares with 1 stallion, 15 of 20 mares expelled their IUD (75% retention rate). Of those expelling their IUD, 3 mares were from one pod and 2 mares were from the other, indicating no stallion or breeding pod effect. All five mares which expelled their IUD became pregnant and those pregnancies persisted beyond the early embryonic loss phase of 60 days of gestation. After which they entered into the final fertility breeding trial.

The results for the post- IUD removal field trial phase:

Histopathology:

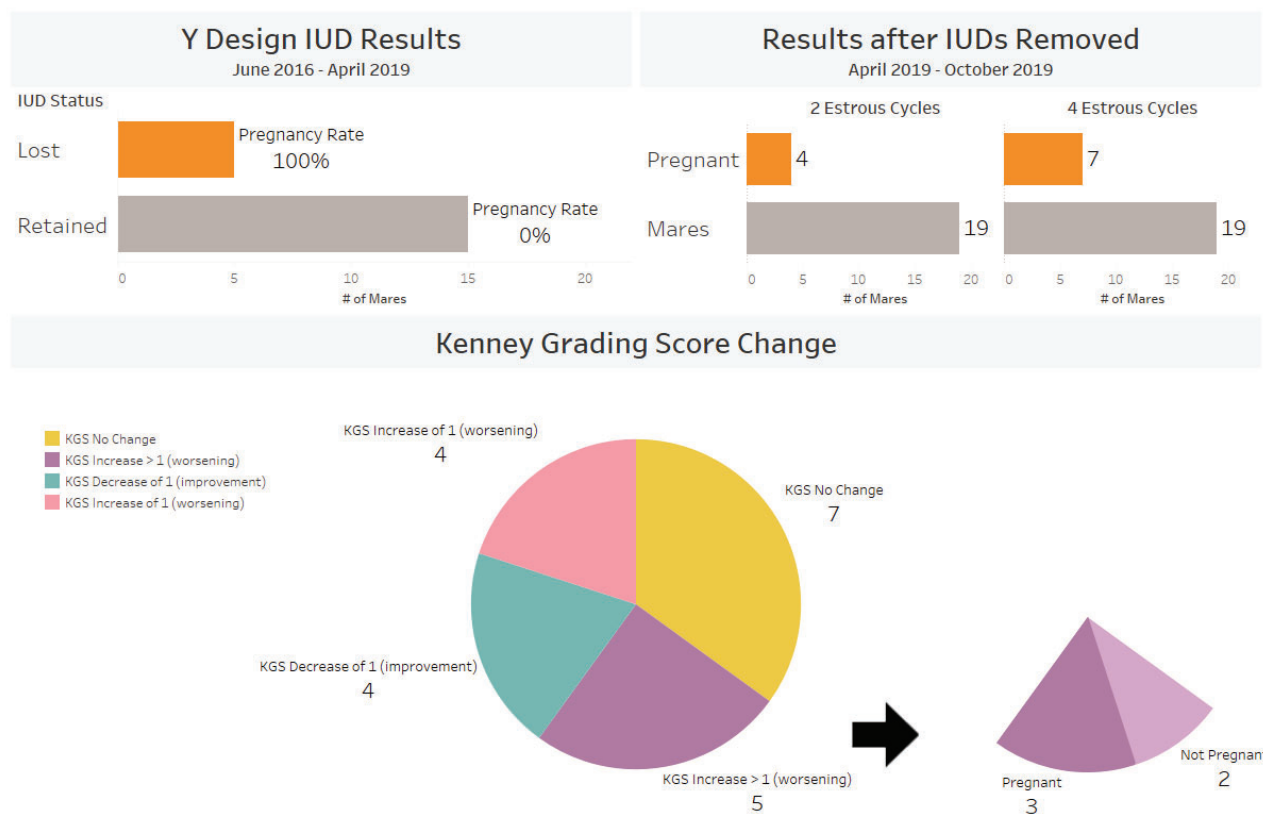
- 7 mares - the Kenney endometrial biopsy grade was unchanged; the inflammation score on all mares indicated more numbers or variety of inflammatory cells in 2019 than 2016.
- 4 mares had an increase (worsening) of one Kenney grade mostly due to an increase and variety of inflammatory cells, with only mild increase in fibrosis.
- 5 mares had an increase (worsening) by more than one Kenney grade; increase was primarily in inflammation and variety of cells but also a moderate amount in fibrosis and glandular changes.
- 4 mares showed decrease (improvement) in one Kenney grade

Field Trial Fertility (after all IUDs were removed to assess continued fertility):

- After 2 cycles 9 mares were pregnant, including the 5 who had lost their IUD previously in the study.
- After 2 cycles 2 more, and after 4 cycles 1 more, mares had become pregnant bringing the total number of pregnant mares to 12.
- No correlation between change in endometrial score and pregnancy was identified.
- 7 mares included within the study did not become pregnant at any time. Interestingly, these mares all had normal I or IIA Kenney grade biopsies at the beginning of the study; of those 7 mares, 5 maintained their biopsy score and 1 worsened by one grade and 1 worsened by two grades. (see below)

- 151 I & I
- 603 I & IIA
- 604 I & IIB
- 607 IIA & I
- 609 I & I
- 611 IIA & IIA
- 615 I & I
- 618 IIA & IIA

Therefore there is no indication that the inability of these 7 mares to become pregnant is solely a result of endometrial environment. Each of these mares had regular estrous cycles during the post-IUD removal phase of the study and were evenly dispersed between both breeding pods. The reason for these mares not becoming pregnant is most likely a result of recovery time of their uterine environment following IUD removal and other currently unidentified intrinsic factors not associated with the IUD.



Of the 3 mares that became pregnant (with >1 KGS increase in endometrial biopsy score - purple):
 Mare # 606 – pregnant within 30 days
 Mare # 602 – pregnant within 60 days
 Mare #613 – pregnant w/in 5 months

Histopathology compared to pregnancy:

Of the 5 mares that had an increase (worsening) by more than one Kenney grade during the study:

Mare #:	June 2016:	April 2019:	3/22 Fluid:	4/11 Fluid:	4/17 Fluid:	IUD Removed:	Pregnancy:
606	IIA	III	3	3	3	4/17/2019	105 days (8/8/19)
607	IIA	III	1	1	Trace	4/17/2019	Negative
612	IIA	III	3	3	3	5/7/2019	50 days (8/8/19)
613	IIA	III	Trace	Trace	3	5/7/2019	120 days (9/5/19)
616	IIB	III	1	1	Trace	4/17/2019	10 days (6/6/19)

Conclusions:

Based on histopathologic and pregnancy results –

Goals of the US Geological Services (USGS) A safe and efficient manner to control pregnancies in feral horses

1.

Appear to have been achieved.

Final Remarks:

This work is important to the US feral horse population in reducing unwanted pregnancies, while still allowing for a return to fertility after IUD removal.

Keywords: intrauterine device, IUD, feral horse, Y-design, O-ring, histopathology, fertility

Citations:

1. Daels PF, Hughes JP. Fertility control using intrauterine devices: An alternative for population control in wild horses. Theriogenology 44:629-639, 1995.
2. Baldrighi JM, Lyman CC, Hornberger K, et al: Evaluating the efficacy and safety of silicone O- ring intrauterine devices as a horse contraceptive through a captive breeding trial. Clinical Theriogenology 2017;9:417.

Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and intrauterine devices

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Abstract. We evaluated the multiyear contraceptive efficacy of the gonadotrophin-releasing hormone (GnRH) vaccine GonaCon, the porcine zona pellucida (PZP) vaccine SpayVac and the human intrauterine device (IUD) 380 Copper ‘T’ in mustang mares provided by the State of Nevada. Eight untreated control mares were compared with 12 mares treated with SpayVac, 16 mares treated with GonaCon and 15 mares treated with the copper-containing IUD. Rates of contraception for Years 1, 2, 3 and 4 respectively for SpayVac were 100% (12 of 12), 83% (10 of 12), 83% (10 of 12) and 83% (10 of 12), rates for GonaCon were 94% (15 of 16), 60% (9 of 15), 60% (9 of 15) and 40% (6 of 15) and rates for IUD-treated mares were 80% (12 of 15), 29% (4 of 14), 14% (2 of 14) and 0% (0 of 14). Antibody titres against PZP and GnRH declined over the four-year study. For mares given SpayVac, uterine oedema was commonly observed. IUDs were visible by ultrasonography in non-pregnant mustang mares, suggesting that pregnant mares did not retain their IUD. IUD retention may be a function of uterine size: pony mares with IUDs had high retention and contraception rates for 4–5 years. We conclude that long-term contraception of mustang mares with a single shot of either the SpayVac or GonaCon vaccine is possible.

Introduction

Overpopulation of wild horses is a significant concern in the western United States (Fisher 1983). In Nevada, where most of the wild horses are located, populations grow at a rate of 15–20% a year on State lands, while their range continues to shrink. Current management strategies of removal and adoption are expensive, logistically challenging, and minimally (if at all) effective in reducing and maintaining wild horse populations at a desired level. Conflicting interests associated with increased movement of people into wild horse ranges, sympathy to maintain wild horse populations because of their historic and cultural importance, competition among horses and indigenous plant and wildlife species, as well as ranching interests, are issues impacted by wild horse overpopulation. Controlling the fertility of free-ranging horses is considered a viable option for population control. However, this approach has many challenges for which solutions have been elusive. Ideally, methods for contraception of wild horses should be safe and potentially reversible, effective for several years, practical to administer and of reasonable cost and have minimal effect on reproductive or harem behaviour. Immunocontraceptive vaccines have garnered considerable attention in recent years as a means to address problems of overabundant wildlife and feral species (Fagerstone *et al.* 2002; Delves and Roitt 2005; Naz *et al.* 2005). Two immunocontraceptive vaccines that have been used in a variety of species and for which data exist on their safety and efficacy are porcine zona pellucida (PZP) vaccine (Kirkpatrick *et al.* 1992, 1995; Miller *et al.* 1999, 2001; Kirkpatrick and Turner 2002; Turner and Kirkpatrick 2002, 2003; Curtis *et al.* 2007) and

gonadotrophin-releasing hormone (GnRH) vaccine (Miller *et al.* 2000, 2004; Killian *et al.* 2006a; Massei *et al.* 2008). Fertility control of mares using existing PZP vaccines has been shown to be safe and effective for up to 10 or more years. However, wild horses vaccinated with PZP preparations have required revaccination every year or two to maintain infertility (Turner *et al.* 2001, 2002). Limited data exist for the use of GnRH vaccines in mares to control fertility, ovarian function or behaviour (Dalin *et al.* 2002; Killian *et al.* 2004, 2006b; Imboden *et al.* 2006; Elhay *et al.* 2007). Information on multiyear efficacy and effects following a single injection of a GnRH vaccine in mares is lacking. Regardless of the contraceptive vaccine considered, most formulations that have been used do not appear to be effective for the long term without revaccination. Revaccination of mustangs involves considerable expense, manpower, and horse handling to maintain infertility. If a single-injection multiyear contraceptive were available it may be possible to achieve effective population reduction and reduce costs and risks associated with frequent horse handling.

We initiated a study in the autumn of 2002 and the spring of 2003 to compare the multiyear contraceptive efficacy of a single-shot contraceptive vaccine directed at GnRH with that of a single-shot vaccine directed at the zona pellucida of the ovum. We selected GonaCon to test as the GnRH vaccine on the basis of our positive experiences with it as a single-injection vaccine in deer and other species (Miller *et al.* 2000, 2004; Miller and Killian 2001; Killian *et al.* 2006c; Fagerstone *et al.* 2008). We selected SpayVac as the PZP vaccine to test in mares on the basis of our unpublished experiences with it as a single-injection vaccine in

white-tailed deer and the reports of multiyear efficacy in harbor seals (Brown *et al.* 1997) and fallow deer (Fraker *et al.* 2002). Both vaccines were administered with AdjuVac, an adjuvant developed at the National Wildlife Research Center. In addition, on the basis of preliminary studies with pony mares, we evaluated the use of an intrauterine contraceptive device (IUD), the 380 copper 'T', which has been shown to be safe and efficacious in humans for multiple years (Fortney *et al.* 1999; Wu *et al.* 2000). Preliminary results of these studies with Nevada mustangs have been reported earlier (Killian *et al.* 2004, 2006b). This paper reports results for these contraceptive approaches after four years of study with Nevada mustangs and five years of observation on the use of IUDs in pony mares.

Materials and methods

Animals

Horses for the study were provided by the State of Nevada and were maintained at the Nevada State Penitentiary, located at Carson City. The studies were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. In total, 51 mares and three stallions were used. The mares weighed 225–360 kg and their ages were estimated to be between 18 months and 12 years of age at the time the treatments were given. All mares except the 18-month-old filly were observed in the wild to foal normal healthy foals.

For jugular blood sampling and vaccinations, the mares were run into a hydraulic chute and haltered. Vaccines were given intramuscularly in the left lateral neck. Mares were chemically restrained for IUD placement and for pregnancy evaluations by ultrasound or palpation. Chemical restraint was achieved with an initial intravenous injection of a mixture of 1 mL Dormosedan (detomidine hydrochloride) 10 mg, 2 mL xylazine 200 mg, and 2 mL acepromazine 20 mg to produce sedation, followed in 5 min with a second intravenous injection of one bottle of Telazol (250 mg Tiletamine base, 250 mg Zolazepam base) resuspended in 3 mL xylazine 300 mg for anaesthesia. This regimen typically gave 15–45 min of anaesthesia.

Ten pony mares and two pony stallions belonging to the Department of Dairy and Animal Science of the Pennsylvania State University were used to develop methods and evaluate the use of IUDs as a means to block fertility. The mares ranged in age from 18 months to 12 years when the treatments were administered. Seven of the mare ponies were pastured with a stallion during the entire five-year study, except when treatments were being administered or when data were collected. They weighed 204–306 kg. Three of the mares were on the study for only one year and were pastured with a stallion for two months during the breeding season. They weighed 281–391 kg.

Ponies were haltered and restrained in a chute for examination and data collection. If sedation was necessary, mares were given intravenous 0.5–1.5 mL xylazine (50–150 mg) and 0.2–0.5 mL butorphanol, or 0.1–0.25 mL Dormosedan (10–25 mg) and 0.2–0.5 mL butorphanol (20–50 mg).

Treatments

For the Nevada study vaccinations were given in March of 2003. Eleven mares were given a single-shot GnRH vaccine containing 1800 µg of GonaCon and four mares were given a single-shot

GnRH vaccine containing 2800 µg GonaCon, 12 mares received a single-shot PZP vaccine containing 400 µg SpayVac and 8 mares were assigned to be untreated controls. Copper-containing 380 'T' IUDs were placed in the uterus of 15 mares transcervically in October of 2002. The SpayVac PZP vaccine was provided Dr Robert Brown (Brown *et al.* 1997), who developed the vaccine. The GonaCon vaccine was provided by the National Wildlife Research Center, USDA-APHIS-WS. Both SpayVac and GonaCon were made into an emulsion with AdjuVac adjuvant (Miller *et al.* 2004) and injected as a 1-mL dose. To evaluate reproductive capacity, treated and control mares were randomly assigned to two breeding groups. 'Band Stallions' that had been observed to sire multiple generations in the wild were selected to be pen stallions. Most mares were maintained in the same pen throughout the study, but if behavioural issues resulted in mares fighting, the less dominant mare was moved to the other pen. Mares were penned with a fertile stallion for a breeding trial typically lasting from June through September of each year. If mares failed to breed, or failed to become pregnant or foal during the breeding season, they were considered infertile.

Ponies were used to evaluate three different types of IUDs for ease of placement, retention and efficacy. The 380 Copper T and the GyneFix IUDs were purchased from Family Planning Sales Limited, Littlemore, Oxford, UK. The ring IUD was fabricated from Sialistic tubing (2.5 mm o.d.), which was used to create a ring size of ~2.5 cm. Prior to closing the ring with Silastic cement, five or six small copper cable clamps were threaded over the tubing for inclusion in the ring.

In preliminary studies we attempted direct finger insertion of the IUDs into the uterus of the mare after dilation of the cervix with two fingers. Because this approach was somewhat cumbersome, time consuming and did not easily ensure placement of the IUD deep within the uterine lumen we attempted to use the IUD insertion devices that were supplied with the IUDs that were intended for humans. However, both of these insertion devices were unsatisfactory for the mare uterus. The insertion device for the copper 'T' was too short to traverse the mare vagina and cervix. Likewise, the insertion device for the GyneFix IUD, which is intended to attach the IUD to the uterine endometrium with a monofilament, was also too small for the mare reproductive tract. Consequently, we modified disposable large animal uterine swabs to accommodate the IUDs. The modified swabs enable the successful deposit of the IUDs transcervically into the uterine lumen of sedated ponies or anaesthetised Nevada mares. The insertion device containing the IUD was placed between the two fingers used to dilate the cervix and guided into the uterine lumen where the IUD was discharged. With minimal practice the time needed to clean the perineum, palpate the cervix by hand, and then insert the device was 2–5 min, with actual insertion of the rod and placement of the device requiring ~30–60 s.

For the research trial, all IUDs were placed into the uterus transcervically following dilation of the cervix with one or two fingers. As detailed in the Results, several animals received more than one type of IUD during the course of the study. If a mare became pregnant after receiving an IUD she was either allowed to go to term or, in the case of two mares in which the pregnancy was 60 days or less, the pregnancy was terminated with prostaglandin F2α. Within 2–3 weeks of termination of pregnancy or

parturition, mares were treated with another IUD. As a result, the GyneFix IUD was evaluated in four mares, the copper ring in four mares and the 380 Copper 'T' in seven mares. Two untreated mares served as a control for each breeding season.

Observations

Blood samples were collected from treated Nevada mares once or twice a year. However, the only observations consistently made on the eight control mares were general health, body condition and foaling. In mid- to late-October of each year treated mares were examined by rectal ultrasonography for pregnancy, IUD retention and uterine inflammation. Pregnancy was established by ultrasonography by observance of an embryonic vesicle, a fetus or in the case of later gestation, rectal palpation of a fetus. These observations were later confirmed by birth of a foal. In a few cases where the behaviour of the mare prevented ultrasonography or rectal palpation, pregnancy was determined later by the birth of a foal. General health and body condition, and uterine oedema that may be associated with oestrous cycle changes or presence of an IUD were noted. Uterine oedema in healthy mares is an indication that she is in heat and that she is under the influence of oestrogen produced by ovarian follicles (Sample 1997). All blood samples were assayed for oestradiol, progesterone and antibody titres to the contraceptive vaccines (Miller *et al.* 2000, 2001). One mare receiving the GonaCon vaccine and one mare with an IUD died after the first breeding season of causes not related to the treatments.

From April through November of 2002–05 ponies were gathered and examined by ultrasonography every 4–6 weeks. During the 2004 breeding season, daily observations were also made from April through August on the breeding and harem behaviour of a group of ponies consisting of one stallion, two control mares, one mare with a GyneFix IUD, two mares with the ring IUD and four mares with the 'T' IUD. For 2006, ponies were pastured for the entire year and the only observations made were for general health status and foaling rates. In April of 2007, ultrasonography was performed to check for IUD placement, pregnancy and any contraindications.

Statistical evaluation

Data from hormone assays were subjected to one-way analysis of variance by treatment. Differences between means were detected by two-sided *t*-test. Mean values are reported plus or minus standard error. Regression analyses were used to evaluate changes in antibody titres during the study.

Results

Nevada mustangs

Foaling data for the eight control mares for Years 1–4 were 75% (6 of 8 mares), 75% (6 of 8 mares), 88% (7 of 8 mares), 100% (8 of 8 mares), respectively. Rates of contraception for SpayVac-treated mares for Years 1, 2, 3 and 4 were 100%, 83%, 83% and 83%, respectively (Fig. 1). Average anti-PZP titres for the autumn bleed for mares that were contracepted were sustained considerably above the titres of mares that became pregnant (Fig. 2). Although regression analysis indicated a significant decline of anti-PZP titres for the first three years of the study ($P < 0.05$), average titres tended to increase in the fourth year. Compared with titres in the

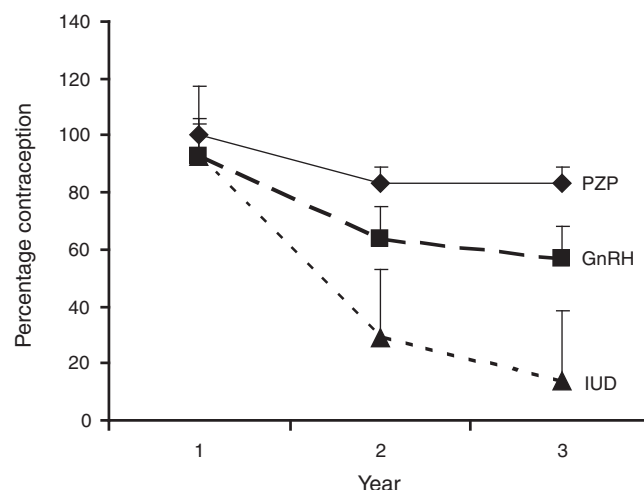


Fig. 1. Comparative rates of contraception for the three methods of contraception for each year of the four-year study. Rates of contraception for SpayVac-treated mares for Years 1, 2, 3 and 4 were 100% (12 of 12 mares), 83% (10 of 12), 83% (10 of 12) and 83% (10 of 12), respectively. Rates of contraception for GonaCon-treated mares were 93% (14 of 15 mares), 64% (9 of 14), 57% (8 of 14) and 43% (6 of 14) for Years 1–4, respectively. Contraception rates for intrauterine device (IUD)-treated mares were 80% (12 of 15 mares), 29% (4 of 14), 14% (2 of 14) and 0% (0 of 14), respectively in Years 1–4 of the study. In Year 2, one mare died in each of the GonaCon- and IUD-treatment groups of causes not related to the treatments.

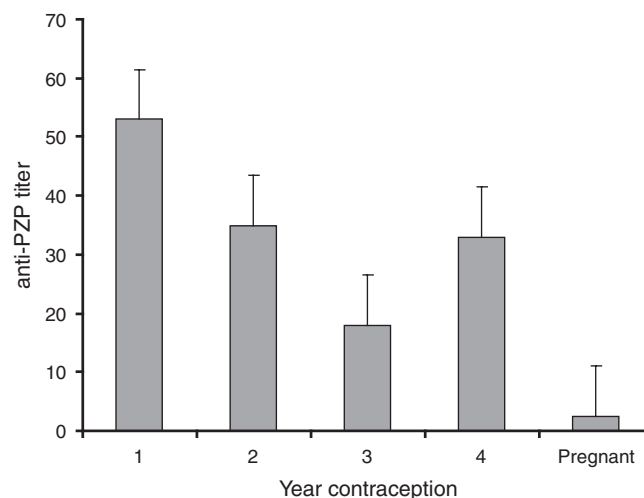


Fig. 2. Average anti-porcine zona pellucida (PZP) titres ($\pm$ s.e.m.) for the autumn bleed for mares that were contracepted compared with titres of mares that became pregnant. The average titres for each of Years 1–4 were for the serum samples for the 10 mares that were infertile. The titre for the pregnant mares was the average for the 2 mares that became pregnant during the study.

third year, individual titres increased in 8 of the 10 contracepted mares, with the remaining 2 mares having titres similar to the titres they had in the third year. For all years, serum progesterone values (ng mL^{-1}) averaged 1.3 ± 0.40 for non-pregnant mares during the October bleed compared with 17.5, the average for two pregnant mares. We were unable to obtain serum oestradiol

values for the two SpayVac-treated mares that became pregnant, but the average serum oestradiol concentrations (ng mL^{-1}) for the cycling females at the autumn bleed was 25.9 ± 2.9 .

Rates of contraception for GonaCon-treated mares were 93%, 64%, 57% and 43% for Years 1–4 (Fig. 1). For contracepted mares, regression analyses indicated that there was significant decline of average antibody titres over the last three years ($P < 0.01$). Nevertheless, anti-GnRH titres of non-fertile mares were significantly greater ($P < 0.01$) than average titres for mares that became pregnant during the study (Fig. 3). Serum progesterone concentrations (ng mL^{-1}) for non-pregnant GonaCon-treated mares at the autumn bleed averaged 0.3 ± 0.1 , which was significantly lower ($P < 0.01$) than progesterone concentrations of pregnant mares treated with GonaCon (7.7 ± 1.5). Average serum oestradiol concentration (pg mL^{-1}) for the non-pregnant GonaCon-treated mares for all years was 32.6 ± 6.7 , which was significantly less ($P < 0.001$) than concentrations detected in pregnant GonaCon-treated mares (1179 ± 225).

Contraception rates for IUD-treated mares were 80%, 29%, 14% and 0%, respectively in Years 1–4 of the study (Fig. 1). Average serum progesterone for all years at the autumn bleed was significantly lower ($P < 0.017$) for non-pregnant IUD-treated mares ($4.3 \pm 1.4 \text{ ng mL}^{-1}$) than for pregnant IUD-treated mares ($11.3 \pm 2.7 \text{ ng mL}^{-1}$). The average serum oestradiol concentration of $62.5 \pm 34.2 \text{ pg mL}^{-1}$ for all years for non-pregnant IUD-treated mares was significantly less ($P < 0.001$) than the oestradiol serum concentrations of IUD-treated mares that became pregnant (1907 ± 505).

Comparing autumn serum progesterone values among treatments for non-pregnant mares, differences between GonaCon- and SpayVac-treated mares were not significant. However, progesterone values in non-pregnant GonaCon-treated mares and SpayVac-treated mares were significantly lower ($P < 0.02$) than values in non-pregnant IUD-treated mares (Fig. 4). In contrast, serum progesterone values at the autumn bleed were lower in pregnant GonaCon-treated mares

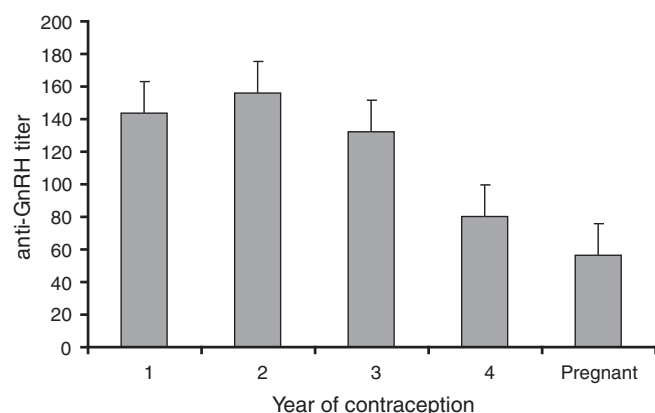


Fig. 3. Average anti-gonadotrophin-releasing hormone (GnRH) titres ($\pm$ s.e.m.) for the autumn bleed for mares that were contracepted compared with titres of mares that became pregnant. The sample sizes for titres for Years 1, 2, 3 and 4 were 14, 9, 8 and 6 respectively. The average titre for the pregnant mares was for the 8 mares that became pregnant during the four-year study.

than in pregnant SpayVac- or IUD-treated mares (Fig. 5). These differences were significant between IUD- and GonaCon-treated mares ($P < 0.025$), but because only two SpayVac-treated mares became pregnant, statistical comparisons could not be made with the other treatments. There were no significant differences in serum oestradiol concentrations in non-pregnant mares among treatments ($P = 0.13$) and differences between serum oestradiol concentrations of pregnant IUD-treated and pregnant GonaCon-treated mares were not significant ($P = 0.1$).

Observations made by ultrasonography usually enabled visualisation of IUD location and the presence of uterine oedema or luminal fluid. In most instances IUDs were not observed in mares that were pregnant. When uterine oedema was observed it was recorded by treatment that the mare received (Table 1). These values were compared with the expected incidence of uterine oedema during oestrus based on a normal mare oestrous cycle. We assumed that within a normal 21-day oestrous cycle, 5–7 days of the cycle would be in oestrus, and the remaining 14–16 days the mare would be in dioestrus (Crowell-Davis 2007). Therefore, in a random sample of mares taken from a normal population, ~25–30% would be expected to be in oestrus

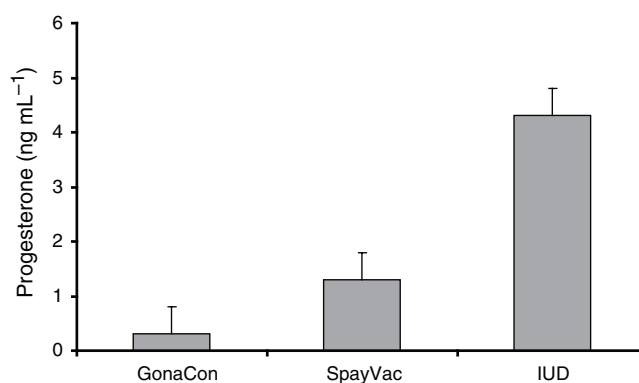


Fig. 4. Average progesterone values (ng mL^{-1}) ($\pm$ s.e.m.) in non-pregnant GonaCon-treated mares ($n = 35$) and SpayVac-treated mares ($n = 46$) and non-pregnant intrauterine device (IUD)-treated mares ($n = 17$) for the autumn bleed for all years of the study. Values for GonaCon- and SpayVac-treated mares were significantly lower ($P < 0.02$) than values for IUD-treated mares.

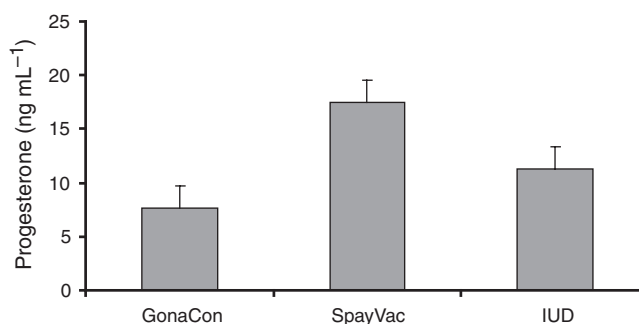


Fig. 5. Average serum progesterone (ng mL^{-1}) at the autumn bleed for pregnant GonaCon-treated-mares ($n = 8$), pregnant SpayVac-treated mares ($n = 2$) and pregnant intrauterine device (IUD)-treated mares ($n = 7$). Values were significantly different between IUD- and GonaCon-treated mares ($P < 0.025$); a comparison could not be run with the SpayVac-treated mares because of only two observations.

Table 1. Percentage of reproductive tracts with oedema revealed by ultrasonography of mares for each of the treatments compared with the predicted number of mares expected to be in oestrus

Numbers in parentheses indicate the number of mares showing uterine oedema out of the total number observed. Not all mares were observed at all time points. If a mare was found to be pregnant in a given year, she was dropped from the trial and not observed in subsequent years. Some mares on the trial could not be evaluated by ultrasonography at a sampling date because their behaviour was unmanageable

Percentage	Year 1	Year 2	Year 3	Year 4
Predicted	25–30%	25–30%	25–30%	25–30%
IUD actual	20% (2 of 10)	0% (0 of 4)	0% (0 of 2)	–
GonCon actual	23% (3 of 13)	25% (2 of 8)	25% (2 of 8)	17% (1 of 6)
SpayVac actual	82% (9 of 11)	91% (10 of 11)	100% (10 of 10)	70% (7 of 10)

and to have oedematous uteri during the breeding season. Non-pregnant mares treated with GonaCon had rates of uterine oedema similar to the expected rate of 25–30% for normal non-pregnant cycling mares (Table 1). Likewise, IUD-treated mares in the first year had rates of uterine oedema similar to the expected rate, although in subsequent years too few non-pregnant mares remained in the IUD-treated group to obtain a reliable estimate. In contrast, SpayVac-treated mares had high rates of uterine oedema during all four years of the study (Table 1).

Ponies

A summary of the IUDs inserted into the pony mares is provided in Table 2. Only one of the four mares successfully retained the GyneFix IUD for a five-year period. Likewise, the copper-containing Sialistic ring was retained in only one mare for at least a year before she was sold. The copper 'T' device provided the greatest rates of contraception and retention, with several mares having the device in place for 3–5 years. We were able to

observe only two of the mares (Table 2: Wanda, Remy) for one year before they were removed from the study.

Regardless of the type of IUD, the usual reason for failure was pregnancy. In most instances, we assumed that the pregnancy resulted from failed retention of the IUD, since we were unable to visualise the IUD by ultrasonography, or to identify it in the afterbirth when observed. In one case, we observed a 60-day pregnancy, but in a subsequent examination six weeks later the mare was not pregnant and the IUD was seen, suggesting that the pregnancy was aborted. In one case, a copper 'T' was removed because of pyometria. The recovery of that mare was unremarkable.

Two mares (Table 2: Libby, Maddie) were tested with all three IUD types. Both mares had the longest contraception rates with the copper 'T'. It is noteworthy that most mares given the copper 'T' IUD remained contracepted for multiple years. However, one mare (Libby) became pregnant within a year, regardless of the IUD type.

Observations made from April through August 2004 on the breeding behaviour of three non-pregnant mature mares equipped with a Copper 'T' IUD (Dewdrop, Maddie, Godiva) indicated that they had four or five oestrous cycles. The one mature mare equipped with a GyneFix IUD (Sprite) had six oestrous cycles and the one filly equipped with a 'T' IUD at 11 months of age had three oestrous cycles. These numbers were within the normal range of oestrous cycles for pony mares at the Penn State University facility.

Table 2. Summary of intrauterine device (IUD) types installed in pony mares, dates of installation, duration of contraception and reason for failure

n.a. = not applicable because the IUD is still intact

IUD type/ mare	Installation date	Date of last observation	Duration of contraception	Reason for failure
GyneFix				
Maddie	24.v.2002	25.vi.2002	30 days	Pregnant
Godiva	24.v.2002	23.vii.2002	60 days	Pregnant
Sprite	24.v.2002	28.iv.2007	5 years	n.a.
Libby (2)	23.ix.2005	23.iv.2006	7 months	Pregnant
Ring				
Maddie	2.vii.2002	23.viii.2002	10 days	Pregnant
Mandy	14.ix.2002	10.xii.2003	>1 year?	No data
Libby	25.v.2004	1.vii.2004	36 days	Pregnant
Libby	9.vii.2004	18.ix.2004	2 months	Pregnant
'T'				
Dewdrop	24.v.2002	1.xi.2006	5 years	Pyometria
Maddie	30.v.2003	28.iv.2007	4 years	n.a.
Godiva	14.vii.2003	28.iv.2007	4 years	n.a.
Libby	24.v.2002	15.v.2003	1 year	Pregnant
Connie	25.v.2004	28.iv.2007	3 years	n.a.
Wanda	4.ix.2002	30.v.2003	>1 year?	No data
Remy	4.ix.2002	30.v.2003	>1 year?	No data

Discussion

As with other species, population management of wild horses presents a specific set of challenges for a contraception method to meet in order for it to be of practical use. Assuming the contraception method does not pose serious problems to the health, behaviour or well being of the animal, two factors of considerable importance for contraceptive application in wild horses are long-term efficacy and whether the approach is easy to use. Wild horses need to be gathered from their range and managed under safe conditions for them to be hand injected with an immunocontraceptive vaccine. This approach has routinely been used for horses in the western United States. However, it is time consuming, expensive and, despite best efforts, not free of risk of injury to the horses or the human handlers. Although remotely darting individuals is possible (Kirkpatrick *et al.* 1990; Turner *et al.* 1996), it is not practical for vaccinating large numbers of horses in the western United States. For example, an internal cost–benefit analysis performed

by the Nevada Department of Agriculture (D. Thain, unpublished) for the Nevada Virginia Range horse-management area, consisting of over 145 000 ha of mountainous arid rangeland, concluded that equine bands did not aggregate in any one area to enable cost-effective darting. Because the Nevada Virginia Range is typical of many horse-management areas in the western United States, we believe that having a single-application contraceptive approach that is effective for multiple years would minimise or remove the need for additional gathers in these areas for revaccination in subsequent years. To date, a single-injection vaccine with multiple years of efficacy has not been available for horses despite considerable efforts of investigators working on the problem (Turner *et al.* 2001, 2002; Liu *et al.* 2005). The present study provides evidence that multiple years of contraceptive efficacy can be achieved with a single-shot immun contraceptive vaccine in the mustang mare. SpayVac was shown to have the greatest contraception rate. For the four years of study, only two of the 12 SpayVac-treated animals had foals. This rate for a single vaccination far exceeds what has been reported by others for wild horses, although SpayVac has been shown to have long-term efficacy in other species (Brown *et al.* 1997; Fraker *et al.* 2002). Mare contraception with SpayVac was associated with antibody titre, since the two mares that became pregnant had titres much below the average titre of contracepted mares. Although the average titre for the non-pregnant SpayVac-treated mares declined in Years 2 and 3 relative to Year 1, the titres actually increased in the fourth year in most of the contracepted mares. This suggests that self boosting of the immune response may occur (Perry *et al.* 2006), perhaps as a result of the seasonality of mare reproduction. It is thought that self boosting occurs in the draining lymph node (Burton *et al.* 1994) as antigen is released from the follicular dendritic cell when the antibody flowing through the draining lymph node drops to a certain level. The released antigen then provides restimulation of antibody production. It is also possible that as the mare returns to breeding condition from a period of anoestrus, new zona pellucida proteins are produced with the initiation of follicle development which restimulate an immune response. If the titre at which mares became pregnant is assumed to be the set point for pregnancy to occur, we would predict that the mares currently contracepted with SpayVac will remain infertile for several more years. It is also possible that some of the mares may remain infertile indefinitely.

Compared with GonaCon and the IUD treatments, mares treated with SpayVac had a greater incidence of uterine oedema than would be predicted for normal cycling mares. Uterine oedema is associated with a predominance of oestradiol (Sample 1997), although there was no significant difference among treatments in oestradiol values for non-pregnant mares. However, expression of uterine oedema during the normal oestrous cycle is also influenced by the absence of serum progesterone (Crowell-Davis 2007). Relative to non-pregnant IUD-treated mares, which we found to have normal cycle lengths in the pony study, the serum progesterone values were significantly lower in SpayVac-treated mares than in IUD-treated mares. Although we were unable to make observations on the length of the oestrous cycle for the Nevada mares in this study, it has been reported that mares treated with PZP vaccine

tended to have normal cycle length (Kirkpatrick *et al.* 1997; Powell 1999) although urinary oestrogen has been reported to be lower (Kirkpatrick *et al.* 1992), along with a short-term high incidence of persistent corpora lutea (Liu *et al.* 2005).

Serum oestradiol concentrations determined for SpayVac-treated mares suggest that some follicular development occurred. However, for SpayVac-treated mares showing some evidence of follicular development based on serum oestradiol, ovarian pathologies may be involved. Ovarian pathologies have been recorded for several species given PZP vaccines (Skinner *et al.* 1984; Mahi-Brown *et al.* 1988; Kirkpatrick *et al.* 1992; Lou *et al.* 1996; Stoops *et al.* 2006; Curtis *et al.* 2007), and are summarised as a disruption of folliculogenesis, depletion of primary oocytes and an infiltration of leucocytes. Given that serum progesterone was significantly lower in SpayVac-treated mares than in IUD-treated pony mares known to be having oestrous cycles of normal length, we suggest that SpayVac-treated mares may undergo some follicular development, but fail to ovulate and/or develop a normal corpus luteum.

Literature reports of circulating progesterone concentrations for untreated mares indicate considerable variation of values. Although concentrations of serum progesterone of $<1 \text{ ng mL}^{-1}$ are generally associated with oestrus, the concentrations progressively increase to high dioestrous values by Days 5–7 and are sustained there until Days 13–14. According to Ginther (1992, pp. 238–240), the range of means for 10 publications assaying serum progesterone during mare dioestrus was $4\text{--}22 \text{ ng mL}^{-1}$. Clearly, an average exceeding 4 ng mL^{-1} for the IUD-treated mares suggests that most IUD-treated mares were in dioestrous when they were sampled. This is what would be expected when randomly sampling a population of mares. In contrast, an average of 1.3 ng mL^{-1} for SpayVac-treated mares suggests that most of those mares were not in dioestrus. Whether they were in oestrus or transitioning into or out of dioestrus cannot be determined with only one sampling point for each mare. Nevertheless, we believe that the published values for average progesterone values during dioestrus, plus the reports of ovarian pathologies associated with PZP vaccines supports the notion that the lower progesterone values in SpayVac-treated mares may be due to a failure to form or maintain a normal corpus luteum. It is possible that antibodies to the zona pellucida prevent follicles from developing to normal ovulatory size and formation of the corpus luteum. Aside from these characteristics, there was no other evidence of contraindications associated with the SpayVac treatment.

Mares receiving a single vaccination of GonaCon showed a high degree of contraception during the first year, but this rate gradually declined to less than half after four years. This decline was associated with a gradual decline in antibody titre to GnRH over the same period. Unlike what was seen with SpayVac titres, there was no evidence for a self-boosting effect that occurs when the native protein is produced. The reason for this difference in response to the two immunogens is unknown, but it may relate to the fact that the PZP immunogen is a large glycoprotein compared with the GnRH decapeptide. Nevertheless, while the contraceptive efficacy of GonaCon was not as impressive as that of SpayVac, the GonaCon results exceed rates of contraception reported by others for mares using other single-injection contraceptive vaccines (Turner *et al.* 2001, 2002).

The incidence of uterine oedema for GonaCon-treated mares was similar to what would be predicted in a population of normal cycling mares. This suggests that these mares may have some degree of oestrous cycle activity, although it is difficult to make firm conclusions. Theoretically, we would predict minimal steroid hormone production in GonaCon-treated mares if we assume that GnRH were inactivated by antibody to the vaccine. However, plasma oestradiol and progesterone concentrations in GonaCon-treated mares were similar to those in the other treatment groups. Because we have no direct observations on the reproductive behaviour of the GonaCon-treated mares, we cannot say whether these females expressed oestrus or showed evidence of an oestrous cycle. However, our unpublished data from white-tailed deer treated with GonaCon suggests that GonaCon does inhibit expression of oestrus at least in the first year or two following vaccination.

The presence of serum oestradiol in GonCon-treated mares is contrary to the notion that the GnRH is the sole regulator of follicle-stimulating hormone (FSH) secretion by the pituitary gland leading to stimulation of follicle development and oestrogen secretion. The GonaCon used in this study is prepared with the luteinising hormone-releasing hormone (LHRH) peptide (Levy *et al.* 2004) which has been shown to stimulate both luteinising hormone (LH) and FSH secretion. However, there is also evidence in several species for a follicle-stimulating hormone-releasing hormone (FSHRH) that specifically stimulates FSH secretion by the anterior pituitary gland (McCann *et al.* 1993, 1998; Yu *et al.* 1997; Padmanabhan and McNeilly 2001; McNeilly *et al.* 2003). If an FSHRH exists in the mare, it could explain the presence of serum oestradiol in infertile GonaCon-treated mares. Antibodies to LHRH in the serum would act to block some follicular development and the LH surge associated with ovulation, but some follicular development and oestrogen production would also occur in response to FSHRH and FSH secretion.

Contraception results for the Nevada mares treated with the IUD were encouraging in the first year of the study, but the performance was poor for the remainder of the study.

When IUDs were visualised, there was no evidence of uterine pathology as assessed by ultrasonography. It has been reported that mares implanted with a Sialistic ring IUDs were infertile for one year, but that device was associated with a uterine inflammatory response (Daels and Hughes 1995). In our study we did not see evidence of a uterine inflammatory response, and mares in the IUD-treatment group had the predicted number of occurrences of uterine oedema for the population size sampled. This, along with the serum progesterone and oestradiol data suggests that the IUD-treated mares were experiencing oestrous cycles.

Studies with ponies enabled more frequent observations of the IUDs and oestrous cycle events. From these observations we concluded that the 380 Copper 'T' IUD was superior to the other IUDs tested for long-term contraception. In addition, observations on oestrous cycle events for one breeding season led us to conclude that cycle length for mares with IUDs was within the normal range. The discrepancy in long-term rates of infertility between the mustang mares and pony mares equipped with a similar IUD is probably due to differences in uterine size. The retention of foreign objects in the mare uterus is related to the size of the object, relative to the size of the uterus. The ability of

glass balls to be retained in the mare uterus has been shown to be related to the size of the glass ball (Nie *et al.* 2001; Thomas 2002). Although we have not found IUDs expelled by the uterus of an IUD-treated mare that became pregnant, we suspect the reason for the decline in efficacy of the Nevada mares was that the IUD was not retained in the uterus. This suggests that if larger 'T' IUDs were used, better rates of retention and contraceptive efficacy may be possible. On the basis of the pony studies, there is also evidence to suggest that shape of the IUD may also be a factor affecting retention, since neither the string GyneFix nor the ring IUDs were retained and performed as well as the 'T'.

The mechanism preventing fertility in IUD-treated females has been argued to be either by interference with attachment of the early embryo to the uterus, or by induction of early abortion (Ortiz *et al.* 1996; Fortney *et al.* 1999). This mechanism may differ among species and no data have been published for the mechanism in the mare. Because the length of the oestrous cycle of pony mares in this study was within the normal range, we suggest that infertility in most instances was the result of the IUD interfering with events occurring between fertilisation and early embryo attachment. However, in one pony mare that was observed to be 50–60 days pregnant, in a subsequent examination she was not pregnant and the IUD was visualised. This indicated that abortion had occurred, but the IUD was retained. This observation raises the possibility that if pregnancy occurs followed by abortion, IUD expulsion may also occur. It is also possible that if pregnancy occurs but abortion does not, the IUD could be expelled with the placenta at parturition. This appears to have occurred with one Nevada mare that we observed to have the IUD when she was pregnant, and she went on to foal.

We believe that these studies provide evidence that long-term contraception of the mare is possible with the SpayVac PZP vaccine. Further improvements to the formulation of GonaCon that are now being tested in white-tailed deer suggest that rates of contraception similar to SpayVac are achievable with GonaCon. Development of larger IUDs that are better suited to the mustang mare may be possible. Regardless of the approach used, if a high rate of contraception is achievable for multiple years, population models suggest that contraception alone or used in conjunction with removal programs have the potential to stabilise and reduce population growth as well as reduce wild horse management costs (Garrott *et al.* 1992; Cameron *et al.* 2001; Bartholow 2004; Ballou *et al.* 2008; Kirkpatrick and Turner 2008). These observations lead us to conclude that population management of wild horses by single-application multiyear contraceptives will probably be possible in the near future for horse populations that can be gathered from their range for treatment and release.

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Marble-induced pyometra in an Appaloosa mare

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Marble-induced pyometras have not been reported previously. This case reports a new possible complication of using intrauterine marbles to suppress estrus in mares. A 16-year old Appaloosa mare presented with a foul-smelling, suppurative vulvar discharge. No reproductive history was available. Pyometra, endometritis or vaginitis were suspected. Transrectal palpation, transrectal ultrasound and fluid cytology were performed. Transrectal ultrasound revealed a corpus luteum and a large fluid-filled uterus. A 34-mm round hyperechoic structure was noted in the left uterine horn. The cervix was firm. Cytology of the draining fluid revealed large numbers of degenerate neutrophils. Pyometra was confirmed and the presence of a mummified fetus or intrauterine marble was suspected. Uterine endoscopy was indicated to directly assess the uterine lumen, which confirmed the structure to be a glass marble. It was recommended to remove the marble during estrus and treat the pyometra. Diestrus was shortened with dinoprost (5 mg IM) and the marble was removed during estrus. Misoprostol (200 µg) was administered intra-cervically to induce cervical dilation and facilitate marble removal. The marble was manipulated towards the cervix transrectally and then through the cervix per vagina. The owners declined treatment of the pyometra. After questioning the owners, it was determined that the marble had been in place for at least two years. This case suggests that pyometra may be a complication of using intrauterine marbles for estrus suppression in mares and stresses the importance of removing the marble once estrus suppression is no longer desired. Pyometra is typically associated with cervical incompetence.¹ This mare's cervix dilated properly during estrus, and estrus was confirmed prior to presentation. It is possible that the marble acted as a foreign body or a nidus for infection. Although this mare's fertility was not tested, it would be reasonable that the pyometra could impact long-term fertility.¹

Keywords: Pyometra, glass marble, mares

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Use of an Intra-uterine Glass Ball Protocol to Extend Luteal Function in Mares

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SUMMARY

A 25- or 35-mm diameter glass ball was placed in the uterus of mares to observe the effect on interovulatory interval, luteal function, estrous behavior, the endometrium, and subsequent fertility. The 25-mm glass ball was spontaneously expelled from the uterus of 6 of 12 mares (50%), whereas none of the 35-mm glass balls was expelled. Teasing results were consistent with the concentration of circulating progesterone. Luteal function was extended in 7 of 18 mares (39%) maintaining a glass ball, whereas an extended luteal period occurred in 4 of 32 mares (13%) observed as controls. Extended luteal function occurred in 7 of 62 diestrus periods (11%) among mares following ball placement, whereas 4 of 50 diestrus periods (8%) were extended in control cycles. The mean luteal life span in mares with a glass ball and extended luteal function was 87 days (range, 76 to 109 days); there were no significant differences in length of luteal function in both groups of mares that received the 2 different ball sizes. Endometrial changes observed between preplacement and postremoval samples were minimal. When mares were bred in the season subsequent to glass ball removal, 17 of 23 (74%) conceived. Placing an intrauterine glass ball in a mare may be an alternative to exogenous hormone therapy to prevent cycling in some mares. Luteal function was extended to nearly 90 days in approximately 40% of mares. The 35-mm diameter glass ball appeared to have an advantage for retention over the 25-mm size. Results of our study could not completely

rule out idiopathic persistence of the corpus luteum as an explanation for the extended luteal function observed in mares with a glass ball. Readers are cautioned that many questions still exist about the use of intra-uterine glass balls in mares. Further work is required to confirm the efficacy of the use of an intra-uterine glass ball for prolonged luteal function in mares and to identify its mechanism of action.

INTRODUCTION

In recent years, there has been a debate among veterinary practitioners concerning the efficacy of various extra-label uses of progestin products (eg, cattle growth implants and human depo-progestin injectables) to modify behavior in mares. Clients who own horses are more frequently seeking means to suppress behavioral signs of estrus, expecting that with such suppression the mare will train or perform better. Requests for these progestin products by mare owners puts veterinary practitioners in the precarious situation of using pharmaceuticals, extra-label, without scientific evidence of efficacy, in mares.

In reality, the only truly effective means of suppressing behavioral signs of estrus in most intact mares is to maintain sufficient concentrations of circulating progesterone or its equivalent. Today the only efficacious way to maintain a sufficient level of progesterone or its equivalent is for the mare to have a functional corpus luteum (CL), administer exogenous progesterone (eg, ≥ 50 mg in oil, intramuscularly, daily), or administer daily synthetic progestins (eg, altrenogest [Regumate], Hoechst Roussel Vet, Warren, NJ).¹⁻³

Recently, placement of a glass ball of 30-mm diameter in the uterus has been suggested as a reversible means of preventing mares from cycling and displaying behavioral signs of estrus (message to Equine Clinicians Network, Dr Randy J. T. de Greef, March 19, 2000). If this technique is effective, it would be of value to mare owners because it would eliminate the need for daily treatments over extended periods.

We have been unable to find literature that would support or refute this idea in horses. However, the effects on ovarian function, body weight gain, and pregnancy rate in

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nulliparous heifers of a copper-bearing intrauterine device were studied.⁴ The researchers reported that the heifers receiving the intrauterine device had lower progesterone concentrations than did control subjects. Nevertheless, nearly all of the treated heifers had better weight gain, were anestrus, and did not become pregnant during the study; however, multiple ovarian follicular cysts developed in many of them. The idea of using an intrauterine device to suppress estrus is said to have originated centuries ago in the Middle East as a common means of keeping camels from cycling and becoming pregnant (personal communication, Dr Ahmed Tibary, College of Veterinary Medicine, Washington State University, Pullman, Wash, May 2000).

To our knowledge, the efficacy and long-term effects of glass ball treatment have not been critically evaluated. Our objectives in this study were to observe the effect of placement of an intra-uterine glass ball on interovulatory interval, luteal function, estrous behavior, the endometrium, and subsequent fertility of mares.

MATERIALS AND METHODS

Animals. A total of 38 light-horse breed mares ranging in age from 3 to 20 years were used for this study. Mares were maintained in accordance with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1st revised edition, January 1999). All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at Auburn University (IACUC Protocol No. 0308-R-2307).

Intra-uterine device. Two glass ball (www.glassmarbles.com) sizes, 25- and 35-mm diameters, were evaluated in this study (Fig 1). In preparation for placement, the glass balls were sterilized by autoclaving. Initial attempts at autoclaving resulted in several broken balls. However, use of a liquid cycle with a temperature of 250°F (121°C) and pressure of 16 psi, with no prevacuum or dry cycle and a slow cool-down phase, did not result in further breakage.

Upon entering the study, mares were monitored daily via transrectal palpation and ultrasonography for their progression through the estrous cycle. A glass ball was placed in the body of the uterus at the first examination following ovulation. The perineum was cleaned with cotton, tap water, and antiseptic dish detergent. A sterilized sleeve was donned and a small amount of sterile lubricant was applied to the back of the hand. Grasping the glass ball, it was manually carried into the vagina. The ball was placed in the cervical lumen and moved forward with the index finger to the caudal uterine body. After removing the hand from the vagina, the ball was located on transrectal palpation and pushed forward to the horn-body junction if it had not already moved to that position. Once the ball was positioned in the uterus, the vulva was again cleaned as previously described. The uterus was infused with 1 g of ticarcillin dis-



Fig 1. Glass ball diameters evaluated during the study were 25 mm (left) and 35 mm (right).

odium (Ticar, SmithKline Beecham Pharmaceuticals, Philadelphia, Pa) in a 35-mL volume and each mare received 250 µg cloprostenol (Estrumate, Bayer Corporation, Shawnee Mission, Kan) intramuscularly to prevent a persistent endometritis if contaminants were introduced with the glass ball.

At the end of the glass ball phase of the experiment for a mare, the ball was removed from the uterus during the following estrus when the cervix was softest. Occasionally a mare would require sedation to allow better manipulation of the ball per rectum. Mares with pendulous horns presented the most difficulty. Removal was accomplished by manipulating the glass ball, per rectum, caudally toward the cervix, through the cervix, and then to the vulva for retrieval. If the cervix was not fully dilated, a gloved hand was taken per vagina to the caudal cervical os and the glass ball was retrieved from the lumen.

Experimental protocol. This study was conducted at Auburn University in southeastern Alabama between May and October 2000. All mares used in this study had ovulated at least once in the season before being assigned to a treatment group. Twelve mares were randomly assigned to each treatment group (25-mm and 35-mm glass balls), representing 24 of 38 study mares. Control data were collected from 32 of 38 study mares during cycles in which no glass ball was in the uterus. Eighteen of the 32 mares used to collect control data were also treated with a glass ball during the study, whereas 14 mares were not treated. Control data were not available from 6 of 24 mares treated with a glass ball for management reasons beyond our control.

An endometrial biopsy was taken from each mare during estrus in the cycle before being assigned to a treatment group, for comparison with a sample following removal of the glass ball. The follow-up samples were taken immedi-



Fig 2. The ultrasonic appearance of a 35-mm glass ball is seen at the left uterine horn-body junction.

ately after removal of the glass ball to ensure detection of inflammation, if present. An attempt was made to take the preplacement and postremoval samples from the same area near the uterine horn-body junction. Endometrial biopsies were evaluated as previously described.⁵ The evaluator was blinded to the group assignment and preplacement results of each mare.

Throughout the study, mares treated with a glass ball were individually teased with a breeding stallion at a rail. Behavior was scored by response of a mare to the stallion using a categorical scale (0 = rejection, 1 = indifference, 2 = receptive) adapted from behavioral signs previously described.⁶

Beginning on the day of glass ball placement, mares were examined daily by ultrasonography and palpation per rectum to monitor changes in the reproductive tract and ball location. The ultrasonic appearance of a glass ball in the uterus is shown in Fig 2. Mares were also teased to a stallion, and blood was taken for determination of progesterone concentrations. Daily evaluation was continued until 2 ovulations had been detected; after this, daily blood sampling and teasing was continued until 2 more ovulations were detected. However, if at any time mares were found to maintain luteal tissue (continued ultrasonic evidence of a CL, palpable tone in the uterus and cervix, absence of estrus signs on ultrasonography, and absence of estrus behavior) for 35 days, daily evaluation was discontinued and the mares were moved to pasture and blood samples were taken weekly to monitor progesterone concentrations.

In mares that did not experience prolonged luteal function (>35 days), the glass ball was removed from the uterus after 4 ovulations had been detected. In mares that experi-

enced prolonged luteal function, the glass ball was removed after progesterone concentrations had fallen to <1 ng/mL followed by a subsequent rise to >4 ng/mL, indicating a subsequent ovulation. Mares that spontaneously expelled the glass ball were removed from the study upon discovery without further sampling.

Immediately following removal of a glass ball, an endometrial biopsy was taken for comparison with the preplacement sample. Following removal of the glass balls, mares were bred during the next season under the protocol of another study. Standard breeding management for artificial insemination and several stallions were used. The results reported are for the season and reflect pregnancy outcome at 15 days after ovulation.

Estrous cycles (n = 50) were observed in 32 of 38 study mares to establish an interovulatory interval and incidence of spontaneous persistence of the CL as a control for the effect of the treatment protocol. Observations were made during separate control cycles when a glass ball was not in the uterus of any mares that were also used in a treatment group during the study. At least one cycle was evaluated for each of the 32 mares, with some contributing a second cycle. The reproductive tract and circulating progesterone concentrations were evaluated in the same fashion and on the same daily schedule as the treatment groups. During the control cycles, the mares were simultaneously being observed to establish estrous cycle control data for another study. Therefore, when spontaneous persistence of a CL occurred and a luteal phase lasted 30 days, the mare was given prostaglandin to lyse the CL.

Progesterone assay. Circulating progesterone concentrations were used to reflect luteal function. Concentrations higher than 1 ng/mL were considered indicative of functional luteal tissue. Plasma was harvested from blood collected from each mare. Plasma samples were frozen and held at -50°C until assayed in batches of approximately 200. Circulating concentrations of progesterone were quantified using a commercial radioimmunoassay kit (COAT-A-COUNT progesterone radioimmunoassay kit, Diagnostic Products Corporation, Los Angeles, Calif).

Statistical analysis. Two measures were derived from each interovulatory period: the interovulatory interval in days and the number of days during which progesterone was >1 ng/mL. The effects of glass ball size, monitoring method, mare and their interactions were tested using the GLM procedure of Statistical Analysis System (SAS Institute, Cary, NC). Ages of mare among groups were compared with use of an unpaired *t* test (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, Calif). The proportion of mares experiencing extended luteal function or spontaneous persistence of a CL during the treatment and control cycles was determined. In addition, the proportion of diestrus periods that resulted in

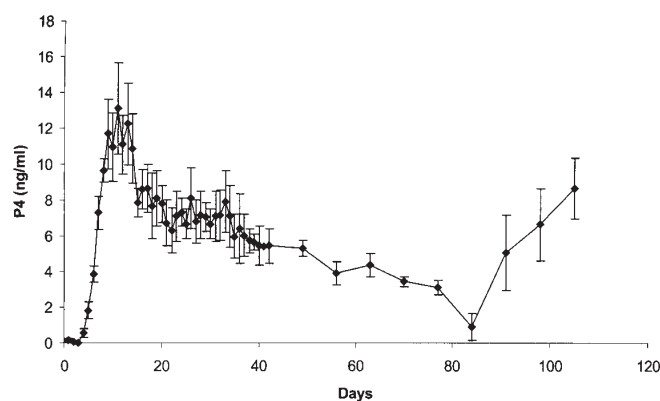


Fig 3. Mean ($\pm$ SEM) progesterone (P4) concentration in 4 mares experiencing extended luteal function during the first diestrus period following placement of a 35-mm ($n = 3$ mares) or 25-mm ($n = 1$ mare) diameter glass ball in the uterus.

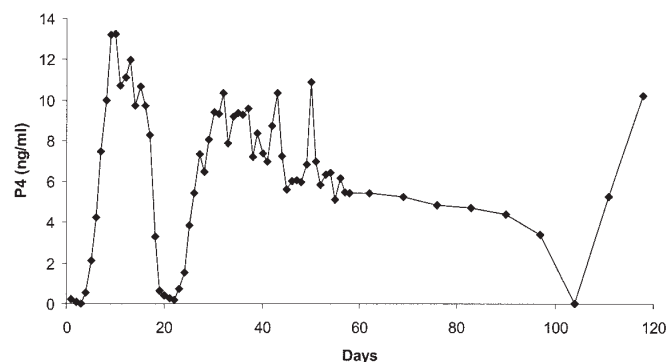


Fig 4. Mean progesterone (P4) concentration in the one mare that experienced extended luteal function during the second diestrus period following placement of a 25-mm diameter glass ball in the uterus.

extended luteal function or spontaneous persistence of a CL was determined. The proportion of mares and diestrus periods in which extended luteal function occurred during treatment and control cycles were compared using a Fisher Exact Test (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, Calif).

RESULTS

A very small amount of uterine fluid (<1 cm depth) was observed via ultrasonography in 3 mares for 2 days following placement of the glass ball. By day 3, however, the fluid was no longer visible in any of the mares, one of which did go on to maintain luteal function for an extended period. None of the mares that developed uterine fluid experienced spontaneous loss of the glass ball.

A total of 24 mares had a glass ball of either 25 mm ($n = 12$) or 35 mm ($n = 12$) diameter placed in the uterus. The 25-mm glass ball was spontaneously expelled in 6 of 12 mares (50%). Five were expelled within 24 hours of placement and a sixth during a subsequent estrus period, 11 days following placement. None of the 35-mm glass balls was spontaneously expelled.

The glass ball was observed to randomly alternate between the left and right uterine horn-body junctions. Movement was observed in every mare except two. In those 2 mares, the 35-mm glass ball was consistently observed at the same site during each examination. One of the mares experienced extended luteal function and the other did not.

Overall, 7 of 18 mares (39%) that maintained the glass ball experienced extended (>35 day) luteal function. Extended luteal function was detected during the first diestrus after ball placement in 4 mares, during the second diestrus in one mare, and during the third diestrus in 2 mares. Mean ($\pm$ SEM) progesterone concentrations during

the extended luteal periods are reported in Figs 3, 4, and 5. There was no difference between the 25- and 35-mm balls in terms of proportion of mares having extended luteal function (2 of 6, 33%, and 5 of 12, 42%; $P > .05$). Extended luteal function occurred in 7 of 62 diestrus periods (11%) among mares following ball placement. Again, there was no difference between 25- and 35-mm balls in terms of proportion of diestrus periods resulting in extended luteal function (2 of 20, 10%, and 5 of 42, 12%, $P > .05$).

An extended luteal period occurred in 4 of 32 mares (13%) observed for control data. Progesterone concentrations remained above 1 ng/mL for 30 days after ovulation in 4 of 50 control diestrus periods (8.0%) observed. Of the 4 mares that experienced extended luteal function during the control cycle, a glass ball was placed in the uterus of 3 of the mares during the treatment cycles. However, none of the 3 mares experienced extended luteal function while the glass ball was in the uterus. The proportion of mares that experienced extended luteal function (7 of 18, 39%) while a glass ball was in the uterus was greater than the proportion of mares that experienced an extended luteal period (4 of 32, 13%) during the control cycle ($P = .04$). The proportion of diestrus periods in which extended luteal function occurred was the same whether a glass ball was present in the uterus (7 of 62, 11%) or not (4 of 50, 8%; $P = .75$).

The interovulatory interval was $23.0 (\pm 0.43)$ days for the control cycles ($n = 46$) in which an extended luteal period did not occur. This was longer than the interovulatory interval (20.2 ± 0.41 days) for the cycles ($n = 55$) that occurred subsequent to glass ball placement without apparent extension of luteal function ($P < .001$). A functional CL was maintained (15.5 ± 0.35 days, range 11 to 23 days) longer in control cycles than in cycles with glass balls (13.2 ± 0.42 days, range 7 to 18 days) in which extended luteal function was not apparent ($P < .001$). Mean ($\pm$ SEM) pro-

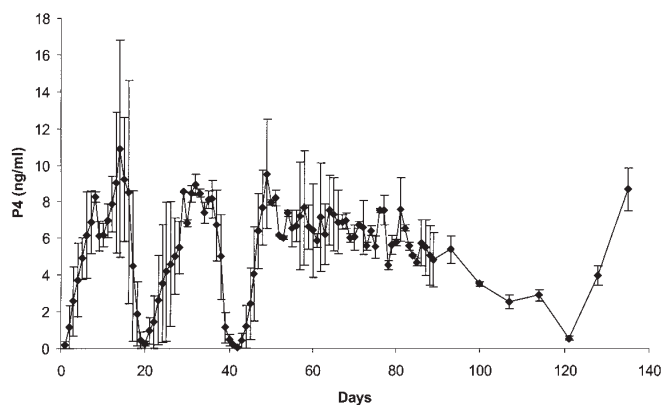


Fig 5. Mean ($\pm$ SEM) progesterone (P4) concentration in mares experiencing extended luteal function during the third diestrus period following placement of a 35-mm ($n = 2$ mares) diameter glass ball in the uterus.

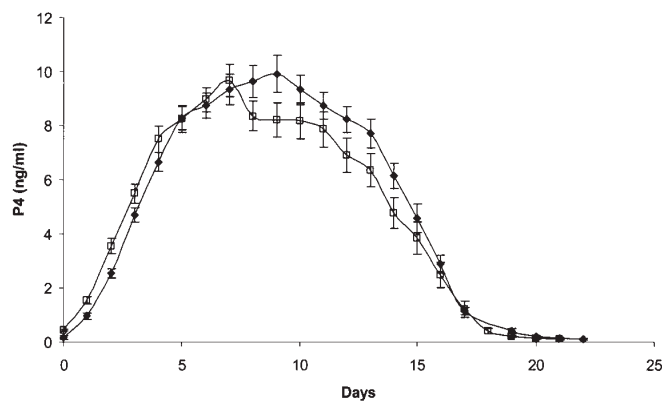


Fig 6. Mean ($\pm$ SEM) progesterone (P4) concentration in mares during control cycles ($\text{—}\blacklozenge\text{—}$; $n = 46$) without a glass ball and treatment cycles ($\text{—}\square\text{—}$; $n = 55$) with a glass ball in which an extended luteal period did not occur.

gesterone concentrations for the control cycles ($n = 46$) and treatment cycles in which an extended luteal period did not occur are presented in Fig 6.

The mean age of all mares in the study was 9.6 years (range, 3 to 20 years). Mares that experienced extended luteal function were younger, at 8.3 years (± 0.87), than mares that did not experience extended luteal function, at 12.6 years (± 1.05 , $P = .012$). Mares that spontaneously expelled the glass ball were younger, at 6.3 years (± 2.0), than mares that did not expel the glass ball, at 10.9 years (± 0.87 , $P = .024$).

The interassay and intra-assay coefficient of variation for the progesterone assay was 7% and 3%, respectively. The sensitivity of the assay was 0.02 ng/mL. Estrous behavior observed during the study accurately reflected circulating progesterone concentrations. All mares with a glass ball invariably displayed behavioral estrus scores of 1 or 2 when progesterone concentrations were <1 ng/mL, while scores of 0 were observed when concentrations were >1 ng/mL. Those that experienced extended luteal function also displayed scores of 0 throughout the period while progesterone concentrations were >1 ng/mL and estrus behavior was monitored. A single CL was observed at the primary ovulation site throughout the observation period. No additional CLs were observed in any mare with a glass ball following the primary ovulation.

No change in endometrial category was observed between the preplacement and postremoval endometrial samples in any of the mares in the 25-mm glass ball group. In the 35-mm group, the score declined by a category in one mare, improved by a category in 2 mares, and was unchanged in the other 9 mares. The difference in category assigned in those 3 mares was attributed to mild changes, up or down, in the amount of lymphocytic inflammation ob-

served. The mare that declined by one category did not experience extended luteal function, whereas 1 of 2 mares with improvement in endometrial category experienced an extended luteal period.

During the season following glass ball removal, 23 of 24 mares were bred, including all of the mares that had experienced extended luteal function. During the season, 17 of 23 (74%) of the mares bred subsequently conceived, including 5 of 7 (71%) of those that had experienced extended luteal function.

DISCUSSION

Placement of the glass ball through the cervix was relatively easy in most of the mares. Occasionally the cervix of a mare would require some degree of manual dilation to push a 35-mm diameter ball through its lumen; however, the 25-mm balls generally passed with ease. During preliminary work, we had found that the glass ball was more likely to be expelled from the uterus if it was placed a day or two before ovulation. Based on the anecdotal information from the Netherlands (message to Equine Clinicians Network, Dr Randy J. T. de Greef, March 19, 2000) and our preliminary experience, we decided to place the glass ball in the uterus at the examination following ovulation. Our assumption when placing the glass ball following ovulation was that the cervix would have started to close under the influence of rising progesterone, which might help prevent it from being expelled. We found that younger mares were more likely to expel the glass ball, perhaps because younger mares have more effective uterine clearance (in estrus and the periovulatory period) and a less dependent uterine position than do older mares. The smaller diameter and lighter weight of the 25-mm glass ball also may have contributed to the spontaneous expulsion from 6 mares, al-

though it is also possible the cloprostenol contributed to the loss in some mares. However, losses observed during preliminary work were not associated with cloprostenol administration, and neither was the loss in this study from the mare that expelled the ball during the subsequent estrus at 11 days following placement.

Idiopathic persistence of the primary CL, also known as spontaneous persistence of the CL,⁷ cannot be completely ruled out as an explanation for the extended luteal function observed in this study. The incidence of idiopathic persistence is reported to vary widely.^{8,9} Ginther and Pierson⁸ did not observe idiopathic persistence in any of 69 interovulatory intervals, while Stabenfeldt and Hughes⁹ suggest it can occur in as many as 25% of estrous cycles. However, acceptance of inadequate evidence for the condition may have led to an overestimation of the incidence in some reports.⁷

The proportions of diestrus periods that resulted in an extended luteal period were not different between the control (4 of 50) and glass ball (7 of 62) cycles. However, the proportion of cycles in mares with glass balls inserted is heavily biased by the 4 cycles from each mare that did not experience extended luteal function. A greater proportion of mares (7 of 18) experienced extended luteal function when a glass ball was in the uterus than without a ball (4 of 32) during the control cycles. Four of the mares that experienced extended luteal function with a glass ball in the uterus did so during the first diestrus following placement and did not experience subsequent ovulations. These mares are consequently underrepresented in the proportion of diestrus periods among the mares that had a glass ball inserted, especially if all or some of the mares had experienced additional periods of extended luteal function following subsequent ovulations. This would seem to make the proportion of mares a more valuable indicator of glass ball efficacy. However, in all fairness, we should point out that 2 of 7 mares that experienced extended luteal function did so after the third ovulation following glass ball placement (Fig 5). The control data were collected from 32 mares in 50 estrous cycles; only 18 of the mares were observed in more than one cycle. This may have biased our results for fewer occurrences of idiopathic persistence of the CL, although we believe it is unlikely.

Five of the 7 mares in this study that experienced extended luteal function when a glass ball was in the uterus had never previously been observed to have prolonged interovulatory intervals over multiple seasons. Historic data were not available for the other 2 mares. The average length of the luteal period reported to be associated with idiopathic persistence of the CL is approximately 2 months.⁷ The average length of luteal function observed in this study was 3 months. These several points would seem to support the idea that the extended luteal function observed in this

study was indeed affected by the glass ball protocol rather than idiopathic persistence. Therefore, although it is not possible to positively distinguish idiopathic persistence of the primary CL from extended luteal function influenced by the glass ball protocol, we believe that our observations in this study suggest a genuine effect. An explanation is not readily apparent for our observation that mares experiencing extended luteal function were younger than those that did not experience extended luteal function.

Two possible explanations for an effect of the glass ball have been discussed (Equine Clinicians Network archives). First, the glass ball simulates a conceptus and through movement and physical contact prevents prostaglandin release from the endometrium, in turn maintaining the CL (an endogenous progesterone source) indefinitely. A second theory suggested that the glass ball would stimulate mild inflammation, in turn causing release of small amounts of prostaglandin that would be inadequate to achieve luteolysis. As long as the glass ball was present, the endometrium would remain in a prostaglandin-depleted state and the CL would be maintained.

In the event that the first theory discussed was correct, two glass ball sizes were evaluated in this study to account for a range in vesicle diameter that would be expected to occur naturally during the early stages of pregnancy. However, this theory seems to assume that the physical presence of a spherical structure, in this case a glass ball, in the uterine lumen will prevent prostaglandin release. This is contrary to conventional logic that would assume, as has been demonstrated in other species, that a chemical messenger is produced by the equine conceptus to allow maternal recognition of pregnancy and avoid prostaglandin release.¹⁰

The second theory is as equally confusing, considering that there are countless reasons why a mare may have low-grade endometrial inflammation, yet clinically we do not recognize scores of mares that maintain their luteal tissue indefinitely. Both theories suggest that the glass ball is mobile enough to contact the majority of the endometrium or cause low-grade endometrial inflammation throughout the uterus. Our findings did not support the idea that the glass ball was particularly mobile in the uterus. Although the ball did move between the uterine horn-body junctions in most mares, the distance moved was only a few centimeters. In 2 mares, the glass ball did not move at all. One of the mares experienced extended luteal function in spite of the lack of ball movement. It probably also would be more logical to assume that an irritant to the endometrium would cause low-grade inflammation, which in turn would likely trigger sufficient release of endogenous prostaglandin $F_{2\alpha}$ to cause luteolysis.^{11,12} Uterine biopsy results did not reflect an increase in endometrial inflammation. Further, the interovulatory interval and functional life of the CL was >2 days

longer during control cycles than in cycles when a glass ball was in the uterus. This would suggest that the ball was more likely to cause early regression of the CL.

Placing the glass ball following ovulation could predispose a mare to endometritis, considering the procedure involved passing a foreign object, although sterilized, through the cervical lumen after a mare has entered diestrus. A mare susceptible to endometritis may not have time to clear her uterus of contaminants before closing the cervical lumen completely. Based on this rationale and experience during our preliminary work, we decided to provide treatments simultaneous to glass ball placement that were intended to help prevent a persistent postplacement endometritis. Hence, each mare in this study was infused with ticarcillin disodium (Ticar) and treated with cloprostenol (Estrumate) following glass ball placement. Uterine fluid was only observed in a few mares in the first few days following glass ball placement, and it resolved quickly. Nevertheless, it is advisable to re-examine a mare with ultrasonography following placement of a glass ball to ensure a detectable endometritis has not developed.

Discussions on the Equine Clinicians Network suggested that the glass ball had no long-term detrimental effects on the uterus. The relatively minor ultrasonic changes detected in the uterus following glass ball placement, the endometrial biopsy results, and a conception in 74% of the mares following glass ball removal would seem to support this claim.

It is interesting that the mares experiencing extended luteal function were able to maintain progesterone concentrations above 1 ng/mL for an average of nearly 3 months. This is the period in which we would expect the fetoplacental unit to begin assuming maintenance of pregnancy through the production of pregnanes in an ever-increasing number of pregnant mares. The progesterone profiles in these mares were very similar to those reported for mares hysterectomized 3 days following ovulation.¹³ The primary CL was present 70 days following ovulation in hysterectomized mares, but disappeared by 140 days.¹³ We found this was also true of mares that experienced extended luteal function with a glass ball in the uterus.

The efficacy of an intrauterine glass ball for maintaining luteal function and thus preventing cycling and behavioral estrus in mares appears to be moderate. Our results were not quite as good as those reported from the Netherlands (Dr Randy J. T. de Greef, message to Equine Clinicians Network, March 19, 2000). The Dutch veterinarian indicated that the technique works in at least 75% of cases; in contrast, we found the technique to be effective in only approximately 40% of mares. Perhaps a placebo effect for mare owners, as is suspected with the use of progestin implants, would explain the additional success reported from the Netherlands.

The glass ball protocol takes advantage of endogenous progesterone production to suppress behavioral estrus. Some variation in estrus behavior will be observed in any group of mares teased to a stallion throughout the cycle. However, mares are typically expected to reject a stallion when a functional CL is present and to change from indifferent to receptive as estrogens rise in the absence of a functional CL. Teasing results in this study were consistent with the behavior expected for the concentration of circulating progesterone detected. We did not monitor the mares that experienced extended luteal function beyond their subsequent ovulation, although we speculate that if the glass ball had been left in the uterus, some of the mares may have experienced another extended luteal period. Another researcher related information to us about 2 mares in which he had placed a glass ball (personal communication, Dr Peter Daels, National Institute of Agricultural Research, Nouisilly, France, April 2000). The mares experienced extended luteal function, then, following administration of prostaglandin, both mares returned to estrus, retained the glass ball, ovulated, and again experienced extended luteal function.

This technique offers the advantage of suppressing behavioral estrus because of endogenous progesterone production over an extended period following a single administration of a glass ball. The disadvantage is that it does not work in every mare nor does it appear to have an immediate effect in every mare following intra-uterine placement of the glass ball. However, when it is effective it may serve as an alternative method for suppressing estrous cycle and/or behavior and thus avoiding the need for administration of exogenous progestin products. Readers are cautioned that many questions still exist about the use of intra-uterine glass balls in mares. Further work is required to confirm the efficacy of the use of an intra-uterine glass ball for prolonged luteal function in mares and to identify its mechanism of action.

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Case Report

Complications associated with the presence of two intrauterine glass balls used for oestrus suppression in a mare**R. M. Turner*, D. K. Vanderwall[†] and R. Stawicki[‡]**

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Keywords: horse; mare; uterus; glass ball; oestrus suppression; complication

Summary

This report describes the identification and removal of 2 glass balls, both of which had fragmented, from the uterus of a mare. The glass balls had been placed into the uterine lumen to prolong function of the corpus luteum for suppression of oestrous behaviour. This report adds to a growing body of evidence that severe complications can result from the use of intrauterine glass balls for oestrus suppression in mares, particularly if they are retained for a prolonged time.

Introduction

Suppression of oestrous behaviour is commonly performed in mares to prevent the behaviour from adversely affecting performance activities. General methods of suppressing oestrous behaviour include: 1) administration of exogenous progesterone or synthetic progestins; 2) extending the duration of corpus luteum (CL) function (i.e. maintaining secretion of endogenous progesterone); 3) suppressing ovarian follicular activity; and 4) ovariectomy (Vanderwall and Nie 2011). Of these, administration of progesterone or synthetic progestins has been the most widely used method of suppressing oestrous behaviour, with the orally active synthetic progestin altrenogest (ReguMate¹ or Altresyn²) widely considered to be the 'gold standard' for blocking oestrous behaviour in mares. Although daily administration of altrenogest at a dose of 0.044 mg/kg bwt is very efficacious for suppressing oestrus in mares (Squires *et al.* 1979; Webel and Squires 1982), its expense, need for long-term daily administration, and safety risks for personnel (particularly women) during handling and administration (Vanderwall 2012) are drawbacks to its use. Although there have been anecdotal reports on the use of synthetic progestins other than altrenogest for oestrus suppression, none have been found to be efficacious when rigorously tested (Vanderwall and Nie 2011).

An alternative method of suppressing oestrus that does not require administration of exogenous progesterone/progestins is prolonging the function of the CL, which is the endogenous source of progesterone that naturally keeps mares from displaying oestrus. In nonpregnant mares, the CL secretes progesterone for approximately 2 weeks after ovulation and then stops functioning as a result of endometrial secretion of prostaglandin F_{2α} (PGF_{2α}), which causes regression of the CL (i.e. luteolysis) by destroying the progesterone-producing cells of the CL. Luteolysis causes progesterone concentrations to fall and the subsequent return to oestrus. Luteolysis does not occur

in pregnant mares because the embryo blocks secretion of PGF_{2α}, therefore maintaining continued progesterone secretion that prevents the mare from coming back into oestrus. Therapeutically preventing luteolysis to maintain progesterone secretion from the CL, essentially mimicking pregnancy, is an alternative method of suppressing oestrus in mares.

The most common method of prolonging CL function in mares has been intrauterine insertion of a glass ball (i.e. marble). Nie *et al.* (2003) reported that placement of a 25 or 35 mm sterile glass ball into the uterine body immediately following ovulation resulted in prolonged CL function in 7 of 18 (39%) mares that retained the glass ball (6 of 12 mares expelled the 25 mm glass ball soon after insertion). In mares that developed prolonged CL function following placement of the glass ball, CL function was maintained for approximately 90 days, during which time progesterone concentrations remained above 1.0 µg/l and oestrous behaviour was not displayed. In nontreated control mares, spontaneous prolongation of CL function occurred in 4 of 32 (13%) mares. Although placement of a glass ball appeared to be an efficacious means of blocking oestrous behaviour for an extended period, it should be noted that in addition to the 61% mares that retained the glass ball and never developed extended CL function, 3 of 7 mares had one or 2 oestrous cycles of normal duration after placement of the glass ball before CL function was prolonged. Therefore, on a 'per cycle' basis the incidence of prolonged CL function was only 11% (7/62 cycles) in the mares that received a glass ball compared to 8% (4/50 cycles) in the nontreated control mares, which was not significantly different between groups. Although the original report describing the use of an intrauterine glass ball for oestrus suppression found no adverse effects of the glass ball on the endometrium or subsequent fertility (Nie *et al.* 2003), it is becoming evident there are risks associated with the use of an intrauterine glass ball for oestrus suppression in mares. Recently, pyometra associated with long-term retention of a glass ball was reported (Klabnik-Bradford *et al.* 2013), and here we describe a case in which 2 intrauterine glass balls were identified in a mare. Both glass balls had fragmented and one had fragmented into multiple pieces.

Case history, clinical findings and treatment

An 8-year-old maiden Quarter Horse mare was presented for removal of 2 intrauterine glass balls, at least one of which had apparently fragmented. It was reported by the current owner

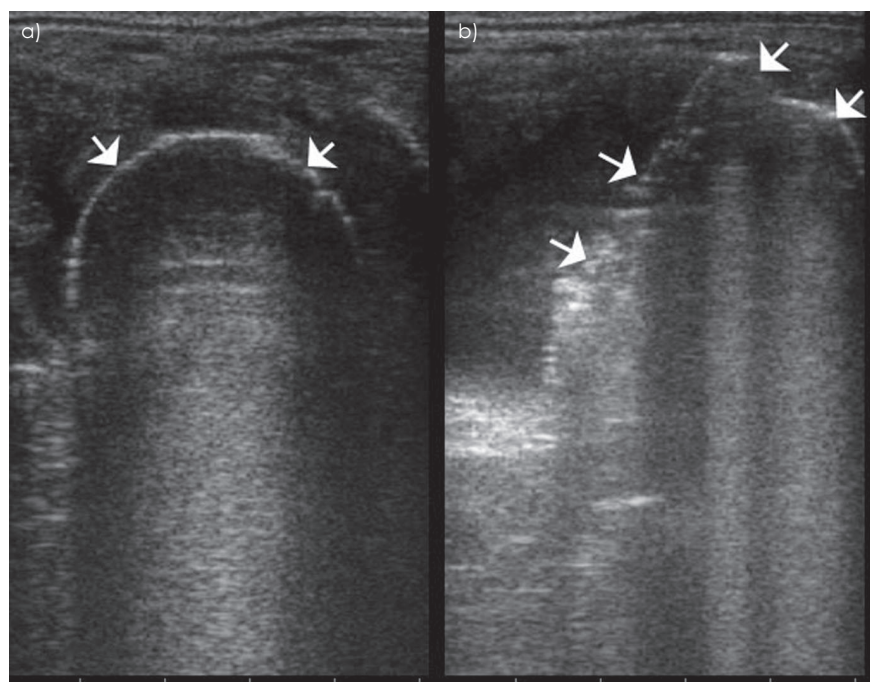


Fig 1: a) Transrectal ultrasonographic image showing an apparently intact glass ball in the uterine lumen. Arrows point to the smooth, curved surface. b) Transrectal ultrasonographic image showing a second, apparently fragmented glass ball in the uterine lumen. Note the irregular, flattened surface delineated by arrows. 7.5 MHz linear array transducer. Each marker line equals 1 cm.

that several years previously one glass ball had been placed into the mare's uterine lumen to suppress oestrus and, when that ball stopped 'working', a second glass ball was placed into the uterine lumen, apparently without knowledge that the first glass ball was still present in the uterus. Subsequently, the current owner acquired the mare and made plans to breed her. In preparation for breeding, the current owner's veterinarian examined the mare with transrectal palpation and ultrasonography, at which time the presence of at least one intrauterine glass ball was noted. The owner's veterinarian made an attempt to retrieve the glass ball transcervically, but found the cervix to be adhered closed, so the mare was referred for additional diagnostic evaluation and treatment.

At the time of presentation, palpation and ultrasonographic examination of the reproductive tract *per rectum* revealed the presence of 2 structures consistent with glass balls in the uterine body (Fig 1). One glass ball appeared to be intact, while the second structure was irregular and appeared to be broken. Several other echogenic structures were identified in the uterine lumen, consistent with multiple glass fragments. Examination *per vagina* revealed a presumptive piece of glass embedded within the cervical tissue surrounding the external cervical os. When assessed *per vagina*, the cervical canal was fibrosed closed. Attempts were made to manually reduce the cervical adhesions and enter the uterus, but the mare became uncomfortable and the attempts were temporarily discontinued. Plans were made to sedate the mare and perform an endoscopic examination of the cervix and uterus.

The mare was sedated with 0.01 mg/kg bwt detomidine hydrochloride (Dormosedan)³, and 0.02 mg/kg bwt butorphanol tartrate (Torbugesic)³, i.v. Mepivacaine hydrochloride (20 ml 2%; Carbocaine-V)³ was infused into the cervical canal to provide local anaesthesia to the cervix. More aggressive attempts to manually break down the adhesions around the cervix were successful and access to the uterus was obtained. Endoscopic examination confirmed the

presence of one largely intact glass ball and a second ball that had fractured into multiple pieces (Fig 2). Two of the fractured pieces were removed with a wire snare through the cervix. However, several small, smooth shards of glass and the larger intact ball could not be grasped for removal as the wire snare could not gain traction and repeatedly fell off once tension was applied. Additional attempts were made to dilate the cervix, and eventually sufficient cervical dilation was achieved to allow passage of the examiner's fingers into the uterine lumen. The apparently intact glass ball and approximately 7–10 additional shards of glass were identified in the uterine body and were removed manually. Following removal of all the accessible glass, the uterus was re-examined endoscopically and found to be in remarkably good condition and free of any gross evidence of glass pieces (Fig 3). The cervix also was re-examined endoscopically and manually and was notably improved. Apparently, the embedded fragment had migrated back into the uterine lumen during manipulation of the reproductive tract and had been removed. All of the adhesions had been reduced, resulting in a patent and palpably normal cervical canal.

Following endoscopy, the mare's uterus was lavaged with 6 l of sterile saline. No additional pieces of glass were noted in the fluid that was recovered. Likewise, ultrasonographic examination of the reproductive tract did not reveal any evidence of glass fragments remaining in the uterus. The uterus was prophylactically infused with 2 g of ampicillin sodium diluted in 60 ml of sterile water. Flunixin meglumine (1 mg/kg bwt i.v.; Banamine)⁴ was administered and a topical cream, consisting of nystatin, neomycin, thiostrepton and triamcinolone (Animax Ointment)⁵, was applied to the cervix prior to discharge. The topical ointment was applied to the cervix once every 2–3 days for 2 weeks following discharge.

The glass fragments were examined following removal. There were 2 approximately 35 mm glass balls. The first ball (blue) was largely intact, with only one relatively small piece shorn off. The second ball (tan) was in multiple pieces,

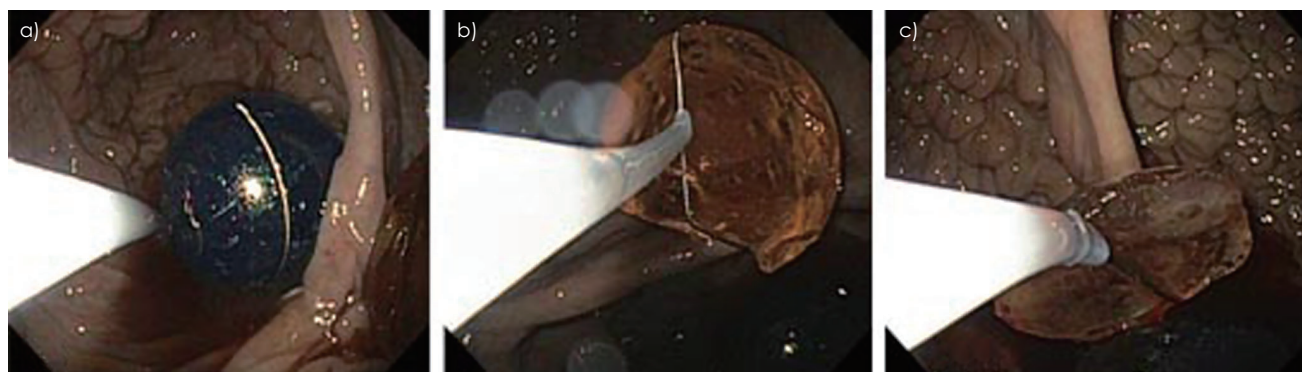


Fig 2: a) Endoscopic view of the uterine lumen containing an apparently intact glass ball. The ball is encircled by a wire snare. However, the smooth surface of the ball caused the snare to slide off each time that traction was applied. b) and c) Endoscopic views of the uterine lumen containing 2 different irregular pieces from the second glass ball. Both pieces are encircled by a wire snare and were successfully removed from the uterus with the snare.



Fig 3: Endoscopic view of the uterine lumen immediately following removal of the glass balls and shards. The endometrium appeared to be in remarkably good condition and there was no gross visual evidence of any additional glass in either uterine horn or in the uterine body. The oviduct papilla is visible at the tip of the uterine horn (arrow).

including 3 larger pieces and at least 10 smaller shards (**Fig 4**). When all of the glass pieces were reassembled, they appeared to form 2 complete 35 mm glass balls. However, particularly for the tan ball, we could not definitively determine if small pieces still were missing, potentially having been left behind embedded in the uterine wall.

The owners reported that the mare recovered uneventfully from the procedure. Two attempts were made to breed the mare in the year following the glass removal. Breedings were performed by the referring veterinarian on the farm using artificial insemination with cooled transported semen from a fertile stallion under good management. The mare did not become pregnant after these breedings and was returned to work under saddle as a Western Pleasure Horse. No additional reproductive evaluations were performed.



Fig 4: Glass balls and associated shards removed from the mare's uterus. The blue glass ball on the left was largely intact with only one small piece shorn off. The tan glass ball on the right was in multiple pieces including 3 larger pieces and multiple smaller shards.

Discussion

This report adds to a growing body of evidence that severe complications can result from the use of intrauterine glass balls for oestrus suppression in mares, particularly if they are retained for a prolonged period of time. It seems plausible that the mare in this report came to have 2 glass balls in her uterus because it was assumed the first glass ball had been spontaneously expelled from the uterine lumen when cyclical oestrous behaviour returned, leading to the placement of the second glass ball without knowledge the first ball was still present in the uterine lumen. The presence of 2 glass balls in the uterine lumen of a mare has been previously reported (Vanderwall 2013) and, although both glass balls were intact, they both showed noticeable pitting on their surfaces when they were removed. It seems plausible that when 2 balls are present, repeated contact of the 2 balls within the uterine lumen eventually results in shearing and fragmentation. Fragmentation of the glass ball in this mare raises serious concerns for future fertility, since there is no way to know with

certainty that all of the fragments were successfully removed (particularly very small pieces). If fragments were left behind, they could impair fertility by serving as a nidus for inflammation/infection and/or by physically damaging a conceptus, particularly during the mobility phase of early gestation.

Although this mare's problems may have been caused by the interaction of 2 glass balls in the uterus, even a single glass ball can result in significant complications, including pyometra (Klabnik-Bradford *et al.* 2013) and fragmentation (M. LeBlanc, personal communication, 2009). Surprisingly, there are anecdotal reports of mares becoming pregnant despite the presence of an intact intrauterine glass ball, with some of these pregnancies going to term, while others have been aborted presumably due to adverse consequences of the glass ball (personal communications through the American College of Theriogenologists List Server).

Because of the spectrum of complications that have now been associated with the use of intrauterine glass balls (pyometra, multiple glass balls with fragmentation, pregnancy/pregnancy failure), it would seem prudent to re-evaluate the use of these devices for oestrus suppression in mares. Although the specific complication of glass fragmentation can be avoided by using a device made of an alternative material such as plastic (Rivera Del Alamo *et al.* 2008), it is important to note that regardless of the composition of the device, if it is not eventually removed (spontaneously or manually), some mares may retain the device for an extended period of time (i.e. years), such that its presence in the uterine lumen may not be known to individuals who do not have access to the animal's complete history. This could lead to other complications such as pyometra, placement of a second device, breeding in spite of the presence of the ball etc. Given the potential for severe consequences associated with the use of intrauterine glass balls for oestrus suppression, there is a need for other methods of extending CL function that are practical, efficacious and safe. Alternative methods of prolonging function of the CL for oestrus suppression include: 1) oxytocin treatment (Vanderwall *et al.* 2007); 2) inducing a late-dioestrus ovulation (Hedberg *et al.* 2006); 3) intrauterine infusion of plant oils (Wilsher and Allen 2011); and 4) manually terminating pregnancy after maternal recognition of pregnancy (i.e. after the embryo has blocked endometrial PGF_{2α} secretion; LeFranc and Allen 2004). For a complete review of these methods see Vanderwall (2013).

Authors' declaration of interests

No conflicts of interest have been declared.

Manufacturers' addresses

¹Intervet, Millsboro, Delaware, USA.

²CEVA Animal Health, Lenexa, Kansas, USA.

³Zoetis, Florham Park, New Jersey, USA.

⁴Merck Animal Health, Whitehouse Station, New Jersey, USA.

⁵Dechra USA, Overland Park, Kansas, USA.

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United States Department of Agriculture

Science Framework for Conservation and Restoration of the Sagebrush Biome: Linking the Department of the Interior's Integrated Rangeland Fire Management Strategy to Long-Term Strategic Conservation Actions

Part 2. Management Applications



Forest
Service

**Rocky Mountain
Research Station**

General Technical Report
RMRS-GTR-389

April 2019

Crist, Michele R.; Chambers, Jeanne C.; Phillips, Susan L.; Prentice, Karen L.; Wiechman, Lief A., eds. 2019. Science framework for conservation and restoration of the sagebrush biome: Linking the Department of the Interior's Integrated Rangeland Fire Management Strategy to long-term strategic conservation actions. Part 2. Management applications. Gen. Tech. Rep. RMRS-GTR-389. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 237 p.

Abstract

The Science Framework is intended to link the Department of the Interior's Integrated Rangeland Fire Management Strategy with long-term strategic conservation and restoration actions in the sagebrush biome. The focus is on sagebrush (*Artemisia* spp.) ecosystems and sagebrush dependent species with an emphasis on Greater sage-grouse (*Centrocercus urophasianus*). Part 1 of the Science Framework, published in 2017, provides the scientific information and decision-support tools for prioritizing areas for management and determining effective management strategies across the sagebrush biome. Part 2, this document, provides the management considerations for applying the information and tools in Part 1. Part 2 is intended to facilitate implementation of resource management priorities and use of management strategies that increase ecosystem resilience to disturbance and resistance to nonnative invasive annual grasses. The target audience of Part 2 is field managers, resource specialists, and regional and national-level managers. The topics addressed in this volume include adaptive management and monitoring, climate adaptation, wildfire and vegetation management, nonnative invasive plant management, application of National Seed Strategy concepts, livestock grazing management, wild horse and burro considerations, and integration and tradeoffs. Geospatial data, maps, and models for the Science Framework are provided through the U.S. Geological Survey's ScienceBase database and Bureau of Land Management's Landscape Approach Data Portal. The Science Framework is intended to be adaptive and will be updated as additional data become available on other values and species at risk. It is anticipated that the Science Framework will be widely used to: (1) inform emerging strategies to conserve sagebrush ecosystems, sagebrush dependent species, and human uses of the sagebrush system; and (2) assist managers in prioritizing and planning on-the-ground restoration and mitigation actions across the sagebrush biome.

Keywords: sagebrush habitat, Greater sage-grouse, resilience, resistance, conservation, restoration, monitoring, adaptive management, climate adaptation, wildfire, nonnative invasive plants, National Seed Strategy, livestock grazing, wild horses and burros

Front cover photo. Sagebrush ecosystem in the Toiyabe Range, Nevada (photo: Jeanne Chambers, USDA Forest Service). Inset: Greater sage-grouse chick (photo: USDOI Fish and Wildlife Service).

Rear cover photo. Tail feathers of a Greater sage-grouse (photo: USDOI Fish and Wildlife Service).

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8. WILD HORSE AND BURRO CONSIDERATIONS

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Introduction

Wild horses (*Equus caballus*) and wild burros (*E. asinus*), like domestic livestock, can alter sagebrush ecosystem structure and composition and affect habitat quality for sagebrush dependent species (Beever and Aldridge 2011). The presence of Federally protected wild horses and wild burros can also have substantial effects on the capacity for habitat restoration efforts to achieve conservation and restoration goals. In the Conservation Objectives Team Report (USDOI FWS 2013), the presence of wild horses and burros was considered a threat to Greater sage-grouse (*Centrocercus urophasianus*; hereafter, GRSg) habitat quality, particularly in the sage-grouse's western range (USDOI FWS 2013). Four years after the Conservation Objectives Team Report was published, wild horse population sizes on Bureau of Land Management (BLM) and Forest Service lands have almost doubled (USDOI BLM 2017).

Lands with Federally protected wild horses and burros are managed for multiple uses, so it can be difficult to separate their ecological effects. However, scientific studies designed to isolate the effects of various land uses lead to the conclusion that landscapes with greater wild horse and burro abundance tend to have lower resilience to disturbance and resistance to invasive plants than similar landscapes with herds at or below target levels (Beever and Aldridge 2011; Chambers et al. 2017 [hereafter, Part 1], section 5.3.8). Many studies corroborate the general understanding that wild horses can lead to biologically significant changes in sagebrush ecosystems, particularly when their populations are overabundant relative to forage and water resources. In the Great Basin, areas without wild horses had higher shrub cover, plant cover, species richness, native plant cover, and overall plant biomass, and lower cover of grazing-tolerant, unpalatable, and invasive plant species such as cheatgrass (*Bromus tectorum*), when compared to areas with horses (Beever et al. 2008; Boyd et al. 2017; Davies et al. 2014; Smith 1986; Zeigenfuss et al. 2014). There were also measurable increases in soil penetration resistance and erosion, decreases in ant mound and granivorous small mammal densities, and changes in reptile communities (Beever et al. 2003; Beever and Brussard 2004; Beever and Herrick 2006; Ostermann-Kelm et al. 2009).

Top: Wild horses at Cherry Spring in the Maverick-Medicine Herd Management Area, Nevada (photo: USDOI Bureau of Land Management). Middle left: Wild horses in Divide Basin Horse Management Area, Wyoming (photo: USDOI Bureau of Land Management). Middle right: Wild burros at Wood Hills spring in the Elko, Nevada, BLM District (photo: USDOI Bureau of Land Management). Bottom left: Wild Horses at Victoria spring in the Antelope Triple B complex (photo: USDOI Bureau of Land Management). Bottom right: Wild horse gather by the BLM (photo: USDOI Bureau of Land Management).

Wild horses can have severe impacts on water source quality, aquatic ecosystems, and riparian communities (Barnett 2002; Beever and Brussard 2000; Earnst et al. 2012; Kaweck 2016; Nordquist 2011; USDO FWS 2008, 2012) and can sometimes exclude native ungulates from water sources (Gooch et al. 2017; Hall et al. 2016; Ostermann-Kelm et al. 2008; Perry et al. 2015; USDO FWS 2008). Bird nest survival may be lower in areas with wild horses (Zalba and Cozzani 2004), and bird populations have recovered substantially after livestock or wild horses, or both, have been removed (Batchelor et al. 2015; Earnst et al. 2005, 2012). Wild horses can spread nonnative plant species, including cheatgrass, and may limit the effectiveness of reseeding projects (Beever et al. 2003; Couvreur et al. 2004; Jessop and Anderson 2007; Loydi and Zalba 2009). Even after domestic livestock are removed, continued wild horse use above appropriate management levels can cause ongoing detrimental ecosystem effects (Davies et al. 2014; USDO FWS 2008), which may require several decades for recovery (e.g., Anderson and Inouye 2001).

Wild burros can have grazing and trampling impacts that are similar to wild horses (Carothers et al. 1976; Douglas and Hurst 1983; Hanley and Brady 1977) and can substantially affect riparian habitats (e.g., Tiller 1997) and native wildlife (e.g., Seegmiller and Ohmart 1981). Where wild burros and GRSG co-occur, year-round use by burros in low elevation habitats may lead to a high degree of overlap between burros and GRSG (Beever and Aldridge 2011).

In contrast to managed domestic livestock grazing (see section 7), neither the seasonal timing nor the intensity of grazing by Federally protected wild horses and burros can be managed, except through efforts to manage their numbers and distribution. Wild horses roam freely on the range year-round, and wild horse populations have the potential to grow 15 to 20 percent or more per year (Dawson 2005; Eberhardt et al. 1982; Garrott et al. 1991; Roelle et al. 2010; Scorolli and Cazorla 2010; Wolfe 1980). Although annual growth rates may be marginally lower in some areas where mountain lions (*Puma concolor*) can take foals (Turner 2015; Turner and Morrison 2001), horses tend to favor use of more open habitats (Schoenecker et al. 2016) that are dominated by grasses and shrubs and where ambush is less likely. For the majority of wild horse herds, there is little evidence that population growth is significantly affected by predation. As a result of the potential for wild horse populations to grow rapidly, impacts of wild horses on water, soil, vegetation, and native wildlife resources can increase exponentially unless there is active management to limit their population sizes.

On lands administered by the BLM, there were an estimated 72,674 BLM-administered, Federally protected wild horses and burros as of March 1, 2017, not including foals born in 2017 (USDO BLM 2017). Approximately 60 percent of those are present within 13 million acres (5 million hectares) of GRSG habitat. Federal protections exist for an estimated 7,100 wild horses and 900 wild burros that occupy approximately 2 million acres (800,000 hectares) of Forest Service-administered lands. Approximately 446,065 acres (180,523 hectares) of active Territories administered by the Forest Service contain GRSG habitat, which is occupied by an estimated 3,400 wild horses and burros. Some wild horses also inhabit other Federal lands in the sagebrush biome, including lands administered by the National Park Service, U.S. Fish and Wildlife Service, or Department of Defense, and Native American reservations and tribal lands.

Although wild horses and burros can present challenges to achieving desired habitat conditions, wild horse management is a necessary requirement of planning for long-term sagebrush ecosystem and GRSG conservation. This section relates to management of Federal lands and the terms “wild horses”

and “wild burros” are used throughout. However, the specific legal status for any given wild horse or burro population has a large influence on management objectives and the ability to manage wild horse and burro impacts.

In the biological sense, all free-roaming horses and burros in North America are feral, meaning that they are descendants of domesticated animals brought to the Americas by European colonists. Horses went extinct in the Americas by the end of the Pleistocene, about 10,000 years ago (MacFadden 2005; Webb 1984). Burros evolved in Eurasia (Geigl et al. 2016). The published literature refers to free-roaming horses and burros as either feral or wild. In the ecological context the terms are interchangeable, but the term “wild” horse is associated with a specific legal status. Wild and free-roaming horses and burros under the jurisdiction of the BLM and Forest Service are designated “wild” as legally defined by the Wild Free Roaming Horses and Burros Act of 1971 (WFRHBA) as amended (Public Law 92–195), and are under the protection, management, and control of the BLM and Forest Service. Only those horses whose unbranded and unclaimed ancestors were present on BLM and Forest Service lands at the time of the passage of the WFRHBA are managed in accordance with the WFRHBA, and only those lands where wild horses and burros were found when the WFRHBA was passed can be managed to maintain Federally recognized wild horse and burro populations.

Other populations of feral horses and burros on Federal lands (i.e., those on lands administered by the U.S. Fish and Wildlife Service, National Park Service, or Department of Defense; and Native American reservations and tribal trust lands) are generally subject to other Federal regulations and relevant State laws, but are not subject to provisions of the WFRHBA. This section draws on scientific studies of feral horses and burros, some of which also have wild horse or wild burro legal status. Clarification of which horses and burros are considered Federally protected is provided in the BLM regulation (43 CFR 4700 [FR 2011]), BLM wild horse and burro management handbook and manuals (USDOI BLM 2010a,b,c,d), Forest Service manual (FSM 2260.5), and Forest Service regulation (36 CFR 222.20(b)(13), 36 CFR 222.63 [FR 2012]). The legal designation of a particular herd is not expected to change the animals’ ecological effects, but it will influence management options. Discussions about management in this section reflect constraints for Federally designated wild horses and burros.

This section begins with information on wild horse and burro management structure, population estimates and spatial distribution, and management actions to maintain wild horses and burros at appropriate management levels. Then it discusses using resilience and resistance concepts to inform management of wild horses and burros. It concludes with management considerations at the project scale. This section refers mainly to wild horses because wild burros are not nearly as numerous as wild horses in most areas of the sagebrush biome.

Wild Horse and Burro Management Structure

For lands administered by the BLM, Herd Areas (HAs) are defined as areas where wild horses and burros existed at the time of passage of the WFRHBA. Herd Management Areas (HMAs)—the subset of lands designated for active management of wild horses and burros as part of multiple use management—can be designated only within HAs during land use planning activities. In most cases, each HMA is intended to support only wild horses or wild burros, but there are some HMAs that contain both. For HAs that do not have an HMA designation, it generally has been determined that resources are limiting and that wild horse and

burro populations cannot be maintained for the long term. The Forest Service-administered Wild Horse Territories (WHTs), Wild Burro Territories (WBTs), and Wild Horse and Burro Territories (WHBTs) are designated according to the species that occupy the Territory. There are some Territories without any wild horses or burros that are considered “inactive,” where it has been determined that there are not sufficient resources to maintain wild horses and burros, or where wild horses and burros no longer exist. The numbers of wild horses and burros in HMAs, WHTs, WBTs, or WHBTs and the overlap with GRSG habitat are in text box 8.1.

When two or more HMAs, WHTs, WBTs, or WHBTs are located close to one another, with the potential for wild horses and burros to move freely among them, those areas may be managed collectively as a “complex” (or “joint management area”). Complexes sometimes cross administrative boundaries between BLM field or district offices and Forest Service districts.

The spatial scales of wild horse management are the entire population at the West-wide scale; complexes or groups of HMAs or WHTs, WBTs, and WHBTs with interchange for the regional scale; and individual herds for the local scale. A National Academies of Science report (National Research Council 2013) suggested that wild horse management should be focused more broadly on meta-populations, in which HMAs, WHTs, WBTs, and WHBTs are grouped where interchange occurs, regardless of administrative boundaries. Thus, relative to the spatial scales presented in section 1 of this report, the BLM and Forest Service manage wild horses between the regional and local project levels. The actual spatial scale for any given wild horse population should be determined in consultation with the local staff that manages those populations (i.e., BLM wild horse and burro specialist; Forest Service rangeland management specialist).

Importantly, each HMA, WHT, WBT, and WHBT has an established target population size range for wild horses (and a separate target for wild burros, if they are present), known as the appropriate management level (AML). The BLM and Forest Service view AML as a target population size range which, if maintained, should allow for a thriving ecological balance and multiple use relationship (43 CFR 4710.3-1 [USDOI BLM 2010b]; 43 CFR 4770.3(c) [USDOI 2012]; 36 CFR 222.60(b)(3), 36 CFR 222.61(a)(1), 36 CFR 222.69(a)

Text Box 8.1—Wild Horse and Burro Population Sizes

The BLM manages wild horses and burros within a total of 177 Herd Management Areas (HMAs), which range in size from 3.0 square miles (777 hectares) to 2,033.8 square miles (526,754.2 hectares). As of March 1, 2017, the estimated number of wild horses and burros managed by BLM was 72,674. A total of 105 HMAs overlap with approximately 13 million acres (5 million hectares) of GRSG habitat.

The Forest Service manages 34 active and 19 inactive wild horse and burro administrative units that include: Wild Horse Territories (WHTs; 27 active, 16 inactive), Wild Burro Territories (WBTs; 4 active, 3 inactive), and Wild Horse and Burro Territories (WHBTs; 3 active). These range in size from 5.4 square miles (1,398.6 hectares) to 530.4 square miles (137,373.6 hectares). The Forest Service manages approximately 8,000 wild horses and burros. Thirteen active Territories overlap with approximately 446,000 acres (180,000 hectares) of GRSG habitat.

One thousand or more wild horses on three WHTs and five HMAs live on or near Bi-State GRSG habitat (about 70,000 Forest Service acres [28,000 hectares] and 82,403 BLM acres [33,348 hectares]) (Bi-State Technical Advisory Committee 2012). The Bi-State population has been identified as a Distinct Population Segment of GRSG and is managed under a separate conservation Action Plan.

[FR 2012]). This view reflects an assumption that wild horse and burro populations at AML should allow for land health standards to be met (USDOI BLM 2010a). The AML generally is a range between a low and high value, to allow for some variability in population size across years (USDOI BLM 2010a). The AML is typically determined at the activity planning level through site-specific analysis or, in some cases, through the land use planning process. Monitoring information that couples data on wild horse and burro populations and rangeland status and trends is used to establish or adjust AMLs (text box 8.2). Progress toward attainment of site-specific and landscape-level management objectives or multiple use objectives is also considered. Future studies at local scales could test the assumption that wild horse and burro populations at AML allow for land health standards to be met.

Text Box 8.2—Monitoring Considerations for Wild Horses and Burros

Reliable estimates of population sizes and habitat data provide the basis for management decisions regarding wild horses and wild burros. Understanding the annual growth rates of wild horse and burro populations and the status and trends of rangelands occupied by wild horses and burros is essential for making informed management decisions.

Inventory (monitoring) data for wild horse and burro populations include information on the numbers of animals, their use patterns, and spatial distribution. Habitat data include grazing utilization, range ecological condition and trend, actual use, and climate (weather) data. Habitat monitoring data collection should be coordinated with other resource programs (e.g., range, watershed, wildlife) to maximize efficiency and minimize duplication.

Data and analyses of populations and habitats are used in concert to:

- Establish or adjust Appropriate Management Levels (AMLs);
- Make a determination of excess wild horses or burros (i.e., establish the need to gather and remove excess animals in order to reach and stay at AML);
- Develop or revise Herd Management Area (HMA) boundaries; and
- Evaluate conformance with Land Health Standards, Land Use Plan goals and objectives, or other site-specific or landscape-level objectives.

Data and methods used to inform decisions should be scientifically defensible. The public should be able to understand the methods used and how they are implemented and also to access the data used to make decisions.

Data on Population Estimates and Spatial Distribution of Wild Horses and Burros

Population estimates for each HA and HMA are reported annually in the Public Land Statistics (http://www.blm.gov/public_land_statistics/); spatial data are available via the BLM GeoCortex, which is available to managers for analyses and planning and is useful in determining the number of excess animals present on the range (<https://webmaps.blm.gov/Geocortex/Html5Viewer/Index.html?viewer=whb>). The Forest Service reports population estimates for each territory on the Forest Service wild horse and burro program website (<https://www.fs.fed.us/wild-horse-burro/territories/index.shtml>). The BLM and Forest Service have recently adopted a statistically valid, standardized methodology for estimating wild horse population sizes (Lubow and Ransom 2009, 2016; Ransom 2012) that accounts for animals that were present, but not seen by observers. In

most cases, reported population estimates are based on the statistical analysis of aerial survey data; BLM policy calls for each HMA (and complexes that include both BLM lands and Forest Service WHTs, WBTs, or WHBTs) to be surveyed at least once every 2 years (USDOI BLM 2010e). For both agencies, population size estimates are projected for intervening years based on the best available information about expected population growth rates for each area. As previously discussed, wild horse growth rates can typically be assumed to be about 15 percent to 20 percent per year (National Research Council 2013) unless there is a contraceptive project to limit reproduction. However, in some places the annual growth rate may be greater than 20 percent. The range-wide population estimates are used to develop BLM geospatial data (accessible at the BLM GeoCortex site) and the status of a population relative to high AML within a particular HMA.

Although it is the intended management goal that wild horses remain only on HMAs, WHTs, or WHBTs, the current reality is that Federally protected wild horses are also present on many HAs and on other Federal, State, tribal, and private lands outside of these administrative boundaries. As a result, the user must be cautiously aware that the data representing boundaries of and populations within HMAs, HAs, WHTs, WBTs, and WHBTs may not portray the actual spatial distribution of all wild horse and burro populations. Continued increases in wild horse and burro populations, relative to AML, will result in a more widespread distribution of herds, including into areas outside designated boundaries. In areas where road or trail access allows for observations and on-the-ground documentation of horse sign (e.g., trailing, scat piles, evidence of horse grazing and browsing), the local designated staff is likely to have a broad understanding of where the animals tend to go in different seasons, which water sources they rely on, and the general pattern of their movements.

Management Actions to Maintain Wild Horses and Burros at Appropriate Management Levels

The 1971 WFRHBA directs the BLM and Forest Service to remove excess animals from the range (43 CFR 4720.1 and 36 CFR 222.69, respectively) to maintain a thriving natural balance. The number of wild horses or burros greater than a designated high AML for a HMA, WHT, WBT, or WHBT is considered to be the number of “excess” animals in the area. In order to take management action, the agencies must make two determinations: (1) that an overpopulation exists, and (2) whether or not it will be necessary to remove excess animals.

Historically, the BLM and Forest Service reduced herd population sizes to the low value of AML. This was accomplished by removing excess animals from the range. The population would then typically grow to reach the high value of the AML range within 3 to 4 years, unless some form of contraception was used to limit population growth rates. Natural regulation via starvation or dehydration is generally not acceptable to many members of the public (National Research Council 2013).

After removal, animals were placed in holding facilities, offered to the public for adoption, and then kept in holding facilities indefinitely if there was no adoption demand. However, removing all excess wild horses and holding them in off-range facilities for the remainder of their lives would be prohibitively expensive (Garrott and Oli 2013). In many recent years, the BLM has not had the budgetary capacity to remove more than approximately 3,500 animals per year

from the range. Further, the more than 45,000 BLM-administered, captive wild horses currently in long-term holding (of which about 850 are horses from Forest Service Territories) require over \$50 million per year to maintain. As a result, populations of wild horses and burros across all BLM-administered lands (and on some Forest Service Territories) have not been gathered so frequently. Average population sizes are now more than three times greater than the high end of the total AML and these populations are growing.

In 2015, the BLM, the Forest Service, and other agencies identified certain areas as the most important habitats for GRSG and other sagebrush obligates. None of those areas overlapped with Forest Service-administered wild horse or wild burro populations. The BLM developed a 5-year gather schedule to achieve AML by 2020 in 22 HMAs that overlapped areas identified as the most important habitats for GRSG and other sagebrush obligates. However, under budget projections made in FY2017, the BLM will not have the fiscal capacity to conduct gathers within GRSG Priority Habitat Management Areas until 2020 or later, and has no capacity to manage wild horse populations that overlap with GRSG General Habitat Management Areas. Unless there are Congressionally directed changes to the BLM program, it is expected that the number of wild horses within GRSG habitat could surpass 65,000 horses in 2019. Furthermore, maintaining any wild horse population at or below AML will require an active and ongoing program of population growth suppression or scheduled removals (or both) of excess animals. Without such a program, habitat restoration will quickly be at risk as wild horse populations again grow to exceed AML.

Currently used population growth suppression methods include gelding and the immunocontraceptives porcine zona pellucida (PZP) and GonaCon (National Research Council 2013). Both vaccines may be effective for only 1 year, unless booster doses are given (National Research Council 2013). Repeated PZP boosters require annual darting or recapture of the vast majority of wild horses under BLM or Forest Service management, which is infeasible on many HMAs and Territories, would be prohibitively expensive to apply across the range of wild horses and burros, and may lead to more stress for wild horses as a result of frequent capture. The BLM is supporting ongoing research initiatives to develop and test longer-term contraception for wild horses and burros and to improve contraceptive efficacy and production (USDOI BLM 2015). However, planning decisions that propose to remove excess horses or utilize population growth suppression on any BLM lands are often appealed and litigated by interested members of the public. This results in a high degree of uncertainty about the ability of designated Federal agencies to maintain wild horse populations within AML.

Using Resilience and Resistance Concepts and the Science Framework to Inform Management of Wild Horses and Burros

Information on relative ecosystem resilience to disturbance and resistance to invasive annual grasses can be used to help understand the responses of sagebrush ecosystems, species at risk, and other resources to wild horse and burro use and to the interactions of wild horse and burro use with other potential disturbance factors such as wildfire and invasive plants. Information on resilience and resistance to invasive annual grasses, coupled with information on current

and projected wild horse and burro population sizes relative to AML and other predominant threats and disturbance factors, can be used to inform conservation and restoration strategies in sagebrush ecosystems across scales.

Part 1 of the Science Framework provides an approach based on an understanding of ecosystem resilience to disturbance and resistance to invasive annual grasses that uses assessments at the mid-scale (ecoregional or GRSG Management Zone) (fig. 1.1) to help prioritize areas for management and determine effective management strategies (Chambers et al. 2017). The approach is based on: (1) the likely response of an area to disturbance or stress due to threats or management actions (i.e., resilience to disturbance and resistance to invasive annual grasses), (2) the capacity of an area to support target species or resources, and (3) the predominant threats. The geospatial data layers and analyses used in the approach are described in Part 1, sections 8.1 and 8.2. The process involves overlaying key data layers including resilience and resistance to invasive annual grasses as indicated by soil temperature and moisture regimes (Maestas et al. 2016), sage-grouse breeding habitat probabilities (Doherty et al. 2016), the densities or distributions of other sagebrush dependent species, and the primary threats for the ecoregions or Management Zones in the assessment. The maps and analyses that managers derive from this process are an essential component of prioritizing areas for management actions and developing management strategies.

Wild horse and burro densities and AMLs can be used similarly to other threats and disturbance factors in the analyses. Managers can devise categories to evaluate the degree to which wild horse and burro populations are within or exceed AMLs for HMAs, WHTs, WBTs, and WHBTs. Here, three abundance categories relative to AML were developed based on available abundance estimates for BLM lands and Forest Service lands: within AML, more than 100 percent to 200 percent of AML, and more than 200 percent of AML. The wild horse HMAs were overlaid with these three abundance categories (fig. 8.1). Note that this figure also depicts HAs where the target population for wild horses is zero, but where wild horses are present.

The three abundance categories were overlaid with: (1) the three resilience and resistance categories derived from soil temperature and moisture regime information, and (2) GRSG breeding habitat probabilities (see Part 1, sections 8.1 and 8.2). This analysis does not include areas outside the boundaries of HMAs, HAs, WHTs, WBTs, and WHBTs where horses and burros have expanded their use. The data used in the analyses can be found at: <https://www.sciencebase.gov/catalog/item/576bf69ce4b07657d1a26ea2>.

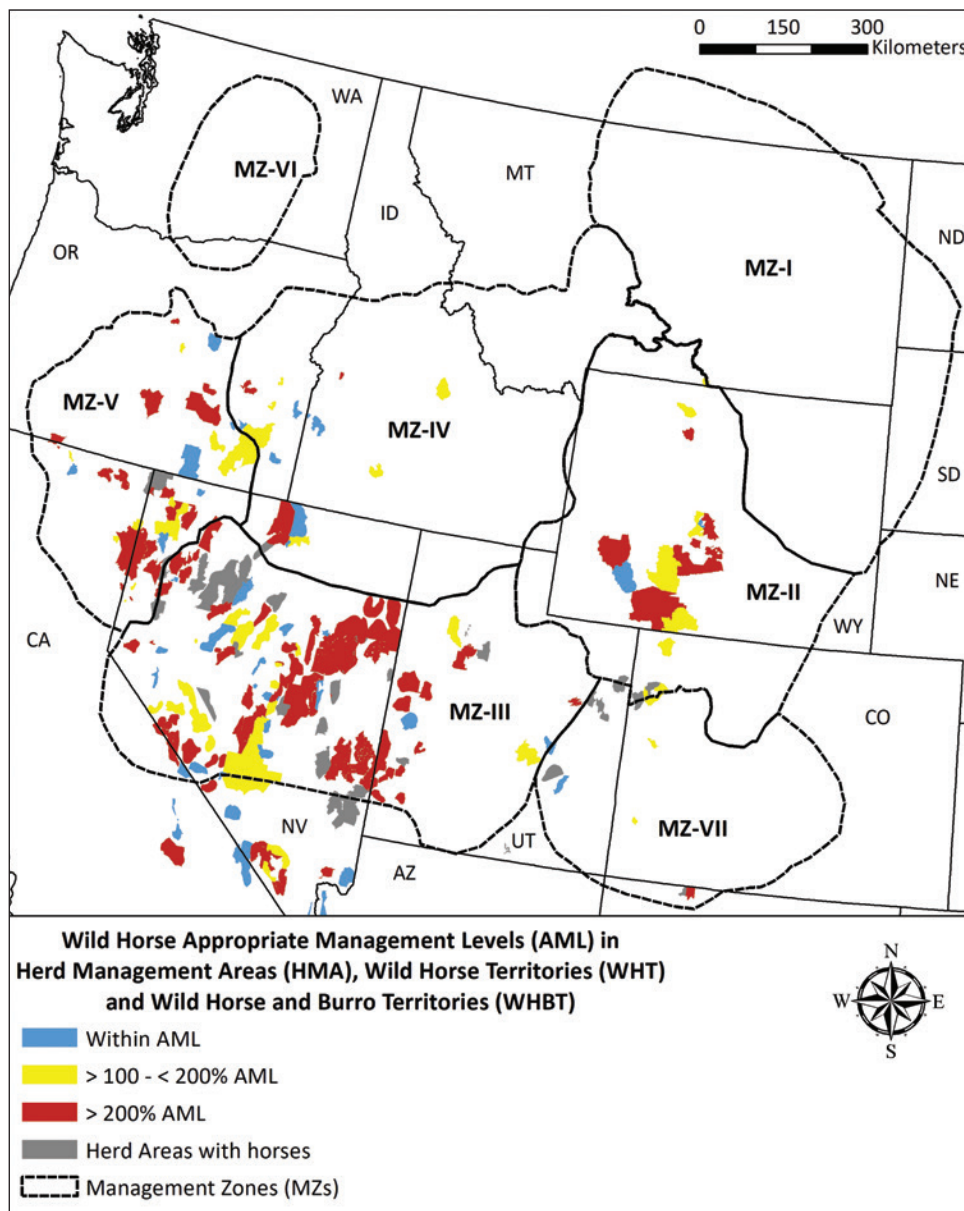


Figure 8.1—Categories of estimated wild horse abundance as of March 1, 2017 relative to Appropriate Management Level (AML) for wild horse Herd Management Areas (HMAs) on BLM lands and Wild Horse Territories (WHTs) and Wild Horse and Burro Territories (WHBTs) on Forest Service lands. Gray polygons indicate Herd Areas where the target population for wild horses is zero, but where wild horses are present. Estimated wild horse abundance exceeds AML in most HMAs, WHTs, and WHBTs.

Analyses of Appropriate Management Levels, Ecosystem Resilience and Resistance, and Breeding Bird Habitat Probabilities

Sixty percent of HMAs, WHTs, and WHBTs managed by the BLM and Forest Service are in areas categorized as having low resilience and resistance (fig. 8.2, table 8.1). In contrast, 33 percent have moderate resilience and resistance and only 7 percent have high resilience and resistance. In the area with low resilience and resistance, 60 percent has wild horse abundance that exceeds 200 percent of the horse AML.

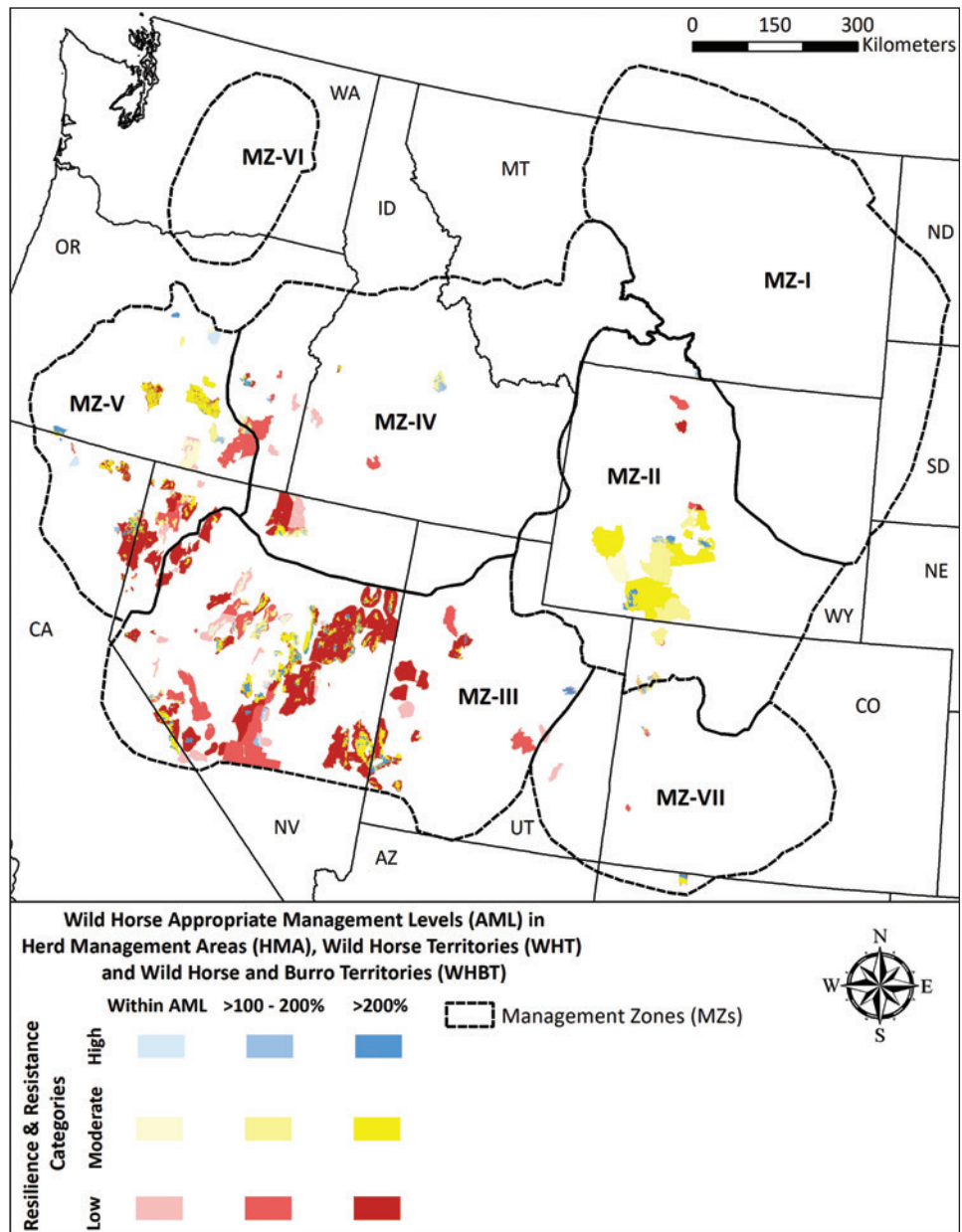


Figure 8.2—Categories of estimated wild horse abundance as of March 1, 2017 relative to Appropriate Management Level (AML), overlaid with the resilience and resistance classes within wild horse Herd Management Areas (HMAs) on BLM lands and Wild Horse Territories (WHTs) and Wild Horse and Burro Territories (WHBTs) on Forest Service lands. Most HMAs, WHTs, and WHBTs are in low to moderate resilience and resistance categories and exceed AML.

Table 8.1—The area and percentage of Herd Management Areas, Wild Horse Territories, and Wild Horse and Burro Territories for the Bureau of Land Management and Forest Service by wild horse Appropriate Management Level (AML) class and resilience and resistance class. Percentages within a Management Zone (MZ) add to 100.

Percent Horse AML class	Resilience and resistance					
	Low		Moderate		High	
	Acres	%	Acres	%	Acres	%
MZ I						
<100	0	0	0	0	0	0
>100–200	0	0	4,326	57	3,200	43
>200	0	0	0	0	0	0
Total	0	0	4,326	57	3,200	43
MZ II						
<100	0	0	414,831	8	2,204	1
>100–200	182,045	4	1,578,883	31	68,236	1
>200	108,086	2	2,548,764	50	166,862	3
Total	290,131	6	4,542,478	89	237,302	5
MZ III						
<100	1,161,465	8	233,713	2	146,235	1
>100–200	2,965,677	19	368,132	2	168,363	1
>200	7,916,216	52	1,743,470	11	618,498	4
Total	12,043,358	79	2,345,315	15	933,096	6
MZ IV						
<100	560,601	27	67,981	3	19,771	1
>100–200	490,895	23	198,977	9	89,076	4
>200	560,706	27	90,401	4	49,144	2
Total	1,612,201	77	357,359	16	157,991	7
MZ V						
<100	193,058	4	426,958	8	186,252	4
>100–200	942,681	18	336,100	6	85,331	2
>200	1,618,840	31	1,119,312	22	276,522	5
Total	2,754,579		1,882,370		548,105	
MZ VII						
<100	130,987	38	0	0	0	0
>100–200	47,132	13	64,758	19	29,502	8
>200	8,427	2	40,236	12	27,286	8
Total	186,546	53	104,994	31	56,788	16
All MZs						
<100	2,046,111	7	1,143,483	4	354,462	1
>100–200	4,628,430	17	2,551,176	9	443,708	2
>200	10,212,274	36	5,542,187	20	1,138,311	4
Total	16,886,815	60	9,236,846	33	1,936,481	7

Differences in both resilience and resistance and the abundance categories exist among Management Zones for wild horses (fig. 8.2, table 8.1). In Management Zone III, where the majority of wild horses are found, lands managed for wild horses are primarily within low resilience and resistance areas (79%). In the area with low resilience and resistance, 52 percent has wild horse abundance in excess of 200 percent of the horse AML. In Management Zones IV and V, lands managed for wild horses also are primarily within low resilience and resistance areas: 77 percent and 53 percent, respectively. In both of these areas, most lands managed for wild horses have horse abundance greater than 100 to 200 percent of the horse AML.

For wild burro populations, most of the land area in HMAs, WBTs, and WHBTs included in this analysis is in low resilience and resistance areas (80 percent), followed by moderate resilience and resistance areas (18 percent) (fig. 8.3, table 8.2). Moreover, 73 percent of the lands managed for wild burros in this analysis have wild burro abundance in excess of 200 percent of the burro AML.

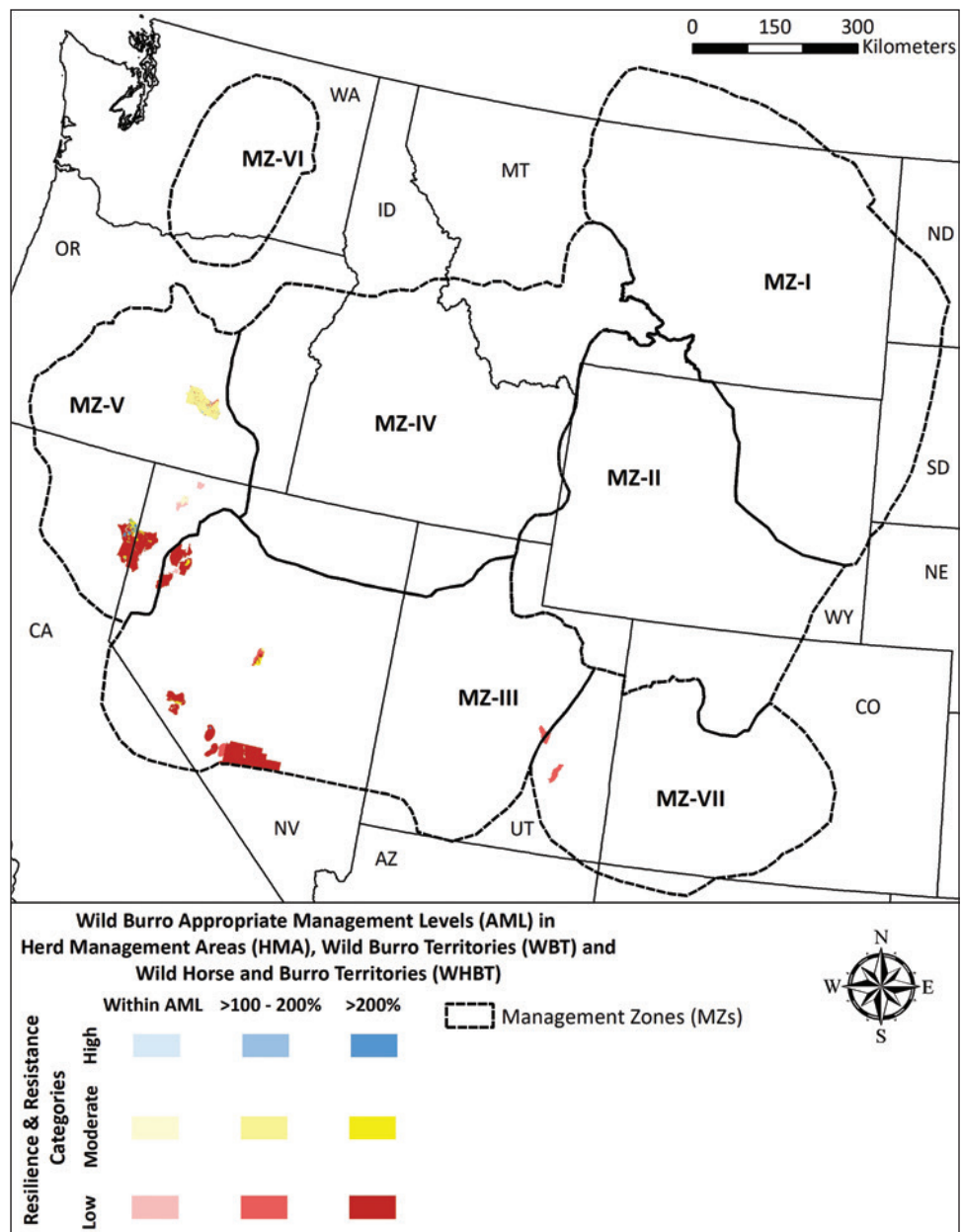


Figure 8.3—Categories of estimated wild burro abundance as of March 1, 2017 relative to Appropriate Management Level (AML), overlaid with the resilience and resistance classes within wild burro Herd Management Areas (HMAs) on BLM lands and Wild Burro Territories (WBTs) and Wild Horse and Burro Territories (WHBTs) on Forest Service lands. Estimated wild burro abundance exceeds AML in most HMAs, WHTs, and WHBTs.

Table 8.2—The area and percentage of Herd Management Areas, Wild Burro Territories, and Wild Horse and Burro Territories for the Bureau of Land Management and Forest Service by wild burro Appropriate Management Level (AML) class and resilience and resistance class. Percentages within a Management Zone (MZ) add to 100.

Percent Burro AML class	Resilience and resistance					
	Low		Moderate		High	
	Acres	%	Acres	%	Acres	%
MZ III						
<100	18,063	1	0	0	0	0
>100–200	162,160	8	9,563	1	0	0
>200	1,655,499	87	59,095	3	4,076	0
Total	1,835,722	96	68,658	4	4,076	0
MZ V						
<100	77,478	5	44,492	3	0	0
>100–200	30,008	2	442,165	29	20,651	1
>200	795,307	52	80,589	5	51,215	3
Total	902,793	59	567,246	37	71,865	4
MZ VII						
<100	0	0	0	0	0	0
>100–200	130,987	100	0	0	0	0
>200	0	0	0	0	0	0
Total	130,987	100	0	0	0	0
All MZs						
<100	95,541	3	44,492	1	0	0
>100–200	323,155	9	451,728	13	20,651	1
>200	2,450,806	68	108,351	4	55,290	1
Total	2,869,502	80	635,940	18	75,941	2

In Management Zones III and V the highest percentage of land is in low resilience and resistance areas with wild burro abundance more than 200 percent of the burro AML. Most of the burros managed by the BLM are located in Arizona and southern Nevada (USDOI 2017), which is outside of the sagebrush biome and the area of this analysis.

Overlaying the categories of wild horse abundance relative to AMLs with the sage-grouse breeding habitat probabilities shows that 42 percent of the lands managed for wild horses occur in the low, 40 percent in the moderate, and 18 percent in the high GRSG breeding habitat probability (fig. 8.4, table 8.3). In the high breeding habitat probability areas, which are the highest priority for protection, and in the moderate breeding habitat probability areas, which often provide opportunities for conservation actions, about two-thirds of the lands managed for wild horses have horse abundance in excess of 200 percent of the horse AML.

Analysis of the sage-grouse breeding habitat probabilities overlaid on categories of wild burro abundance relative to AML shows that 46 percent, 46 percent, and 8 percent of those GRSG breeding habitats managed for wild burros and included in this analysis occur in the low, moderate, and high breeding habitat probability areas, respectively (table 8.4). Within low, moderate, and high GRSG breeding habitat probability areas, 69 percent, 72 percent, and 38 percent, respectively, of the lands managed for wild burros have burro abundance greater than 200 percent of the burro AML. Management Zone V has a higher land area managed for wild burros with GRSG breeding habitat than Management Zone III, and a higher percentage of the wild burro population is in moderate and high GRSG breeding habitat probability areas.

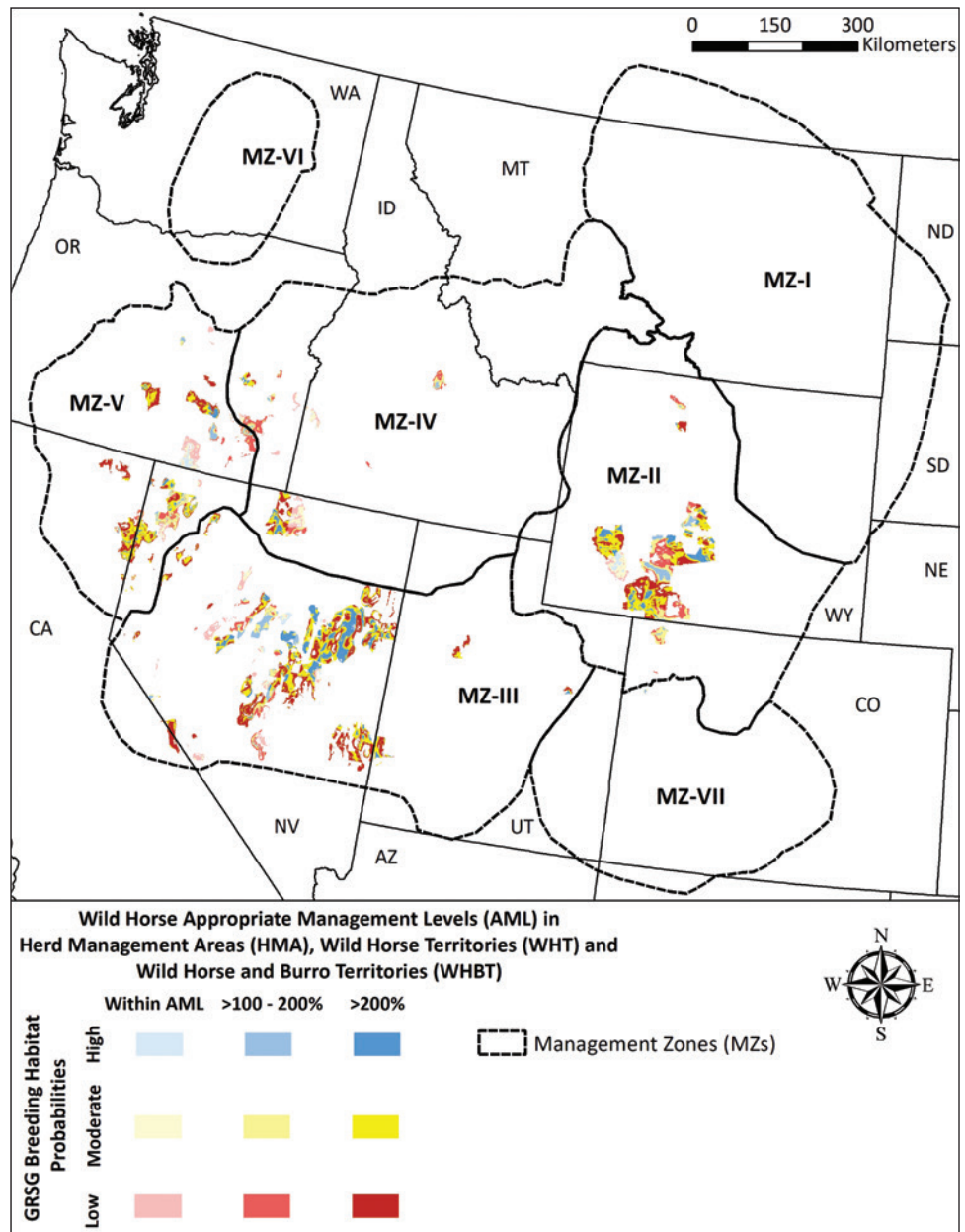


Figure 8.4—Categories of estimated wild horse abundance as of March 1, 2017 relative to Appropriate Management Level (AML), overlaid with the GRSG breeding habitat probabilities within wild horse Herd Management Areas (HMAs) on BLM lands and Wild Horse Territories (WHT) and Wild Horse and Burro Territories (WHBTs) on Forest Service lands. Estimated wild horse abundance exceeds AML in many areas with moderate to high GRSG breeding habitat probabilities.

Table 8.3—The area and percentage of Herd Management Areas, Wild Horse Territories, and Wild Horse and Burro Territories for the Bureau of Land Management and Forest Service by wild horse Appropriate Management Level (AML) class and Greater sage-grouse (GRSG) breeding habitat probability class. Percentages within a Management Zone (MZ) add to 100.

Percent Horse AML class	GRSG breeding habitat probability					
	Low		Moderate		High	
	Acres	%	Acres	%	Acres	%
MZ II						
<100	92,230	2	198,329	4	77,042	2
>100–200	573,836	13	557,183	13	255,275	6
>200	924,545	21	1,298,137	29	462,370	10
Total	1,590,610	36	2,053,649	46	794,686	18
MZ III						
<100	353,147	5	148,052	2	85,319	1
>100–200	312,594	4	319,359	5	273,905	4
>200	2,319,075	33	2,028,561	29	1,185,258	17
Total	2,984,816	42	2,495,972	36	1,544,482	22
MZ IV						
<100	234,091	16	208,371	14	10,955	1
>100–200	293,756	20	160,647	11	33,053	2
>200	212,954	14	224,679	15	95,330	7
Total	740,802	50	593,697	40	139,338	10
MZ V						
<100	281,312	9	161,838	5	94,638	3
>100–200	334,833	10	320,755	10	142,127	4
>200	867,460	27	832,302	26	178,115	6
Total	1,483,605	46	1,314,895	41	414,880	13
MZ VII						
<100	0	0	0	0	0	0
>100–200	252	3	2,494	29	5,748	68
>200	0	0	0	0	0	0
Total	252	3	2,494	29	5,748	68
All MZs						
<100	960,780	6	716,590	4	267,954	2
>100–200	1,515,271	9	1,360,438	8	710,108	4
>200	4,324,034	27	4,383,679	27	1,921,073	12
Total	6,800,085	42	6,460,707	40	2,899,135	18

Using the Science Framework to Inform Management Decisions

Primary considerations for wild horse and burro management from the Science Framework approach are presented next (see tables 1.3, 1.4).

- In general, areas that support medium to high sage-grouse breeding habitat probabilities or other important resources are high priorities for management (table 1.3: cells 2A, 2B, 2C, 3A, 3B, 3C), especially low resilience and resistance categories that lack the potential to recover from disturbances such as excessive wild horse and burro use without significant intervention (table 1.3: cells 2C, 3C). These areas could be considered priorities for wild horse and burro gathers and fertility control where horse and burro abundance exceeds target AMLs and the area is not highly degraded.
- Areas with moderate and, especially, high resilience and resistance often have the potential to recover through successional processes (table 1.3: cells 1B, 1C, 2B, 2C).

Table 8.4—The area and percentage of Herd Management Areas, Wild Burro Territories, and Wild Horse and Burro Territories for the Bureau of Land Management and Forest Service by wild burro Appropriate Management Level (AML) class and Greater sage-grouse (GRSG) breeding habitat probability class. Percentages within a Management Zone (MZ) add to 100.

Percent Burro AML class	GRSG breeding habitat probability					
	Low		Moderate		High	
	Acres	%	Acres	%	Acres	%
MZ III						
<100	107	1	0	0	0	0
>100–200	9,882	3	12,082	4	8,717	3
>200	168,963	58	86,373	30	2,943	1
Total	178,952	62	98,455	34	11,660	4
MZ V						
<100	23,217	2	68,662	7	18,022	2
>100–200	147,908	14	91,557	8	50,412	5
>200	263,516	24	364,745	34	44,423	4
Total	434,640	40	524,964	49	112,857	11
All MZs						
<100	23,217	2	68,662	5	18,022	1
>100–200	157,790	12	103,638	8	59,130	4
>200	432,479	32	451,118	33	47,366	3
Total	613,486	46	623,418	46	124,518	8

- These areas represent significant opportunities to improve habitat and could also be considered priorities for wild horse and burro gathers and fertility control where horse and burro abundance exceeds target AMLs and removals are likely to result in habitat improvement.
- In areas where wild horses and burros exceed target AMLs (including occupied areas outside of HMAs, HAs, WHTs, WBTs, and WHBTs), managers should carefully consider the current spatial extent and growth potential of any nearby wild horse herds and their potential effects on management actions to improve habitat.
- New postfire rehabilitation areas and areas that provide sagebrush habitat connectivity for GRSG and other species at risk are conservation priorities and, thus, could be priorities for wild horse and burro gathers, where abundance exceeds AMLs.

Ecological type or ecological site descriptions and their associated state-and-transition models (STMs) can be used to help evaluate potential effects of wild horse and burro use and the likely success of conservation and restoration actions. In the Science Framework, generalized ecological types and STMs have been developed for the range of environmental conditions in the eastern and western portions of the sagebrush biome (see Part 1, Appendices 5 and 6). The ecological types and STMs are characterized according to their resilience to disturbance and resistance to invasive annual grasses based on soil temperature and moisture regimes and other biophysical characteristics such as plant community composition. They provide information on the alternative states, ranges of variability within states, and processes that cause plant community shifts within states as well as transitions among states. These ecological types and STMs can be used to: (1) identify the different ecological types that exist within the HMA or Territory and determine their relative resilience to disturbance and resistance to invasive annual grasses; (2) evaluate the current ecological dynamics of the

ecological types or ecological sites and, where possible, their restoration pathways; (3) increase understanding of the potential effects of wild horse and burro use; and (4) determine the likelihood of conservation and restoration actions succeeding given ongoing wild horse and burro use (Part 1, section 9).

Section 7 uses these STMs to illustrate potential livestock management strategies for ecological types that support GRS populations and that may benefit from improved livestock grazing management. Information on how to use these resilience-based ecological types and STMs for managing ecosystem threats across the sagebrush biome is in Part 1, section 9.2. Information on how to use resilience-based ecological types and STMs for selecting appropriate treatments for assessing postwildfire recovery and restoration decisions in sagebrush and juniper-piñon ecosystems in the Great Basin is in Miller et al. (2014, 2015) and Pyke et al. (2017), respectively.

Management Considerations at the Project Scale

An assessment of the ecological sites in the project area and their relative resilience to disturbance and resistance to invasive annual grasses can help determine the potential for conservation and restoration treatments to succeed. More detailed information can be obtained from ecological site descriptions for those areas where they have been developed (see <http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/technical/ecoscience/desc/>). Ecological type and ecological site descriptions provide basic information on the climate and soil characteristics of an area and the potential of the area to support a dynamic set of plant communities. The associated STMs provide information on the current states and the potential transitions among them due to disturbances and other drivers such as wild horse and burro use as well as management treatments. Assessing the states and the plant communities within the states based on STMs provides information on both the disturbances and the drivers that have led to the current state and the potential restoration pathways. For example, plant communities within the reference state or within states that have feasible restoration pathways may respond favorably to conservation and restoration actions if the wild horse population can be managed at or below AML. However, plant communities in other states, such as an invaded state or annual state (see figs. 7.2, 7.6) may not respond favorably to conservation and restoration actions if the wild horse population cannot be managed at or below AML. Ecological types or ecological sites with relatively low resilience and resistance to invasive annual grasses often require more than one intervention for restoration efforts to succeed and wild horse and burro use can have significant effects on project success.

Effects of wild horses and burros on project success depend on the number of wild horses and burros that can reach the site. If the project site is located within an HMA, WHT, WBT, or WHBT, then grazing and trampling pressure from wild horses should be expected in most cases. Even if the project area is outside any HMA, WHT, WBT, and WHBT, managers should carefully consider the current spatial extent, and growth potential, of any nearby wild horse population. Higher population sizes tend to lead to an expanded spatial area used by the wild horse population. If the number of wild horses is at AML, and there are measures in place to limit the population's growth rate, then wild horse use across the landscape may be distributed enough that a conservation or restoration project could achieve habitat quality goals. Thus, managers should carefully evaluate the likelihood of success of planned conservation and restoration activities if a local or adjacent wild horse population cannot be kept at AML.

Project success is also likely to be influenced by distance to the nearest drinking water source for wild horses. The greater the distance, the lower the grazing pressure that can be expected. Horses require access to large amounts of water; an individual can drink an average of 7.4 gallons [28.0 liters] of water per day (Groenendyk et al. 1988). Despite a general preference for habitats near water (e.g., Crane et al. 1997), wild horses will routinely commute long distances (e.g., 10+ miles [16 kilometers] per day) between water sources and palatable vegetation (Hampson et al. 2010). Managers should expect that any restoration project less than 5 miles [8 kilometers] from water will be subject to use by wild horses in the area. Riparian and wildlife habitat improvement projects that intend to increase the availability of grasses, forbs, riparian habitats, and water are likely to attract and be subject to heavy grazing and trampling by wild horses that live near the project.

Managers need to understand and consider the potential effects of wild horses and burros on conservation and restoration projects and plan accordingly. For certain habitat restoration projects, managers may want to consider installing fencing to discourage use by wild horses, particularly around riparian areas. On BLM and Forest Service lands, temporary fencing for habitat rehabilitation is generally acceptable on HMAs, HAs, WHTs, WBTs, and WHBTs. But permanent fencing often requires a more in-depth environmental assessment or land use plan revision, and should be designed in a way that allows for wild horse and burro movement throughout the rest of the HMA or Territory. The Forest Service also requires National Environmental Policy Act analysis for fence installation. Fencing that excludes wild horses and burros from riparian areas or water development projects that are designed to disperse both riparian and upland use by wild horses and burros are important management tools to protect riparian habitat. Fencing riparian areas to exclude wild horses and burros is generally acceptable as long as water from the area continues to be available to them, and solid pipe fencing is used that can withstand pressure from wild horses and burros. Continued monitoring to assess changes in plant communities and wild horse and burro abundance should be part of any conservation or restoration project where these animals are found.

If AML cannot be achieved, it may be more reasonable to forego a habitat restoration project entirely instead of spending time and resources on projects with a low probability of success. Managers deciding about any project that is near a wild horse or burro population should consider population sizes of wild horses and burros relative to the AML, including explicit schedules for wild horse and burro removals or population growth suppression treatments that are adequate to limit population growth. Unfortunately, high populations of wild horses or burros can substantially affect the ability of land managers to implement conservation measures in some areas. A potential project area with high current wild horse or burro population sizes may become suitable for restoration if the manager can influence priorities and policies such that wild horse and burro populations in the project area are reduced to and maintained at or below high AML.

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Chronic intermittent colic in a mare attributed to uterine marbles

By: [Freeman, CE](#) (Freeman, C. E.)^[1]; [Lyle, SK](#) (Lyle, S. K.)^[1]

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Abstract

A 13-year-old Quarter Horse mare presented for evaluation of chronic intermittent colic. Following extensive diagnostics, abdominal radiographs revealed two round, radiopaque objects in the caudal abdomen. Palpation per rectum and transrectal ultrasonography of the reproductive tract confirmed that the round objects were uterine marbles. Dinoprost tromethamine (Lutalyse, 5mg i.m. q. 24h for 2days) was administered to bring the mare into oestrus, and both uterine marbles were manually removed from the uterus following digital dilation of the relaxed cervix. Follow-up with the owner 12months after discharge revealed that the mare had shown no further signs of abdominal discomfort since having the uterine marbles removed. To the authors' knowledge, this is the first published report of chronic intermittent colic attributed to uterine marbles in a mare.

Keywords

Author Keywords: [horse](#); [intermittent colic](#); [uterine marble](#); [oestrus suppression](#); [abdominal radiograph](#)**KeyWords Plus:** [INTRAUTERINE-DEVICE](#); [ESTROUS-CYCLE](#); [PONY MARES](#); [GNRH](#); [SUPPRESSION](#); [OXYTOCIN](#); [DIESTRUS](#); [ESTRUS](#); [BEHAVIOR](#); [HORSES](#)

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Original Research

Self-Assembling Intrauterine Device (Upod) Modulation of the Reproductive Cycle in Mares

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ABSTRACT

The present study evaluated a novel intrauterine device for its effect on estrus suppression. The self-assembling intrauterine device (Upod) consists of three 12 mm × 26 mm elliptically shaped units each with a magnetic core and coated with a polymer (total weight 22.5 g). Each magnetic unit is inserted independently from the other. Once inside the uterus, these magnetic elliptical units self-assemble and adapt the lower energy “ring” conformation. In mares, the devices can be inserted at any stage of the estrous cycle without the need for multiple exams. Shatter-proof grade material is used to assure safety for long-term use. The Upod is inserted and retrieved using an applicator and magnetic retriever. In experiment 1, Upods were inserted postovulation in 15 mares during estrus. The mean duration of diestrus was 73.4 ± 36.6 days (mean $\pm$ S.D.). Endometrial biopsies showed no significant differences before and after Upod placement. In experiment 2, Upods were inserted regardless of the stage of the estrous cycle in 14 mares. The mean duration of diestrus was 51.3 ± 22.7 days (mean $\pm$ S.D.). The presence of the Upod was monitored by transrectal ultrasound and an external handheld metal detector. Progesterone was assayed to monitor corpus luteum lifespan, and the mean duration of diestrus was significantly extended in both experiments compared to the control group. Eight mares were randomly selected to be bred the following year, with 100% conception rate post-Upod retrieval.

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1. Introduction

Mares in estrus can behave in ways that can interfere with handling and competition. Current methods to suppress estrus behavior include pharmacological treatments, glass marbles, and negative reinforcement strategies.

The use of pharmacological therapies, a common strategy, is to administer synthetic progesterone compounds to horses on a daily basis [1]. Different methods of application include intramuscular (IM) administration of exogenous progesterone [2] or oral administration of the synthetic progestin altrenogest (Merck, Madison, NJ, 07940) [3]. The U.S. Food and Drug Administration recently

highlighted the potential health risks, including reproductive system disorders and other adverse effects, in people who are exposed to altrenogest through contact with skin or product residue on barn surfaces, equipment, or treated animals [4]. The administration of exogenous oxytocin during diestrus can disrupt luteolysis; however, it requires IM administration every 12 hours [5]. GnRH vaccines, antagonists, or agonists with highly variable individual responses and irreversibility have also been used [6].

Another pharmacological therapy includes injections of medroxyprogesterone acetate [7]; however, this drug is considered a controlled medication and has been prohibited in Federation Equestre Internationale competition since 2011.

All these treatments require frequent administration and generally only result in a short-term effect. Therefore, the use of these pharmacological therapies for behavioral control is limited.

Another strategy is the use of 25 mm or 35 mm glass marbles placed in the uterus of mares to extend the functional lifespan of the corpus luteum [8]. The 25-mm glass ball was spontaneously expelled from the uterus of 6 of 12 mares (50%), whereas the 35-mm glass ball had 100% retention rate. However, an extended mean luteal function of 87 days (range 76–109 days) occurred in

Animal welfare/ethical statement: All experimental procedures were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee.

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only 7 of 62 diestrus periods (11%) among mares following glass ball placement. When mares were bred in the season subsequent to glass ball removal, 17 of 23 (74%) conceived. Complications have been reported with the use of glass marbles, including chronic endometritis, pyometra, and glass shards becoming adhered to the endometrium [9]. Morris et al. described several cases involving fragmentation of marbles in the uterine lumen [10].

This raises the question: Do the control mechanisms of an embryo and a self-assembling intrauterine device (Upod) differ on luteal function? Estrus suppression using Upods extend luteal function, but the biological mechanism is unknown. A luminal inflammatory response in the uterus without observable changes in the endometrium could somehow be involved. It is generally accepted that intrauterine devices (IUDs) produce a local, sterile, inflammatory reaction [11,12]. The Upod releases no chemical compounds but may exert pressure on the uterine walls by physical contact. Embryo mobility is due to uterine contractions that move them in the uterus [13], and unrestricted motility is a prerequisite for pregnancy maintenance [14]. However, IUD movement in the uterus is limited [15], and thus local influence directly on the endometrium is most likely. For example, after mechanical stress, uteroferrin [16] may be involved in luteostasis in the mare and be reflective of chronic inflammation.

Important factors considered in the development of the Upod were reversibility and absence of any lasting detrimental effects on fertility as any mare currently engaged in a sporting career may be required to breed. The parameters set were as follows: (1) efficacy; (2) safe to be used in mares; (3) minimal distortion of the uterine lumen; (4) high retention rate in the uterus; (5) easy for the veterinarian to insert through the cervix with or without an applicator; (6) horse owner ability to monitor if the device is present by noninvasive means (readily available handheld metal detectors); (7) easily visualized by ultrasound; (8) and easy to retrieve.

The purpose of this study was to develop and test an Upod that would suppress the expression of estrus in mares by extending the lifespan of corpora lutea. Specifically, the study's objectives were to evaluate (1) Upod retention rates, (2) the effect of Upods in suppressing the frequency of estrus, (3) uterine integrity following Upod removal, and (4) fertility post-Upod retrieval.

2. Materials and Methods

All experimental procedures were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee.

2.1. Animals

Thirty-eight mares (Upod treatment $n = 38$) of mixed light horse breeds, aged 3–20 years, with no history of reproductive failure, clinically normal and nonpregnant, were used over a five-year period (2013–2018) for the development and testing of the novel Upod device. The mean $\pm$ standard deviation (SD) of age at time of Upod insertion was 11.5 ± 7.1 years. Seven mares (control $n = 7$) were also randomly chosen to serve as controls for the study. The mean $\pm$ SD of age for the control mares was 8.6 ± 5.9 years (range 3–17 years old). Eight mares (fertility $n = 8$) were randomly selected to be bred post-Upod retrieval. The mean $\pm$ SD of age at time of breeding was 11.1 ± 6.2 years (range 4–20 year old).

All mares were housed and cared for at the University of Massachusetts Hadley Farm. Mares were fed free-choice forage (mixed grass hay and pasture), a ration balancer, and had 24/7 access to water and a plain white salt block. The majority of mares lived outside in small herds with access to a three-sided shelter; five of

the mares were individually stalled overnight and received a minimum of 6 hours of daily turnout.

2.2. Intrauterine Device

The intrauterine device used was a frameless, self-assembling, sterilized device (Upod; 12 mm $\times$ 26 mm) made of shatter-proof inert FDA grade material with a mean total weight of 22.5 g (Fig. 1). For comparison, a 35-mm glass marble weighs 53 g.

Three dimensional printing was used to design an ergonomic, magnetic-based Upod that can be adapted to several mammalian species. The Upod consists of three magnetic elliptically shaped units each with a magnetic core coated with a polymer, inserted independently from each other. In mares, the device can be inserted at any stage of the estrous cycle without the need for multiple exams or repeated darting in the case of wild equids. Once inside the uterus, these magnetic elliptical units will self-assemble and adapt the lower energy triad conformation.

The ability of the device to self-assemble after insertion remain in a stable configuration and then disassemble for removal is governed by magnetic forces and required a few physical design considerations that were tested and optimized (Figs. 1 and 2). The “ring” shape formed by the three magnets is a singularly unique configuration that minimizes the magnetic energy of the system. In physics terminology, the system does whatever necessary to achieve a complete “circuit” of magnetic flux closure, ultimately leading to the “ring” shape. The shape of the package material surrounding the magnet provides an additional constraint that modifies this stability. Minimizing uterine lumen (cavity) deformity may be important to reduce device expulsion/rejection.

The field stability is aided by the ring geometry, in that, even if one has a somewhat lower coercivity material, there is a strong energetic tendency to stay in a ring formation. Thus, the magnetic field strength is stable, especially once it is in the ring configuration.

Stability and on-demand disassembly were investigated and optimized using computer modeling and experimental prototypes. Package prototypes of different shapes were made by 3D printing. Package shape was found to also impact biocompatibility, as rounded surfaces that allow for small fluctuations in ring configuration helped to keep the surfaces free of biofilm formation (ongoing research). Because of its biocompatibility, Teflon (PTFE) was identified as a preferred package material, and next generation designs will include either gold-copper 2^+ bands or progesterin eluting polymers.

2.3. Insertion and Retrieval of Upod

Upods for mares in estrus (or within 48 hours postovulation) can be inserted using an Upod applicator with the 3 units loaded in tandem or alternatively can be manually inserted. The 3 units are pushed through the cervix until just clear of the internal cervical os.

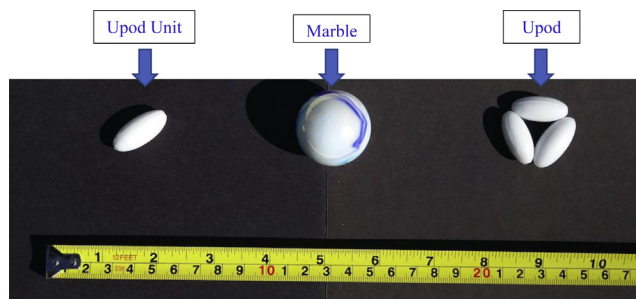


Fig. 1. Upod (7.5 g each = 22.5 g total); 35 mm glass marble (53 g).

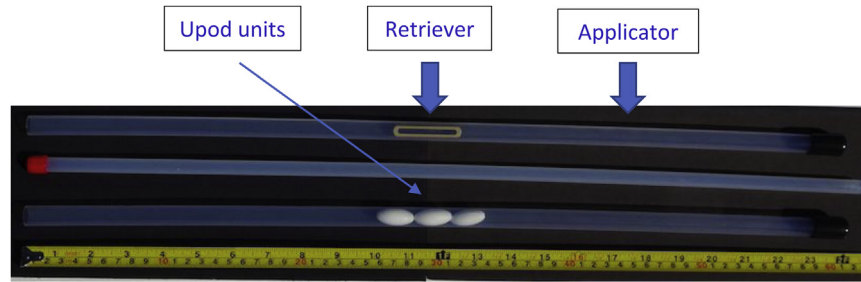


Fig. 2. Upod magnetic retriever and applicator.

The location of the devices should be checked with ultrasound. The Upod will self-aggregate in the uterus in a “ring” configuration (please see [video 1. Upod Insertion Horse Model - Supplementary material](#)). For mares with a closed cervix (e.g., early estrus, diestrus, maiden mares), insert individual units by hand. Place the 3 units of the Upod in one's hand and push them through the cervix one by one.

To retrieve the Upods, induce estrus with 250 µg cloprostenol (Estrumate; Merck Animal Health, Millsboro, DE, 19966-4217) and retrieve the Upod in mid-estrus using the specially designed magnetic retriever with the applicator. The retriever is attached to a fluorocarbon line. Insert the applicator loaded with the retriever through the cervix and push the retriever into the uterus, making sure the retriever drops into the uterus, or sweep the uterus with the applicator and retriever until contact with the Upod. One can either (1) keep the applicator in the cervix as one pulls the tag line and feels the device with the index finger at the end of the applicator, or (2) withdraw the applicator, leaving and holding on to the tag line, before pulling and feeling the weight of the Upod attached to the retriever (please see [video 2. Upod Retriever Horse Model - Supplementary material](#)).

2.4. Experimental Design

Mares were examined at the time of Upod insertion by transrectal palpation and ultrasonography to rule out pregnancy, presence of uterine fluid or other pathologies (e.g., urine pooling, granuloma cell tumor). At the time of Upod insertion, the perineal area was scrubbed with 7.5% povidone-iodine, and mares were sedated with 0.02–0.04 mg/kg IV detomidine hydrochloride (Dormosedan HCL; Zoetis US, Parsippany, NJ, 07057) (please see [video 3. Upod Placement - Supplementary material](#)). After insertion of the Upod, 3.1 g of disodium/clavulanate potassium (Absolute Veterinary Compounding Pharmacy Weatherford, TX, 76086-4779) diluted in 10 mL of sterile water was introduced into the uterus (please see [video 4. Intrauterine Infusion - Supplementary material](#)). Mares were examined by transrectal ultrasonography within 5 minutes postinsertion (please see [video 5. Upod Ultrasound Detection - Supplementary material](#)) and then weekly (Fig. 3). The number and size of follicles, the corpora lutea, and the presence of intra-uterine fluid were assessed with ultrasonography and recorded. Transabdominal detection of the Upod, using a handheld magnetic detector wand, was also performed weekly for the first month and then biweekly.

2.4.1. Pilot Study

Phase one of the study consisted of testing several prototypes of the novel intrauterine device on nine mares ($n = 9$) over a two-year period (2013–2015). Specifically, the techniques for insertion, visualization, transabdominal detection, and retrieval of the device were tested. Estrus was induced with an IM injection of 250 µg

cloprostenol (Estrumate), and the Upod was pushed through the cervix into the uterus 48–72 hours postovulation, using the double-glove technique [17]. Mares also received an IM injection of 250 µg cloprostenol (Estrumate) to induce estrus to facilitate the removal of the Upod.

2.4.2. Experiment 1

Phase two of the study consisted of testing the refined Upod design on 15 mares ($n = 15$) during the 2016 breeding season. Specifically, retention of the Upod and effect on uterine health were investigated. Upods were inserted and retrieved as described previously in the pilot study. The device was retrieved after 3 months (4 mares), 5 months (2 mares), 13 months (3 mares), 14 months (4 mares), 17 months (1 mare), or 18 months (1 mare). Serum samples were collected for progesterone assay at the time of Upod insertion to confirm the mares were in estrus and then assayed every 2 weeks to monitor corpus luteum function. Transabdominal detection was also performed at each blood draw. Samples for uterine biopsies were taken before Upod insertion and at the time of Upod retrieval. Samples were taken during estrus based on edema and vascular congestion. A postinsertion uterine biopsy specimen was obtained from the ventral wall at the base of either horn, ipsilateral to last observed Upod location; if the Upod was located in the uterine body, the biopsy was taken from the base of the right horn.

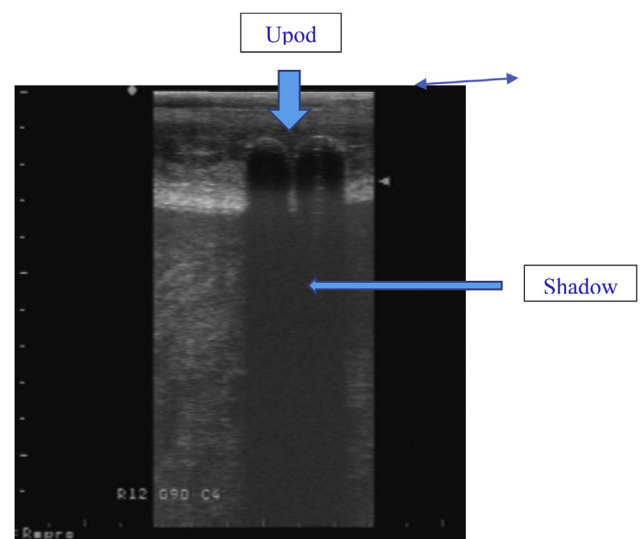


Fig. 3. Ultrasound image of a Upod (down thick arrow) in the uterus; Uterus on top; Bladder at the bottom. Note the absence of fluid in the lumen (cavity) of the uterus, and particularly in the vicinity of the Upod, indicating absence of clinical signs of inflammation. Please notice the shadowing artifact produced by the device (horizontal thin arrow).

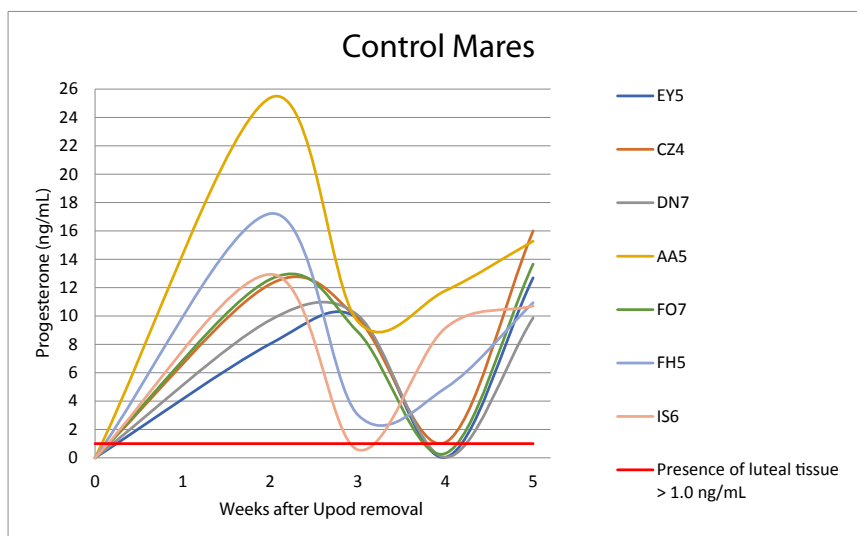


Fig. 4. Progesterone profiles of control mares post-Upod retrieval.

2.4.3. Experiment 2

Phase three of the study consisted of testing the Upod on 14 mares ($n = 14$) during the 2017 breeding season. Mare progesterone profiles were collected on mares with an intrauterine device ($n = 14$) and after the intrauterine device was removed as control data ($n = 7$). Specifically, progesterone was assayed at the time of Upod insertion, then weekly in the first month postinsertion, and then every 2 weeks. Progesterone was assayed weekly from the control mares for an additional five weeks after Upod retrieval. Transabdominal detection was also performed at each blood draw. In addition, the insertion of the device during diestrus was tested. In this phase, mares were not induced into estrus. Upods were inserted late in the breeding season into the uterus of 14 mares, regardless of the stage of the estrous cycle. Four of the mares in this study had nursing foals by their side for the duration of the study (Upods were inserted 3–10 weeks postpartum). The devices were retrieved at 3 months postinsertion (end of September) for 10 mares and at 5 months postinsertion (end of November at time of weaning) for the 4 postpartum mares.

2.4.4. Experiment 3

Phase four of the study consisted of post-Upod fertility testing during the 2018 breeding season. Eight mares ($n = 8$) were randomly assigned to be inseminated with fresh cooled or frozen semen on the subsequent season following Upod retrieval. These mares had carried the devices for at least 3 months before being inseminated, post-Upod removal. Mares were ultrasounded transrectally 14 days postovulation to check for pregnancy.

2.5. Analysis of Blood and Tissue Samples

Serum for progesterone analysis was performed at a reference laboratory using MP Biomedicals Progesterone RIA (Animal Health Diagnostic Center, Ithaca, NY, 14853), without previous knowledge of treatment status. Reference progesterone values were as follows: Absence of active luteal tissue: 0.1–0.5 ng/mL; borderline for the presence of luteal tissue: 0.5–1.0 ng/mL; presence of luteal tissue: > 1.0 ng/mL.

Endometrial biopsies were fixed in 10% neutral-buffered formalin, routinely processed, and stained with hematoxylin and eosin (H&E). Tissue sections were evaluated by a board-certified

veterinary pathologist blinded to treatment history and graded as described by Kenney and Doig [18].

2.6. Statistical Analysis

For analysis of the results, independent samples T-tests were used to detect significant ($P < .05$) differences in mean diestrus durations and interestrus intervals (IEI) between control mares and Upod mares with sustained progesterone levels and persistent diestrus (Upod-P) as estimated by progesterone levels (SPSS; Sta-taCorp. 2017. Stata Statistical Software: Release 15. College Station, TX, 77845). The W/S test for normality ($P > .10$, accepts the null hypothesis that the data were normally distributed) was used to determine normal distribution of the data before statistical analysis; the Kolmogorov–Smirnov test confirmed normal distribution in SPSS.

3. Results

3.1. Control Mares—Duration of Diestrus

Seven mares ($n = 7$) were randomly chosen to serve as controls for the study (Fig. 4). Mean duration of diestrus for control mares was 16.6 ± 7.4 days (mean $\pm$ S.D.).

3.2. Pilot Study

Nine mares ($n = 9$) were used in the pilot study. The techniques for insertion, visualization, transabdominal detection, and retrieval of the device were tested and refined. Stability and on-demand disassembly were investigated and optimized.

Table 1
Average diestrus length and interestrus interval (IEI) in days in study 1 and 2.

Group	Duration of Diestrus (d)	IEI (d)
Control mares ($n = 7$)	16.6 ± 7.4 (7–28)	23.6 ± 7.4 (14–35)
Study 1 ($n = 15$)	73.4 ± 36.6 (20–155) ^a	75.7 ± 36.0 (27–162) ^a
Study 2 ($n = 14$)	51.3 ± 22.7 (21–92) ^a	57.5 ± 23.8 (28–99) ^a

Average diestrus length and interestrus interval (IEI) in days represented as mean $\pm$ SD (range).

Based on serial progesterone concentration value decline to <1 ng/mL indicating absence of luteal tissue. Values in brackets are minimum and maximum values.

^a $P < .05$.

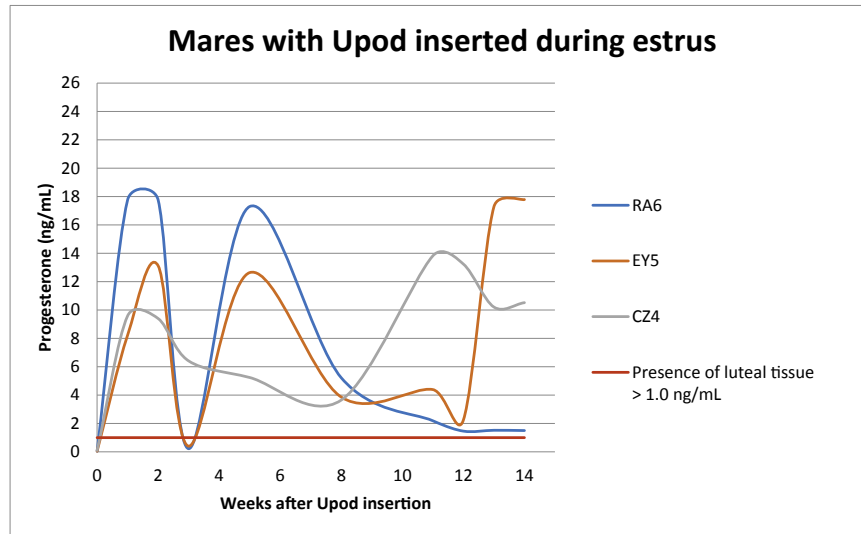


Fig. 5. Progesterone profiles of mares with Upod inserted during estrus.

3.3. Experiment 1—Duration of Diestrus, Retention, and Uterine Biopsies

Mares ($n = 15$) were induced into estrus and had a mean progesterone concentration of 1.7 ± 3.8 ng/mL at time of Upod insertion. Mean duration of extended diestrus for Upod mares was 73.4 ± 36.6 days (mean $\pm$ S.D.), a significant ($P < .05$) difference compared to control group (Table 1). Two mares underwent luteolysis 3 weeks following device insertion (Fig. 5).

Upod retention was 100% and the devices were purposely removed at the end of the breeding season (75–227 days post-insertion). Total Upod retention in this experiment was 2,377 days (equivalent to 113 mare-21 day average cycles).

The results of the histopathological evaluation of the uterine biopsies are shown in Table 2. Before Upod insertion (time 0), 7/15 mares had normal endometrium (Grade I) and 8/15 mares had signs of a mild chronic endometritis (Grade II). After Upod removal (time 1), there were no significant histopathological changes in the grade of the uterine biopsy for all the mares. Changes in inflammation scores in endometrial biopsy specimens obtained at time 0 and time of retrieval were i) increase 4/15 (26.7%); decrease 3/15 (20.0%); no change 8/15 (53.3%). None of the mares showed fluid accumulations in the uterus during diestrus in the cycle preceding Upod insertion. In 2 Upod mares, small accumulation of non-echogenic intrauterine fluid ($\leq 5 \times 5$ mm) that dispersed over time was seen during estrus but not during the extended luteal phase. Uterine edema was observed in Upod mares in estrus in the presence of a preovulatory follicle. No vaginal discharge was observed in any mare carrying Upods or at time of removal.

3.4. Experiment 2—Duration of Diestrus, Retention, and Progesterone Profiles

Mares ($n = 14$) were not induced into estrus and had a mean progesterone concentration of 5.6 ± 5.6 ng/mL at time of Upod insertion. Mean duration of diestrus for Upod mares was 54.6 ± 24.1 days in mares that were in diestrus ($n = 10$) and 42.9 ± 18.9 days for mares in estrus ($n = 2$) or without ovarian activity at time of insertion ($n = 2$). Mean duration of diestrus for all 14 mares was 51.3 ± 22.7 days. A significant ($P < .05$) difference was found compared to control group (Table 1). Two mares underwent luteolysis 1 week following device insertion (Fig. 6).

Upod retention was 100% for the duration of the experiment (98–161 days). Total Upod retention in this experiment was 1,491 days (equivalent to 71 mare-21 day average cycles). Progesterone profiles are shown in Figs. 4–6.

Twelve of the mares (85.7%) experienced extended luteal function, and the average duration of diestrus was 74.1 ± 11.1 days (range 64–92). Nineteen estrous cycles were observed over the 98-day study; 63.2% (12/19) of those cycles had prolonged luteal function of more than 35 days (Table 3).

We observed that Upod-treated mares ≤ 15 years of age had a slightly longer extended luteal function (56.3 ± 15.9 days) than mares > 15 years of age (44.5 ± 29.8 days); however, this difference was not statistically significant ($P = .35$).

3.5. Experiment 3—Fertility

Eight mares (3 maiden and 5 multiparous) were randomly selected to test post-Upod fertility. Eight of the eight mares conceived using artificial insemination with fresh cooled or frozen semen for 100% conception rate. The oldest mare in the group successfully conceived post-Upod retrieval using frozen semen at the age of 20 years.

Table 2
Histopathology category^a of endometrial biopsy specimens in study 1.

Mare ID	Time 0 (Upod Insertion)	Time 1 (Upod Retrieval)
AA5	IIA	I
CZ4	IIA	IIA
EY5	I	IIA
FH5	I	I
IL6	I	IIA
IS6	I	I
JR6	I	I
LI6	I	I
LY5	I	IIA
MA4	IIA	IIA
NE4	IIA	I
NS5	IIA	I
RA6	IIA	IIB
SL5	IIA	IIA
TK6	IIA	IIA

Grade I (normal) or IIA (mild lesions).

^a Kenney/Doig grading. Samples taken at estrus based on edema and vascular congestion.

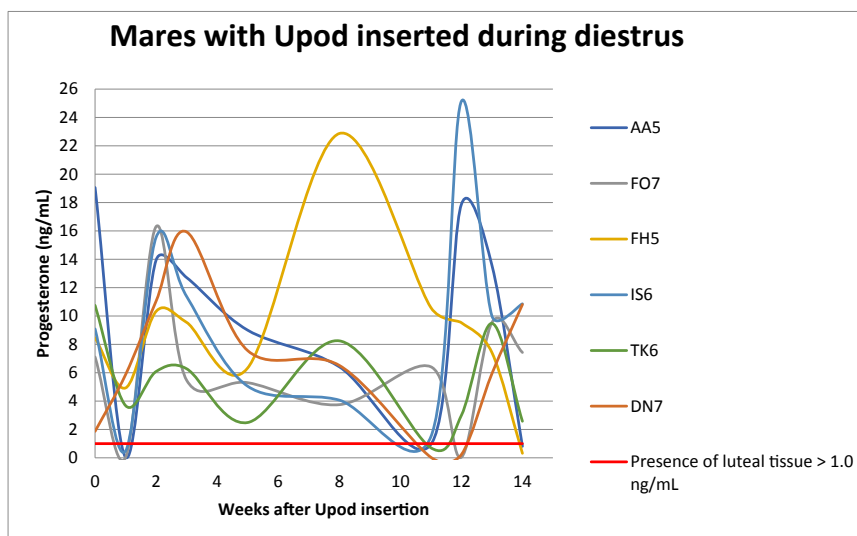


Fig. 6. Progesterone profiles of mares with Upod inserted during diestrus.

4. Discussion

This is a novel device manufactured with inert FDA shatter-proof grade material. The Upod's long-term safety and minimal distortion of the uterine lumen (cavity) decrease the risk for discomfort/pain/inflammation resulting in a high retention rate. Because this device has elastic potential energy, the three vertices will dissipate forces against the uterine walls. This decreases potential trauma as they deform ergonomically and adapt to the uterine cavity's ever-changing conformation. The core of this Upod contains a magnet of high coercivity material. The magnetic field strength will not degrade over time, provided it is made with a sufficiently "hard" (i.e., high coercivity) ferromagnetic material. The detection of the device in the uterus by ultrasound is very easy, particularly in diestrus mares. Another consideration of the design was that a layperson can monitor, by noninvasive means (common handheld metal detectors), if the device is present (please see [video 6. Upod Metal Detection - Supplementary material](#)). Insertion and retrieval of the device can be accomplished quickly, with minimal effort. Disassembly is initiated by introducing a magnetic probe, which disrupts the stability of the ring ([Fig. 2](#)). The initial retrieval prototype included a stainless-steel metal stylet with a magnetic tip with an inverted north pole. In mares with a pendulous uterus, it required bending the stylet over the pubic brim.

Time of insertion, Upod properties, and individual animal differences all seem to affect the outcome. Mare age may play a role. Nie et al. [8] showed that intrauterine glass ball-treated mares with extended luteal function were younger than those that did not exhibit prolonged luteal lifespan. Klein et al. [16] also observed that the IUD (water-filled, 3.6 g, 20 mm plastic ball) in mares with extended luteal function were younger than IUD mares with normal luteal function. Luteolysis seemed more likely

in Upod mares that exhibited more severe endometrial angio-pathies. Young age and normality of the endometrial blood vessels may improve the efficacy of Upods, suggesting that adequate perfusion and drainage of the endometrium may be important requirements for Upod effectiveness. It is possible that the "signal" from the Upod to prevent luteolysis is mediated through endometrial-ovarian vascular system. Two mares underwent luteolysis 3 and 1 week, respectively, following device insertion ([Figs. 5 and 6](#)). The devices were not able to block luteolysis in the first cycle subsequent to device insertion (experiment 1); however, diestrus periods were extended afterward. In the two mares with the Upod inserted during diestrus (experiment 2), the devices were not able to block luteolysis. The insertion procedure could have triggered an endogenous peak of prostaglandin and/or enable to block it. Timing of insertion, relative to endogenous spike in prostaglandin release on day 13–14 of the estrous cycle, has to be considered.

Upod mares exhibited subdued estrous-related behaviors and improved rideability. There was a general demeanor shift, even when in estrus. This was especially apparent when the Upod was retrieved. However, judging behavior is subjective, and to avoid prejudice, further studies with blinded evaluators and objective scoring system are needed to assess changes in behavior.

In experiment 2, Upods were inserted into the uterus of mares, (1) regardless of the stage of the estrous cycle and, (2) included postpartum mares. The rationale for was (1) to be able to insert the device as one visit outpatient procedure, without the need for repeat exams to determine stage of the estrous cycle; and (2) to envision future application in feral horses, where the device would be inserted in mares first seen with a young foal, within 30 days postpartum, without the need for additional exams to rule out pregnancy and disturb harem social fabric.

Table 3

Extended luteal function in mares with Upod versus control mares.

Group	Length of Study	Cycles Per Mare	Extended Diestrus ^a		
			Mares	Cycles	Length
Control mares (n = 7)	35 d	1.0 ± 0.0	0/7 (0%)	0/7 (0%)	n/a
Upod mares (n = 14)	98 d	1.4 ± 0.5	12/14 (85.7%)	12/19 (63.2%)	74.1 ± 11.1 (64–92)

^a Prolonged luteal function >35 days (Nie, 2003).

The potential use of this device is not restricted to horses. Inert nonmedicated, medicated (levonorgestrel-eluting), or copper Upods could potentially be used in other mammalian species as a reversible contraceptive device. Additional applications include control of feral populations of equids and wildlife. This would alleviate the need for repeated administration of ovotoxic industrial chemicals [19], immunogens to disrupt folliculogenesis [20], or GnRH vaccines [21] with highly variable individual responses and irreversibility. Field trials are ongoing to test retention and contraception in feral horse populations with a stallion harem. Ongoing research is being conducted on time-releasing progesterone-eluting Upods that may have application in transition and embryo transfer mares. We recommend that these devices are used by a licensed veterinarian only.

5. Conclusions

The inert, frameless, self-assembling Upods used in this study show a high retention rate. Endometrial integrity was not significantly affected by the device. Fertility post-Upod retrieval was not affected by the device and 100% of mares bred in the following breeding season successfully conceived. Hormone-free estrous behavior regulators have significant advantages over other methods, including (1) high efficacy, (2) 100% retention rate, (3) safe for health of the uterus, (4) easy to insert and retrieve, and (5) easy to detect with external detector and visualize with ultrasound. The Upod design decreased the risk of damage to the cervix with a streamline magnetic retrieval technique. Even with a high retention rate, the mares do eventually recycle without Upod expulsion, possibly decreasing the risk of infection from long-term use.

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Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jevs.2019.02.009>.

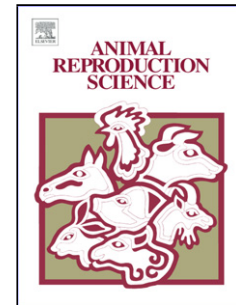
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Journal Pre-proof

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An intrauterine device with potential to control fertility in feral equids

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Highlights

- Retention rate with 40x16 mm size self-assembling uterine device iUPOD was 100%.
- The iUPOD immediate contraceptive efficacy was 100%.
- Reversibility was observed within 30 days post device retrieval.

ABSTRACT

Fertility control of feral equids is difficult. A 4-month pilot study was conducted with a hormone-free intrauterine device (iUPOD). There was evaluation of i) device retention; ii) contraceptive efficacy; iii) fertility following device removal; iv) effects of device on estrous cycle periodicity and; v) abundance of biofilm on devices after removal from the uterus. The iUPODs were inserted trans-cervically in eight mares at random stages of the estrous cycle. Mares were confined in a paddock with a stallion the following day and remained with the stallion for 120 days. Transabdominal detection of the iUPOD, using a non-invasive handheld magnetic detector wand, was performed weekly. Mares were examined using transrectal ultrasonography on days 0 (Time at insertion = day 0), 14, and 30, and subsequently every third week to assess number and size of follicles, corpora lutea, and whether there was intrauterine fluid (IUF) present. The mares and stallion were observed daily for mating behavior. Weekly samples were assayed for progesterone (P₄) at day 0 and until 3 weeks subsequent to stallion removal. None of the mares became pregnant while fitted with the iUPOD. Two of four mares conceived within 30 days subsequent to iUPOD removal. Three of eight mares fitted with the device had periods greater than 14 days with P₄ concentrations <1 ng/mL, and seven of eight mares had periods greater than 14 days with P₄ concentrations >1 ng/mL. There was a marked abundance of biofilm on devices of two mares at the time of device removal.

Keywords

Contraception; Feral horses; Fertility control; iUPOD; Reversibility; Self-assembling.

1. Introduction

At this time, there are not effective, inexpensive, safe, reversible and practical long-term contraceptive for treatment of feral equids in the American West, Australia and New Zealand. Free-roaming populations, having exceeded carrying capacity, have historically been managed by culling and

removal of animals for adoption (Tryon, 2019). Although contraceptive vaccines are promising, use has been limited primarily due to the requirement for ongoing vaccinations to sustain immunity (Kirkpatrick et al., 2011; Baker et al., 2018; Hobbs and Hinds, 2018). Likewise, surgical sterilization has had limited application in field settings for controlling reproduction in feral equids (Kane, 2018).

Daels and Hughes (1995), examined the use of an O-ring shaped intrauterine device (IUD) for a 1-year period; and Killian et al. (2006), examined efficacy of a copper T-shaped IUD for a 3 year period. Results from both studies indicated IUDs prevented pregnancy in mares while the IUDs remained in place. It is generally accepted that IUDs produce a local inflammatory reaction that disrupts the microenvironment in the uterine lumen (Daels and Hughes, 1995). The presence of relatively larger quantities of intrauterine fluid (IUF) may be indicative of chronic inflammation (Klein et al., 2016). Rivera del Alamo et al. (2008) evaluated the mechanisms leading to intrauterine device-induced luteal persistence in mares with intrauterine devices. Results indicated there was an inhibition of COX-2 production by the endometrium. Furthermore, there is a larger quantity of Annexin A1 in intrauterine fluid from mares with an intrauterine device in place, especially in those mares in which the presence of devices led to an inhibition of luteolysis (Rivera del Alamo, et al., 2018), suggesting that inflammation is the mechanism that leads to device efficacy for contraception of equids.

More than 150 million women use IUDs worldwide for fertility control. In women, IUDs primarily in the form of the copper 2+ intrauterine copper devices (IUCD), are the most effective and safe forms of Long Acting Reversible Contraceptive (LARC) birth control available (Kaneshiro and Aeby, 2010). Over time, LARC methods are 20 times more effective than birth control pills, progesterone (P₄)/estrogen patches, or the cervical/vaginal ring (Wu and Pickle, 2014; United Nations, 2019; NICE, 2019. Clinical Guidelines, No. 30).

There has been development of a novel-type intrauterine device (self-contained unit pod; iUPOD; Fig. 1), prototype for use in horses with a magnetic core that allows self-assembly inside the uterus. This property facilitates device insertion and retrieval. There is a highly acceptable device retention rate, with endometrial histological integrity and fertility post-device removal not being affected during the period the

device is in place (Gradil et al., 2017; 2019). This inexpensive (estimated unit cost: \$11.00, US) magnetic-based iUPOD was (0/8; 100%) effective as a contraceptive in mares that were inseminated artificially during periods of estrous expression (Joone et al., 2021).

Magnetic intensity leads to retention of device assembly (Gradil et al., 2019). There was an alternative device with lesser magnetic intensity (<3 newtons) evaluated in a 3-year old mare. The device was voided within 5 weeks after insertion.

It was hypothesized that the iUPOD could be a practical, effective, reversible contraceptive for use in pastured-bred equids. There, therefore, was evaluation for i) device retention; ii) contraceptive efficacy; iii) fertility following device removal; iv) effects of the device on estrous cycle periodicity and; v) presence of biofilm on the devices.

2. Materials and methods

Experimental procedures were approved by the Institutional Animal Care and Use Committee, at the University of Massachusetts (protocol # 2017-0046). Mares were current on all the vaccinations and deworming and reproductively sound, based on transrectal palpation and ultrasonic assessment evaluation that indicated mares had a normal reproductive tract and absence of uterine fluid.

2.1. Objectives

Eight mares of mixed light horse breeds, aged 3 to 15 years, clinically normal and non-pregnant were selected for this study that was 120 days in duration (April-August, 2019). The mean $\pm$ standard deviation (SD) at day 0 (Time at insertion = day 0), was 8 ± 4 years. Five mares were nulliparous and three were multiparous. The latter had no history of reproductive failure. After 90 days of being in a paddock with the stallion, mares were randomly allocated to two equal groups: Groups A and B. The devices were removed from Group A mares – that continued to be in the paddocks with the stallion subsequent to device removal. In Group B mares, the devices were left in place until day 120, when the stallion was removed from both groups. The stallion was of light horse breeding that was 5-year-old and was of proven fertility.

All mares were housed and cared for at the UMass Hadley Farm. Mares were fed free choice forage (mixed grass hay and pasture), a ration balancer, and had access to water and a white salt block at all times. The mares and stallion were maintained in a paddock of 0.6 hectares and had access to a three-sided shelter for the duration of the study.

2.2. Intrauterine Device (*self-contained unit; iUPOD*)

The intrauterine device used was a frameless, self-assembling, sterilized device made of a shatterproof, hormone-free inert polymer. The device was similar to the device used by Gradil et al. (2017 and 2019), but was larger (40 x 16 mm) with a mean unit weight of 18.9 g (Fig. 1). The magnetic force between the units was 3.8 newtons and between the devices and the retriever 4.2 newtons. The retriever was an oblong magnet 10 x 15 mm long attached to a fluorocarbon line and inserted trans-cervically through an applicator.

2.3. Insertion and removal of iUPODs

To mimic a catch and release scenario in the range, iUPODs were inserted regardless of the stage of estrous cycle, even in mares with a non-dilated cervix (e.g., early estrous period, diestrus, and nulliparous mares). Mares were placed in the paddock with a stallion of known fertility on the day after iUPOD removal and remained in paddock with the stallion for the duration of the study. At day 0, mares were sedated with (0.2-0.4 mL) IV detomidinehydrochloride (Dormosedan® HCL, 10 mg/mL) and the perineal area was scrubbed with 7.5% povidone- iodine. At day 0 the cervix was thoroughly evaluated manually by palpation per vaginum for lacerations and adhesions (Katila, 2012). For iUPOD insertion, the three sterilized units of the iUPOD were placed in the operator's hand and pushed through the cervix one by one using the double-glove technique (Portus et al., 2005), until the device was immediately cranial to the internal cervical os. Mares were examined using transrectal ultrasonography within 5 minutes subsequent to iUPOD insertion to confirm the location of the devices.

At the end of the study, the mares were administered an IM injection of 250 µg cloprostenol (Estrumate®) to facilitate the removal of the IUD during the early estrous period using a specially designed magnetic retriever with the applicator. The retriever was attached to a fluorocarbon line. The device was located in the uterus using ultrasonic procedures. The applicator loaded with the retriever was inserted through the cervix into the uterus, and then moved side to side until the uterine endometrial layer was in contact with the iUPOD. The retriever was pushed into the uterus, taking care to ensure the retriever was inserted into the uterus. The applicator was subsequently withdrawn, before pulling the tag line and ascertaining that the device was attached to the retriever. The devices were retrieved at 90 days subsequent to the day of insertion in mares of Group A; (third week of July) and at 120 days subsequent to the day of device insertion in mares of Group B; (third week of August).

2.4. Monitoring device retention

Transrectal ultrasonography (Aloka SSD 900, 5 MHZ linear probe; Hitachi Aloka Medical America, Inc.) was conducted on days 0, 14, 30, and subsequently every third week in mares fitted with the devices. Transabdominal detection of the iUPOD using an external handheld magnetic detector wand (PD240CB; CEIA USA Ltd.), was performed weekly. The Detection reliability – true alarm alerts without producing false alarm indications – of the handheld metal detector was 100% (Gradil et al., 2019).

2.5. Contraceptive efficacy

To determine fertility post-device insertion, transrectal ultrasonography was conducted on days 0, 14, 30, and subsequently every three weeks in mares fitted with the devices. To determine fertility after device removal in mares of Group A, mares were examined 2, 4, 5 and 6 weeks subsequent to iUPOD removal.

2.6. Monitoring the estrous cycle

Mares and the stallion were observed daily by two individuals for behavioral estrus and mating behavior.

ior for at least 15 minutes in the morning and afternoon after feeding. In addition to these dedicated times for estrous detection, the mares were observed during daily husbandry operations for a minimum of 30 minutes. Being a small university herd, any behavior such as estrus and mating observed throughout the day, including at night assessments, were recorded (Fig. 2). In addition to daily observations, the estrous cycle was monitored by assessing the number and size of ovarian follicles, corpora lutea, iUPODs and the presence of IUF – dorso-ventral depth measurements – using ultrasonic procedures.

Blood samples for P₄ quantitation were collected at the time 0, to confirm stage of the estrous cycle and then every week until 3 weeks post-stallion removal – for a total of 20 weeks – to monitor CL function. Blood was collected from the occluded jugular veins using plain 10 mL BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, NJ). Blood was centrifuged at 1500 x *g* for 15 minutes within 1 hour of collection and serum was frozen at -20 °C until laboratory analysis.

2.7. Analysis of blood

The P₄ quantitation was performed at a reference laboratory using an enzyme immunoassay (Munro and Stabenfeldt, 1984) with the technical staff conducting the assays having no knowledge of mare treatment status. All samples were analyzed in a single batch. The intra-assay CV was 16.6%. Reference P₄ values were as follows: Absence of active luteal tissue: 0.1 to 0.5 ng/mL; Non-discriminating for the presence of luteal tissue: 0.5 to 1.0 ng/mL; Presence of luteal tissue: > 1.0ng/mL.

2.8. Biofilm evaluations on iUPODs after removal from the uterus

The presence of biofilm on the devices after retrieval was assayed using the procedures described by Kadouri and Tran (2013), Merritt et al. (2005), and Taha et al. (2018). With this assay, there was use of unattached microbes which were initially removed from the samples with washing; then the attached biofilm microbes were stained with crystal violet, solubilized in 70% ethanol and the absorbance of the resulting solution was measured in OD units at 465 nm. The values represent the average of triplicate units of each iUPOD.

2.9. Statistical analysis

To address iUPOD retention and contraception efficacy, no formal statistical methods was used. There was $\geq 75\%$ retention rate and contraceptive efficacy, with a “pass or fail” outcome based on observed device retention rates for all the mares ($n = 8$). The benchmark for reversibility was $\geq 50\%$ for ($n = 4$) mares.

For analysis of the presence of biofilm on the devices after retrieval, Welch’s t test was used to determine if there were mean differences ($P < .05$) between biofilm formation on removed iUPODs compared with sterilized, unused control devices.

3. Results

3.1. Device retention

After insertion, devices self-aggregated in the uterus in a “ring” configuration (Figure 1, 3). The iUPOD (40 x 16 mm; 3.8 newtons) retention was 100% (eight of eight mares). The devices were purposely removed at 90 (Group A) and 120 (Group B) days subsequent to the day of insertion.

3.2. Fertility subsequent to iUPOD removal

None of the mares fitted with iUPOD for 90 (Group A) or 120 (Group B) days were pregnant (none of eight; 100% contraceptive efficacy) at the time of device removal. Two of four mares (Group A) used to test fertility subsequent to device removal were pregnant as a result of mating during the second estrous period associated with the second estrous cycle subsequent to device removal. The period without the contraceptive iUPOD in the presence of the stallion was 30 days. This group had two nulliparous (both 5 year-olds) and two multiparous (ten and 12 year-old) mares. The nulliparous mares conceived as a result of mating during the period of estrus that was associated with the second estrous cycle, as determined by ultrasonic evaluations subsequent to iUPOD removal – the multiparous mares did not conceive during the same interval.

3.3. Monitoring of the estrous cycle and mating

There were no serious injuries that occurred as a result of sexual behaviors associated with mating in this study. Because of the stallion being in the paddock with the mares continuously and during the extended period of time for conducting this study, it was not feasible to visually observe all stallion interactions with the mare that was associated with mating, especially during the nighttime hours. There were no stallion-mare matings observed for one mare and the maximum number of matings was six for another mare (Fig. 2).

Transrectal ultrasonography after device insertion was conducted on days 0, 14, 30, and subsequently every three weeks. Corpora lutea (CL) were observed in both ovaries of all except one mare, in which the CL were only observed in the right ovary. In another mare, there were two ovulations during one estrous cycle and in another mare there were two ovulations during two estrous cycles. Pre-ovulatory follicles were detected with concomitant uterine edema in all the mares. Even though there was an infrequent of ultrasonic exams (i.e., every three weeks) it was apparent based on the presence and location of corpora lutea, uterine edema and IUF, that there was disruption of timing of some typical reproductive physiological occurrences during some estrous cycles.

3.4. Estrous cycle periodicity/ P_4 patterns

At day 0, five of eight mares were in estrus ($P_4 = 0.07 \pm 0.02$ ng/mL) and three of eight mares were in diestrus ($P_4 = 5.99 \pm 0.91$ ng/mL). There were three of the eight mares fitted with the device that has sustained luteal functions for periods of greater than 14 days with $P_4 < 1$ ng/mL, and in seven of eight mares there were periods of greater than 14 days with P_4 concentrations of > 1 ng/mL (Fig. 2).

3.5. Uterine response to iUPODs

The information validating presence of IUF in the uterine lumen as determined by ultrasonic assessments is reported in Table 1. Five of eight mares treated with an iUPOD had an accumulation of IUF,

with sparsely hyperechoic specks detectable using ultrasonography, following intrauterine insertion of iUPODs. Fluid, when present, was associated with the iUPOD (Fig. 3), but occasionally there was a mass of uterine lumen fluid that was not in the proximity of the iUPODs. The IUF volume was less during the period of estrus and around the time of ovulation. There was no IUF detected in mares at the time of removal of the iUPOD, except for in one mare. The IUF was not detected in this mare 2 weeks subsequently to removal of the iUPOD.

3.6. Biofilm on iUPODs

Data are depicted in Figure 4 for biofilm quantities detected using the Crystal Violet Assay on iUPODs in four mares of group B in which these devices remained in the uterine lumen for 120 days. There was a marked abundance of biofilm on the devices in two mares. Interestingly, one of these mares mated with the stallion more frequently than the other mares in this study (Fig. 2).

Neither biofilm forming bacteria detected on the devices nor samples for endometrial culture were submitted for culture. The assay provides a “semi- quantitative” measure of relative biofilm formation on different iUPODs. Samples from all mares were evaluated in triplicate, except for samples from one mare that were evaluated in duplicate. Mean and standard deviation absorbance in the biofilm samples from iUPODS inserted into mares in this study were: $0.058 \pm 0.0046^{**}$; 0.033 ± 0.0055 ; 0.033 ± 0.0132 ; $0.044 \pm 0.0049^{*}$; and for the Control IUPOD was 0.033 ± 0.0011 . There was a difference in values for absorbance ($P < .05^{*}$; $< .01^{**}$) as compared with values of absorbance with the control sample.

4. Discussion

In the present study, there was evaluation of a novel IUD design for: intra-uterine retention, contraceptive efficacy, fertility following device removal, effect on estrous cycle periodicity and the presence of biofilm on the devices after devices were intra-uterine for 120 days. Killian et al. (2008) reported that device retention failure was the primary reason for contraceptive failure when IUDs were used for this purpose (380 Copper ‘T’) in mustang mares in Nevada. All mares except an 18-month-old filly

were observed in their natural habitat to produce normal healthy foals in this previous study. As number of parities increase, this may contribute to the more ventral orientation of the uterus. The caudal-ventral angle of the uterus and cervix relative to horizontal is less marked in nulliparous than multiparous mares (LeBlanc et al., 1998). Weight of the devices may be a contributing factor for uterine retention. The combined weight of the three units of the iUPOD is 56.7 g. The weight of the ‘T’ Copper IUDs used in the Nevada studies was < 1 g. The dimension of the self-assembled iUPOD is 47 x 49 mm. The dimension of the ‘T’ Copper IUDs was 32 mm horizontally and 36 mm vertically, with a 3 mm diameter bulb at the tip of the vertical stem. In the present study, the size, weight, and magnetic pull (3.8 newtons) were increased as compared to that in previous studies to assure iUPOD retention rates $\geq 75\%$. All the devices remained in the uterus until time of removal, confirming findings for previously reported retention rates (Gradil et al., 2019).

With use of the iUPOD, there was a contraceptive failure rate of 0%; and 50% reversibility within 30 days subsequent to device removal from the uterine lumen, confirming results from previous findings on fertility subsequent to device removal (Gradil et al., 2019). The present study was a pilot study with a limited number of mares. Mares served as their own controls. Mares had no previous history of reproductive failure. The mares in the present study were carefully selected from a closely monitored university brood mare herd. The stallion was a 5-year-old which when used previously was determined to be fertile, and that had been used regularly for breeding and teaching. Aborting a contemporary cohort of control mares, was not an option in the present study considering the use of mares from this herd for other purposes.

From most studies of IUD’s in mares, there are reports that there is minimal uterine fluid accumulation in response to the IUD (Nie et al., 2003; Rivera del Alamo et al., 2008). Although all mares were reproductively normal at the start of the present study, it is possible that at least some may have been predisposed to intra-uterine fluid accumulation and endometritis prior to intrauterine insertion of the iUPODs. In the current study some mares, (most prevalent in older mares; Fig. 2; Table 1), had occasional uterine fluid accumulation of ≥ 10 mm column that was no longer present after mares initiated estrous cycling subsequent to iUPOD removal. A uterus that tilts ventrally in relation to the pelvic brim area may contribute to the lack of capacity or delay in uterine clearance of fluids from the lumen, and thus fluid

accumulation. Relationships of age to uterine function and reproductive efficiency in mares indicate that mares which are older have an associated greater endometrial inflammation, less uterine contractility and less uterine tone (LeBlanc et al., 1998). All IUDs, when in place intravaginally, induce a local inflammatory reaction that disrupts the functioning of the endometrium and myometrium and leads to changes in the microenvironment in the uterine lumen (Ortiz et. al., 1996). Considering a contraceptive failure rate of 0%, the shortened or prolonged luteal function and the extent of IUF associated with the presence of the devices, it is likely that the contraceptive effect involved i) interference with sperm transport/viability and/or integrity of an embryo within the uterus; and prevention of implantation (Stanford et al., 2002). The limited number of mares used in the current study is not adequate to establish long term effects of iUPODs on fertility control in feral horses. The results from the present study, however, are considered to establish the proof of principle for further evaluations in studies conducted for relatively longer periods than that of the present study in rangeland settings where feral horses are prevalent.

In the present study, after mares were fitted with the iUPOD the normal estrous periods were disrupted (please see Fig. 2). Do the devices have the potential for contraception by simply altering estrous periodicity, for example resulting in the sustaining of P_4 concentrations at 0.1 to 0.5 ng/mL or 0.5 to 1.0 ng/mL for extended periods of time? Also, in seven of eight mares there were periods of luteal function for greater than 14 days during which P_4 concentrations were >1 ng/mL, with a reduction in the number of times behavioral estrus was expressed during the experimental period. In a concurrent Australian study (Joone et al., 2021) in non-pastured mares inseminated using fresh semen, estrous periods were disrupted. Diestrus was extended (median 52.5 days) in all the mares in which there had been insertion of iUPODs. The devices were 100% contraceptive in this Australian study. Rivera del Alamo et al. (2008), considering only estrous cycles without an extended luteal phase, reported that inter-ovulatory intervals were not shorter in IUD-treated mares in comparison to control mares. The iUPODs used in previous studies in non-pasture-bred mares were much smaller i.e., 26 x 12 mm in size weighed 7.5 g (Gradil, 2017; Gradil et al., 2019), yet mares had disruption of the regular estrous cycle periodicity. The mean duration of diestrus in these previous studies was 51.3 ± 22.7 days (mean $\pm$ S.D).

The iUPOD shape has effects on biocompatibility because rounded surfaces that allow for small fluctuations in ring configuration facilitate the lack of accumulation of biofilm on the iUPOD surface (Lagree et al., 2018). There, nevertheless, was a marked abundance of biofilm on the devices that were removed from two mares (Fig. 4), particularly that of one of the mares, that was observed to be mated more often, compared to the other mares that were included in the study (Fig. 2). Samples for endometrial culture were not submitted. It is possible that bacteria cultured from uterine swabs, if present, would differ from biofilm forming bacteria on the devices. In a previous study, bacteria isolated from similar devices after these were removed from the uterus of mares, however, were mainly common commensal species (Joone et al., 2021).

The self-assembling magnetic device consists of three magnetic elliptical shape units each with a magnetic core coated with a polymer, inserted independently from each other. The “ring” shape formed by the three magnets is a singularly unique configuration that minimizes the magnetic energy of the system and prevents expulsion of the device from the uterus subsequent to intravaginal insertion (Gradil, 2017). The magnetic field strength will not decrease during the period the devices are intravaginal. Because this device has elastic potential energy, the three vertices will dissipate forces against the uterine walls. This device capacity decreases potential trauma, as the magnets deform ergonomically and adapt to the shape of the uterine lumen’s dynamic conformational changes. If necessary, for example in mares that are included in adoption programs, disassembly is initiated by inserting a magnetic probe, which disrupts the stability of the ring.

What is the relevance of this methodology for fertility control? The iUPOD is hormone-free. Infertility is essentially immediate after intravaginal device insertion. Device costs are inexpensive (estimated unit cost: \$11.00, US). The iUPOD can be inserted anytime regardless of the stage of the estrous cycle. Utilization of the device involves the need for only one animal handling regimen for device insertion, minimizing the number of times animals have to be confined and managed for iUPOD use, and facilitating scheduling device placement in catch and release scenarios. It is recommended with the iUPOD treatment that mares which are clinically sound from a uterine structure and function perspective have the device

inserted and should not be placed with stallions until the following day subsequent to the intrauterine procedure, to allow mares a period for full recovery from sedation. A single solution will not ever be possible to control fertility of feral equids. Rather a combined approach for management of feral populations will likely be most efficacious such as short-term (vaccines) and long-term (IUDs) contraceptive alternatives.

5. Conclusion

The iUPOD, a self-assembling uterine device designed for use in mares represents a promising inexpensive means of a long-term, reversible contraceptive for equids. The iUPOD contraceptive efficacy was 100% in eight mares treated with the devices in the present study. Reversibility of the contraceptive effect was observed within 30 days post device removal from the uterine lumen. If mares retain a hormone-free iUPOD for an extended period in a pasture-bred management system - a contraceptive eluting agent, for example copper 2^+ IUCD (Fig. 5), will induce immediate infertility and for as long as the device is *in situ*, putatively for 3 to 5 years, a significant period of the reproductive life span of most feral horses.

Author contributions

Carlos Gradil: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization, Project administration, Funding acquisition. Carolynne Joone: Methodology, Writing - review & editing. Teresa Haire: Project administration, Resources. Bradley Fowler: Methodology, Visualization. Jacquelyn Zinchuk: Methodology, Visualization. Chris Davies: Writing - review and editing. Barry Ball: Writing - review and editing.

Declaration of Competing Interest

Pending patent application for the intrauterine device by the University of Massachusetts, Amherst.

The authors declare that they have no conflict of interest.

Declaration of Competing Interest

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Fig. 1. Novel intrauterine device (self-contained unit pod; iUPOD), prototype for use in horses with a magnetic core that allows self-assembly inside the uterus; Size of the devices is 40 x 16 mm with a mean unit weight of 18.9 g; Magnetic force between the units is 3.8 newtons.

Fig. 2. Depiction of observed estrus/mating/week, progesterone (ng/mL), and intrauterine fluid (IUF) in mares fitted with a frameless self-assembling intrauterine device (iUPOD);

Device was inserted into the uterus of eight mares in varying stages of the estrous cycle.

Device was removed at 90 (Group A -) or 120 (Group B -) days subsequent to insertion.

Progesterone baseline <1 ng/mL; Time of device insertion = day 0.

Two of the mares were pregnant within 4 weeks subsequent to iUPOD removal, as determined by ultrasonic procedures.

Fig. 3. Ultrasonic image of a self-assembled iUPOD in the uterus.

Note the intrauterine fluid in close proximity to the device.

Scale on the left is in increments of 10 mm.

Fig. 4. Biofilm detected by the Crystal Violet Assay on iUPODs in four mares (Group B) with devices *in situ* for 120 days.

^aSamples from all mares were evaluated in triplicate, except for one mare for which there was a duplicate evaluation.

^bMare with the most recorded matings $P < .05^*$; $< .01^{**}$.

Fig. 5. A 2⁺ IUCD - Intrauterine copper Device prototype.

Copper self-assembling iUPOD unit could potentially be used to suppress reproduction in feral populations of equids.



Fig. 1.

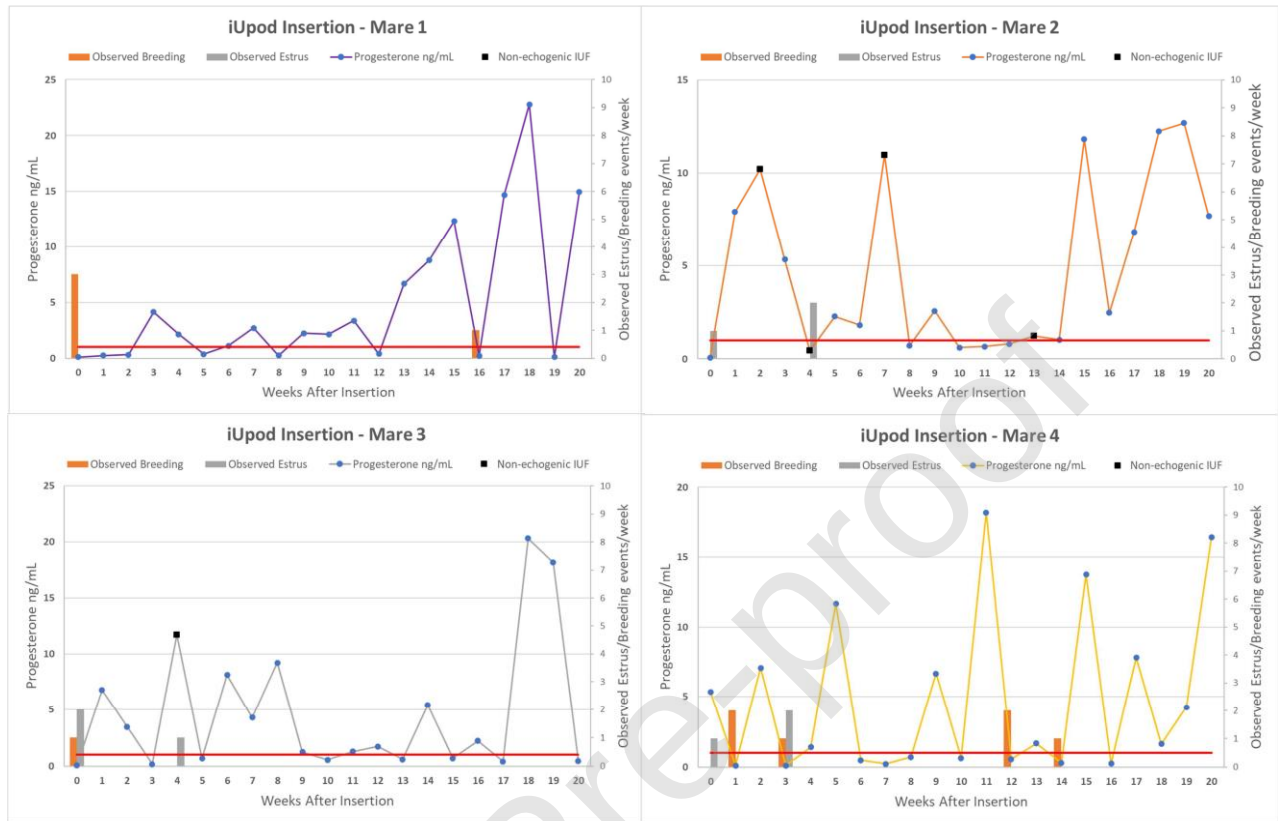




Fig. 2

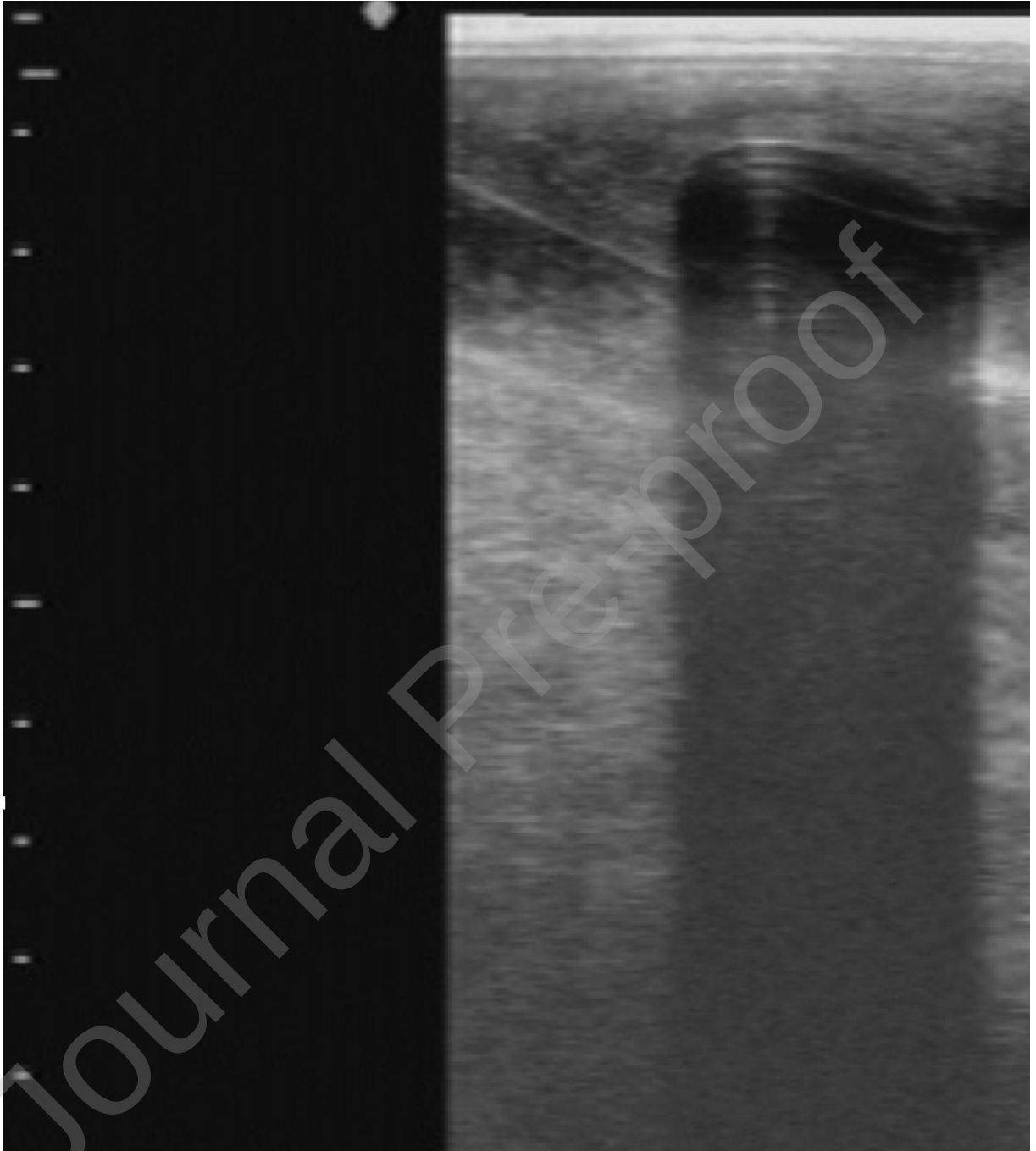


Fig. 3.

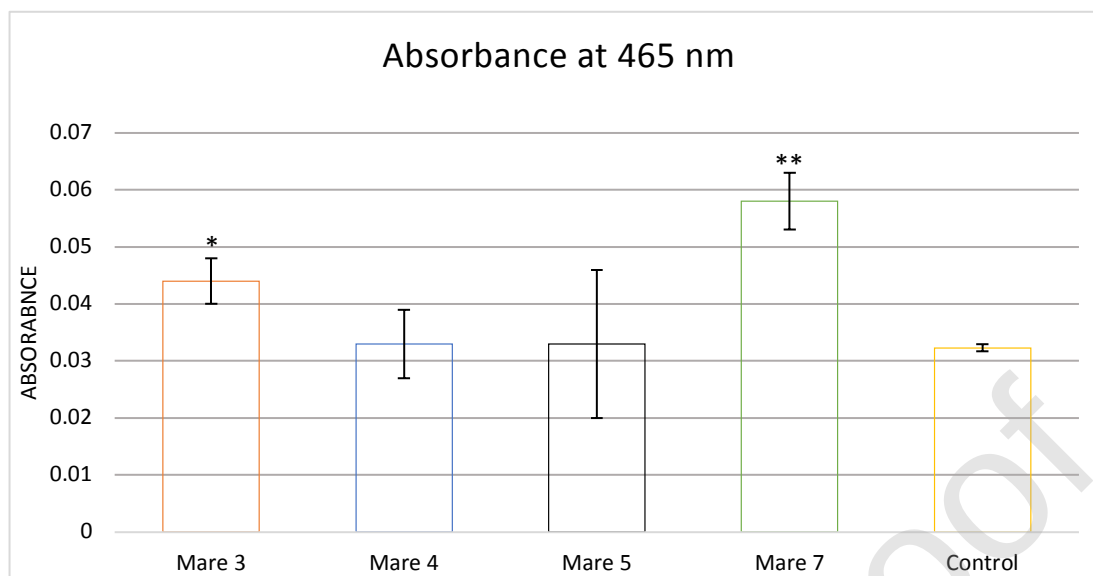


Fig. 4.

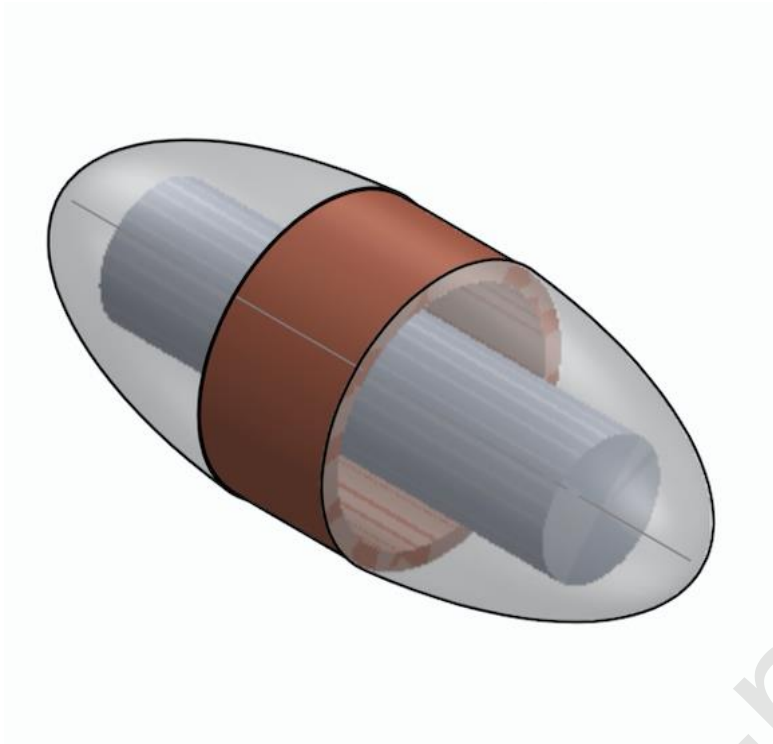


Fig. 5.

Table 1

Mares with ultrasonically visible intrauterine fluid (IUF).

	Non-echogenic IUF
Group A – Mare Age	
Mare 1 5	NO ^a
Mare 2 10	d14, 28, 49, 91
Mare 8 5	d70 ^{ab}
Mare 6 12	d49, 70
Group B – Mare Age	
Mare 3 15	d28
Mare 4 3	NO
Mare 5 3	NO
Mare 7 10	d70, 91, 105, 120

d = days subsequent to iUPOD insertion

NO = Not observed

^aPregnant in the post-removal period. Mares conceived within 2 cycles (30 days) post-iUPOD retrieval.

^bEchogenic fluid



Special Section on Management of Feral Equids

Efficacy of a Y-Design Silastic Elastomer Intrauterine Device as a Horse Contraceptive

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ABSTRACT To successfully manage population growth rates of free-roaming horses (*Equus ferus caballus*), an effective, long-acting contraceptive could be beneficial. We evaluated the efficacy and safety of an intrauterine device (IUD) in a breeding trial using seasonal pasture assemblages of 2 males individually pastured in large enclosures (~81 ha) each with 10 females per male. The study took place at a large animal research facility at Oklahoma State University in Stillwater, Oklahoma, USA. Specific objectives of the study were to evaluate the effectiveness of IUDs in preventing pregnancy over 20.5 months with return to fertility assessed over the subsequent 6 months and to evaluate potential effects of this IUD on ovarian function and uterine health (e.g., endometrial inflammation, infection, fibrosis). We developed a Y-design IUD, made of silastic elastomer, and set the desired retention rate at 75%. After procurement of endometrial biopsies, we inserted IUDs into the uteri of 20 females and examined them via transrectal ultrasound every 2 weeks for detection of the device and assessment of uterine health. After 20.5 months we removed the remaining 15 IUDs, obtained a second endometrial biopsy for comparison, and returned the females to their respective males for continued breeding in efforts to assess uterine return to health as indicated by pregnancy rates. The Y-design IUD met the 75% retention rate goal, with 100% contraception in females that retained the device; 12 of 20 treated females became pregnant (i.e. returned to fertility) after removal of the IUD. Based on these results, we conclude that the Y-design IUD is a safe and effective device to control fertility in free-roaming horses. © 2021 The Wildlife Society.

KEY WORDS contraception, *Equus ferus caballus*, feral horses, fertility, histopathology, intrauterine device.

Because of unrestricted population growth on western rangelands in the United States there are populations of free-roaming horses (*Equus ferus caballus*) administered by the Bureau of Land Management (BLM) that exceed the desired population size established by range managers (Fisher 1983, Beever and Brussard 2004, Beever and Herrick 2006, Scasta et al. 2018, BLM 2019). Therefore, an effective, long-lasting contraceptive for free-roaming horses on the western range that does not require the animal be recaptured frequently would be beneficial.

We previously conducted a series of studies examining the efficacy and safety of several sizes and degrees of hardness (durometer) of an O-ring intrauterine device (IUD) composed of a silastic elastomer polymer (Baldrighi et al. 2017). These studies were based on earlier results indicating a >80% retention rate of an O-ring IUD (Daels and

Hughes 1995, Killian et al. 2006). In our early trials, we observed that a majority of the O-ring IUDs were expelled within 14 days of insertion; this was likely a result of either uterine expulsive forces or frequent breeding behavior caused by an abbreviated diestrus resulting from placement of the IUD (Baldrighi et al. 2017). Manufacturers of IUDs for humans have described their devices as having a fundus-seeking design (Mirena® Bayer HealthCare Pharmaceuticals Whippany, NJ, USA, or the Flexi-T®, Prosan International, Arnhem, Netherlands). The Mirena® IUD is a flexible T- or Y-type design intended to match the luminal space in the fundus and upper uterine body between the promontory bulges of the myometrium. The intent is that the closer the placement of this IUD to the fundus, where the arms sit snugly above the myometrial promontory, the more likely the device would remain in place. As a result of our initial findings, we concluded that we would need to formulate an alternative IUD design that would result in a significantly increased rate of uterine retention (Vemula 2016).

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Therefore, we developed a Y-design, silastic prototype IUD based on the human IUD fundus-seeking design; this design is intended to ameliorate normal uterine expulsion forces (Fig. 1). Although we recognized that the studies we used as the basis for the developmental Y-design IUD were performed in humans, where a unicornuate uterus rather than a bicornual uterus is present, the human-based findings did shed light on IUD movement (Goldstuck and Wildemeersch 2014) that we believed could be extrapolated to the equine uterus. One major factor that is unique to equines, and affects the stability of a uterine IUD, is that during a breeding event, the male's flared glans penis securely contacts, and actively dilates, an already relaxing cervix. The dialation ensures that semen deposition can occur directly into the uterine body rather than within the vagina, as is common in many other species. Because of this unique joint effort between the male's glans penis and the female's dilated cervix, an IUD design that withstands the forces of equine breeding is essential for long-term retention. The most effective and tolerated IUD design should cause a minimum amount of distortion of the uterine

lumen during the most active phases of contraction (Vemula 2016).

The main goal of this study was to develop a long-lasting contraceptive IUD for use within a free-roaming horse population that was not detrimental to their health, the health of other animals or predators, or the environment. We set the desired rate of retention for the IUD at 75%.

STUDY AREA

Our study took place in Stillwater, Oklahoma, USA, from 1 August 2017 to 15 April 2019, with return to fertility assessed from 15 April 2019 to 15 October 2019. Climate in this part of the United States is described as humid subtropical, possesses 4 seasons annually, and has an average high temperature of about 34°C (typically achieved in Jul) and an average low of -3°C (typically achieved in Jan). Winter months relative to the equine breeding season in Oklahoma are from November through February. Vegetation consumed by the animals in this study was primarily a mix of mostly native bluestem grasses (*Andropogon* spp.) and Bermuda grasses (*Cynodon dactylon*) on 162 ha, divided into 2 fenced pastures of open plains with gradual hills of roughly 81 ha each.

METHODS

We used 20 light-breed females (~400–500 kg) between the ages of 4 years and 10 years for this study. Housing consisted of a pasture environment (162 ha divided into 2 pastures of approximately equal size) where the horses socialized in groups of 10 females and 1 male mimicking harem bands found in natural living conditions. For examination purposes, we rounded up the females from their pastures using a utility vehicle and funneled them into a fenced alley that led to a catch pen just outside the barn where we conducted bi-weekly reproductive ultrasound examinations. We used a halter and lead rope to lead each female into a set of standing stocks with a chest rope and rear door. Even though these females previously received very little hands-on interactions, they were readily amenable to handling and examination without the use of tranquilizers. Each female received an initial breeding soundness examination consisting of a physical examination and an external and internal genital tract examination (including an endometrial swab for microbiological culture and endometrial biopsy). Only females with a normal culture result and an endometrial biopsy score of Kenney-Doig grade I, IIA, or IIB were included in the study (Kenney and Doig 1986).

We randomly divided the 20 females into 2 groups and assigned 1 male per group. Prior to placement in a group that formed a natural breeding pod, each male received a standard equine breeding soundness examination that included measurement and evaluation of characteristics such as total sperm number, sperm motility, and sperm morphology; both males had >1 billion progressively motile morphologically normal sperm cells in the second ejaculate collected 1 hour after the first semen collection, per breeding soundness examination standards. After receiving an IUD,

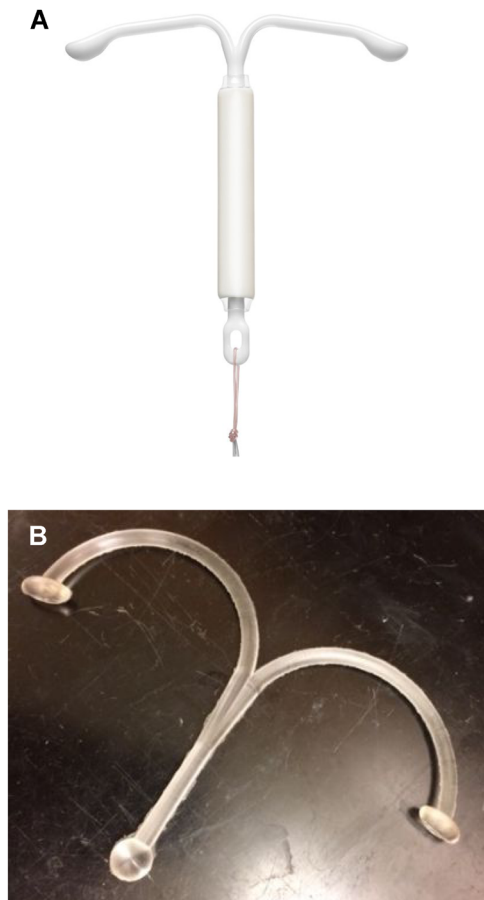


Figure 1. A) An example of a human intrauterine device (IUD) design described as being fundus seeking to ameliorate normal uterine expulsion forces. This model served as a template for developing a Y-design IUD for the horse. Mirena®. Bayer HealthCare Pharmaceuticals Whippany, NJ, USA; image used with permission <http://www.mirena.com>. B) Oklahoma State University silastic Y-design intrauterine device specific for a female's uterine shape.

each female received a transrectal ultrasonographic examination of the uterus to look for the presence of the IUD or a pregnancy every other week. We described uterine fluid using a numeric scale of 0–3; an assigned value of zero indicated that we did not detect fluid within the uterus on ultrasonography.

We initiated the first phase of our study on 1 August 2017. We compared 50- and 60-durometer Y-design IUDs by randomly assigning 10 females to receive a 50-durometer IUD and 10 females to receive a 60-durometer IUD. Additionally, 5 females in each of the 50-durometer and 60-durometer treatment groups received an intramuscular injection (5 mL) of BioRelease LA P4 300 (BET Pharm, Lexington, KY, USA) at the time of IUD insertion; non-P4-treated females received an intramuscular injection of saline. BioRelease LA P4 300 is a microparticle formulation of progesterone at a concentration of 300 mg/mL designed to release progesterone over 10 days. The decision for co-administration of a bio-release progesterone was to reduce myometrial contractility in response to a luminal foreign body and to suppress any estrus behavior that would promote breeding activity in the immediate time frame following IUD insertion (Baldrighi et al. 2017). This arrangement allowed for an equally randomized, split-plot design of equal numbers of females receiving each treatment for IUD hardness and co-administration of progesterone. The predetermined goal for IUD retention was $\geq 75\%$ over the study period. We performed IUD placement after thorough washing of the perineum with mild soap. We inserted each IUD transcervically using a customized polyvinyl chloride (PVC) tube, with plunger, that had a rounded tip (1.27 cm outside diameter, 63.5 cm in length, with a 0.95-cm-diameter plunger). We manually guided the PVC device through the cervix via a sterile gloved arm.

Evaluation of results after 60 days suggested that females fitted with 60-durometer Y-design IUDs (9/10), or females that received the Bio-Release LA P4 300 (10/10), were more inclined to retain their IUDs. Even though the difference was not significant, we chose to move forward with the positive trend to optimize treatment group numbers, by removing the remaining 50-durometer IUDs and replacing them with 60-durometer IUDs following manual cervical dilation. Females initially treated with 60-durometer IUDs (5 of which had received Bio-Release LA P4 300 and 5 of which had not) did not receive a second treatment of any kind. This change in study design initiated the start of a second phase of our study. We returned the 20 females back to their original breeding pods and continued to monitor them via a transrectal ultrasonographic examination of the uterus every other week for the remaining duration of the study.

On 15 April 2019, we removed all IUDs and the females entered a third and final phase of the study wherein they could active breed without contraception and we could assess their return to fertility via pregnancy rates under continued natural breeding conditions. At the initiation of this third phase of the study, we obtained an endometrial biopsy from all females once again for assessment of histopathology.

We returned the 2 breeding pods to their respective pastures until 15 October 2019.

We examined ovarian cyclicity and function, location of the IUDs, retention rates (of both 50- and 60-durometer IUDs), pregnancy rates after IUD removal, endometrial histopathology changes in grade, endometrial histopathology as it relates to pregnancy rates, and uterine fluid as it relates to pregnancy rates. We performed comparisons of data between treated groups using a Fisher's exact test. We considered 2-tailed *P* values of 0.05 to be significant. We used the statistical software SAS (SAS Institute, Cary, NC, USA) for statistical analyses. Animal use and care procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (IACUC VM15-29).

RESULTS

Ovarian function and cyclicity experienced no consequential changes as a result of the IUD as compared to a female's typical estrous cycle: females continued to cycle and be receptive to the male during estrus. No effect on ovarian follicular dynamics as a result of the exogenous progesterone administration at the time of IUD insertion was evident. The time frame of expected efficacy for the progesterone product used was 10 days and because the next examination did not take place until 14 days after administration, we may not have been able to document the exogenous hormone's effect on ovarian follicular dynamics. We documented evidence of ovulation within females that retained their IUD, as exhibited by the development and regression of corpora lutea (CL), for every female over the duration of the study with the exception of winter (Dec–Feb) where all but 1 female experienced anestrus. We identified CL on both ovaries of each female in the study. A minority of females (3/20) appeared to enter autumnal transition, as evidenced by the appearance of anovulatory hemorrhagic follicles, starting in late September; the majority of non-pregnant females (10/16) continued to ovulate through October and into November.

The IUDs did not appear to have notable movement from their deposited site (i.e., at the bifurcation of the uterus) unless expulsion occurred. In phase 1 of the study, we used 50- and 60-durometer Y-design IUDs and 17 of the 20 females retained the Y-design IUD, giving an overall 85% retention rate. More specifically, the 50-durometer IUDs had a retention rate of 8/10, whereas the 60-durometer IUDs had a retention rate of 9/10. The co-administration of Bio-Release LA P4 300 also resulted in a higher retention rate (10/10) compared to those females that received saline (7/10), although this was not statistically significant (Fisher's exact test $P = 0.21$).

At the initiation of phase 2 of the study, all 20 females possessed a 60-durometer IUD. Five females expelled their IUD and 15 of 20 females retained the IUD, resulting in an overall 75% retention rate. The 15 females that retained the IUD did not become pregnant ($P < 0.001$). Of the 5 IUDs that were expelled, 1 was expelled after 1 month of insertion, 1 after 3 months, 2 after 4 months, and 1 after

10 months. Of those females that expelled their IUD, 3 females were from 1 pod and 2 females were from the other, indicating no male or breeding pod effect ($P=0.60$).

All 5 females that expelled their IUD became pregnant between 1 and 3 months later with the exception of 1 female that took 6 months to become pregnant (the majority of those 6 months were spent in winter anestrus). When pregnant, the females continued to be maintained in their pods. The existing pregnancies persisted beyond the early embryonic loss phase of 60 days of gestation; we terminated the pregnancies shortly thereafter with administration of an injectable prostaglandin. Because we allowed pregnancy to progress beyond the formation of endometrial cups in the 5 females that expelled their IUDs, repeat pregnancies did not immediately occur after we terminated the pregnancies. Their ability to become pregnant again during the upcoming third phase of the project was not compromised because resolution of endometrial cups did occur prior to the commencement of phase 3.

During phase 3 of the study, after we removed all IUDs on 15 April 2019, 9 females were pregnant after 2 estrous cycles; included in this number were the original 5 females that had lost their IUDs and became pregnant in the earlier months of the study. After 3 estrous cycles, we diagnosed 2 more females pregnant and after 4 cycles 1 additional female had become pregnant, bringing the number of pregnant females to 12/20. We found no differences between the number of pregnancies per estrous cycle ($P=0.34$).

We determined the safety of the device, as it pertains to uterine health, by endometrial histopathology and reflected in the Kenney-Doig endometrial grading score changes. The Kenney-Doig endometrial biopsy score was unchanged for 7/20 females after comparing their final score to their original biopsy score. Even though inflammation seemed more apparent on final biopsies, as indicated by either a higher number or variety of inflammatory cells, this did not affect their overall resulting grade. Four females had an increase (or worsening) by 1 Kenney-Doig grade; this was largely due to an increase in the number and variety of inflammatory cells, in addition to a mild increase in fibrosis. Five females had an increase by >1 Kenney-Doig grade; these females had more inflammatory cell infiltrates, with a moderate amount of fibrosis and glandular changes. Three of these 5 females became pregnant after IUD removal: female 606 was pregnant within 30 days, female 602 was pregnant within 60 days, and female 613 was pregnant within 5 months; this indicates a relatively quick resolution of the inflammatory effect of the IUD. Finally, 4 of the 20 females actually showed a decrease by 1 Kenney-Doig grade (i.e., improvement), indicating that they may have had a very low mucosal immune response to the presence of the IUD. Overall, in regards to uterine health as indicted by endometrial biopsies, over half of the females either stayed the same or improved over the course of the study; the distribution of the increase or decrease in Kenney-Doig grades were randomly distributed among the 2 horse groups ($P=0.66$).

We did not detect a relationship between a change in individual Kenney-Doig grade and the female becoming

Table 1. Female endometrial biopsy scores of 7 female horses (out of 20) that did not become pregnant in the 6 months following removal of an intrauterine device that was in place for 20.5 months, including 2 breeding seasons at Oklahoma State University, Oklahoma, USA, 2017–2019. We used the Kenney-Doig endometrial biopsy grading system. A change in endometrial biopsy score did not provide an explanation for the lack of identified pregnancies in these 7 females that did not become pregnant at any time during the study.

Female identification	Endometrial biopsy score	
	Initial grade	Final grade
151	I	I
603	I	IIA
607	IIA	I
609	I	I
611	I	IIA
615	I	I
618	IIA	IIA

pregnant. Seven females did not become pregnant at any time. All of these females had ideal Kenney-Doig grade biopsies of I or IIA at the beginning of the study. By the end of the study 5 maintained their biopsy score, 1 worsened by 1 grade, and 1 worsened by 2 grades. These changes did not affect the probability of the female achieving a positive pregnancy result ($P=0.60$; Table 1) and there was no indication that the inability of these 7 females to become pregnant was solely a result of endometrial environment. Each of these females had regular estrous cycles during the post-IUD removal phase of the study as evidenced by continued development, and regression, of CLs. Reasons for females not becoming pregnant likely includes need for increased recovery time for their uterine environment following IUD removal, or other currently unidentified intrinsic factors not associated with the IUD such as oviductal pathology and uterine fluid clearance abnormalities following breeding events. There was no relationship between intrauterine fluid found during the bi-weekly examinations and pregnancy, or between the endometrial biopsy grade and pregnancy outcome (Table 2).

DISCUSSION

Our study indicates that inserted Y-design IUDs in association with an intramuscular injection of a bio-release progesterone, can result in a nearly 2-year retention rate of 75% with 100% efficacy at preventing pregnancy when retained. Based on ultrasonographic and histopathologic examinations, and pregnancy results, our goal of developing a safe and efficacious method to control fertility in free-roaming horses appear to have been achieved. We recognize that application of an IUD method of contraception would be best accomplished in free-roaming females that were not pregnant. Although the effect that an IUD would have on a lactating female is not documented, literature from humans indicates that presence of an IUD does not influence breastfeeding performance or increase risk for adverse events (Berry-Bibee et al. 2016).

Further generalization of our results to a population of free-roaming horses living in natural conditions, with harem groups and potentially multiple mature males nearby,

Table 2. Female endometrial biopsy scores of the 5 female horses that had an increase (worsening) by >1 Kenney-Doig grade after removal of intrauterine devices that had been in place for 20.5 months, including 2 breeding seasons. We did not identify a relationship between the presence of intrauterine fluid and the ability to become pregnant. We measured biopsy scores using the Kenney-Doig endometrial biopsy grading system, and uterine fluid scores by transrectal ultrasonography, Oklahoma State University, Oklahoma, USA, 2017–2019.

Female identification	Endometrial biopsy score		Fluid score			Pregnancy or gestational length
	Jun 2016	Apr 2019	Mar 2019	Apr 2019	Apr 2019	
606	IIA	III	3	3	3	105 days
607	IIA	III	1	1	Trace	Negative
612	IIA	III	3	3	3	50 days
613	IIA	III	Trace	Trace	3	120 days
616	IIB	III	1	1	Trace	10 days

should be done with caution because our females were housed with only 1 male. It is also possible that absence from the normal pregnant state of free-roaming females would have a social effect on the family unit because females may continue to be cyclic and allow breeding behavior.

Other contraceptive solutions for the problem of unrestricted population growth on western rangelands do exist, with some being quite effective. Many of these methods either have potential to pose a risk to the ecosystem (steroids or reproductive hormone protocols), are in conflict with the goal of protecting the fertility of genetically valuable groups of horses (anti-fertility vaccines), or require a higher than desired frequency of handling for the drug protocol to be effective (steroid or hormone protocols, vaccination against the zona pellucida of the embryo). Two notable, and highly effective, methods of estrus suppression include the use of altrenogest (a synthetic progestin) and an exogenous oxytocin treatment regimen; however, the increased amount of handling required for both methods, not to mention durations of efficacy that last anywhere from a few days to a few months, would not be practical or advisable for use within a group of free-roaming horses (Vanderwall et al. 2007, 2012, 2016; Hornberger et al. 2017). The use of a non-hormone impregnated contraceptive device could be considered advantageous when compared to the above methods in that persisting steroids or other reproductive drugs, such as gonadotropin releasing hormone superagonists (i.e., a slow-release deslorelin implant) run the risk of being consumed by wildlife. Vaccination against the oocyte's zona pellucida, which is effective as long as antibody titers exist, may also not be ideal for a group of free-roaming horses where managers want to retain fertility of horses long-term. Initial boosters, followed by annual booster vaccinations, are required for consistent duration of efficacy and would require repeated capture events (Gupta and Minhas 2017).

Efforts at specifically preventing ovulation without the use of hormones include other methods such as uterine IUDs (glass marbles, self-assembling magnets), vaccination against gonadotropin releasing hormone (GnRH vaccines), or ovariectomy (removal of the ovaries). Although effective at suppressing follicular recruitment and ovulation, these methods also possess the downside of either requiring more frequent than desired handling of the horses for repeated dosing, run the risk of causing permanent infertility in a herd where fertility may wish to be preserved, require invasive surgery where a degree of mortality should be

expected in a group of free-roaming horses unable to receive appropriate post-operative care, or risk inducing females to display paradoxical estrus behaviors (Asa et al. 1980).

The concept of using IUDs as a method of estrus suppression or contraception is not new but has been met with controversy because of inconsistent results and induction of uterine pathology (i.e., shattering intrauterine marbles; Diel de Amorim et al. 2016); however, some researchers report no complications with high efficacy (Nie et al. 2003, Rivera Del Alamo et al. 2008, Turner et al. 2015, Gradil et al. 2019). In contradiction with these reports, the use of our Y-design IUD did not result in the suppression of estrus or breeding behaviors as evidenced by the continued detection of CLs and witnessed breeding events. One distinct benefit to our study was being able to observe the same group of females fitted with IUDs for a prolonged period.

MANAGEMENT IMPLICATIONS

Use of a Y-shaped IUD provides a long-lasting, effective, and safe alternative to hormones and antifertility immunizations, which require repeated captures and injections or darting. To apply the Y-design IUD in free-roaming horses in semi-field conditions, the females would need to be examined via transrectal palpation. After harems were rounded up, females would be sorted and put into a squeeze chute for an initial pregnancy examination (performed with or without an ultrasound machine depending on the experience of personnel). Those females not pregnant would undergo a quick perineal and external genitalia wash and prep prior to the transcervical insertion of the IUD using an applicator similar to the one made by our group. Females could then be immediately released back into their harems.

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Preliminary study of the contraceptive effect of a self-assembling intrauterine device (iUPODs) in mares maintained in a paddock with a fertile stallion

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**Preliminary study of the contraceptive effect of a self-assembling intrauterine device
(iUPODs) in mares maintained in a paddock with a fertile stallion**

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**Preliminary study of the contraceptive effect of a self-assembling intrauterine device
(iUPODs) in mares in a paddock breeding setting**

Animal welfare/ethical statement: All animal procedures were approved and conducted
following the guidelines of the (University) Institutional Animal Care and Use Committee
(Protocol #10234).

Conflict of interest statement: Dr. Carlo Gradil's employer, the University of Massachusetts has
applied for a patent on the iUPOD.

ABSTRACT

There is an urgent need for practical methods of population control (i.e., contraception and/or sterilization) for free-roaming (i.e., “wild” or “feral”) horses and burros on Western Public Lands in the United States. The objective of this study was to evaluate the contraceptive efficacy of a novel self-assembling three-part polymer-coated magnetic intrauterine device termed as an intrauterine POD (self-assembling; iUPOD) when there are natural breeding conditions when iUPOD use was managed by veterinary professionals with no prior experience with the device. Six mares were administered an iUPOD and were then housed continuously with a fertile stallion for 91 days. The intrauterine POD retention and contraceptive efficacy were 100%. Two mares had prolonged corpus luteum function (for 37 and 91 days) immediately after iUPOD placement. For the estrous cycles of the other mares, the duration of diestrus was 7.8 ± 2.7 days (mean $\pm$ S.D.). Four of the mares (67%) became pregnant when in a paddock with the same stallion the year after iUPOD removal. These results are encouraging for use of the iUPOD as a practical and reversible method of fertility control in free-roaming horses and burros.

Keywords: Equine; Mare; Self-assembling intrauterine device (iUPOD); Contraceptive; Reversibility

1. Introduction

The current status and management of free-roaming (i.e., “wild” or “feral”) horses and burros (FRHB) on Western Public Lands in the United States is a very contentious political issue (Scasta et al. 2018). There is a pressing need for safe, reliable and practical population control methods (i.e., contraception and/or sterilization) for FRHB, because the current number of animals far exceeds the Federally-mandated “Appropriate Management Level” of animals on Public Lands, and numbers of these animals continue to increase relatively unchecked

(Rittenhouse 2018). An efficacious device for population management of FRHB would include practices of one-time handling of animals, efficacy in utilization, long duration of effects, reversibility of effect, and safety regarding overall health and reproductive status of the horse. Efforts for efficacious contraception/sterilization have primarily been directed at mares, and methods that have been used and/or are in development include: 1) vaccination with/against zona pellucida antigen (PZP) or gonadotropin releasing hormone (GnRH), 2) surgical sterilization utilizing ovariectomy, or tubal ligation procedures and 3) use of an intrauterine device (Kane 2018). Of these approaches, the PZP vaccination has been the most widely used method, but to achieve an optimum effect (i.e., 10% to 20% foaling rate), multiple doses must be administered, and even more problematic is the need to revaccinate mares every year for lasting efficacy (Kane 2018). The PZP vaccination could be used to prevent pregnancy in the short term, allowing the use of other longer lasting contraceptive techniques such as intrauterine devices (IUDs) that require administration to a non-pregnant animal.

Intrauterine devices have been widely used for estrous suppression in performance mares by prolonging function of the corpus luteum (CL), which results in continued secretion of progesterone from the CL, therefore, inhibiting the onset of behavioral estrus (Vanderwall 2013). The most widely used IUD for estrous suppression has been a glass ball (i.e., “marble”) that was first reported to be utilized in 2003 (Nie et al. 2003); however, during the past several years numerous reports of deleterious consequences associated with the use of intrauterine glass balls have resulted in a request for discontinuation of the use of these devices for control of reproductive functions in mares and jennies (Vanderwall 2015). Alternatives to a glass ball IUD include plastic water-filled balls (Rivera Del Alamo et al. 2008), a silastic O-ring IUD (Daels

and Hughs 1995; Baldrighi et al. 2017) and a novel self-assembling three-part polymer-coated magnetic intrauterine POD (self-contained; iUPOD) (Gradil et al. 2019; Gradil et al. 2021).

Regarding the use of any type of IUD for contraception, these devices have the potential when there is intrauterine placement to inhibit reproduction through two mechanisms: 1) estrous suppression, by inducing prolonged function of the CL that will inhibit expression of behavioral estrus precluding mating and the establishment of pregnancy and/or 2) impairing the capacity of the uterus to maintain pregnancy resulting in early embryonic loss within the first 10 days of the gestational period (i.e., before the embryo can be identified with ultrasound) (Kane 2018). There, however, is limited information on the use of IUDs for contraception in mares. Recently, the novel iUPOD was evaluated for contraceptive efficacy in an artificial insemination study (Joone et al. 2021). The results from that study indicated 100% efficacy as a contraceptive when there was artificial insemination. The objective of the present study, therefore, was to evaluate the contraceptive efficacy of the novel iUPOD when mares were maintained with a fertile stallion in a paddock with the use the iUPOD being managed by veterinary professionals with no prior experience with the device.

2. Materials and methods

2.1. Animals

All experimental procedures were approved through the Utah State University Institutional Animal Care and Use Committee. The study included the use of six Quarter Horse-type mares with no known reproductive and/or health problems, ranging from 5 to 19 years of age (median mare age was 13.5 years old) and a 10-year-old Haflinger stallion known to be fertile when breeding mares in a paddock setting. Throughout the period the experiment was

being conducted, the mares and the stallion were residing in a 0.162-hectare dry paddock with a three-sided shelter available. The mares and stallion were provided grass hay *ad libitum* in two large feeders, as well as water and a salt mineral block *ad libitum*. During the period the study was being conducted, there were observations to detect estrous behavior and mating and findings were recorded. Staff were instructed regarding details for detection of symptoms of estrous behavior in horses and there was recording of the symptoms of estrous and mating behaviors when these were detected.

2.2. Experimental protocol

One week prior to the study, all of the mares were examined utilizing transrectal palpation and ultrasonography procedures to assess mare reproductive status. At 7 days subsequent to when these procedures were conducted, there was trans-cervical insertion of the self-assembling iUPOD into all six mares regardless of the stage of estrous cycle. Three mares were randomly assigned to be administered an iUPOD comprised of three individual pods that were each 16 x 38 mm, and the other three mares were administered an iUPOD comprised of pods that were each 16 x 40 mm. The insertion of the iUPOD was performed manually as previously described (Gradil et al. 2019) with the exception that no intrauterine therapy was performed after insertion. Mare preparation included tail wrapping, initial removal of fecal debris (if any) from the perineum with warm water and mild soap, followed by a 5-minute scrub with povidone and there was a subsequent final rinsing with tap water. Sterile sleeves were placed over the arm of the veterinarian and iUPOD devices were inserted through the cervix. The mares were examined utilizing transrectal palpation and ultrasonography procedures immediately after placement of the iUPOD to confirm there was placement in the

uterus. In addition, the mares were examined using a hand-held metal detector (Model PD240CB; CEIA USA, Ltd., Twinsburg, OH, USA) placed against the flank to confirm detection of the iUPOD. The mares were then placed in a paddock with the stallion where all animals remained continuously for 91 days (August 5, 2019 – November 4, 2019). At the completion of the study, each iUPOD was manually removed as previously described (Gradil et al. 2019).

The mares were examined using transrectal palpation and ultrasonography procedures 7 and 14 days after iUPOD insertion, and then every 2 weeks for the duration of the study. The following endpoints were evaluated at each examination: 1) presence and location of the iUPOD, 2) presence of a conceptus, 3) extent of uterine edema (0 = no edema to 3 = prominent edema), 4) presence of intrauterine fluid (subjective amount was graded on the scale of none, trace, moderate, and abundant; and extent of echogenicity was rated on a scale of 1 – 4, 1 being relatively greater echogenicity and 4 being no echogenicity), and 5) ovarian follicular and corpora luteal status. The mares were also examined transabdominally with the metal detector on the same schedule.

Blood collection tubes with no anti-coagulant were used to obtain jugular vein blood samples on the day of iUPOD insertion, and then every Monday, Wednesday, and Friday throughout the time the mares were maintained in the paddock with the stallion. Blood samples were allowed to clot at room temperature before being centrifuged at $300 \times g$ for 20 minutes at 21 °C. Serum was collected from each sample and stored at -20 °C until being analyzed for progesterone concentration.

2.3. Progesterone assay

Serum samples were processed and evaluated for progesterone concentration using a commercial kit (Immulite Progesterone, Siemens, Malvern, PA, USA) according to the manufacturer's instructions. The sensitivity of this assay was 0.2 ng/mL. Intra- and inter-assay coefficients of variation were 4.0% and 4.8%, respectively. The length of diestrus was calculated as the number of days from when the progesterone concentration first increased to a value greater than 1.0 ng/mL until the time when there was a decrease in concentration to less than 1.0 ng/mL.

2.4. Assessment of fertility

Fertility was assessed in all six mares the following breeding season by placing the mares in a paddock with the same stallion for 90 days, during which time transrectal palpation and ultrasonography procedures were performed every 2 weeks to determine pregnancy status. The stallion was again placed with the mares 6 months after the removal of the iUPOD. Mating occurred as evidenced by subsequent pregnancies. A Fishers Exact Test comparing pregnancy rate with and without iUPODs was performed to compare the difference between the two periods when there was a stallion in the paddock with the mares (with and without iUPODs).

3. Results

All the novel intrauterine devices remained in the uterine lumen for the duration of the study, and there was no evidence of the establishment of pregnancy in any of the mares during this period. The novel intrauterine devices remained in the uterine body during 67% of the examinations, and was located in one of the uterine horns at the time of the other examinations. The novel intrauterine device was identified in each mare with the metal detector at every examination regardless of the iUPOD position within the uterus. None of the

mares had any intrauterine fluid present at the time of iUPOD insertion. During the remainder of the study period, however, intrauterine fluid was identified at the time of 77% of the examinations, and quantity varied from a trace amount of non-echogenic fluid to an abundant amount of echogenic fluid. The values for quantity and characterization of uterine fluid in each mare during the period of transrectal palpation and ultrasonography examinations are reported in Table 1. Three mares had intrauterine fluid present at every examination throughout the study period. Vaginal discharge, evidenced by discharge accumulated on tails, was noted on three of the six mares during the course of the study with no scalding being evident. Four mares (Mares B, C, E, and F) had intrauterine fluid present at the time of iUPOD removal, that ranged from a modest to an abundant quantity of echogenic fluid. Samples for microbial testing were obtained from the uterine lumen of the four mares with intrauterine fluid at the time of iUPOD removal and submitted for routine laboratory analysis. *Streptococcus equi subspecies zooepidemicus*, a common uterine pathogen, was identified in all four samples, and a second *Streptococcus* microbial species was identified in samples from three mares that was not deemed to be clinically significant. Other than removing the iUPOD, no therapy was performed in these mares. At the time of removal there was no visual evidence of change in the iUPOD. Transrectal palpation and ultrasonographic examination 2 weeks after the removal of the iUPOD indicated there was no intrauterine fluid in any of the mares.

The values for blood progesterone concentrations for all six mares are depicted individually in Figure 1. In one mare (Fig. 1, Panel A), the progesterone concentration never decreased to less than 1.0 ng/mL during the study period (91 days), so the progesterone data from that mare was not used for the statistical analysis of the duration of diestrus. In another

mare (Fig. 1, Panel B), the progesterone concentration remained >1.0 ng/mL for 37 days after insertion of the iUPOD; therefore, the progesterone data for that period of time was not used for the statistical analysis of the duration of diestrus. For the other mares/estrous cycles, the duration of diestrus was 7.8 ± 2.7 days (mean $\pm$ S.D.).

None of the six mares were detected to be pregnant during the time that the iUPODs were in the uterus. Four of the six mares (mares A, D, E, and F) became pregnant the year after iUPOD removal when the mares were in a paddock with the same stallion utilized when the iUPODs were *in utero* for 90 days. Two of the six mares (mares D and E) were confirmed pregnant 21 days after placement in the paddock with the stallion, and an additional two mares (mares A and F) were confirmed pregnant within 38 days after placement in the paddock with the stallion. A Fishers Exact Test comparing pregnancy rate with and without iUPODs was performed. The probability that the difference between the two periods of breeding (with and without iUPODs) occurred by chance is $P = 0.03$. Two of the pregnant mares were from the group that had a positive uterine culture when the iUPOD was removed. With the exception of Mare A, in which there was prolonged CL function throughout the research period, there was detection of estrous behavior and confirmation of mating between each mare and the stallion documented.

4. Discussion

When there were the breeding conditions utilized in the present study, iUPOD retention and the contraceptive effect were 100%. Due to the small sample size, the statistical analysis utilized was a Fishers Exact Test comparing pregnancy rate with and without iUPODs being *in utero*. The probability that the difference between the two period of breeding (with and

without iUPODs) occurred by chance is $P = 0.03$. This result means that if having iUPODs does not affect mare pregnancy then the chance of the observed 0 out of 6 non-pregnancy is 3%, indicating iUPODs do have an effect on pregnancy occurrence. This is very encouraging for the potential application of iUPODs for fertility control in FRHB. Importantly, the veterinary personnel who performed all of the procedures in this study had no prior experience with the iUPOD system, which is indicative that there could be widespread adoption of the device for fertility control in horses and burros. The contraceptive effect of the iUPOD was 100%, which is consistent with the results reported by Joone et al. (2021). The contraceptive efficacy of the iUPOD was comparable to that previously reported when there was use of a 40-mm silicone O-ring IUD (Daels and Hughs 1995; Baldrighi et al. 2017); and appeared to be efficacious than when two human IUDs and a 25-mm silicone/copper IUD (Killian et al. 2008) were utilized in mares when there were natural breeding conditions.

Two mares in the present study had a prolonged CL function for 37 and 91 days immediately after insertion of the iUPOD. For the other estrous cycles, the average duration of diestrus was approximately 8 days. Although a contemporary control group of mares was not available for direct comparison, in a prior study with a similar group of mares that were estrous cyclic without any reproductive manipulation, the average duration of diestrus was 14.7 days (± 1.1 S.D.; unpublished data). It, therefore, appears that the presence of the iUPOD and breeding resulted in an abbreviated duration of diestrus, which was likely due to premature release of endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) due to endometritis. Notably, this is in contrast to results from previous studies with the iUPOD in estrous cycling mares that were not placed in a paddock with a fertile stallion where the average duration of diestrus was 73.4 days and 51.3

days when the iUPOD was inserted during an induced estrus or randomly during the estrous cycle, respectively (Gradil et al. 2019). There were two primary differences between the present study and the one reported by Gradil et al. (2019): 1) whether or not breeding occurred and 2) the size of the devices used, 12 x 26 mm in the previous study, compared to 16 x 38 and 16 x 40 mm in the present study. It seems likely the occurrence of breeding was an important contributing factor to the apparent shortening of diestrus in the present study, and it is plausible that the presence of the iUPOD in the uterine lumen predisposed the mares to the development of persistent mating-induced endometritis (PMIE), which occurs when there is delayed clearance of inflammatory material from the uterine lumen after breeding (Canisso et al., 2020; Leblanc 2010). A potential outcome of PMIE is spontaneous “short-estrous cycling” due to release of endometrial PGF_{2α} that is associated with the extended duration of the uterine inflammatory response (Canisso et al., 2020). The presence of free intrauterine fluid at 77% of the reproductive examinations during the study period is consistent with the occurrence of PMIE, particularly because the amount of fluid present was greater than 2.0 cm 86% of the time, which is an indicator of susceptibility to PMIE (Leblanc 2010). None of the mares in the present study were known to be susceptible to PMIE, so it seems likely that the presence of the iUPOD in the uterine lumen was the primary factor contributing to this occurrence.

Furthermore, when PMIE fails to resolve (spontaneously or with treatment), it typically leads to the development of infectious endometritis (Leblanc and Causey 2009), which is consistent with the positive culture results in the four mares with echogenic uterine fluid present at the time of iUPOD removal. Removal of the iUPOD was deemed likely to be sufficient to allow the mares to resolve the uterine infection spontaneously, which is supported by the absence of free uterine

fluid 2 weeks later (Leblanc 2010), as well as the fact that two of the mares became pregnant when placed in a paddock with a fertile stallion. The development of PMIE may have been in part due to the iUPOD and gravity changing the position of the uterus. Further studies are required to determine what size of iUPOD is most effective. Larger iUPODs, such as the device used in the present study, resulted in 100% retention in the mare but may have contributed to PMIE and spontaneous “short-cycling”. The utilization of smaller iUPODs may not lead to PMIE and spontaneous “short-estrous cycling”.

The presence of the iUPOD in the uterine lumen for 3 months did not appear to adversely affect subsequent fertility, because four of the six mares became pregnant the year after removal of the iUPOD. This is consistent with the previous findings of Gradil et al. (2019) where there was a 100% pregnancy rate in eight mares that were mated by a stallion the year after removal of the iUPOD. Similar to the present study, the mares in the Gradil et al. (2019) study had maintenance of an iUPOD in the uterine lumen for 3 months. Further research will be needed to assess the fertility of mares that have maintained an iUPOD for longer periods of time.

Indeed, the limited number of mares evaluated in the present study is not adequate to establish long term efficacy of IUDs on fertility control in feral horses. The results, however, are considered to establish the proof of principle for further evaluations in future studies for longer periods and with larger numbers of horses. With a contraceptive efficacy of 100%, shortened or prolonged diestrus and IUF associated with the devices, it is likely that the contraceptive effect includes interference with sperm viability and embryo integrity as well as a negative effect on embryo implantation.

5. Conclusion

In conclusion, placement of an iUPOD in the uterine lumen of six mares resulted in a complete contraceptive effect for 3 months during the period when mares in a paddock with a fertile stallion. The contraceptive effect was reversible as indicated by the pregnancy occurrence in four of the six mares after removal of the iUPOD. Further assessment of the contraceptive effect of the iUPOD, however, is warranted because there is a report documenting the establishment of pregnancy in a mare when there was a glass ball IUD in the uterus of a mare (Grady 2015), so it will be important to determine whether that adverse outcome can occur in a mare when there is an iUPOD *in utero*.

The novel intrauterine device used in this study could be used to facilitate population management of FRHB. Female FRHB that are captured and determined to be non-pregnant can have an iUPOD inserted immediately. The FRHB that are determined to be pregnant may be isolated from stallions until after they have foaled, then the iUPOD can be inserted. This device could also be used in combination with other population management procedures such as PZP vaccination, which could be used to prevent pregnancy in the short term, allowing the insertion of an iUPOD for a longer lasting contraceptive effect. The prime target could be the fillies 1 to 2 years old, because these animals have a lesser likelihood of already being pregnant when reproductive management procedures are being implemented.

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Table 1 Uterine fluid detected when transrectal palpation and ultrasonography examinations were conducted in six female horses implanted with a novel self-assembling three-part polymer-coated magnetic intrauterine devices (iUPODs) that were in paddocks with a fertile stallion for 91 days.

	Mare A	Mare B	Mare C	Mare D	Mare E	Mare F
Day	Age 19	Age 13	Age ~15	Age 5	Age 14	Age 13
0	0	0	0	0	0	0
7	M4	M2	A1	A3	M3	T1
14	M3	M2	A1	M2	M2	M3
29	M3	M1	A1	0	0	M3
42	M4	T4	A1	0	0	M1
56	T1	A2	A1	0	0	T2
70	T4	M1	A1	0	M2	M2
84	0	M1	A2	0	M2	M3
91	0	M2	A2	0	M2	M3

Uterine fluid amount was categorized by amount and echogenicity.

A = Abundant M = Moderate T = Trace 0 = No fluid present

1 = Relatively greater echogenicity 2 = Moderate echogenicity 3 = Relatively lesser echogenicity 4 = No echogenicity

The presence of free intrauterine fluid at 77% of the reproductive examinations during the study period is consistent with the occurrence of persistent mating-induced endometritis.

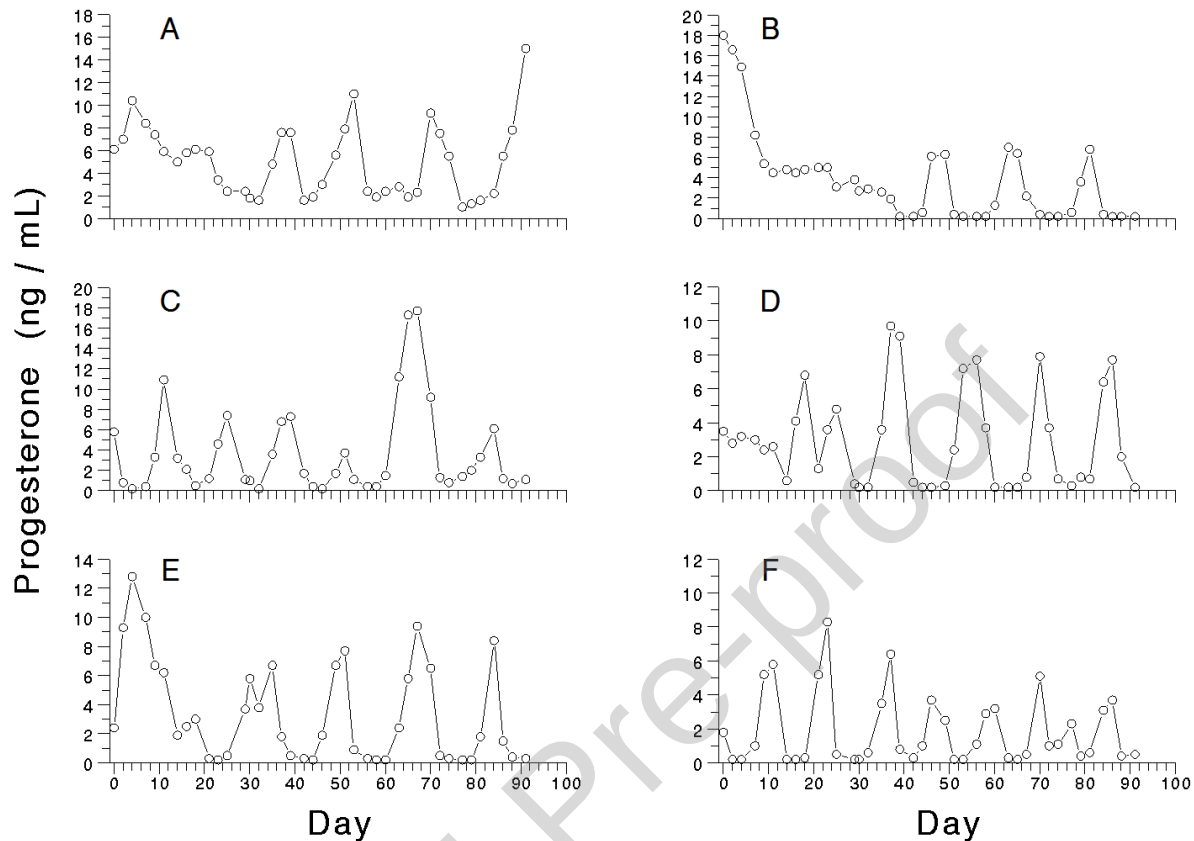


Fig. 1. Serum progesterone concentration in six female horses implanted with a polymer-coated magnetic intrauterine devices (iUPODs) that were in a paddock with a fertile stallion for 91 days. The length of diestrus was calculated as the number of days from when the progesterone concentration increased to a value greater than 1.0 ng/mL to when it subsequently decreased to a value of less than 1.0 ng/mL.

Immediately after insertion of an iUPOD, Mare B and Mare A had prolonged corpus luteum function for 37 and 91 days, respectively. For the other estrous cycles, the average duration of diestrus was approximately 7.8 ± 2.7 days (mean $\pm$ S.D.).

Conflict of Interest

Dr. Carlo Gradil's employer, the University of Massachusetts has applied for a patent on the iUPOD.

Highlights

- A self-assembling 3-part polymer-coated magnetic IUD (Upod) is an effective contraceptive under pasture breeding conditions.
- Veterinary professionals with no prior experience with the Upod IUDs can successfully manage their use in equines.
- Upod IUDs can be successfully removed from mares and breeding sounds can return.

ORIGINAL ARTICLE

The contraceptive efficacy of a self-assembling intra-uterine device in domestic mares

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Objective The primary aim of this study was to investigate the contraceptive efficacy of a self-assembling uterine device (iUPOD™) in the mare. In addition, the effects of iUPODs on oestrous cyclicity, uterine health and circulating concentrations of cortisol were evaluated.

Methods Domestic mares underwent oestrous monitoring and artificial insemination. After subsequent ovulation, mares underwent either placement (n = 7) or sham placement (n = 7; controls) of an iUPOD device. Devices were left in place for at least 3 months. Pregnancy diagnoses were carried out 14 days post-ovulation, with any pregnancies terminated at 28 days post-ovulation. All mares underwent weekly blood sampling with or without reproductive examinations throughout the study. Towards the end of the study, multiple serum samples collected over three consecutive days were analysed for concentrations of cortisol. Endometrial biopsies were collected before artificial insemination and during the subsequent breeding season. Endometrial cytology and bacterial cultures were performed before device removal (iUPOD mares) or at the end of the study (control mares).

Results Pregnancies were diagnosed in 0 of 7 iUPOD mares versus 7 of 7 control mares. Placement of iUPODs was associated with extended luteal phases and variable accumulations of intra-uterine fluid. Bacterial culture results suggested that the mild endometritis associated with iUPODs was sterile in six of seven mares. Short-term placement of iUPODs had no detrimental effects on endometrial architecture. Mean serum cortisol concentrations were significantly lower in iUPOD mares than control mares.

Conclusion iUPODs represent a promising means of fertility control in the mare.

Keywords cortisol; endometritis; fertility control; horse; immunocontraception

Abbreviations AI, artificial insemination; GnRH, gonadotrophin-releasing hormone; IUD, intra-uterine device; PGF2α, prostaglandin-F2α; PZP, porcine zona pellucida

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There is a growing need for a humane but practical means of population control of feral horses in Australia, where an anecdotally estimated one million brumbies compete with livestock for grazing and water supplies and disrupt native ecosystems.¹ Most populations of feral horses in Australia are shy of human contact and are dispersed over extensive, often inaccessible, regions.² This limits the practicality of fertility control tools such as immunocontraception, where repeated treatments are invariably required. Currently, the porcine zona pellucida (PZP) and gonadotrophin-releasing hormone (GnRH) vaccines are most effective when administered as two treatments approximately 5 weeks apart, followed by annual boosters.^{3,4} A long-acting, single-shot formulation of PZP (SpayVac), when delivered by hand injection, maintained infertility in approximately half of treated mares over a 3-year period.⁵ Similarly, GonaCon-Equine, a single-shot formulation of the anti-GnRH vaccine, produced equivocal results in the first 4 years after treatment, although results after a booster in the fourth year showed promise.⁶ Nevertheless, major drawbacks of immunocontraception as a means of population control in feral horses remain as follows: (1) treated horses must be individually identified and retraced to maintain the vaccination schedule, (2) current long-acting formulations must be hand-injected, and (3) learned aversion may add to the difficulties of repeat treatments.²

Intra-uterine devices (IUDs) are the contraceptive of choice for over 150 million women worldwide.⁷ Considered safe, reliable and cost-effective, IUDs in women maintain contraceptive efficacy for up to 12 years; a mechanical contraceptive effect is likely to persist for even longer.⁸ Recently, a self-assembling intra-uterine device (iUPOD) was developed for use in mares.⁹ Although the delivery of these devices to feral mares will be an important practical hurdle, a major advantage is the duration of contraceptive efficacy – which may be indefinite – after a single intervention.

The aims of this study were to evaluate the contraceptive efficacy of iUPODs in domestic mares and their effect on oestrous cyclicity, uterine health and concentrations of cortisol in serum.

Materials and methods

The study was approved by the Animal Ethics Committee, James Cook University (A2582).

Mare management and study design

Seven Standardbred and seven Thoroughbred mares, aged between 10 and 17 years, were studied from October 2018 to March 2019 during the physiological breeding season in the southern

hemisphere. Only clinically normal, non-pregnant mares were selected. Mares were housed in outdoor camps with free access to water, *Chloris gayana* (Rhodes grass) hay and a mineral lick. Mares were stratified by breed and age and randomly assigned to either the control (n = 7) or iUPOD (n = 7) group.

The design and structure of the iUPOD has been previously described.⁹ Briefly, iUPODs consist of three elliptically shaped units (40 × 16 mm), each composed of a magnetic core coated in an inert polymer. Once in free contact, the three units naturally assemble into a ring formation. The shape of each unit facilitates its insertion and retrieval through a mare's cervix, whereas the ring formation aims to prevent expulsion of the device from the uterus after placement.

Initial oestrous monitoring and artificial insemination

Trans-rectal palpation and ultrasonography (M7Vet with 5–8 MHz linear rectal transducer; Mindray, Shenzhen, China) of the reproductive tract was conducted on mares every 3–4 days when mares were in dioestrus and daily when mares were in oestrus. Ovulation was induced by the administration of 1500 IU human chorionic gonadotrophin (Chorulon; MSD Animal Health, NSW, Aust) once the dominant follicle reached a size of ≥35 mm in the presence of maximal or decreasing endometrial oedema. On the same day, mares were artificially inseminated with at least 500 million progressively motile spermatozoa from a Thoroughbred stallion of proven fertility. Semen was extended at a ratio of one part semen to one part extender (Equiplus; Minitube, VIC, Aust) and inseminated according to standard practices.

Placement and sham placement of iUPODs

After artificial insemination (AI), mares underwent successive reproductive examinations to identify the day of ovulation, designated as D0 and defined as a newly identified corpus haemorrhagicum correlated with the absence of a dominant follicle recorded at the previous examination. An iUPOD was placed (iUPOD group) or sham placed (control group) on D0. The maximum interval between D0 and the preceding examination was 72 h.

An iUPOD was placed by manually passing each unit through the cervix individually, under aseptic technique. Sham placement aimed to mimic the placement of iUPODs; the index finger was briefly passed into the cervix three times, under aseptic technique. After placement or sham placement of a device, 2 g of ampicillin (Austrapen, Alphapharm Pty Ltd, QLD, Aust) in 10 mL of sterile water was instilled into the uterus via an insemination pipette.

Ongoing monitoring of oestrous cyclicity

Mares underwent reproductive examinations on D3 and D7 to monitor initial uterine response and device retention. Pregnancy diagnoses were performed at D14. Mares diagnosed as pregnant were re-examined weekly until D28, at which point pregnancy was terminated by the administration of prostaglandinF2α (PGF2α; 375 µg cloprostenol im; Juramate, Jurox Animal Health, NSW, Aust). Non-pregnant mares underwent weekly or bi-weekly monitoring, involving either reproductive examinations and blood sampling or blood sampling only, until the end of the study period. Variables recorded included the presence of follicles, corpora lutea, iUPODs and intra-

uterine fluid, as well as the degree of endometrial oedema. Follicles estimated to be ≥30 mm in diameter were measured using the electronic calliper function of the ultrasound machine, taking the average of two perpendicular measurements where one of these measurements represented the maximal diameter of the follicle. The maximum dorsoventral depth of any intra-uterine fluid was also recorded. Ovulations were identified based on the replacement of a previously identified dominant follicle by a corpus luteum over successive reproductive examinations.

Blood sampling for progesterone and cortisol monitoring

Blood samples were collected from all mares by jugular venipuncture at approximately 7-day intervals, starting on D0 post-breeding and aligning with subsequent ovulations as far as possible. Samples were allowed to clot, centrifuged and serum decanted for storage at –20°C until assayed for concentrations of progesterone.

To assess the effect of iUPODs on concentrations of cortisol in serum, three blood samples per day (early morning, noon, late afternoon) were collected from all mares for 3 days (26–28 February 2019). Samples were allowed to clot, centrifuged and serum decanted for storage at –20°C until assayed for cortisol concentration. The most central sample (noon, day two of three) was additionally assayed for the concentration of progesterone in serum.

Serum progesterone and cortisol assays

Serum concentrations of progesterone were determined using chemiluminescence immunoassay (Immulite 1000, Siemens Healthcare Pty Ltd, VIC, Aust), performed according to the manufacturer's recommendations. All samples drawn before the cortisol study were analysed for progesterone in a single batch, for which the intra-assay coefficient of variation, determined by running a single mid-range sample 10 times consecutively within the same batch, was 5.1%. The limit of detection of the assay was 0.64 nmol/L. Serum concentrations of cortisol were similarly determined using chemiluminescence immunoassay (Immulite 1000), performed according to the manufacturer's recommendations and run in a single batch. The intra-assay coefficient of variation, determined by running a single sample 10 times consecutively within the same batch, was 6.1%. The limit of detection was 5.52 nmol/L.

Device removal

At the conclusion of the study (March 2019), all iUPODs were manually retrieved during natural or induced oestrus using a magnet attached to a nylon thread, with adherence to aseptic technique.

Endometrial cytology and biopsy

Guarded endometrial swabs were used to obtain a sample for endometrial cytology in all mares before placement or sham placement of iUPODs once mares were identified as being in early oestrus. Endometrial cytology was repeated at the end of the study period in control mares and immediately before iUPOD removal in iUPOD mares. An endometrial cytology smear was considered positive for endometritis when ≥0.5% of cells were polymorphonuclear cells; a minimum of 200 cells was counted.¹⁰

Guarded swabs were similarly used to obtain a sample of the endometrium for bacterial culture at the end of the study period in control mares and immediately before iUPOD removal in iUPOD mares. In iUPOD mares, aseptically-retrieved iUPODs were also swabbed for microbiological analyses.

Endometrial biopsies were obtained from all mares before placement or sham placement of iUPODs, during early oestrus. A second biopsy was obtained from all mares during the subsequent breeding season (December 2019). Samples were fixed in Bouin's solution for 24–48 h followed by storage in 70% ethanol. De-identified samples were graded, in a single batch, according to Kenney–Doig classification.

Microbiological analyses

Endometrial and iUPOD swabs were dilution streaked out onto Columbia agar (Code 7734, Acumedia, Neogen, MI, USA) enriched with 7% sheep blood and onto MacConkey agar without crystal violet (7236, Acumedia) and incubated in 5% CO₂ in air. They were also dilution streaked onto pre-reduced Wilkins-Chalgren anaerobic agar (AM217, Amyl, Biolone, Aust) and placed in anaerobic conditions using an anaerobic gas generator (AnaeroGenTMCompact, AN0020D, ThermoScientific, Basingstoke, UK). A metronidazole-impregnated disk (CT0466B, Oxoid, Basingstoke, UK) was placed on the primary streak to check for susceptible obligate anaerobic bacteria. All agar plates were incubated at 37°C for up to 4 days.

The abundance of each colony type on blood agars was graded with a score from 0 to 4. The scoring was qualitative, as follows: 0 indicated 1–5 colonies; 1 was >5 colonies, but with only growth on the primary streak line; 2 was given where there was abundant growth on the primary streak line, with some colonies present on the second streak line; 3 indicated abundant growth on both the primary and secondary streak lines, with some colonies on the third set of streak lines; and 4 was allocated to cultures that had abundant growth on the primary, second and third streak lines and a variable amount of colonies on the most diluted lines.

For purification, representative colonies were dilution streaked onto fresh Columbia or Wilkins-Chalgren blood agars. The purified colonies were subsequently identified using a set of preliminary identification tests, including Gram's stain (212525, Becton Dickinson, MD, USA), catalase (3% hydrogen peroxide, 0144001/5, Biotech Pharmaceuticals, Victoria, Aust) and oxidase (BactidropTM, 21540, Remel, Kent, UK) tests. Depending on the initial results, the bacteria were identified fully using either Gen III MicroPlate™ and AN MicroPlate™ microarrays (1030 and 1007, Biolog, Haywood, NC, USA), API®20E or API®20NE (20100 and 20050, BioMérieux, Marcy-l'Étoile, France).

Data analysis

Categorical data were compared among treatment groups using Fisher's exact test. Continuous data were assessed for normality through the calculation of descriptive statistics, the plotting of histograms and the Shapiro–Wilk test. Non-normal data were compared among treatment groups using the Mann–Whitney *U*-test. Data satisfying the normality assumption were compared among treatment groups using the independent samples *t*-test. Pearson's correlation was used

to assess potential correlations between continuous variables. Data analyses were performed using commercially available statistical software (IBM SPSS Statistics Version 25, International Business Machines Corp., Armonk, NY, USA), with significance set at $P \leq 0.05$.

Results

Pregnancy rate and oestrous cyclicity

All seven control mares (100%) were pregnant after AI and sham IUD-placement. In one control mare, a single embryo was found to be fixed within the uterine body at D14 and D21 and had undergone spontaneous demise by D28. This mare failed to ovulate for the remainder of the study. All other control mares maintained their pregnancies until treatment with prostaglandin-F2 α at approximately D28. Of these, four showed apparently normal oestrous cyclicity, characterised by ovulations every 18–24 days, for the duration of the study. One mare developed persistent corpora lutea and failed to show oestrus for the remainder of the study. The final mare showed anoestrus, characterised by bilaterally small, inactive ovaries and baseline serum concentrations of progesterone (<0.64 nmol/L), for at least 24 days after pregnancy termination, followed by a spontaneous resumption of oestrous cyclicity.

In contrast, none of the seven mares in the iUPOD group (0%) were diagnosed as pregnant at D14 after AI and IUD placement. All mares in this group had extended inter-ovulatory periods (median 52.5 days; range 36–91 days; Figure 1). One mare developed marked endometrial oedema and ovulated approximately 70 days after placement of the IUD, despite serum concentrations of progesterone being above 10 nmol/L at the time of first detection of this ovulation and also at a sampling point 4 days previously. All iUPODs were successfully retained until they were deliberately removed.

Uterine response to iUPODs

After placement, all iUPOD-treated mares showed an accumulation of intra-uterine fluid, which appeared anechoic with hyperechoic specks on trans-rectal ultrasonography. Fluid was invariably accumulated in a pocket around the iUPOD but was also occasionally found at sites far from the iUPOD. Fluid accumulation often improved markedly in line with oestrus and ovulation (Figure 1).

The results of pre- and post-treatment endometrial cytology and culture are shown in Table 1. Bacterial growth, consisting of predominantly mixed populations, was obtained from all iUPODs post-retrieval. Cultures of endometrial swabs demonstrated bacterial growth in seven of 14 mares, of which four were control mares and three were iUPOD mares. Excluding bacterial growth graded as $\leq 1+$, clinically relevant bacterial growth was obtained from four of 14 mares, of which three were control mares and one was an iUPOD mare. The species of bacteria cultured from iUPODs differed from that cultured from endometrial swabs in all mares except one.

Effect of iUPODs on endometrial architecture

Changes in endometrial grading over time were not related to the presence of iUPODs ($U = 21$, $z = -0.515$, $P = 0.71$; Table 2).



Figure 1. Graphs showing serum progesterone concentrations (nmol/L) as a blue line and the maximal dorsoventral depth of any intra-uterine fluid (cm) as a red line and area, over time for each iUPOD mare ($n = 7$) and one control mare (Sophie). Week 0 represents the time of placement or sham placement of iUPOD devices. Asterisks indicate reproductive findings consistent with oestrus, characterised by the presence of endometrial oedema and follicular development, with ovulation identified at the successive examination.

Effect of iUPODs on serum cortisol levels

Mean serum concentrations of cortisol measured over a 3-day period were lower in iUPOD mares than in control mares ($P = 0.03$; Figure 2A). In order to investigate whether this finding was related to stage of the oestrous cycle, progesterone concentrations of the

most central serum sample (noon, day two of three) were determined. There was no correlation between serum progesterone levels at this timepoint and mean serum cortisol levels over the 3-day period (Pearson's correlation coefficient, $r = 0.056$, $P = 0.85$; Figure 2B).

Table 1. Results of endometrial cytology taken before placement (iUPOD group, n = 7) or sham placement (control group, n = 7) of iUPODs and results of endometrial cytology and culture of endometrial swabs and swabs of retrieved iUPODs performed at the end of the study

Mare	Group	Pretreatment		Post-treatment	
		Endometrial cytology	Endometrial cytology	Culture of endometrial swab ^a	Culture of iUPOD ^a
Sox	Control	Negative	Negative	No growth	-
Paddington	Control	Negative	Negative	1+ <i>Routella terrigena</i> 1+ <i>Bacillus</i> species	-
Embers	Control	Negative	Positive 1+ bacteria	5+ <i>Acinetobacter towneri</i>	-
Sophie	Control	Negative	Negative	No growth	-
Ribbon	Control	Negative	Negative	No growth	-
Florence	Control	Negative	Positive 1+ bacteria	1+ <i>Bacillus</i> sp. 1+ <i>Enterococcus</i> sp. 3+ <i>Escherichia coli</i> 1+ yeast	-
Little Georgette	Control	Negative	Negative	1+ <i>Corynebacterium pseudotuberculosis</i> 1+ <i>Staphylococcus coagulase negative</i> 2+ viridans group <i>Streptococcus</i>	-
Detroit	iUPOD	Negative	Positive: Inactive neutrophils, macrophages, no bacteria	1+ <i>Acinetobacter towneri</i>	1+ <i>Streptococcus</i> non-groupable
Qewy	iUPOD	Negative	Positive: Inactive neutrophils, macrophages, no bacteria	No growth	1+ <i>Corynebacterium pseudotuberculosis</i> 1+ <i>Acinetobacter towneri</i> 1+ <i>Streptococcus</i> non-groupable
Express	iUPOD	Negative	Positive: Inactive neutrophils, no bacteria	No growth	1+ <i>Bacillus</i> sp. 1+ <i>Streptococcus</i> non-groupable 1+ <i>Escherichia coli</i>
Violet	iUPOD	Negative	Negative	1+ mould 3+ <i>Bacillus</i> sp.	1+ <i>Streptococcus</i> non-groupable 1+ <i>Acinetobacter towneri</i> 1+ <i>Staphylococcus coagulase negative</i>
Santiago	iUPOD	Negative	Positive: Inactive neutrophils, no bacteria	1+ <i>Acinetobacter towneri</i>	3+ <i>Acinetobacter towneri</i> 1+ <i>Bacillus</i> sp.
Danielle	iUPOD	Negative	Negative	No growth	2+ <i>Bacillus</i> sp. 1+ <i>Corynebacterium pseudotuberculosis</i> 1+ <i>Acinetobacter towneri</i>
Anne	iUPOD	Negative	Positive: Inactive neutrophils, macrophages, no bacteria	No growth	1+ <i>Corynebacterium pseudotuberculosis</i> 1+ <i>Staphylococcus coagulase negative</i> 1+ <i>Escherichia coli</i>

^a ≤1+ considered non-clinically relevant growth.

Discussion

The iUPOD was found to prevent the establishment of pregnancy in all seven treated mares when placed during a single breeding attempt. Although further study using larger sample sizes is warranted, these results suggest that the iUPOD is effective as a contraceptive in this species. Importantly, all iUPODs were successfully retained *in utero* until deliberately removed. These results confirm a previous report of a 100% retention rate for iUPODs in domestic mares.⁹ Failure of device retention has previously been implicated as the primary reason for contraceptive failure of IUDs in mares.¹¹

In the current study, the presence of iUPODs lengthened the luteal phase in all mares. This result is again consistent with a previous report assessing iUPODs in domestic mares.⁹ In practice, it is generally accepted that IUDs deliver an improvement in behaviour and performance for a significant proportion of treated mares by suppressing oestrus, despite unclear clinical evidence of this effect. An initial report found that a 35-mm glass marble extended dioestrus to a mean length of 88.8 days in 42% of treated mares.¹² A follow-up study found that approximately 40% of treated mares demonstrated at least one extended luteal phase; however, extended dioestrus periods were recorded in only 11% of oestrous cycles in treated mares versus 8% of cycles in control mares.¹³ Argo and

Table 2. Results of blinded histopathological evaluations of biopsy samples taken pretreatment (before placement [iUPOD group] or sham placement [control group] of iUPODs) and post-treatment (after removal of iUPODs, during the subsequent breeding season [iUPOD group] or during the subsequent breeding season [control group]). There were no biopsy samples classified at Grade IIB or higher

Kenney–Doig classification	iUPOD group (n = 7)		Control group (n = 7)	
	Pretreatment	Post-treatment ^a	Pretreatment	Post-treatment ^a
Grade I	7	3	7	4
Grade IIA	0	4	0	3

^aThere was no significant association between treatment group and Kenney–Doig classification post-treatment, as assessed by Fisher's exact test ($P = 1.0$).

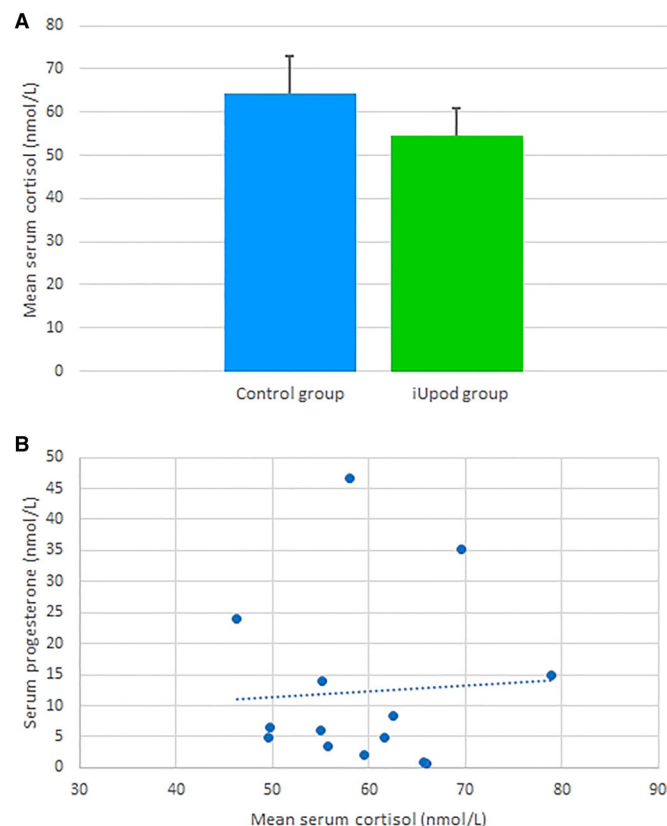


Figure 2. (A) Graph showing mean serum cortisol concentrations (nmol/L) in control and iUPOD group mares, derived from a total of nine blood samples per mare drawn in the early morning, at noon and the late afternoon over three consecutive days towards the end of the study. Error bars represent SD. (B) Scatter chart showing the negligible correlation between mean serum cortisol concentration (nmol/L), derived from a total of nine blood samples (drawn in the early morning, noon and late afternoon for three consecutive days), and serum progesterone concentration (nmol/L), derived from a single sample drawn at noon on the second day of sampling for cortisol analysis, for each mare (Pearson correlation coefficient, $r = 0.056$, $P = 0.85$).

Turnbull¹⁴ found that 35-mm glass IUDs failed to have any discernible effect on oestrous cyclicity in pony mares. In contrast, a 20-mm, water-filled plastic ball inserted 2–4 days after ovulation extended the luteal phase in 75% of mares.¹⁵ Hypotheses regarding the extended luteal phases associated with IUDs in some mares have been advanced. These include a mimicking of the maternal recognition of pregnancy induced by the mobile equine conceptus and a chronic uterine inflammatory response, leading to either absent or continuous but ineffective PGF2 α release and subsequent failure of luteolysis.^{13–15} In mares showing extended luteal phases after placement of an IUD, PGF2 α metabolite levels remained basal from 14 days post-ovulation, supporting the hypothesis that the presence of an IUD inhibits PGF2 α release from the endometrium.¹⁵ Interestingly, oestrous cycles without an extended luteal phase may be significantly shorter in IUD-treated mares in comparison with control mares,^{13,15} reminiscent of the shortened luteal phases associated with endometritis in the mare.¹⁶

In women, the possible mechanisms of the action of IUDs have been classified according to whether they take their effect before or after fertilisation, particularly as some women may object to post-fertilisation effects.¹⁷ Potential pre-fertilisation effects include interference with sperm transport through the cervix, uterus and uterine tubes and interference with oocyte transport or oocyte integrity within the uterine tubes. Potential post-fertilisation effects include an interference with transport or integrity of an embryo within the uterine tubes and prevention of implantation.¹⁷ In the current study, insemination occurred before iUPOD placement. Assuming that, for the majority of iUPOD mares, fertilisation was essentially complete before placement of an iUPOD, and assuming that rates of fertilisation in control and iUPOD mares were similar, this study supports the post-fertilisation contraceptive effect of iUPODs. Given the extent of intra-luminal fluid associated with the presence of these devices, it is likely that this contraceptive effect involves an interference with embryonic development and/or maintenance. Most studies of IUDs in mares report minimal uterine fluid accumulation in response to the IUD.^{12,13,15} Similarly, other investigators of iUPODs report negligible to mild uterine responses to the devices when used in primarily young, maiden mares (D Hanlon personal communication). In contrast, mares used in the current study were all aged ≥ 10 years and were retired embryo transfer recipient mares with uncertain reproductive histories. Although all mares were reproductively normal at the start of the study, it is possible that at least some may have been predisposed to intra-uterine fluid accumulation and endometritis before placement of the iUPODs.

Bacterial and fungal cultures of endometrial swabs taken from iUPOD mares at the time of iUPOD removal demonstrated no growth in four of seven mares, with mixed growth, suggestive of contamination, in one mare. Two mares demonstrated growth of a pure culture of *Acinetobacter towneri*. *A. towneri* is found naturally in activated sewage sludge and plays a role in nitrogen and sulphide removal¹⁸; its source in the current study is unknown. In both mares, growth of this organism was at low levels that were considered unlikely to be clinically relevant. This suggests that the endometritis associated with iUPODs could be considered essentially sterile, in agreement with similar findings in women fitted with IUDs.¹⁹

Interestingly, bacterial culture of swabs from individual iUPODs after their removal revealed mixed populations on six of seven iUPODs. Only one mare showed evidence of potential seeding from the device to the endometrium (Santiago). These results suggest that, for most mares, bacterial contamination of an iUPOD remains isolated to the device. Moreover, bacteria isolated from the devices were mainly common commensal species in mares. As such, a biofilm of these bacteria on an iUPOD may be not only benign but potentially beneficial in preventing the growth of more pathogenic species.

Given the extent of uterine fluid accumulation in mares carrying iUPODs, a study was carried out to assess the impact of iUPODs on cortisol concentrations in serum as an indication of the impact of these devices on mare welfare. Interestingly, circulating concentrations of cortisol in iUPOD mares were significantly lower than those in control mares, and this effect did not appear to be related to a mare's stage within the oestrous cycle. Further research to clarify the significance of this finding is warranted.

Limitations of the current study include the small sample size and the relatively short period of time (3–5 months) during which mares were exposed to the iUPODs. Further research involving larger numbers of mares and placement of iUPODs for longer durations is warranted. Furthermore, the mares in this study had no contact with a stallion and were exposed to only one insemination before iUPOD placement. Studies investigating ovarian and uterine responses to iUPODs in mares exposed to a stallion, in a manner mimicking a feral herd, are warranted. In women, copper is frequently incorporated into IUDs in order to take advantage of the antibacterial²⁰ and spermicidal²¹ effects of copper ions. Further research into the effects of copper-containing iUPODs in mares is also warranted.

Conclusion

The iUPOD, a self-assembling intra-uterine device designed for use in mares, represents a promising means of reversible fertility control in this species. The iUPOD successfully prevented the establishment of potential pregnancies in all seven treated mares. Treated mares showed lengthened luteal phases and a predominantly sterile endometritis in association with iUPODs. Mean serum cortisol concentrations were lower in mares carrying iUPODs than control mares.

Conflicts of interest and sources of funding

C. Gradil is the original inventor of the iUPOD device. The authors declare no sources of funding for the work presented here.

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Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and intrauterine devices

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Abstract. We evaluated the multiyear contraceptive efficacy of the gonadotrophin-releasing hormone (GnRH) vaccine GonaCon, the porcine zona pellucida (PZP) vaccine SpayVac and the human intrauterine device (IUD) 380 Copper ‘T’ in mustang mares provided by the State of Nevada. Eight untreated control mares were compared with 12 mares treated with SpayVac, 16 mares treated with GonaCon and 15 mares treated with the copper-containing IUD. Rates of contraception for Years 1, 2, 3 and 4 respectively for SpayVac were 100% (12 of 12), 83% (10 of 12), 83% (10 of 12) and 83% (10 of 12), rates for GonaCon were 94% (15 of 16), 60% (9 of 15), 60% (9 of 15) and 40% (6 of 15) and rates for IUD-treated mares were 80% (12 of 15), 29% (4 of 14), 14% (2 of 14) and 0% (0 of 14). Antibody titres against PZP and GnRH declined over the four-year study. For mares given SpayVac, uterine oedema was commonly observed. IUDs were visible by ultrasonography in non-pregnant mustang mares, suggesting that pregnant mares did not retain their IUD. IUD retention may be a function of uterine size: pony mares with IUDs had high retention and contraception rates for 4–5 years. We conclude that long-term contraception of mustang mares with a single shot of either the SpayVac or GonaCon vaccine is possible.

Introduction

Overpopulation of wild horses is a significant concern in the western United States (Fisher 1983). In Nevada, where most of the wild horses are located, populations grow at a rate of 15–20% a year on State lands, while their range continues to shrink. Current management strategies of removal and adoption are expensive, logistically challenging, and minimally (if at all) effective in reducing and maintaining wild horse populations at a desired level. Conflicting interests associated with increased movement of people into wild horse ranges, sympathy to maintain wild horse populations because of their historic and cultural importance, competition among horses and indigenous plant and wildlife species, as well as ranching interests, are issues impacted by wild horse overpopulation. Controlling the fertility of free-ranging horses is considered a viable option for population control. However, this approach has many challenges for which solutions have been elusive. Ideally, methods for contraception of wild horses should be safe and potentially reversible, effective for several years, practical to administer and of reasonable cost and have minimal effect on reproductive or harem behaviour. Immunocontraceptive vaccines have garnered considerable attention in recent years as a means to address problems of overabundant wildlife and feral species (Fagerstone *et al.* 2002; Delves and Roitt 2005; Naz *et al.* 2005). Two immunocontraceptive vaccines that have been used in a variety of species and for which data exist on their safety and efficacy are porcine zona pellucida (PZP) vaccine (Kirkpatrick *et al.* 1992, 1995; Miller *et al.* 1999, 2001; Kirkpatrick and Turner 2002; Turner and Kirkpatrick 2002, 2003; Curtis *et al.* 2007) and

gonadotrophin-releasing hormone (GnRH) vaccine (Miller *et al.* 2000, 2004; Killian *et al.* 2006a; Massei *et al.* 2008). Fertility control of mares using existing PZP vaccines has been shown to be safe and effective for up to 10 or more years. However, wild horses vaccinated with PZP preparations have required revaccination every year or two to maintain infertility (Turner *et al.* 2001, 2002). Limited data exist for the use of GnRH vaccines in mares to control fertility, ovarian function or behaviour (Dalin *et al.* 2002; Killian *et al.* 2004, 2006b; Imboden *et al.* 2006; Elhay *et al.* 2007). Information on multiyear efficacy and effects following a single injection of a GnRH vaccine in mares is lacking. Regardless of the contraceptive vaccine considered, most formulations that have been used do not appear to be effective for the long term without revaccination. Revaccination of mustangs involves considerable expense, manpower, and horse handling to maintain infertility. If a single-injection multiyear contraceptive were available it may be possible to achieve effective population reduction and reduce costs and risks associated with frequent horse handling.

We initiated a study in the autumn of 2002 and the spring of 2003 to compare the multiyear contraceptive efficacy of a single-shot contraceptive vaccine directed at GnRH with that of a single-shot vaccine directed at the zona pellucida of the ovum. We selected GonaCon to test as the GnRH vaccine on the basis of our positive experiences with it as a single-injection vaccine in deer and other species (Miller *et al.* 2000, 2004; Miller and Killian 2001; Killian *et al.* 2006c; Fagerstone *et al.* 2008). We selected SpayVac as the PZP vaccine to test in mares on the basis of our unpublished experiences with it as a single-injection vaccine in

white-tailed deer and the reports of multiyear efficacy in harbor seals (Brown *et al.* 1997) and fallow deer (Fraker *et al.* 2002). Both vaccines were administered with AdjuVac, an adjuvant developed at the National Wildlife Research Center. In addition, on the basis of preliminary studies with pony mares, we evaluated the use of an intrauterine contraceptive device (IUD), the 380 copper 'T', which has been shown to be safe and efficacious in humans for multiple years (Fortney *et al.* 1999; Wu *et al.* 2000). Preliminary results of these studies with Nevada mustangs have been reported earlier (Killian *et al.* 2004, 2006b). This paper reports results for these contraceptive approaches after four years of study with Nevada mustangs and five years of observation on the use of IUDs in pony mares.

Materials and methods

Animals

Horses for the study were provided by the State of Nevada and were maintained at the Nevada State Penitentiary, located at Carson City. The studies were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. In total, 51 mares and three stallions were used. The mares weighed 225–360 kg and their ages were estimated to be between 18 months and 12 years of age at the time the treatments were given. All mares except the 18-month-old filly were observed in the wild to foal normal healthy foals.

For jugular blood sampling and vaccinations, the mares were run into a hydraulic chute and haltered. Vaccines were given intramuscularly in the left lateral neck. Mares were chemically restrained for IUD placement and for pregnancy evaluations by ultrasound or palpation. Chemical restraint was achieved with an initial intravenous injection of a mixture of 1 mL Dormosedan (detomidine hydrochloride) 10 mg, 2 mL xylazine 200 mg, and 2 mL acepromazine 20 mg to produce sedation, followed in 5 min with a second intravenous injection of one bottle of Telazol (250 mg Tiletamine base, 250 mg Zolazepam base) resuspended in 3 mL xylazine 300 mg for anaesthesia. This regimen typically gave 15–45 min of anaesthesia.

Ten pony mares and two pony stallions belonging to the Department of Dairy and Animal Science of the Pennsylvania State University were used to develop methods and evaluate the use of IUDs as a means to block fertility. The mares ranged in age from 18 months to 12 years when the treatments were administered. Seven of the mare ponies were pastured with a stallion during the entire five-year study, except when treatments were being administered or when data were collected. They weighed 204–306 kg. Three of the mares were on the study for only one year and were pastured with a stallion for two months during the breeding season. They weighed 281–391 kg.

Ponies were haltered and restrained in a chute for examination and data collection. If sedation was necessary, mares were given intravenous 0.5–1.5 mL xylazine (50–150 mg) and 0.2–0.5 mL butorphanol, or 0.1–0.25 mL Dormosedan (10–25 mg) and 0.2–0.5 mL butorphanol (20–50 mg).

Treatments

For the Nevada study vaccinations were given in March of 2003. Eleven mares were given a single-shot GnRH vaccine containing 1800 µg of GonaCon and four mares were given a single-shot

GnRH vaccine containing 2800 µg GonaCon, 12 mares received a single-shot PZP vaccine containing 400 µg SpayVac and 8 mares were assigned to be untreated controls. Copper-containing 380 'T' IUDs were placed in the uterus of 15 mares transcervically in October of 2002. The SpayVac PZP vaccine was provided Dr Robert Brown (Brown *et al.* 1997), who developed the vaccine. The GonaCon vaccine was provided by the National Wildlife Research Center, USDA-APHIS-WS. Both SpayVac and GonaCon were made into an emulsion with AdjuVac adjuvant (Miller *et al.* 2004) and injected as a 1-mL dose. To evaluate reproductive capacity, treated and control mares were randomly assigned to two breeding groups. 'Band Stallions' that had been observed to sire multiple generations in the wild were selected to be pen stallions. Most mares were maintained in the same pen throughout the study, but if behavioural issues resulted in mares fighting, the less dominant mare was moved to the other pen. Mares were penned with a fertile stallion for a breeding trial typically lasting from June through September of each year. If mares failed to breed, or failed to become pregnant or foal during the breeding season, they were considered infertile.

Ponies were used to evaluate three different types of IUDs for ease of placement, retention and efficacy. The 380 Copper T and the GyneFix IUDs were purchased from Family Planning Sales Limited, Littlemore, Oxford, UK. The ring IUD was fabricated from Silastic tubing (2.5 mm o.d.), which was used to create a ring size of ~2.5 cm. Prior to closing the ring with Silastic cement, five or six small copper cable clamps were threaded over the tubing for inclusion in the ring.

In preliminary studies we attempted direct finger insertion of the IUDs into the uterus of the mare after dilation of the cervix with two fingers. Because this approach was somewhat cumbersome, time consuming and did not easily ensure placement of the IUD deep within the uterine lumen we attempted to use the IUD insertion devices that were supplied with the IUDs that were intended for humans. However, both of these insertion devices were unsatisfactory for the mare uterus. The insertion device for the copper 'T' was too short to traverse the mare vagina and cervix. Likewise, the insertion device for the GyneFix IUD, which is intended to attach the IUD to the uterine endometrium with a monofilament, was also too small for the mare reproductive tract. Consequently, we modified disposable large animal uterine swabs to accommodate the IUDs. The modified swabs enable the successful deposit of the IUDs transcervically into the uterine lumen of sedated ponies or anaesthetised Nevada mares. The insertion device containing the IUD was placed between the two fingers used to dilate the cervix and guided into the uterine lumen where the IUD was discharged. With minimal practice the time needed to clean the perineum, palpate the cervix by hand, and then insert the device was 2–5 min, with actual insertion of the rod and placement of the device requiring ~30–60 s.

For the research trial, all IUDs were placed into the uterus transcervically following dilation of the cervix with one or two fingers. As detailed in the Results, several animals received more than one type of IUD during the course of the study. If a mare became pregnant after receiving an IUD she was either allowed to go to term or, in the case of two mares in which the pregnancy was 60 days or less, the pregnancy was terminated with prostaglandin F2α. Within 2–3 weeks of termination of pregnancy or

parturition, mares were treated with another IUD. As a result, the GyneFix IUD was evaluated in four mares, the copper ring in four mares and the 380 Copper 'T' in seven mares. Two untreated mares served as a control for each breeding season.

Observations

Blood samples were collected from treated Nevada mares once or twice a year. However, the only observations consistently made on the eight control mares were general health, body condition and foaling. In mid- to late-October of each year treated mares were examined by rectal ultrasonography for pregnancy, IUD retention and uterine inflammation. Pregnancy was established by ultrasonography by observance of an embryonic vesicle, a fetus or in the case of later gestation, rectal palpation of a fetus. These observations were later confirmed by birth of a foal. In a few cases where the behaviour of the mare prevented ultrasonography or rectal palpation, pregnancy was determined later by the birth of a foal. General health and body condition, and uterine oedema that may be associated with oestrous cycle changes or presence of an IUD were noted. Uterine oedema in healthy mares is an indication that she is in heat and that she is under the influence of oestrogen produced by ovarian follicles (Sample 1997). All blood samples were assayed for oestradiol, progesterone and antibody titres to the contraceptive vaccines (Miller *et al.* 2000, 2001). One mare receiving the GonaCon vaccine and one mare with an IUD died after the first breeding season of causes not related to the treatments.

From April through November of 2002–05 ponies were gathered and examined by ultrasonography every 4–6 weeks. During the 2004 breeding season, daily observations were also made from April through August on the breeding and harem behaviour of a group of ponies consisting of one stallion, two control mares, one mare with a GyneFix IUD, two mares with the ring IUD and four mares with the 'T' IUD. For 2006, ponies were pastured for the entire year and the only observations made were for general health status and foaling rates. In April of 2007, ultrasonography was performed to check for IUD placement, pregnancy and any contraindications.

Statistical evaluation

Data from hormone assays were subjected to one-way analysis of variance by treatment. Differences between means were detected by two-sided *t*-test. Mean values are reported plus or minus standard error. Regression analyses were used to evaluate changes in antibody titres during the study.

Results

Nevada mustangs

Foaling data for the eight control mares for Years 1–4 were 75% (6 of 8 mares), 75% (6 of 8 mares) 88% (7 of 8 mares) 100% (8 of 8 mares), respectively. Rates of contraception for SpayVac-treated mares for Years 1, 2, 3 and 4 were 100%, 83%, 83% and 83%, respectively (Fig. 1). Average anti-PZP titres for the autumn bleed for mares that were contracepted were sustained considerably above the titres of mares that became pregnant (Fig. 2). Although regression analysis indicated a significant decline of anti-PZP titres for the first three years of the study ($P < 0.05$), average titres tended to increase in the fourth year. Compared with titres in the

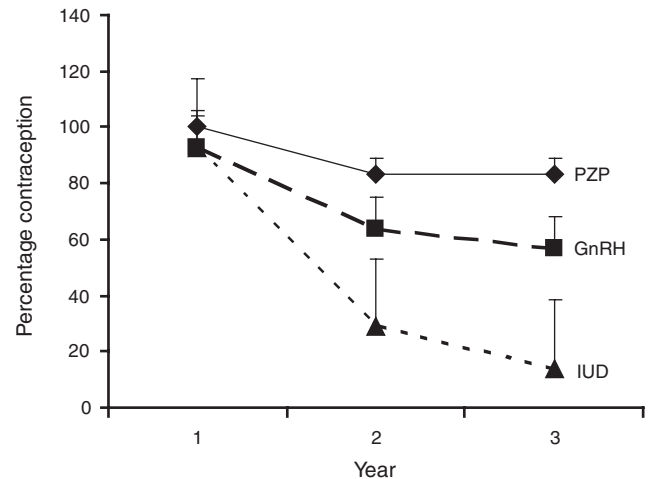


Fig. 1. Comparative rates of contraception for the three methods of contraception for each year of the four-year study. Rates of contraception for SpayVac-treated mares for Years 1, 2, 3 and 4 were 100% (12 of 12 mares), 83% (10 of 12), 83% (10 of 12) and 83% (10 of 12), respectively. Rates of contraception for GonaCon-treated mares were 93% (14 of 15 mares), 64% (9 of 14), 57% (8 of 14) and 43% (6 of 14) for Years 1–4, respectively. Contraception rates for intrauterine device (IUD)-treated mares were 80% (12 of 15 mares), 29% (4 of 14), 14% (2 of 14) and 0% (0 of 14), respectively in Years 1–4 of the study. In Year 2, one mare died in each of the GonaCon- and IUD-treatment groups of causes not related to the treatments.

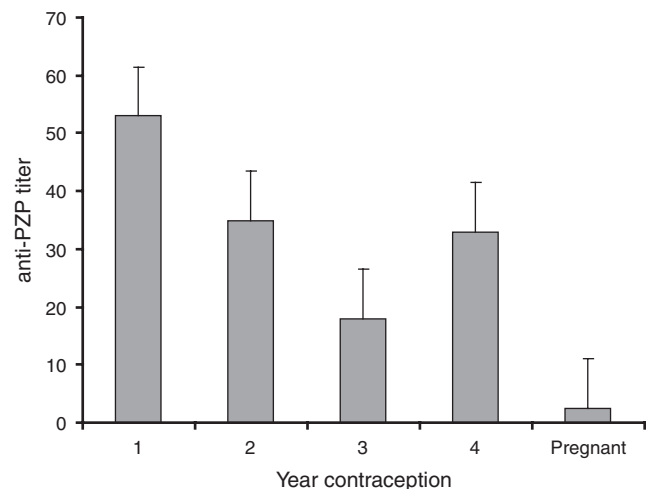


Fig. 2. Average anti-porcine zona pellucida (PZP) titres ($\pm$ s.e.m.) for the autumn bleed for mares that were contracepted compared with titres of mares that became pregnant. The average titres for each of Years 1–4 were for the serum samples for the 10 mares that were infertile. The titre for the pregnant mares was the average for the 2 mares that became pregnant during the study.

third year, individual titres increased in 8 of the 10 contracepted mares, with the remaining 2 mares having titres similar to the titres they had in the third year. For all years, serum progesterone values (ng mL^{-1}) averaged 1.3 ± 0.40 for non-pregnant mares during the October bleed compared with 17.5, the average for two pregnant mares. We were unable to obtain serum oestradiol

values for the two SpayVac-treated mares that became pregnant, but the average serum oestradiol concentrations (ng mL^{-1}) for the cycling females at the autumn bleed was 25.9 ± 2.9 .

Rates of contraception for GonaCon-treated mares were 93%, 64%, 57% and 43% for Years 1–4 (Fig. 1). For contracepted mares, regression analyses indicated that there was significant decline of average antibody titres over the last three years ($P < 0.01$). Nevertheless, anti-GnRH titres of non-fertile mares were significantly greater ($P < 0.01$) than average titres for mares that became pregnant during the study (Fig. 3). Serum progesterone concentrations (ng mL^{-1}) for non-pregnant GonaCon-treated mares at the autumn bleed averaged 0.3 ± 0.1 , which was significantly lower ($P < 0.01$) than progesterone concentrations of pregnant mares treated with GonaCon (7.7 ± 1.5). Average serum oestradiol concentration (pg mL^{-1}) for the non-pregnant GonaCon-treated mares for all years was 32.6 ± 6.7 , which was significantly less ($P < 0.001$) than concentrations detected in pregnant GonaCon-treated mares (1179 ± 225).

Contraception rates for IUD-treated mares were 80%, 29%, 14% and 0%, respectively in Years 1–4 of the study (Fig. 1). Average serum progesterone for all years at the autumn bleed was significantly lower ($P < 0.017$) for non-pregnant IUD-treated mares ($4.3 \pm 1.4 \text{ ng mL}^{-1}$) than for pregnant IUD-treated mares ($11.3 \pm 2.7 \text{ ng mL}^{-1}$). The average serum oestradiol concentration of $62.5 \pm 34.2 \text{ pg mL}^{-1}$ for all years for non-pregnant IUD-treated mares was significantly less ($P < 0.001$) than the oestradiol serum concentrations of IUD-treated mares that became pregnant (1907 ± 505).

Comparing autumn serum progesterone values among treatments for non-pregnant mares, differences between GonaCon- and SpayVac-treated mares were not significant. However, progesterone values in non-pregnant GonaCon-treated mares and SpayVac-treated mares were significantly lower ($P < 0.02$) than values in non-pregnant IUD-treated mares (Fig. 4). In contrast, serum progesterone values at the autumn bleed were lower in pregnant GonaCon-treated mares



Fig. 3. Average anti-gonadotrophin-releasing hormone (GnRH) titres ($\pm$ s.e.m.) for the autumn bleed for mares that were contracepted compared with titres of mares that became pregnant. The sample sizes for titres for Years 1, 2, 3 and 4 were 14, 9, 8 and 6 respectively. The average titre for the pregnant mares was for the 8 mares that became pregnant during the four-year study.

than in pregnant SpayVac- or IUD-treated mares (Fig. 5). These differences were significant between IUD- and GonaCon-treated mares ($P < 0.025$), but because only two SpayVac-treated mares became pregnant, statistical comparisons could not be made with the other treatments. There were no significant differences in serum oestradiol concentrations in non-pregnant mares among treatments ($P = 0.13$) and differences between serum oestradiol concentrations of pregnant IUD-treated and pregnant GonaCon-treated mares were not significant ($P = 0.1$).

Observations made by ultrasonography usually enabled visualisation of IUD location and the presence of uterine oedema or luminal fluid. In most instances IUDs were not observed in mares that were pregnant. When uterine oedema was observed it was recorded by treatment that the mare received (Table 1). These values were compared with the expected incidence of uterine oedema during oestrus based on a normal mare oestrous cycle. We assumed that within a normal 21-day oestrous cycle, 5–7 days of the cycle would be in oestrus, and the remaining 14–16 days the mare would be in dioestrus (Crowell-Davis 2007). Therefore, in a random sample of mares taken from a normal population, ~25–30% would be expected to be in oestrus

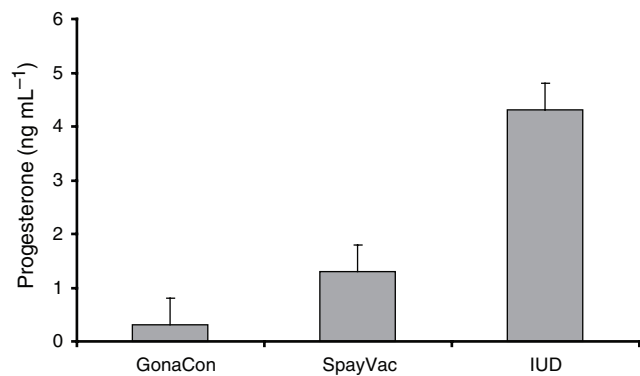


Fig. 4. Average progesterone values (ng mL^{-1}) ($\pm$ s.e.m.) in non-pregnant GonaCon-treated mares ($n = 35$) and SpayVac-treated mares ($n = 46$) and non-pregnant intrauterine device (IUD)-treated mares ($n = 17$) for the autumn bleed for all years of the study. Values for GonaCon- and SpayVac-treated mares were significantly lower ($P < 0.02$) than values for IUD-treated mares.

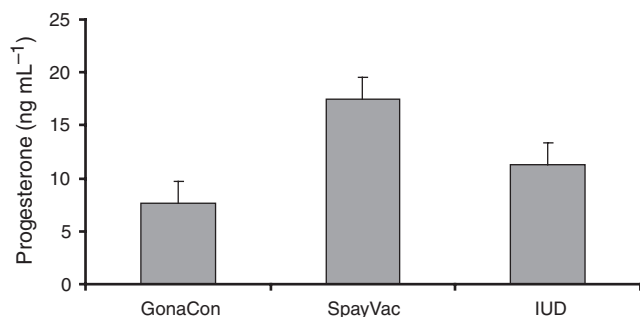


Fig. 5. Average serum progesterone (ng mL^{-1}) at the autumn bleed for pregnant GonaCon-treated-mares ($n = 8$), pregnant SpayVac-treated mares ($n = 2$) and pregnant intrauterine device (IUD)-treated mares ($n = 7$). Values were significantly different between IUD- and GonaCon-treated mares ($P < 0.025$); a comparison could not be run with the SpayVac-treated mares because of only two observations.

Table 1. Percentage of reproductive tracts with oedema revealed by ultrasonography of mares for each of the treatments compared with the predicted number of mares expected to be in oestrus

Numbers in parentheses indicate the number of mares showing uterine oedema out of the total number observed. Not all mares were observed at all time points. If a mare was found to be pregnant in a given year, she was dropped from the trial and not observed in subsequent years. Some mares on the trial could not be evaluated by ultrasonography at a sampling date because their behaviour was unmanageable

Percentage	Year 1	Year 2	Year 3	Year 4
Predicted	25–30%	25–30%	25–30%	25–30%
IUD actual	20% (2 of 10)	0% (0 of 4)	0% (0 of 2)	–
GonCon actual	23% (3 of 13)	25% (2 of 8)	25% (2 of 8)	17% (1 of 6)
SpayVac actual	82% (9 of 11)	91% (10 of 11)	100% (10 of 10)	70% (7 of 10)

and to have oedematous uteri during the breeding season. Non-pregnant mares treated with GonaCon had rates of uterine oedema similar to the expected rate of 25–30% for normal non-pregnant cycling mares (Table 1). Likewise, IUD-treated mares in the first year had rates of uterine oedema similar to the expected rate, although in subsequent years too few non-pregnant mares remained in the IUD-treated group to obtain a reliable estimate. In contrast, SpayVac-treated mares had high rates of uterine oedema during all four years of the study (Table 1).

Ponies

A summary of the IUDs inserted into the pony mares is provided in Table 2. Only one of the four mares successfully retained the GyneFix IUD for a five-year period. Likewise, the copper-containing Sialistic ring was retained in only one mare for at least a year before she was sold. The copper 'T' device provided the greatest rates of contraception and retention, with several mares having the device in place for 3–5 years. We were able to

observe only two of the mares (Table 2: Wanda, Remy) for one year before they were removed from the study.

Regardless of the type of IUD, the usual reason for failure was pregnancy. In most instances, we assumed that the pregnancy resulted from failed retention of the IUD, since we were unable to visualise the IUD by ultrasonography, or to identify it in the afterbirth when observed. In one case, we observed a 60-day pregnancy, but in a subsequent examination six weeks later the mare was not pregnant and the IUD was seen, suggesting that the pregnancy was aborted. In one case, a copper 'T' was removed because of pyometria. The recovery of that mare was unremarkable.

Two mares (Table 2: Libby, Maddie) were tested with all three IUD types. Both mares had the longest contraception rates with the copper 'T'. It is noteworthy that most mares given the copper 'T' IUD remained contracepted for multiple years. However, one mare (Libby) became pregnant within a year, regardless of the IUD type.

Observations made from April through August 2004 on the breeding behaviour of three non-pregnant mature mares equipped with a Copper 'T' IUD (Dewdrop, Maddie, Godiva) indicated that they had four or five oestrous cycles. The one mature mare equipped with a GyneFix IUD (Sprite) had six oestrous cycles and the one filly equipped with a 'T' IUD at 11 months of age had three oestrous cycles. These numbers were within the normal range of oestrous cycles for pony mares at the Penn State University facility.

Table 2. Summary of intrauterine device (IUD) types installed in pony mares, dates of installation, duration of contraception and reason for failure

n.a. = not applicable because the IUD is still intact

IUD type/ mare	Installation date	Date of last observation	Duration of contraception	Reason for failure
GyneFix				
Maddie	24.v.2002	25.vi.2002	30 days	Pregnant
Godiva	24.v.2002	23.vii.2002	60 days	Pregnant
Sprite	24.v.2002	28.iv.2007	5 years	n.a.
Libby (2)	23.ix.2005	23.iv.2006	7 months	Pregnant
Ring				
Maddie	2.vii.2002	23.viii.2002	10 days	Pregnant
Mandy	14.ix.2002	10.xii.2003	>1 year?	No data
Libby	25.v.2004	1.vii.2004	36 days	Pregnant
Libby	9.vii.2004	18.ix.2004	2 months	Pregnant
'T'				
Dewdrop	24.v.2002	1.xi.2006	5 years	Pyometria
Maddie	30.v.2003	28.iv.2007	4 years	n.a.
Godiva	14.vii.2003	28.iv.2007	4 years	n.a.
Libby	24.v.2002	15.v.2003	1 year	Pregnant
Connie	25.v.2004	28.iv.2007	3 years	n.a.
Wanda	4.ix.2002	30.v.2003	>1 year?	No data
Remy	4.ix.2002	30.v.2003	>1 year?	No data

Discussion

As with other species, population management of wild horses presents a specific set of challenges for a contraception method to meet in order for it to be of practical use. Assuming the contraception method does not pose serious problems to the health, behaviour or well being of the animal, two factors of considerable importance for contraceptive application in wild horses are long-term efficacy and whether the approach is easy to use. Wild horses need to be gathered from their range and managed under safe conditions for them to be hand injected with an immunocontraceptive vaccine. This approach has routinely been used for horses in the western United States. However, it is time consuming, expensive and, despite best efforts, not free of risk of injury to the horses or the human handlers. Although remotely darting individuals is possible (Kirkpatrick *et al.* 1990; Turner *et al.* 1996), it is not practical for vaccinating large numbers of horses in the western United States. For example, an internal cost–benefit analysis performed

by the Nevada Department of Agriculture (D. Thain, unpublished) for the Nevada Virginia Range horse-management area, consisting of over 145 000 ha of mountainous arid rangeland, concluded that equine bands did not aggregate in any one area to enable cost-effective darting. Because the Nevada Virginia Range is typical of many horse-management areas in the western United States, we believe that having a single-application contraceptive approach that is effective for multiple years would minimise or remove the need for additional gathers in these areas for revaccination in subsequent years. To date, a single-injection vaccine with multiple years of efficacy has not been available for horses despite considerable efforts of investigators working on the problem (Turner *et al.* 2001, 2002; Liu *et al.* 2005). The present study provides evidence that multiple years of contraceptive efficacy can be achieved with a single-shot immunocontraceptive vaccine in the mustang mare. SpayVac was shown to have the greatest contraception rate. For the four years of study, only two of the 12 SpayVac-treated animals had foals. This rate for a single vaccination far exceeds what has been reported by others for wild horses, although SpayVac has been shown to have long-term efficacy in other species (Brown *et al.* 1997; Fraker *et al.* 2002). Mare contraception with SpayVac was associated with antibody titre, since the two mares that became pregnant had titres much below the average titre of contracepted mares. Although the average titre for the non-pregnant SpayVac-treated mares declined in Years 2 and 3 relative to Year 1, the titres actually increased in the fourth year in most of the contracepted mares. This suggests that self-boosting of the immune response may occur (Perry *et al.* 2006), perhaps as a result of the seasonality of mare reproduction. It is thought that self-boosting occurs in the draining lymph node (Burton *et al.* 1994) as antigen is released from the follicular dendritic cell when the antibody flowing through the draining lymph node drops to a certain level. The released antigen then provides restimulation of antibody production. It is also possible that as the mare returns to breeding condition from a period of anoestrus, new zona pellucida proteins are produced with the initiation of follicle development which restimulate an immune response. If the titre at which mares became pregnant is assumed to be the set point for pregnancy to occur, we would predict that the mares currently contracepted with SpayVac will remain infertile for several more years. It is also possible that some of the mares may remain infertile indefinitely.

Compared with GonaCon and the IUD treatments, mares treated with SpayVac had a greater incidence of uterine oedema than would be predicted for normal cycling mares. Uterine oedema is associated with a predominance of oestradiol (Sample 1997), although there was no significant difference among treatments in oestradiol values for non-pregnant mares. However, expression of uterine oedema during the normal oestrous cycle is also influenced by the absence of serum progesterone (Crowell-Davis 2007). Relative to non-pregnant IUD-treated mares, which we found to have normal cycle lengths in the pony study, the serum progesterone values were significantly lower in SpayVac-treated mares than in IUD-treated mares. Although we were unable to make observations on the length of the oestrous cycle for the Nevada mares in this study, it has been reported that mares treated with PZP vaccine

tended to have normal cycle length (Kirkpatrick *et al.* 1997; Powell 1999) although urinary oestrogen has been reported to be lower (Kirkpatrick *et al.* 1992), along with a short-term high incidence of persistent corpora lutea (Liu *et al.* 2005).

Serum oestradiol concentrations determined for SpayVac-treated mares suggest that some follicular development occurred. However, for SpayVac-treated mares showing some evidence of follicular development based on serum oestradiol, ovarian pathologies may be involved. Ovarian pathologies have been recorded for several species given PZP vaccines (Skinner *et al.* 1984; Mahi-Brown *et al.* 1988; Kirkpatrick *et al.* 1992; Lou *et al.* 1996; Stoops *et al.* 2006; Curtis *et al.* 2007), and are summarised as a disruption of folliculogenesis, depletion of primary oocytes and an infiltration of leucocytes. Given that serum progesterone was significantly lower in SpayVac-treated mares than in IUD-treated pony mares known to be having oestrous cycles of normal length, we suggest that SpayVac-treated mares may undergo some follicular development, but fail to ovulate and/or develop a normal corpus luteum.

Literature reports of circulating progesterone concentrations for untreated mares indicate considerable variation of values. Although concentrations of serum progesterone of $<1 \text{ ng mL}^{-1}$ are generally associated with oestrus, the concentrations progressively increase to high dioestrous values by Days 5–7 and are sustained there until Days 13–14. According to Ginther (1992, pp. 238–240), the range of means for 10 publications assaying serum progesterone during mare dioestrus was $4\text{--}22 \text{ ng mL}^{-1}$. Clearly, an average exceeding 4 ng mL^{-1} for the IUD-treated mares suggests that most IUD-treated mares were in dioestrus when they were sampled. This is what would be expected when randomly sampling a population of mares. In contrast, an average of 1.3 ng mL^{-1} for SpayVac-treated mares suggests that most of those mares were not in dioestrus. Whether they were in oestrus or transitioning into or out of dioestrus cannot be determined with only one sampling point for each mare. Nevertheless, we believe that the published values for average progesterone values during dioestrus, plus the reports of ovarian pathologies associated with PZP vaccines supports the notion that the lower progesterone values in SpayVac-treated mares may be due to a failure to form or maintain a normal corpus luteum. It is possible that antibodies to the zona pellucida prevent follicles from developing to normal ovulatory size and formation of the corpus luteum. Aside from these characteristics, there was no other evidence of contraindications associated with the SpayVac treatment.

Mares receiving a single vaccination of GonaCon showed a high degree of contraception during the first year, but this rate gradually declined to less than half after four years. This decline was associated with a gradual decline in antibody titre to GnRH over the same period. Unlike what was seen with SpayVac titres, there was no evidence for a self-boosting effect that occurs when the native protein is produced. The reason for this difference in response to the two immunogens is unknown, but it may relate to the fact that the PZP immunogen is a large glycoprotein compared with the GnRH decapeptide. Nevertheless, while the contraceptive efficacy of GonaCon was not as impressive as that of SpayVac, the GonaCon results exceed rates of contraception reported by others for mares using other single-injection contraceptive vaccines (Turner *et al.* 2001, 2002).

The incidence of uterine oedema for GonaCon-treated mares was similar to what would be predicted in a population of normal cycling mares. This suggests that these mares may have some degree of oestrous cycle activity, although it is difficult to make firm conclusions. Theoretically, we would predict minimal steroid hormone production in GonaCon-treated mares if we assume that GnRH were inactivated by antibody to the vaccine. However, plasma oestradiol and progesterone concentrations in GonaCon-treated mares were similar to those in the other treatment groups. Because we have no direct observations on the reproductive behaviour of the GonaCon-treated mares, we cannot say whether these females expressed oestrus or showed evidence of an oestrous cycle. However, our unpublished data from white-tailed deer treated with GonaCon suggests that GonaCon does inhibit expression of oestrus at least in the first year or two following vaccination.

The presence of serum oestradiol in GonCon-treated mares is contrary to the notion that the GnRH is the sole regulator of follicle-stimulating hormone (FSH) secretion by the pituitary gland leading to stimulation of follicle development and oestrogen secretion. The GonaCon used in this study is prepared with the luteinising hormone-releasing hormone (LHRH) peptide (Levy *et al.* 2004) which has been shown to stimulate both luteinising hormone (LH) and FSH secretion. However, there is also evidence in several species for a follicle-stimulating hormone-releasing hormone (FSHRH) that specifically stimulates FSH secretion by the anterior pituitary gland (McCann *et al.* 1993, 1998; Yu *et al.* 1997; Padmanabhan and McNeilly 2001; McNeilly *et al.* 2003). If an FSHRH exists in the mare, it could explain the presence of serum oestradiol in infertile GonaCon-treated mares. Antibodies to LHRH in the serum would act to block some follicular development and the LH surge associated with ovulation, but some follicular development and oestrogen production would also occur in response to FSHRH and FSH secretion.

Contraception results for the Nevada mares treated with the IUD were encouraging in the first year of the study, but the performance was poor for the remainder of the study.

When IUDs were visualised, there was no evidence of uterine pathology as assessed by ultrasonography. It has been reported that mares implanted with a Sialistic ring IUDs were infertile for one year, but that device was associated with a uterine inflammatory response (Daels and Hughes 1995). In our study we did not see evidence of a uterine inflammatory response, and mares in the IUD-treatment group had the predicted number of occurrences of uterine oedema for the population size sampled. This, along with the serum progesterone and oestradiol data suggests that the IUD-treated mares were experiencing oestrous cycles.

Studies with ponies enabled more frequent observations of the IUDs and oestrous cycle events. From these observations we concluded that the 380 Copper 'T' IUD was superior to the other IUDs tested for long-term contraception. In addition, observations on oestrous cycle events for one breeding season led us to conclude that cycle length for mares with IUDs was within the normal range. The discrepancy in long-term rates of infertility between the mustang mares and pony mares equipped with a similar IUD is probably due to differences in uterine size. The retention of foreign objects in the mare uterus is related to the size of the object, relative to the size of the uterus. The ability of

glass balls to be retained in the mare uterus has been shown to be related to the size of the glass ball (Nie *et al.* 2001; Thomas 2002). Although we have not found IUDs expelled by the uterus of an IUD-treated mare that became pregnant, we suspect the reason for the decline in efficacy of the Nevada mares was that the IUD was not retained in the uterus. This suggests that if larger 'T' IUDs were used, better rates of retention and contraceptive efficacy may be possible. On the basis of the pony studies, there is also evidence to suggest that shape of the IUD may also be a factor affecting retention, since neither the string GyneFix nor the ring IUDs were retained and performed as well as the 'T'.

The mechanism preventing fertility in IUD-treated females has been argued to be either by interference with attachment of the early embryo to the uterus, or by induction of early abortion (Ortiz *et al.* 1996; Fortney *et al.* 1999). This mechanism may differ among species and no data have been published for the mechanism in the mare. Because the length of the oestrous cycle of pony mares in this study was within the normal range, we suggest that infertility in most instances was the result of the IUD interfering with events occurring between fertilisation and early embryo attachment. However, in one pony mare that was observed to be 50–60 days pregnant, in a subsequent examination she was not pregnant and the IUD was visualised. This indicated that abortion had occurred, but the IUD was retained. This observation raises the possibility that if pregnancy occurs followed by abortion, IUD expulsion may also occur. It is also possible that if pregnancy occurs but abortion does not, the IUD could be expelled with the placenta at parturition. This appears to have occurred with one Nevada mare that we observed to have the IUD when she was pregnant, and she went on to foal.

We believe that these studies provide evidence that long-term contraception of the mare is possible with the SpayVac PZP vaccine. Further improvements to the formulation of GonaCon that are now being tested in white-tailed deer suggest that rates of contraception similar to SpayVac are achievable with GonaCon. Development of larger IUDs that are better suited to the mustang mare may be possible. Regardless of the approach used, if a high rate of contraception is achievable for multiple years, population models suggest that contraception alone or used in conjunction with removal programs have the potential to stabilise and reduce population growth as well as reduce wild horse management costs (Garrott *et al.* 1992; Cameron *et al.* 2001; Bartholow 2004; Ballou *et al.* 2008; Kirkpatrick and Turner 2008). These observations lead us to conclude that population management of wild horses by single-application multiyear contraceptives will probably be possible in the near future for horse populations that can be gathered from their range for treatment and release.

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Marble-induced pyometra in an Appaloosa mare

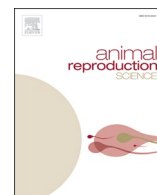
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Marble-induced pyometras have not been reported previously. This case reports a new possible complication of using intrauterine marbles to suppress estrus in mares. A 16-year old Appaloosa mare presented with a foul-smelling, suppurative vulvar discharge. No reproductive history was available. Pyometra, endometritis or vaginitis were suspected. Transrectal palpation, transrectal ultrasound and fluid cytology were performed. Transrectal ultrasound revealed a corpus luteum and a large fluid-filled uterus. A 34-mm round hyperechoic structure was noted in the left uterine horn. The cervix was firm. Cytology of the draining fluid revealed large numbers of degenerate neutrophils. Pyometra was confirmed and the presence of a mummified fetus or intrauterine marble was suspected. Uterine endoscopy was indicated to directly assess the uterine lumen, which confirmed the structure to be a glass marble. It was recommended to remove the marble during estrus and treat the pyometra. Diestrus was shortened with dinoprost (5 mg IM) and the marble was removed during estrus. Misoprostol (200 µg) was administered intra-cervically to induce cervical dilation and facilitate marble removal. The marble was manipulated towards the cervix transrectally and then through the cervix per vagina. The owners declined treatment of the pyometra. After questioning the owners, it was determined that the marble had been in place for at least two years. This case suggests that pyometra may be a complication of using intrauterine marbles for estrus suppression in mares and stresses the importance of removing the marble once estrus suppression is no longer desired. Pyometra is typically associated with cervical incompetence.¹ This mare's cervix dilated properly during estrus, and estrus was confirmed prior to presentation. It is possible that the marble acted as a foreign body or a nidus for infection. Although this mare's fertility was not tested, it would be reasonable that the pyometra could impact long-term fertility.¹

Keywords: Pyometra, glass marble, mares

Reference

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Modification of O-ring intrauterine devices (IUDs) in mares: Contraception without estrus suppression

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ABSTRACT

A significant welfare concern for those within the horse industry is that of the overpopulation in free-roaming horses in the western US. The goal of the present study was to identify a humane and inexpensive means of providing contraception and reducing pregnancy rates in a way that could be applied to free-roaming horses on the western U.S. open range lands. A series of studies was conducted utilizing the previously-described silastic O-ring intrauterine devices (IUDs). Throughout these studies pregnancy was successfully prevented in mares with an O-ring IUD, but collective retention rates failed to exceed 50% regardless of size or durometer (material hardness/malleability). When the O-ring IUDs were compared to the recently-described Y-design IUDs (Holyoak et al., 2021) the Y-design IUDs had a markedly greater rate of retention; therefore, the Y-design IUDs would be most appropriate for potential use in free-roaming horses. Assessment of mare records revealed that the vast majority of mares continued to have ovulations and mating continued to occur while the IUDs were in place. A few mares had multiple small ovarian follicles when the IUD was *in utero*. Findings indicate that utilizing an IUD with the expectation of estrus suppression in mares would fail to be fruitful.

1. Introduction

A significant welfare concern for those within the equine industry is that of the overpopulation in free-roaming horses in the western US. On-range population growth of horses on western rangelands in the US has resulted in approximately 82,000 free-roaming horses in an area where range ecologists have established 27,000 as the desired size (based on ecological sustainability) of the population (Bureau of Land Management BLM, 2019). Overpopulation of lands in the western US leading to overgrazing and forage depletion raises significant animal welfare (Fisher, 1983; Scasta et al., 2018; BLM, 2019) and environmental concerns (Levin et al., 2002; Beaver and Brussard, 2004; Beaver and Herrick, 2006).

A second challenge for the horse industry is that of the “difficult” or “misbehaving” performance of mares when in behavioral estrus. Albeit a less severe problem in comparison to free-roaming horse overpopulation, singularly this problem should be considered a welfare concern for those mares ridden by misinformed and frustrated owners or trainers deeming these mares as simply uncooperative. In cases where mares appear to be refractory to behavior modification efforts, uninformed owners may be persistent in seeking

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an ovariectomy and insist that veterinarians to perform the surgical procedure before documentation between the undesired behavior and estrus exists. This occurs even though there is evidence that estrous behaviors increase in mares after ovarian removal (Whitesell and McDonnell, 2013). For veterinarians, it can be extremely frustrating in attempting to differentiate a lack of “chemistry” between horse and rider, a deficiency of targeted horse training, or a legitimate change in mare behavioral physiology as there are changes in the mare’s estrous cycle.

In addition to the described welfare concerns, another commonality between the issue of the free-roaming horse overpopulation and “misbehaving” mares in behavioral estrus lies in a proposed treatment with intrauterine devices (IUDs). The IUDs have been investigated for a number of years as a solution to both the overpopulation of horses in western rangelands as well as for “misbehaving” performance mares (Daels and Hughes, 1995; Nie et al., 2003; Killian et al., 2006; Vanderwall, 2015; Diehl de Amorim et al., 2016; Campbell, 2018; Gradil et al., 2019). Inconsistent research results reported in the literature that is easily available to owners continues to emphasize ovariectomy as a potential solution for the “difficult estrus mare” (Campbell, 2018; Melgaard et al., 2020).

The initial focus of this study was to develop a humane and inexpensive means of reducing pregnancy rates within the US free-roaming horse population. Furthermore, during the period this study was being conducted data were collected for which there was subsequently some important points of discussion concerning the effectiveness of an IUD as a method of suppression of behavioral estrus in the mare.

Based on previous results from research where there was insertion of O-ring IUDs into the uteri of six mares with a greater than 80% retention rate (Daels and Hughes, 1995), there was a series of studies conducted in which there was examination of the efficacy and safety of several sizes and hardness increments (i.e., durometer) of an O-ring IUD composed of a silastic elastomer polymer (Baldridge et al., 2017). There was a proof of concept study designed and conducted with the hypothesis being silicone O-ring IUDs would be retained within the uterus and as a result providing an approach for preventing pregnancy in mares in pasture breeding conditions. A desired rate for IUD retention was established as >75%.

2. Materials and methods

2.1. Animals

As many as 24 Quarter Horse mares and two Quarter Horse stallions were utilized for three phases of the present studies that were conducted in 2015 and 2016. The research conducted in 2015 was a pilot study in which there was utilization of only five mares with there being no stallions utilized in the pilot study.

2.2. Ethics approval

Animal use and care procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (VM15–29).

Table 1

In utero IUD retention rates during summer of 2016; IUDs were inserted on 18 July 2016; remaining IUDs were removed on 9 September 2016; *IUDs were maintained *in utero* for the complete observation period of 53 days; ($P = 0.09$).

Mare ID	IUD size (mm)	Date IUD expulsion	Days IUD retained	Total number IUDs lost
143	50–75	7/22	4	4/4
130	50–75	7/26	8	
618	50–75	7/28	10	
606	50–75	8/5	18	
612	55–40	7/26	8	4/4
609	55–40	8/2	15	
610	55–40	8/12	25	
616	55–40	8/16	29	
605	55–75	7/19	1	2/4
150	55–75	9/2	46	
601	55–75		*	
603	55–75		*	
123	60–40	7/22	4	1/4
620	60–40		*	
607	60–40		*	
619	60–40		*	
614	60–75	7/22	4	2/4
613	60–75	8/2	15	
611	60–75		*	
615	60–75		*	

2.3. Pilot study design

In the fall of 2015, a pilot study was performed where 50 durometer O-ring IUDs (outside diameter of 40 mm) were placed into the uteri of five mares. Transrectal palpation and ultrasonographic examinations (ibex® EVO® with 5–7 MHz linear transducer, E.I. Medical Imaging®) were performed twice weekly to determine whether the IUD remained *in utero*. After there were unsatisfactory retention rates utilizing the 40 mm, 50 durometer O-ring IUD, the decision was made to increase the durometer scale value for and the diameter of the IUDs in efforts to increase uterine retention rates.

2.4. O-ring IUD mating study design

In the summer of 2016, there continued to be evaluation of the 40 mm diameter IUD but with increased durometers of 55 and 60. Additionally, 75 mm diameter IUDs were also evaluated that had a durometer scale value of 50, 55, and 60. There were 20 mares that were randomly assigned to be administered IUDs of each size (40 or 75 mm) and with a durometer scale value of 50, 55, or 60 that were inserted into four uteri. The 40 mm IUD with a durometer scale value of 50 was not utilized in this portion of the study because it was previously evaluated in the fall of 2015. The 20 mares were then assigned to two groups and mares of each group were placed in a paddock with a breeding stallion that had undergone a standard breeding soundness examination with there being no abnormalities identified at the time of physical examination, reproductive examination, or semen analysis. After administering an IUD, the mares were immediately placed in a paddock with a previously assigned stallion to allow for behavioral estrus and mating to occur while there continued to be transrectal palpation and ultrasonographic examinations performed twice weekly for the ensuing 53-day study duration.

There was determination that the IUDs with a scale value of 60 durometers had a greater rate of *in utero* retention after 53 days (Table 1). It, therefore, was decided to re-evaluate the IUDs with the durometer scale value of 60 durometer in a larger number of mares. Additionally, two batches of IUDs were manufactured with larger durometer scale values of 70 and 80. Because results were similar for both the 40 and 75 mm diameter IUDs, where more than half of the IUDs were not retained *in utero* for a 30 day period, the decision was to produce the IUDs with a 70 and 80 durometer scale value that were 40 mm in diameter and observe for IUD *in utero* retention rates for a period of 28 days. The working hypothesis was that, with the IUDs with a greater durometer scale value, these devices would be less malleable during uterine contractions (potentially an important characteristic for an IUD when there is estrus and during the process of mating) and, therefore, the IUD would be less likely to be expelled. There was utilization of 24 mares by assigning them to three groups with each group having eight mares with different IUDs with a different durometer scale value (60, 70, and 80). The mares were then placed back into a paddock with a breeding stallion.

Table 2

In utero IUD retention rates for 60, 70, and 80 durometer scale values for IUDs; IUDs were inserted on 20 September 2016 and removed 18 October 2016; *IUD was retained *in utero* for the complete observation period of 28 days; ($P = 0.51$).

Mare ID	IUD size (mm)	Date IUD expulsion	Days IUD retained	Total number IUDs lost
602	60–40	9/27	7	5/8
605	60–40	9/27	7	
614	60–40	9/30	10	
620	60–40	10/4	14	
611	60–40	10/18	27	
601	60–40		*	
607	60–40		*	
619	60–40		*	
604	70–40	9/21	1	3/8
609	70–40	9/23	3	
603	70–40	9/27	7	
150	70–40		*	
612	70–40		*	
615	70–40		*	
618	70–40		*	
617	70–40		*	
610	80–40	9/23	3	3/8
123	80–40	9/27	7	
613	80–40	10/11	21	
143	80–40		*	
151	80–40		*	
606	80–40		*	
130	80–40		*	
616	80–40		*	

2.5. Statistical analysis

Analysis of retention rates for IUDs was performed using the Chi square test utilizing Prism analysis software. There were considered to be mean differences when there was a $P = <0.05$ and a tendency for differences when there was a $P \leq 0.10$ and > 0.5 .

3. Results

3.1. Pilot study IUD retention rate

Results when there was evaluation of the O-ring IUDs with 40 mm (outer diameter), 50 durometer scale values indicated 40% (2/5) of mares retained the IUD *in utero* during the 2-month period. Of the mares that did not retain the IUD, there was expulsion of the IUD from two-thirds of the mares within 21 days of insertion.

3.2. Mating experiment IUD retention rates

Retention rates for the 40 and 75 mm diameter O-ring IUDs that had durometer score values of 50, 55, and 60 were similar to each other and only 7 of 20 IUDs were retained during the period when mares were in paddocks with stallions and there was mating occurring (Table 1). There were 12 of 13 IUDs that were not retained *in utero* for the 1-month period subsequent to insertion. When there were greater durometer scale values, there tended to be a difference in uterine retention of IUDs ($P = 0.09$). Three mares that were detected to be pregnant were ascertained to have conceived as a result of mating during the first period of behavioral estrus after the expulsion of the IUD from the uterus.

There was retention of 13 of 24 IUDs with durometer scale values of 60 (repeat trial), 70, or 80 on the durometer scale during the experimental period when there was stallion mating with mares (Table 2) with 10 of the 11 IUDs being expelled within 21 days of insertion. Overall, for the IUDs with greater durometer scale values there was no difference in uterine retention rates ($P = 0.51$). Unfortunately, it appeared that even with the adjustments made to the O-ring IUDs, there continued to be a lack of retention of IUDs at the 75% *in utero* retention rate goal.

Pregnancy was not identified in mares with an IUD *in utero*. There were data from only three mares in regards to pregnancy but it is possible that pregnancy rates after IUD loss were greater. This is due to the fact that embryonic vesicles are not visible using ultrasonographic procedures until about 11 days after ovulation and in performing the present studies early-stage pregnancies may have been disrupted by the insertion of IUDs when mares were assigned to a subsequent study. At no time were any of the mares observed to have evidence of vaginal or vestibular irritation (as may be symptomatically evidenced by frequent bouts of urination or vulvar discharge) after the placement of IUDs while 6 of 24 mares were reported to have echogenic fluid within the uterus that was detected when an ultrasonographic examination was performed, at some point during these studies.

3.3. Comparison of O-ring and Y-design IUD retention rates

Holyoak et al. (2021) reported that IUDs with a 60-durometer scale value that were composed of silastic and were of a Y-shaped design met the contraception criteria established for application in free-roaming horses for population. This was most notable with the desired retention rate being $>75\%$ in the absence of induced uterine pathology. With the 60-durometer scale score, there was a retention of the Y-design IUDs in 15 of 20 mares for a period of 463 days. There was expulsion of five IUDs between days 43 and 294 of the experimental period (Holyoak et al., 2021). Retention rates for the IUDs with a 60, 70, or 80 durometer scale score ($n = 24$) were compared with the Y-design IUDs ($=20$) and the *in utero* retention of the Y-design IUDs was markedly greater during the first 28 days of the experimental period (i.e., period the O-ring IUDs with 60, 70, or 80 durometer scale values were evaluated in the current study; $P = 0.001$).

3.4. Effect of IUD on reproductive cyclicity

Results gleaned from reproductive record data, indicated that the use of IUDs did not result in suppression of behavioral estrus or mating behaviors as evidenced by intermittent mating (which were observed during daily feeding by the ranch manager and by veterinarians at times of group processing for ultrasonographic examinations) and as a result of documented ovulations as evidenced by the detection of corpora lutea (CLs) in mares that were administered an O-ring or Y-design IUD. Exceptions to this include three mares which did not have ovulations during the period the O-ring IUDs were *in situ*. The ovaries of these mares contained populations of follicles ranging from “NSF” (no significant follicles) or “MSF” (multiple significant follicles) during the twice weekly ultrasonographic examinations. It would also appear that one mare (i.e., 615) might have had a CL with a prolonged persistence that was still present on a day where an IUD placement occurred (20 September) and that was maintained until IUD removal (18 October). In this mare, a dominant follicle/ovulation was not detected during the 28 day-period the study was conducted (Table 2).

Unfortunately, due to the original study goal of simply identifying retention rates of IUDs, the original study design did not result in collection of quantifiable data that could be evaluated to closely describe day to day ovarian follicular dynamics during the multiple month period. There, however, was three mares (i.e., 616, 616, and 618) that did not have ovulations during the period of time when the O-ring IUDs were *in utero* and during this period there were populations of follicles ranging from “NSF” (no significant follicles) or “MSF” (multiple significant follicles) when there were the twice weekly ultrasonographic examinations performed. With follicle

populations of this type, there would not be expected to be “suppression of behavioral estrus” because there were only basal concentrations of systemic progesterone resulting from no luteal presence during this time period when there IUDs were *in utero*. This is important because when there is luteal production of progesterone, this hormone is a very potent suppressor of behavioral estrus.

4. Discussion

The original intent of the O-ring IUD studies was to identify a contraceptive device that could be utilized in free-roaming horse herds, and while the results from the O-ring IUD studies did not indicate the minimum desired 75% *in utero* retention rate was met, as a consequence, these results did provide impetus to conduct studies using silastic elastomer Y-design IUDs (Holyoak et al., 2021). At the conclusion of the studies with the O-ring IUDs, it was decided to alter the silastic IUD design to mimic those utilized in women and that are described as ‘fundus-seeking.’ Additionally, there was administration of a single injection of BioRelease LA P₄ 300 (BET Pharm, Lexington, KY, USA) at the time of IUD insertion to circumvent breeding behaviors from occurring in the days immediately subsequent to IUD insertions because this is a time period when there is expulsion of many O-ring IUDs from the uterus of mares. There was a 75% retention rate of the Y-design IUDs, with 100% contraception in females where there was retention of the device *in utero* during a 20.5 month study period (Holyoak et al., 2021). Holyoak et al. (2021) speculated that it would appear that an acceptable and potentially long-lasting IUD contraceptive had been developed in a semi-field environment but the thought was that there needed to be evaluation of this type of IUD in field conditions utilizing free-roaming horses.

It has become obvious based on findings in the present study that the optimal combination of size, shape and durometer scale value must be identified for an efficacious IUD *in utero* retention rate. These characteristics are likely very important because one major factor that is unique is the belled glans of the stallion penis that likely induces a high-pressure occurrence when the stallion penis penetrates the cervix during mating (i.e., stallion penis emits an ejaculate through a soft, dilated cervix directly into the uterus).

There has been an enhanced interest in the topic of behavior modification in mares after administration of IUDs subsequent to when there was the initial publication of results gleaned from the data reported in the present study. This renewed interest provided an impetus for further retrospective inspection of individual mare records collected during the evaluation of both the O-ring and the Y-design IUDs. The study design did not allow for collection of quantifiable data that would precisely describe daily changes in follicular dynamics during the multiple month period during which data were collected. There were rather reproductive ultrasonographic examinations conducted twice weekly (for less than 2 months) or bi-weekly for 20 months depending on whether there was O-ring or Y-design evaluations occurring. Even with these schedules, it could be gleaned from the records for the mares included in both the O-ring and Y-design studies that the use of IUDs did not result in suppression of estrus or breeding behaviors as evidenced by observed intermittent mating occurrences, documented ovulations, and development of CL in mares that had an IUD *in utero*. There have been similar results reported in mares with marbles and other intrauterine devices (Daels and Hughes, 1995; Nie et al., 2003; Argo and Turnbull, 2010). From these results, it was ascertained that behavioral estrus suppression should not be expected to occur following insertion of an IUD in either a free-roaming mare or a domesticated performance mare.

Ovarian follicular dynamics in mares where the Y-design IUD was evaluated were more difficult to describe due to the bi-weekly examination interval conducted during much of the study period (Holyoak et al., 2021). It was suspected that few mares had a deviation from normal patterns of ovarian follicular dynamics after IUD insertions into the uterus but this evaluation was even further complicated by individual mare variation with the initiation (or cessation) of the estrous cycle transition phases before and after seasonally-induced anestrus. It is also suspected that variations in mare estrous cyclicity could have occurred as a result of induced endometritis following placement of the IUD (and retention) but, as previously described, the frequency of the bi-weekly ultrasonographic examinations did not lend to detailed documentation of follicular dynamics. In the Holyoak et al. (2021) study, it was documented that in one mare there was development of three CLs (evidenced by the CL development in alternate ovaries) in less than a 30-day period. This mare also had a large amount of uterine fluid present, indicating that an inflammation induced short-estrous cycling effect may have contributed to development of these multiple CL during the study period. It's possible that a second mare could have had a persistent CL on the right ovary for several months because a CL was documented on the right ovary during four consecutive examinations before a CL developed on the left ovary. Because this mare also had a large amount of uterine fluid, however, it cannot be ascertained if this mare continued to have ovulations from the same ovary during this time period or if this mare had retention of a CL that resulted from a spontaneous ovulation with there being a prolonged luteal function as compared to that which typically occurs in mares.

The number of mares in a group that will have spontaneous prolongation of a CL functions has been reported to be 8% in untreated control mares (Nie et al., 2003). Similar to indications in previous studies (Nie et al., 2003; Klein, 2016), it was interesting that in the present study mares having apparent deviations from normal ovarian cyclic patterns were the youngest mares. It was deduced that the vast majority of mares continued to have ovulations throughout the duration of the studies with the O-ring and Y-designed IUDs as evidenced by the presence of CL in ovaries on alternating sides of the reproductive tract. Those mares described (previously in this manuscript) as having these patterns of deviation from normal estrous cyclicity also had basal endogenous progesterone, resulting in an enhanced occurrence of estrus-like behavior, similar to a mare during the winter anestrus period.

Successful suppression of behavioral estrus in mares is problematic because of owner-requested outcomes for equine veterinarians. The owner expectations are to address inhibition of a vast array of mare behaviors such as poor athletic performance, kicking, biting, aggression, excessive urination, vocalizing, tail twitching, etc. (Hooper et al., 1993; Nie et al., 2003; Hedberg et al., 2007; Argo and Turnbull, 2010; Gradil et al., 2019; Melgaard et al., 2020). It may be true that, similar to women athletes, mares can experience a change in neuromuscular performance as a result of ligamentous laxity resulting from changes in reproductive hormone concentrations throughout an estrous cycle (Hewett, 2000). Additionally, actual ovarian pain manifested in the region of the epaxial muscles

during the peri-ovulation period, termed “mittelschmerz” in women, should be considered as a potential reason for lameness or lack of performance in “misbehaving” mares expressing behavioral estrus. “Problem” mares should be examined by a veterinarian at the time at which the owner perceives the behavior to be occurring and examination procedures need to include, not only evaluation of the reproductive tract, but must also include a complete lameness evaluation to identify issues that may or may not be estrogen related.

Confusion between contraception and suppression of behavioral estrus in mares, especially by laypeople, may exist in part due to the public’s experience with the spayed bitch and the dogma that “ovarian removal results in removal of natural breeding behavior” (Pets WebMD <https://pets.webmd.com/reasons-spay-neuter-pet#2-6>). In contrast to the bitch, however, mares that have been ovariectomized express “paradoxical” behavioral estrus and there is often an increase in the frequency of behaviors associated with estrus when compared to ovary-intact mares in which there are cyclical phases of diestrus (Ginther, 1974; Asa et al., 1980; Hooper et al., 1993; Crowell-Davis, 2007). While the concept of utilizing IUDs, as a method of estrous suppression or contraception is not new, there is considerable skepticism due to inconsistent results and induction of uterine pathologies [i.e., shattering intrauterine marbles (Klabnik-Bradford et al., 2013; Turner et al., 2015; Vanderwall, 2015; Diel de Amorim et al., 2016)]. It should be taken seriously that placement of any form of IUD into the uterus of a valuable mare can result in acute endometritis as evidenced by uterine fluid accumulation and edema at the site of the IUD in both the present study and as reported previously (Rivera Del Alamo et al., 2008; Joonè et al., 2021). It, however, is equally important to note that there were no adverse effects on the health or welfare of the mares following *in utero* insertions of any of the silastic IUDs during the present study.

Lending to further confusion in the differences between suppression of behavioral estrus and contraception in mares, is the availability of summaries of results or literature reviews that include information on both topics within the same report (Hornberger et al., 2017). On a more specific note, publications or legislative efforts focused on identification of methods for population control in the western US (e.g., GnRH vaccination, PZP vaccination, ovariectomy) describe methods of contraception that may also inadvertently induce behavioral estrus in mares (Garza et al., 1986; Dalin et al., 2002; Killian et al., 2006). Unfortunately, implementation of such methods also lead to the risk of inducing increased frequencies of mating on a long-term basis, reduced mare band-fidelity to the stallion, and lengthening of breeding seasons in free-roaming mares (Powell, 1999; Ransom et al., 2010; Nuñez et al., 2017). Such an increase in behavioral estrus could have an effect on mare and stallion welfare and herd dynamics.

It is recognized that there are two limitations in the present IUD-oriented studies. These include the inability to quantify the number of stallion mounts of each mare and documentation of more accurate inter-ovulation intervals that would have led to a more precise description of estrous cyclicity deviations. Documentation of accurate inter-ovulation intervals would require either ultrasonographic examinations more frequently than every 2 weeks or even weekly serum progesterone analysis; both of which were outside the scope of this project in which the main goal was investigation of IUDs for contraception purposes. It can be presumed the number of mating occurrences during a period of months in mares with an IUD *in utero* is greater than what would occur in a non-treated fertile, free-roaming mares during the same time period due to eventual conception and pregnancy in mares without an IUD. Excessive mating of mares is a concern when mares are not pregnant for a prolonged period of time; however, because the mares in the present study had documented luteal phases (as indicated by the presence of CLs) excessive mating during a 3-week period was not a concern in the present study.

5. Conclusion

While the O-ring IUDs failed to meet the retention rate goal, the subsequently manufactured Y-design IUDs resulted in adequate *in utero* retention and contraception in semi-field conditions (Holyoak et al., 2021). The results from these sequentially-performed studies involving both O-ring and Y-design IUDs indicated the ideal IUD size, shape and malleability must be identified to obtain an acceptable IUD *in utero* retention rates. Continued ovulations were documented in the vast majority of mares in which there was *in utero* retention of O-ring and Y-design IUDs during the experimental period.

Veterinarians have an ethical responsibility for educating owners of the conflicting evidence that exists in regard to IUDs resulting in enhanced mare performance as a result of suppression of behavioral estrus. Other significant reasons for subpar mare performance should not be overlooked and full lameness and reproductive examinations should be performed in combination before resorting to invasive procedures (e.g., ovariectomy) or methods that lack evidence-based information (e.g., IUDs) in the performance mare.

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CRedit authorship contribution statement

CCL, SSG, AJK, GRH participated in the conceptualization of the study, analysis of data, and approval of the manuscript. CCL, JMB, COA, and GRH participated in data collection.

Declarations of Competing Interest

The authors report no declarations of interest.

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REFEREED

Use of an Intra-uterine Glass Ball Protocol to Extend Luteal Function in Mares

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SUMMARY

A 25- or 35-mm diameter glass ball was placed in the uterus of mares to observe the effect on interovulatory interval, luteal function, estrous behavior, the endometrium, and subsequent fertility. The 25-mm glass ball was spontaneously expelled from the uterus of 6 of 12 mares (50%), whereas none of the 35-mm glass balls was expelled. Teasing results were consistent with the concentration of circulating progesterone. Luteal function was extended in 7 of 18 mares (39%) maintaining a glass ball, whereas an extended luteal period occurred in 4 of 32 mares (13%) observed as controls. Extended luteal function occurred in 7 of 62 diestrus periods (11%) among mares following ball placement, whereas 4 of 50 diestrus periods (8%) were extended in control cycles. The mean luteal life span in mares with a glass ball and extended luteal function was 87 days (range, 76 to 109 days); there were no significant differences in length of luteal function in both groups of mares that received the 2 different ball sizes. Endometrial changes observed between preplacement and postremoval samples were minimal. When mares were bred in the season subsequent to glass ball removal, 17 of 23 (74%) conceived. Placing an intrauterine glass ball in a mare may be an alternative to exogenous hormone therapy to prevent cycling in some mares. Luteal function was extended to nearly 90 days in approximately 40% of mares. The 35-mm diameter glass ball appeared to have an advantage for retention over the 25-mm size. Results of our study could not completely

rule out idiopathic persistence of the corpus luteum as an explanation for the extended luteal function observed in mares with a glass ball. Readers are cautioned that many questions still exist about the use of intra-uterine glass balls in mares. Further work is required to confirm the efficacy of the use of an intra-uterine glass ball for prolonged luteal function in mares and to identify its mechanism of action.

INTRODUCTION

In recent years, there has been a debate among veterinary practitioners concerning the efficacy of various extra-label uses of progestin products (eg, cattle growth implants and human depo-progestin injectables) to modify behavior in mares. Clients who own horses are more frequently seeking means to suppress behavioral signs of estrus, expecting that with such suppression the mare will train or perform better. Requests for these progestin products by mare owners puts veterinary practitioners in the precarious situation of using pharmaceuticals, extra-label, without scientific evidence of efficacy, in mares.

In reality, the only truly effective means of suppressing behavioral signs of estrus in most intact mares is to maintain sufficient concentrations of circulating progesterone or its equivalent. Today the only efficacious way to maintain a sufficient level of progesterone or its equivalent is for the mare to have a functional corpus luteum (CL), administer exogenous progesterone (eg, ≥ 50 mg in oil, intramuscularly, daily), or administer daily synthetic progestins (eg, altrenogest [Regumate], Hoechst Roussel Vet, Warren, NJ).¹⁻³

Recently, placement of a glass ball of 30-mm diameter in the uterus has been suggested as a reversible means of preventing mares from cycling and displaying behavioral signs of estrus (message to Equine Clinicians Network, Dr Randy J. T. de Greef, March 19, 2000). If this technique is effective, it would be of value to mare owners because it would eliminate the need for daily treatments over extended periods.

We have been unable to find literature that would support or refute this idea in horses. However, the effects on ovarian function, body weight gain, and pregnancy rate in

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nulliparous heifers of a copper-bearing intrauterine device were studied.⁴ The researchers reported that the heifers receiving the intrauterine device had lower progesterone concentrations than did control subjects. Nevertheless, nearly all of the treated heifers had better weight gain, were anestrus, and did not become pregnant during the study; however, multiple ovarian follicular cysts developed in many of them. The idea of using an intrauterine device to suppress estrus is said to have originated centuries ago in the Middle East as a common means of keeping camels from cycling and becoming pregnant (personal communication, Dr Ahmed Tibary, College of Veterinary Medicine, Washington State University, Pullman, Wash, May 2000).

To our knowledge, the efficacy and long-term effects of glass ball treatment have not been critically evaluated. Our objectives in this study were to observe the effect of placement of an intra-uterine glass ball on interovulatory interval, luteal function, estrous behavior, the endometrium, and subsequent fertility of mares.

MATERIALS AND METHODS

Animals. A total of 38 light-horse breed mares ranging in age from 3 to 20 years were used for this study. Mares were maintained in accordance with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1st revised edition, January 1999). All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at Auburn University (IACUC Protocol No. 0308-R-2307).

Intra-uterine device. Two glass ball (www.glassmarbles.com) sizes, 25- and 35-mm diameters, were evaluated in this study (Fig 1). In preparation for placement, the glass balls were sterilized by autoclaving. Initial attempts at autoclaving resulted in several broken balls. However, use of a liquid cycle with a temperature of 250°F (121°C) and pressure of 16 psi, with no prevacuum or dry cycle and a slow cool-down phase, did not result in further breakage.

Upon entering the study, mares were monitored daily via transrectal palpation and ultrasonography for their progression through the estrous cycle. A glass ball was placed in the body of the uterus at the first examination following ovulation. The perineum was cleaned with cotton, tap water, and antiseptic dish detergent. A sterilized sleeve was donned and a small amount of sterile lubricant was applied to the back of the hand. Grasping the glass ball, it was manually carried into the vagina. The ball was placed in the cervical lumen and moved forward with the index finger to the caudal uterine body. After removing the hand from the vagina, the ball was located on transrectal palpation and pushed forward to the horn-body junction if it had not already moved to that position. Once the ball was positioned in the uterus, the vulva was again cleaned as previously described. The uterus was infused with 1 g of ticarcillin dis-

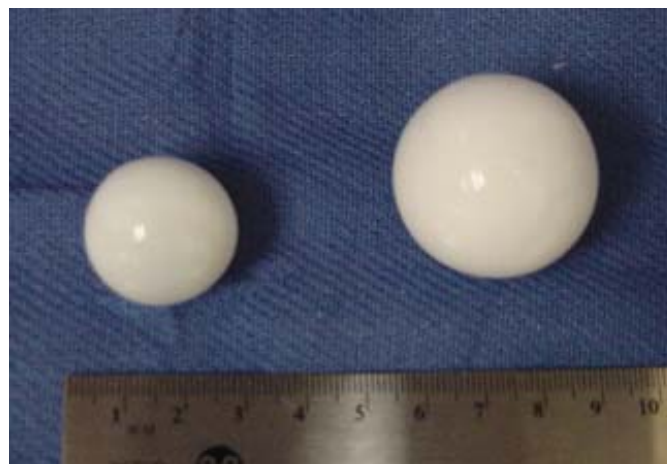


Fig 1. Glass ball diameters evaluated during the study were 25 mm (left) and 35 mm (right).

odium (Ticar, SmithKline Beecham Pharmaceuticals, Philadelphia, Pa) in a 35-mL volume and each mare received 250 µg cloprostenol (Estrumate, Bayer Corporation, Shawnee Mission, Kan) intramuscularly to prevent a persistent endometritis if contaminants were introduced with the glass ball.

At the end of the glass ball phase of the experiment for a mare, the ball was removed from the uterus during the following estrus when the cervix was softest. Occasionally a mare would require sedation to allow better manipulation of the ball per rectum. Mares with pendulous horns presented the most difficulty. Removal was accomplished by manipulating the glass ball, per rectum, caudally toward the cervix, through the cervix, and then to the vulva for retrieval. If the cervix was not fully dilated, a gloved hand was taken per vagina to the caudal cervical os and the glass ball was retrieved from the lumen.

Experimental protocol. This study was conducted at Auburn University in southeastern Alabama between May and October 2000. All mares used in this study had ovulated at least once in the season before being assigned to a treatment group. Twelve mares were randomly assigned to each treatment group (25-mm and 35-mm glass balls), representing 24 of 38 study mares. Control data were collected from 32 of 38 study mares during cycles in which no glass ball was in the uterus. Eighteen of the 32 mares used to collect control data were also treated with a glass ball during the study, whereas 14 mares were not treated. Control data were not available from 6 of 24 mares treated with a glass ball for management reasons beyond our control.

An endometrial biopsy was taken from each mare during estrus in the cycle before being assigned to a treatment group, for comparison with a sample following removal of the glass ball. The follow-up samples were taken immedi-



Fig 2. The ultrasonic appearance of a 35-mm glass ball is seen at the left uterine horn-body junction.

ately after removal of the glass ball to ensure detection of inflammation, if present. An attempt was made to take the preplacement and postremoval samples from the same area near the uterine horn-body junction. Endometrial biopsies were evaluated as previously described.⁵ The evaluator was blinded to the group assignment and preplacement results of each mare.

Throughout the study, mares treated with a glass ball were individually teased with a breeding stallion at a rail. Behavior was scored by response of a mare to the stallion using a categorical scale (0 = rejection, 1 = indifference, 2 = receptive) adapted from behavioral signs previously described.⁶

Beginning on the day of glass ball placement, mares were examined daily by ultrasonography and palpation per rectum to monitor changes in the reproductive tract and ball location. The ultrasonic appearance of a glass ball in the uterus is shown in Fig 2. Mares were also teased to a stallion, and blood was taken for determination of progesterone concentrations. Daily evaluation was continued until 2 ovulations had been detected; after this, daily blood sampling and teasing was continued until 2 more ovulations were detected. However, if at any time mares were found to maintain luteal tissue (continued ultrasonic evidence of a CL, palpable tone in the uterus and cervix, absence of estrus signs on ultrasonography, and absence of estrus behavior) for 35 days, daily evaluation was discontinued and the mares were moved to pasture and blood samples were taken weekly to monitor progesterone concentrations.

In mares that did not experience prolonged luteal function (>35 days), the glass ball was removed from the uterus after 4 ovulations had been detected. In mares that experi-

enced prolonged luteal function, the glass ball was removed after progesterone concentrations had fallen to <1 ng/mL followed by a subsequent rise to >4 ng/mL, indicating a subsequent ovulation. Mares that spontaneously expelled the glass ball were removed from the study upon discovery without further sampling.

Immediately following removal of a glass ball, an endometrial biopsy was taken for comparison with the preplacement sample. Following removal of the glass balls, mares were bred during the next season under the protocol of another study. Standard breeding management for artificial insemination and several stallions were used. The results reported are for the season and reflect pregnancy outcome at 15 days after ovulation.

Estrous cycles (n = 50) were observed in 32 of 38 study mares to establish an interovulatory interval and incidence of spontaneous persistence of the CL as a control for the effect of the treatment protocol. Observations were made during separate control cycles when a glass ball was not in the uterus of any mares that were also used in a treatment group during the study. At least one cycle was evaluated for each of the 32 mares, with some contributing a second cycle. The reproductive tract and circulating progesterone concentrations were evaluated in the same fashion and on the same daily schedule as the treatment groups. During the control cycles, the mares were simultaneously being observed to establish estrous cycle control data for another study. Therefore, when spontaneous persistence of a CL occurred and a luteal phase lasted 30 days, the mare was given prostaglandin to lyse the CL.

Progesterone assay. Circulating progesterone concentrations were used to reflect luteal function. Concentrations higher than 1 ng/mL were considered indicative of functional luteal tissue. Plasma was harvested from blood collected from each mare. Plasma samples were frozen and held at -50°C until assayed in batches of approximately 200. Circulating concentrations of progesterone were quantified using a commercial radioimmunoassay kit (COAT-A-COUNT progesterone radioimmunoassay kit, Diagnostic Products Corporation, Los Angeles, Calif).

Statistical analysis. Two measures were derived from each interovulatory period: the interovulatory interval in days and the number of days during which progesterone was >1 ng/mL. The effects of glass ball size, monitoring method, mare and their interactions were tested using the GLM procedure of Statistical Analysis System (SAS Institute, Cary, NC). Ages of mare among groups were compared with use of an unpaired *t* test (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, Calif). The proportion of mares experiencing extended luteal function or spontaneous persistence of a CL during the treatment and control cycles was determined. In addition, the proportion of diestrus periods that resulted in

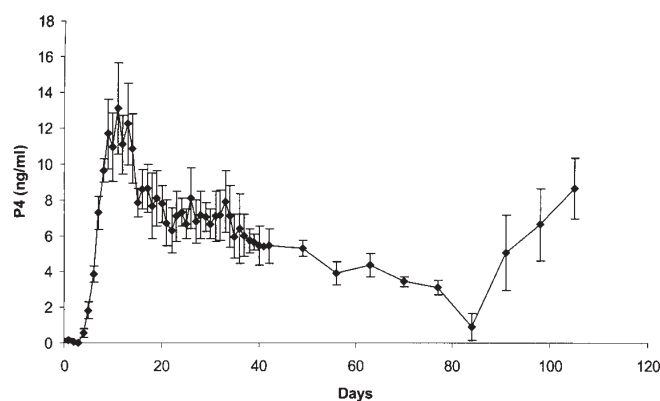


Fig 3. Mean ($\pm$ SEM) progesterone (P4) concentration in 4 mares experiencing extended luteal function during the first diestrus period following placement of a 35-mm ($n = 3$ mares) or 25-mm ($n = 1$ mare) diameter glass ball in the uterus.

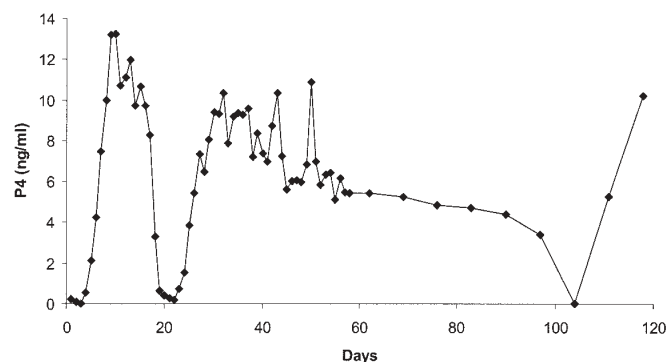


Fig 4. Mean progesterone (P4) concentration in the one mare that experienced extended luteal function during the second diestrus period following placement of a 25-mm diameter glass ball in the uterus.

extended luteal function or spontaneous persistence of a CL was determined. The proportion of mares and diestrus periods in which extended luteal function occurred during treatment and control cycles were compared using a Fisher Exact Test (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, Calif).

RESULTS

A very small amount of uterine fluid (<1 cm depth) was observed via ultrasonography in 3 mares for 2 days following placement of the glass ball. By day 3, however, the fluid was no longer visible in any of the mares, one of which did go on to maintain luteal function for an extended period. None of the mares that developed uterine fluid experienced spontaneous loss of the glass ball.

A total of 24 mares had a glass ball of either 25 mm ($n = 12$) or 35 mm ($n = 12$) diameter placed in the uterus. The 25-mm glass ball was spontaneously expelled in 6 of 12 mares (50%). Five were expelled within 24 hours of placement and a sixth during a subsequent estrus period, 11 days following placement. None of the 35-mm glass balls was spontaneously expelled.

The glass ball was observed to randomly alternate between the left and right uterine horn-body junctions. Movement was observed in every mare except two. In those 2 mares, the 35-mm glass ball was consistently observed at the same site during each examination. One of the mares experienced extended luteal function and the other did not.

Overall, 7 of 18 mares (39%) that maintained the glass ball experienced extended (>35 day) luteal function. Extended luteal function was detected during the first diestrus after ball placement in 4 mares, during the second diestrus in one mare, and during the third diestrus in 2 mares. Mean ($\pm$ SEM) progesterone concentrations during

the extended luteal periods are reported in Figs 3, 4, and 5. There was no difference between the 25- and 35-mm balls in terms of proportion of mares having extended luteal function (2 of 6, 33%, and 5 of 12, 42%; $P > .05$). Extended luteal function occurred in 7 of 62 diestrus periods (11%) among mares following ball placement. Again, there was no difference between 25- and 35-mm balls in terms of proportion of diestrus periods resulting in extended luteal function (2 of 20, 10%, and 5 of 42, 12%, $P > .05$).

An extended luteal period occurred in 4 of 32 mares (13%) observed for control data. Progesterone concentrations remained above 1 ng/mL for 30 days after ovulation in 4 of 50 control diestrus periods (8.0%) observed. Of the 4 mares that experienced extended luteal function during the control cycle, a glass ball was placed in the uterus of 3 of the mares during the treatment cycles. However, none of the 3 mares experienced extended luteal function while the glass ball was in the uterus. The proportion of mares that experienced extended luteal function (7 of 18, 39%) while a glass ball was in the uterus was greater than the proportion of mares that experienced an extended luteal period (4 of 32, 13%) during the control cycle ($P = .04$). The proportion of diestrus periods in which extended luteal function occurred was the same whether a glass ball was present in the uterus (7 of 62, 11%) or not (4 of 50, 8%; $P = .75$).

The interovulatory interval was $23.0 (\pm 0.43)$ days for the control cycles ($n = 46$) in which an extended luteal period did not occur. This was longer than the interovulatory interval (20.2 ± 0.41 days) for the cycles ($n = 55$) that occurred subsequent to glass ball placement without apparent extension of luteal function ($P < .001$). A functional CL was maintained (15.5 ± 0.35 days, range 11 to 23 days) longer in control cycles than in cycles with glass balls (13.2 ± 0.42 days, range 7 to 18 days) in which extended luteal function was not apparent ($P < .001$). Mean ($\pm$ SEM) pro-

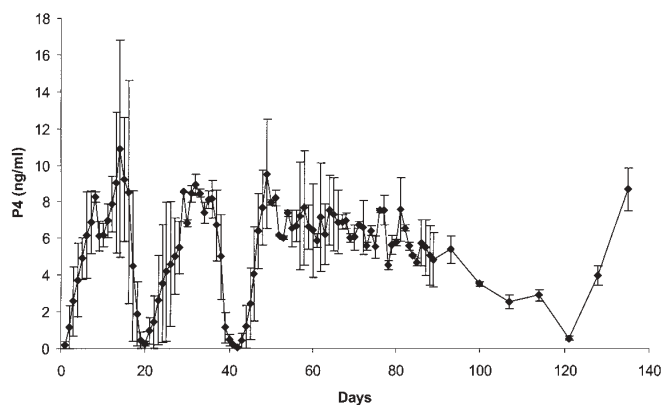


Fig 5. Mean ($\pm$ SEM) progesterone (P4) concentration in mares experiencing extended luteal function during the third diestrus period following placement of a 35-mm ($n = 2$ mares) diameter glass ball in the uterus.

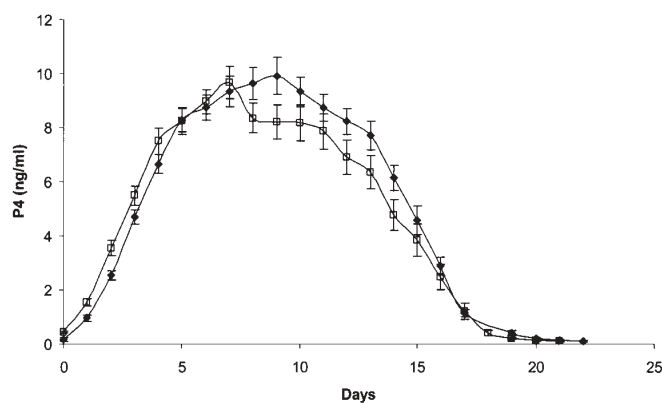


Fig 6. Mean ($\pm$ SEM) progesterone (P4) concentration in mares during control cycles ($-\blacklozenge-$; $n = 46$) without a glass ball and treatment cycles ($-\square-$; $n = 55$) with a glass ball in which an extended luteal period did not occur.

gesterone concentrations for the control cycles ($n = 46$) and treatment cycles in which an extended luteal period did not occur are presented in Fig 6.

The mean age of all mares in the study was 9.6 years (range, 3 to 20 years). Mares that experienced extended luteal function were younger, at 8.3 years (± 0.87), than mares that did not experience extended luteal function, at 12.6 years (± 1.05 , $P = .012$). Mares that spontaneously expelled the glass ball were younger, at 6.3 years (± 2.0), than mares that did not expel the glass ball, at 10.9 years (± 0.87 , $P = .024$).

The interassay and intra-assay coefficient of variation for the progesterone assay was 7% and 3%, respectively. The sensitivity of the assay was 0.02 ng/mL. Estrous behavior observed during the study accurately reflected circulating progesterone concentrations. All mares with a glass ball invariably displayed behavioral estrus scores of 1 or 2 when progesterone concentrations were <1 ng/mL, while scores of 0 were observed when concentrations were >1 ng/mL. Those that experienced extended luteal function also displayed scores of 0 throughout the period while progesterone concentrations were >1 ng/mL and estrus behavior was monitored. A single CL was observed at the primary ovulation site throughout the observation period. No additional CLs were observed in any mare with a glass ball following the primary ovulation.

No change in endometrial category was observed between the preplacement and postremoval endometrial samples in any of the mares in the 25-mm glass ball group. In the 35-mm group, the score declined by a category in one mare, improved by a category in 2 mares, and was unchanged in the other 9 mares. The difference in category assigned in those 3 mares was attributed to mild changes, up or down, in the amount of lymphocytic inflammation ob-

served. The mare that declined by one category did not experience extended luteal function, whereas 1 of 2 mares with improvement in endometrial category experienced an extended luteal period.

During the season following glass ball removal, 23 of 24 mares were bred, including all of the mares that had experienced extended luteal function. During the season, 17 of 23 (74%) of the mares bred subsequently conceived, including 5 of 7 (71%) of those that had experienced extended luteal function.

DISCUSSION

Placement of the glass ball through the cervix was relatively easy in most of the mares. Occasionally the cervix of a mare would require some degree of manual dilation to push a 35-mm diameter ball through its lumen; however, the 25-mm balls generally passed with ease. During preliminary work, we had found that the glass ball was more likely to be expelled from the uterus if it was placed a day or two before ovulation. Based on the anecdotal information from the Netherlands (message to Equine Clinicians Network, Dr Randy J. T. de Greef, March 19, 2000) and our preliminary experience, we decided to place the glass ball in the uterus at the examination following ovulation. Our assumption when placing the glass ball following ovulation was that the cervix would have started to close under the influence of rising progesterone, which might help prevent it from being expelled. We found that younger mares were more likely to expel the glass ball, perhaps because younger mares have more effective uterine clearance (in estrus and the periovulatory period) and a less dependent uterine position than do older mares. The smaller diameter and lighter weight of the 25-mm glass ball also may have contributed to the spontaneous expulsion from 6 mares, al-

though it is also possible the cloprostenol contributed to the loss in some mares. However, losses observed during preliminary work were not associated with cloprostenol administration, and neither was the loss in this study from the mare that expelled the ball during the subsequent estrus at 11 days following placement.

Idiopathic persistence of the primary CL, also known as spontaneous persistence of the CL,⁷ cannot be completely ruled out as an explanation for the extended luteal function observed in this study. The incidence of idiopathic persistence is reported to vary widely.^{8,9} Ginther and Pierson⁸ did not observe idiopathic persistence in any of 69 interovulatory intervals, while Stabenfeldt and Hughes⁹ suggest it can occur in as many as 25% of estrous cycles. However, acceptance of inadequate evidence for the condition may have led to an overestimation of the incidence in some reports.⁷

The proportions of diestrus periods that resulted in an extended luteal period were not different between the control (4 of 50) and glass ball (7 of 62) cycles. However, the proportion of cycles in mares with glass balls inserted is heavily biased by the 4 cycles from each mare that did not experience extended luteal function. A greater proportion of mares (7 of 18) experienced extended luteal function when a glass ball was in the uterus than without a ball (4 of 32) during the control cycles. Four of the mares that experienced extended luteal function with a glass ball in the uterus did so during the first diestrus following placement and did not experience subsequent ovulations. These mares are consequently underrepresented in the proportion of diestrus periods among the mares that had a glass ball inserted, especially if all or some of the mares had experienced additional periods of extended luteal function following subsequent ovulations. This would seem to make the proportion of mares a more valuable indicator of glass ball efficacy. However, in all fairness, we should point out that 2 of 7 mares that experienced extended luteal function did so after the third ovulation following glass ball placement (Fig 5). The control data were collected from 32 mares in 50 estrous cycles; only 18 of the mares were observed in more than one cycle. This may have biased our results for fewer occurrences of idiopathic persistence of the CL, although we believe it is unlikely.

Five of the 7 mares in this study that experienced extended luteal function when a glass ball was in the uterus had never previously been observed to have prolonged interovulatory intervals over multiple seasons. Historic data were not available for the other 2 mares. The average length of the luteal period reported to be associated with idiopathic persistence of the CL is approximately 2 months.⁷ The average length of luteal function observed in this study was 3 months. These several points would seem to support the idea that the extended luteal function observed in this

study was indeed affected by the glass ball protocol rather than idiopathic persistence. Therefore, although it is not possible to positively distinguish idiopathic persistence of the primary CL from extended luteal function influenced by the glass ball protocol, we believe that our observations in this study suggest a genuine effect. An explanation is not readily apparent for our observation that mares experiencing extended luteal function were younger than those that did not experience extended luteal function.

Two possible explanations for an effect of the glass ball have been discussed (Equine Clinicians Network archives). First, the glass ball simulates a conceptus and through movement and physical contact prevents prostaglandin release from the endometrium, in turn maintaining the CL (an endogenous progesterone source) indefinitely. A second theory suggested that the glass ball would stimulate mild inflammation, in turn causing release of small amounts of prostaglandin that would be inadequate to achieve luteolysis. As long as the glass ball was present, the endometrium would remain in a prostaglandin-depleted state and the CL would be maintained.

In the event that the first theory discussed was correct, two glass ball sizes were evaluated in this study to account for a range in vesicle diameter that would be expected to occur naturally during the early stages of pregnancy. However, this theory seems to assume that the physical presence of a spherical structure, in this case a glass ball, in the uterine lumen will prevent prostaglandin release. This is contrary to conventional logic that would assume, as has been demonstrated in other species, that a chemical messenger is produced by the equine conceptus to allow maternal recognition of pregnancy and avoid prostaglandin release.¹⁰

The second theory is as equally confusing, considering that there are countless reasons why a mare may have low-grade endometrial inflammation, yet clinically we do not recognize scores of mares that maintain their luteal tissue indefinitely. Both theories suggest that the glass ball is mobile enough to contact the majority of the endometrium or cause low-grade endometrial inflammation throughout the uterus. Our findings did not support the idea that the glass ball was particularly mobile in the uterus. Although the ball did move between the uterine horn-body junctions in most mares, the distance moved was only a few centimeters. In 2 mares, the glass ball did not move at all. One of the mares experienced extended luteal function in spite of the lack of ball movement. It probably also would be more logical to assume that an irritant to the endometrium would cause low-grade inflammation, which in turn would likely trigger sufficient release of endogenous prostaglandin $F_{2\alpha}$ to cause luteolysis.^{11,12} Uterine biopsy results did not reflect an increase in endometrial inflammation. Further, the interovulatory interval and functional life of the CL was >2 days

longer during control cycles than in cycles when a glass ball was in the uterus. This would suggest that the ball was more likely to cause early regression of the CL.

Placing the glass ball following ovulation could predispose a mare to endometritis, considering the procedure involved passing a foreign object, although sterilized, through the cervical lumen after a mare has entered diestrus. A mare susceptible to endometritis may not have time to clear her uterus of contaminants before closing the cervical lumen completely. Based on this rationale and experience during our preliminary work, we decided to provide treatments simultaneous to glass ball placement that were intended to help prevent a persistent postplacement endometritis. Hence, each mare in this study was infused with ticarcillin disodium (Ticar) and treated with cloprostenol (Estrumate) following glass ball placement. Uterine fluid was only observed in a few mares in the first few days following glass ball placement, and it resolved quickly. Nevertheless, it is advisable to re-examine a mare with ultrasonography following placement of a glass ball to ensure a detectable endometritis has not developed.

Discussions on the Equine Clinicians Network suggested that the glass ball had no long-term detrimental effects on the uterus. The relatively minor ultrasonic changes detected in the uterus following glass ball placement, the endometrial biopsy results, and a conception in 74% of the mares following glass ball removal would seem to support this claim.

It is interesting that the mares experiencing extended luteal function were able to maintain progesterone concentrations above 1 ng/mL for an average of nearly 3 months. This is the period in which we would expect the fetoplacental unit to begin assuming maintenance of pregnancy through the production of pregnanes in an ever-increasing number of pregnant mares. The progesterone profiles in these mares were very similar to those reported for mares hysterectomized 3 days following ovulation.¹³ The primary CL was present 70 days following ovulation in hysterectomized mares, but disappeared by 140 days.¹³ We found this was also true of mares that experienced extended luteal function with a glass ball in the uterus.

The efficacy of an intrauterine glass ball for maintaining luteal function and thus preventing cycling and behavioral estrus in mares appears to be moderate. Our results were not quite as good as those reported from the Netherlands (Dr Randy J. T. de Greef, message to Equine Clinicians Network, March 19, 2000). The Dutch veterinarian indicated that the technique works in at least 75% of cases; in contrast, we found the technique to be effective in only approximately 40% of mares. Perhaps a placebo effect for mare owners, as is suspected with the use of progestin implants, would explain the additional success reported from the Netherlands.

The glass ball protocol takes advantage of endogenous progesterone production to suppress behavioral estrus. Some variation in estrus behavior will be observed in any group of mares teased to a stallion throughout the cycle. However, mares are typically expected to reject a stallion when a functional CL is present and to change from indifferent to receptive as estrogens rise in the absence of a functional CL. Teasing results in this study were consistent with the behavior expected for the concentration of circulating progesterone detected. We did not monitor the mares that experienced extended luteal function beyond their subsequent ovulation, although we speculate that if the glass ball had been left in the uterus, some of the mares may have experienced another extended luteal period. Another researcher related information to us about 2 mares in which he had placed a glass ball (personal communication, Dr Peter Daels, National Institute of Agricultural Research, Nouisilly, France, April 2000). The mares experienced extended luteal function, then, following administration of prostaglandin, both mares returned to estrus, retained the glass ball, ovulated, and again experienced extended luteal function.

This technique offers the advantage of suppressing behavioral estrus because of endogenous progesterone production over an extended period following a single administration of a glass ball. The disadvantage is that it does not work in every mare nor does it appear to have an immediate effect in every mare following intra-uterine placement of the glass ball. However, when it is effective it may serve as an alternative method for suppressing estrous cycle and/or behavior and thus avoiding the need for administration of exogenous progestin products. Readers are cautioned that many questions still exist about the use of intra-uterine glass balls in mares. Further work is required to confirm the efficacy of the use of an intra-uterine glass ball for prolonged luteal function in mares and to identify its mechanism of action.

We thank the ERC technicians and veterinary students who participated in this study.

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Case Report

Complications associated with the presence of two intrauterine glass balls used for oestrus suppression in a mare**R. M. Turner*, D. K. Vanderwall[†] and R. Stawicki[‡]**

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Summary

This report describes the identification and removal of 2 glass balls, both of which had fragmented, from the uterus of a mare. The glass balls had been placed into the uterine lumen to prolong function of the corpus luteum for suppression of oestrous behaviour. This report adds to a growing body of evidence that severe complications can result from the use of intrauterine glass balls for oestrus suppression in mares, particularly if they are retained for a prolonged time.

Introduction

Suppression of oestrous behaviour is commonly performed in mares to prevent the behaviour from adversely affecting performance activities. General methods of suppressing oestrous behaviour include: 1) administration of exogenous progesterone or synthetic progestins; 2) extending the duration of corpus luteum (CL) function (i.e. maintaining secretion of endogenous progesterone); 3) suppressing ovarian follicular activity; and 4) ovariectomy (Vanderwall and Nie 2011). Of these, administration of progesterone or synthetic progestins has been the most widely used method of suppressing oestrous behaviour, with the orally active synthetic progestin altrenogest (ReguMate¹ or Altresyn²) widely considered to be the 'gold standard' for blocking oestrous behaviour in mares. Although daily administration of altrenogest at a dose of 0.044 mg/kg bwt is very efficacious for suppressing oestrus in mares (Squires *et al.* 1979; Webel and Squires 1982), its expense, need for long-term daily administration, and safety risks for personnel (particularly women) during handling and administration (Vanderwall 2012) are drawbacks to its use. Although there have been anecdotal reports on the use of synthetic progestins other than altrenogest for oestrus suppression, none have been found to be efficacious when rigorously tested (Vanderwall and Nie 2011).

An alternative method of suppressing oestrus that does not require administration of exogenous progesterone/progestins is prolonging the function of the CL, which is the endogenous source of progesterone that naturally keeps mares from displaying oestrus. In nonpregnant mares, the CL secretes progesterone for approximately 2 weeks after ovulation and then stops functioning as a result of endometrial secretion of prostaglandin F_{2α} (PGF_{2α}), which causes regression of the CL (i.e. luteolysis) by destroying the progesterone-producing cells of the CL. Luteolysis causes progesterone concentrations to fall and the subsequent return to oestrus. Luteolysis does not occur

in pregnant mares because the embryo blocks secretion of PGF_{2α}, therefore maintaining continued progesterone secretion that prevents the mare from coming back into oestrus. Therapeutically preventing luteolysis to maintain progesterone secretion from the CL, essentially mimicking pregnancy, is an alternative method of suppressing oestrus in mares.

The most common method of prolonging CL function in mares has been intrauterine insertion of a glass ball (i.e. marble). Nie *et al.* (2003) reported that placement of a 25 or 35 mm sterile glass ball into the uterine body immediately following ovulation resulted in prolonged CL function in 7 of 18 (39%) mares that retained the glass ball (6 of 12 mares expelled the 25 mm glass ball soon after insertion). In mares that developed prolonged CL function following placement of the glass ball, CL function was maintained for approximately 90 days, during which time progesterone concentrations remained above 1.0 µg/l and oestrous behaviour was not displayed. In nontreated control mares, spontaneous prolongation of CL function occurred in 4 of 32 (13%) mares. Although placement of a glass ball appeared to be an efficacious means of blocking oestrous behaviour for an extended period, it should be noted that in addition to the 61% mares that retained the glass ball and never developed extended CL function, 3 of 7 mares had one or 2 oestrous cycles of normal duration after placement of the glass ball before CL function was prolonged. Therefore, on a 'per cycle' basis the incidence of prolonged CL function was only 11% (7/62 cycles) in the mares that received a glass ball compared to 8% (4/50 cycles) in the nontreated control mares, which was not significantly different between groups. Although the original report describing the use of an intrauterine glass ball for oestrus suppression found no adverse effects of the glass ball on the endometrium or subsequent fertility (Nie *et al.* 2003), it is becoming evident there are risks associated with the use of an intrauterine glass ball for oestrus suppression in mares. Recently, pyometra associated with long-term retention of a glass ball was reported (Klabnik-Bradford *et al.* 2013), and here we describe a case in which 2 intrauterine glass balls were identified in a mare. Both glass balls had fragmented and one had fragmented into multiple pieces.

Case history, clinical findings and treatment

An 8-year-old maiden Quarter Horse mare was presented for removal of 2 intrauterine glass balls, at least one of which had apparently fragmented. It was reported by the current owner

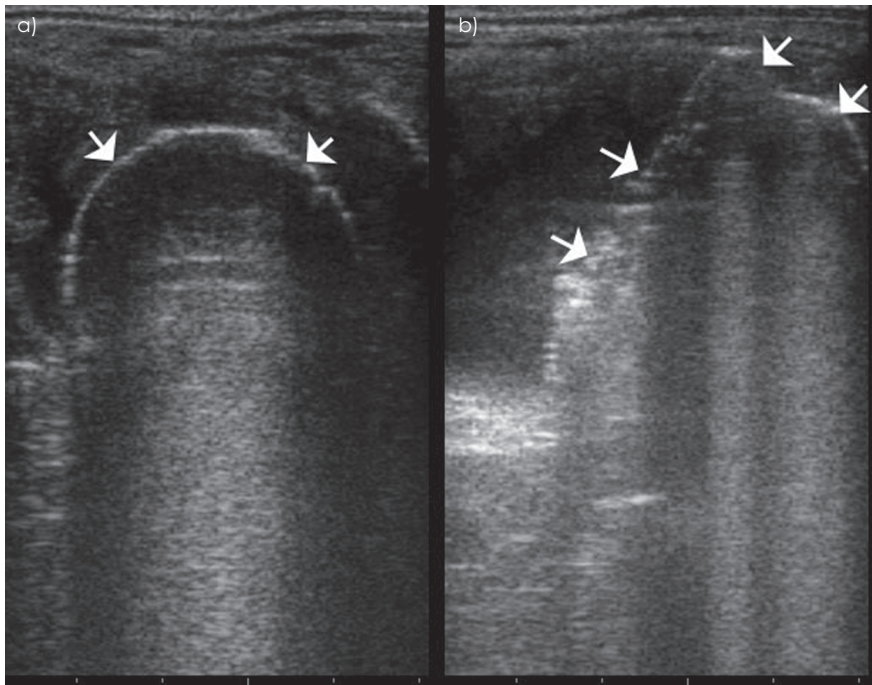


Fig 1: a) Transrectal ultrasonographic image showing an apparently intact glass ball in the uterine lumen. Arrows point to the smooth, curved surface. b) Transrectal ultrasonographic image showing a second, apparently fragmented glass ball in the uterine lumen. Note the irregular, flattened surface delineated by arrows. 7.5 MHz linear array transducer. Each marker line equals 1 cm.

that several years previously one glass ball had been placed into the mare's uterine lumen to suppress oestrus and, when that ball stopped 'working', a second glass ball was placed into the uterine lumen, apparently without knowledge that the first glass ball was still present in the uterus. Subsequently, the current owner acquired the mare and made plans to breed her. In preparation for breeding, the current owner's veterinarian examined the mare with transrectal palpation and ultrasonography, at which time the presence of at least one intrauterine glass ball was noted. The owner's veterinarian made an attempt to retrieve the glass ball transcervically, but found the cervix to be adhered closed, so the mare was referred for additional diagnostic evaluation and treatment.

At the time of presentation, palpation and ultrasonographic examination of the reproductive tract *per rectum* revealed the presence of 2 structures consistent with glass balls in the uterine body (**Fig 1**). One glass ball appeared to be intact, while the second structure was irregular and appeared to be broken. Several other echogenic structures were identified in the uterine lumen, consistent with multiple glass fragments. Examination *per vagina* revealed a presumptive piece of glass embedded within the cervical tissue surrounding the external cervical os. When assessed *per vagina*, the cervical canal was fibrosed closed. Attempts were made to manually reduce the cervical adhesions and enter the uterus, but the mare became uncomfortable and the attempts were temporarily discontinued. Plans were made to sedate the mare and perform an endoscopic examination of the cervix and uterus.

The mare was sedated with 0.01 mg/kg bwt detomidine hydrochloride (Dormosedan)³, and 0.02 mg/kg bwt butorphanol tartrate (Torbugesic)³, i.v. Mepivacaine hydrochloride (20 ml 2%; Carbocaine-V)³ was infused into the cervical canal to provide local anaesthesia to the cervix. More aggressive attempts to manually break down the adhesions around the cervix were successful and access to the uterus was obtained. Endoscopic examination confirmed the

presence of one largely intact glass ball and a second ball that had fractured into multiple pieces (**Fig 2**). Two of the fractured pieces were removed with a wire snare through the cervix. However, several small, smooth shards of glass and the larger intact ball could not be grasped for removal as the wire snare could not gain traction and repeatedly fell off once tension was applied. Additional attempts were made to dilate the cervix, and eventually sufficient cervical dilation was achieved to allow passage of the examiner's fingers into the uterine lumen. The apparently intact glass ball and approximately 7–10 additional shards of glass were identified in the uterine body and were removed manually. Following removal of all the accessible glass, the uterus was re-examined endoscopically and found to be in remarkably good condition and free of any gross evidence of glass pieces (**Fig 3**). The cervix also was re-examined endoscopically and manually and was notably improved. Apparently, the embedded fragment had migrated back into the uterine lumen during manipulation of the reproductive tract and had been removed. All of the adhesions had been reduced, resulting in a patent and palpably normal cervical canal.

Following endoscopy, the mare's uterus was lavaged with 6 l of sterile saline. No additional pieces of glass were noted in the fluid that was recovered. Likewise, ultrasonographic examination of the reproductive tract did not reveal any evidence of glass fragments remaining in the uterus. The uterus was prophylactically infused with 2 g of ampicillin sodium diluted in 60 ml of sterile water. Flunixin meglumine (1 mg/kg bwt i.v.; Banamine)⁴ was administered and a topical cream, consisting of nystatin, neomycin, thiostrepton and triamcinolone (Animax Ointment)⁵, was applied to the cervix prior to discharge. The topical ointment was applied to the cervix once every 2–3 days for 2 weeks following discharge.

The glass fragments were examined following removal. There were 2 approximately 35 mm glass balls. The first ball (blue) was largely intact, with only one relatively small piece shorn off. The second ball (tan) was in multiple pieces,

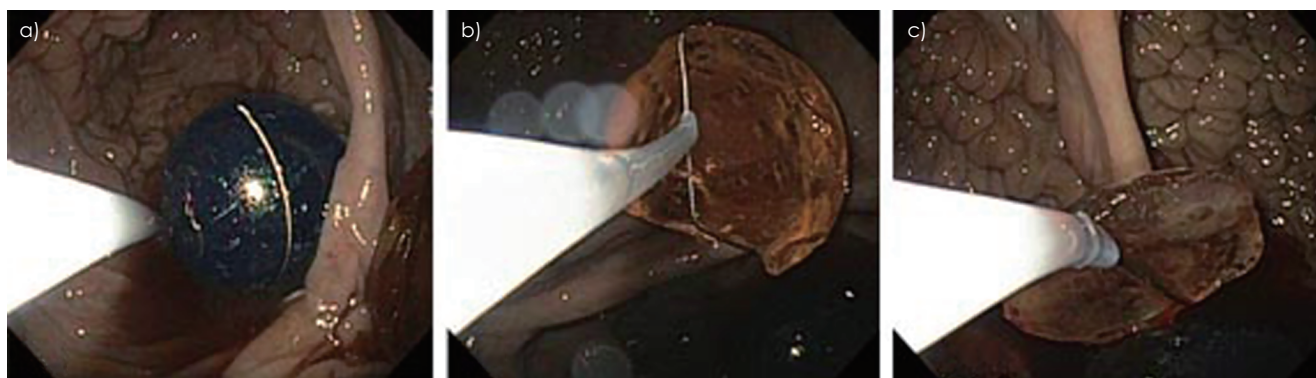


Fig 2: a) Endoscopic view of the uterine lumen containing an apparently intact glass ball. The ball is encircled by a wire snare. However, the smooth surface of the ball caused the snare to slide off each time that traction was applied. b) and c) Endoscopic views of the uterine lumen containing 2 different irregular pieces from the second glass ball. Both pieces are encircled by a wire snare and were successfully removed from the uterus with the snare.



Fig 3: Endoscopic view of the uterine lumen immediately following removal of the glass balls and shards. The endometrium appeared to be in remarkably good condition and there was no gross visual evidence of any additional glass in either uterine horn or in the uterine body. The oviduct papilla is visible at the tip of the uterine horn (arrow).

including 3 larger pieces and at least 10 smaller shards (**Fig 4**). When all of the glass pieces were reassembled, they appeared to form 2 complete 35 mm glass balls. However, particularly for the tan ball, we could not definitively determine if small pieces still were missing, potentially having been left behind embedded in the uterine wall.

The owners reported that the mare recovered uneventfully from the procedure. Two attempts were made to breed the mare in the year following the glass removal. Breedings were performed by the referring veterinarian on the farm using artificial insemination with cooled transported semen from a fertile stallion under good management. The mare did not become pregnant after these breedings and was returned to work under saddle as a Western Pleasure Horse. No additional reproductive evaluations were performed.



Fig 4: Glass balls and associated shards removed from the mare's uterus. The blue glass ball on the left was largely intact with only one small piece shorn off. The tan glass ball on the right was in multiple pieces including 3 larger pieces and multiple smaller shards.

Discussion

This report adds to a growing body of evidence that severe complications can result from the use of intrauterine glass balls for oestrus suppression in mares, particularly if they are retained for a prolonged period of time. It seems plausible that the mare in this report came to have 2 glass balls in her uterus because it was assumed the first glass ball had been spontaneously expelled from the uterine lumen when cyclical oestrous behaviour returned, leading to the placement of the second glass ball without knowledge the first ball was still present in the uterine lumen. The presence of 2 glass balls in the uterine lumen of a mare has been previously reported (Vanderwall 2013) and, although both glass balls were intact, they both showed noticeable pitting on their surfaces when they were removed. It seems plausible that when 2 balls are present, repeated contact of the 2 balls within the uterine lumen eventually results in shearing and fragmentation. Fragmentation of the glass ball in this mare raises serious concerns for future fertility, since there is no way to know with

certainty that all of the fragments were successfully removed (particularly very small pieces). If fragments were left behind, they could impair fertility by serving as a nidus for inflammation/infection and/or by physically damaging a conceptus, particularly during the mobility phase of early gestation.

Although this mare's problems may have been caused by the interaction of 2 glass balls in the uterus, even a single glass ball can result in significant complications, including pyometra (Klabnik-Bradford *et al.* 2013) and fragmentation (M. LeBlanc, personal communication, 2009). Surprisingly, there are anecdotal reports of mares becoming pregnant despite the presence of an intact intrauterine glass ball, with some of these pregnancies going to term, while others have been aborted presumably due to adverse consequences of the glass ball (personal communications through the American College of Theriogenologists List Server).

Because of the spectrum of complications that have now been associated with the use of intrauterine glass balls (pyometra, multiple glass balls with fragmentation, pregnancy/pregnancy failure), it would seem prudent to re-evaluate the use of these devices for oestrus suppression in mares. Although the specific complication of glass fragmentation can be avoided by using a device made of an alternative material such as plastic (Rivera Del Alamo *et al.* 2008), it is important to note that regardless of the composition of the device, if it is not eventually removed (spontaneously or manually), some mares may retain the device for an extended period of time (i.e. years), such that its presence in the uterine lumen may not be known to individuals who do not have access to the animal's complete history. This could lead to other complications such as pyometra, placement of a second device, breeding in spite of the presence of the ball etc. Given the potential for severe consequences associated with the use of intrauterine glass balls for oestrus suppression, there is a need for other methods of extending CL function that are practical, efficacious and safe. Alternative methods of prolonging function of the CL for oestrus suppression include: 1) oxytocin treatment (Vanderwall *et al.* 2007); 2) inducing a late-dioestrus ovulation (Hedberg *et al.* 2006); 3) intrauterine infusion of plant oils (Wilsher and Allen 2011); and 4) manually terminating pregnancy after maternal recognition of pregnancy (i.e. after the embryo has blocked endometrial PGF_{2α} secretion; LeFranc and Allen 2004). For a complete review of these methods see Vanderwall (2013).

Authors' declaration of interests

No conflicts of interest have been declared.

Manufacturers' addresses

¹Intervet, Millsboro, Delaware, USA.

²CEVA Animal Health, Lenexa, Kansas, USA.

³Zoetis, Florham Park, New Jersey, USA.

⁴Merck Animal Health, Whitehouse Station, New Jersey, USA.

⁵Dechra USA, Overland Park, Kansas, USA.

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