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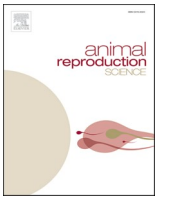

An intrauterine device with potential to control fertility in feral equids

Carlos Gradil [a,*], Carolynne Joone [b], Teresa Haire [c], Bradley Fowler [d], Jacquelyn Zinchuk [c], Christopher J. Davies [e], Barry Ball [f]

[a] Cummings Tufts School of Veterinary Medicine/Vet&AnSci, UMass Amherst, MA, USA
[b] James Cook University, Townsville, QLD, Australia
[c] Vet&AnSci, UMass, Amherst, MA, USA
[d] Cummings Tufts School of Veterinary Medicine, N Grafton, MA, USA
[e] Animal, Dairy and Vet Sci, USU, Logan, UT, USA
[f] Gluck Equine Research Center, Lexington, KY, USA

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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. Trans-abdominal 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 (P_4) 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 P_4 concentrations <1 ng/mL, and seven of eight mares had periods greater than 14 days with P_4 concentrations>1 ng/mL. There was a marked abundance of biofilm on devices of two mares at the time of device removal.

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.,

* Corresponding author at: Cummings Tufts School of Veterinary Medicine/Vet&AnSci, UMass, 661 N, Pleasant Street, Amherst, MA, 01003, USA.
E-mail address: gradil@umass.edu (C. Gradil).

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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 × 16 mm with a mean unit weight of 18.9 g; Magnetic force between the units is 3.8 newtons.

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 (P_4)/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 3–15 years, clinically normal and non-pregnant were selected for this study that was 120 days in duration (April-August, 2019). The mean ± 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

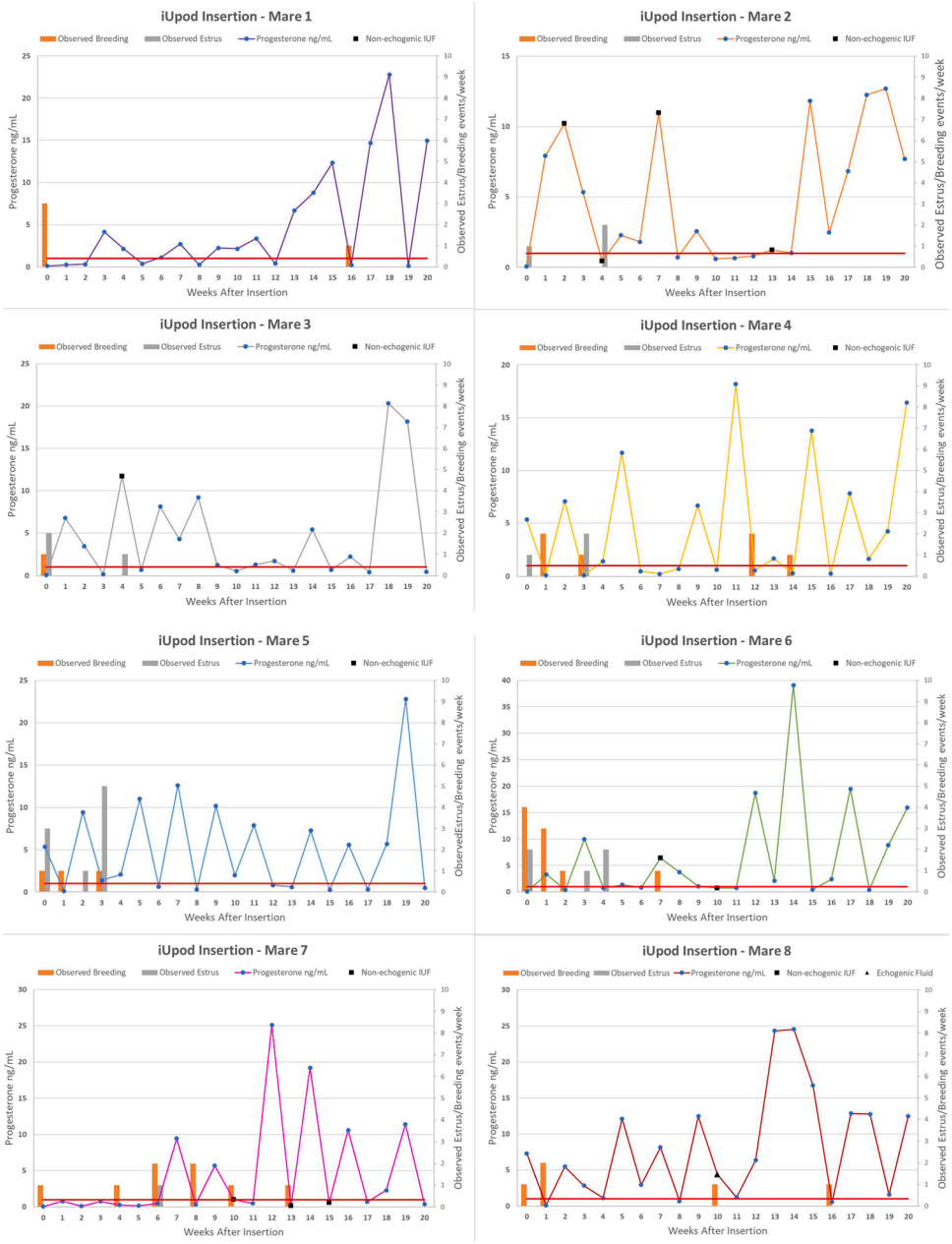

Fig. 2. Depiction of observed estrus/mating/week, progesterone (ng/mL), and intrauterine fluid (IUF) in mares fitted with a frameless self-assembling intrauterine device (iUPOD);
Device was inserted into the uterus of eight mares in varying stages of the estrous cycle.
Device was removed at 90 (Group 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.

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 ha 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 × 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 × 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 detomidine hydrochloride (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 min 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 for at least 15 min 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 min. 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 P_4 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 min within 1 h of collection and serum was frozen at −20 °C until laboratory analysis.

2.7. Analysis of blood

The P_4 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 P_4 values were as follows: Absence of active luteal tissue: 0.1 to 0.5 ng/mL; Non-discriminating for the presence of luteal tissue: 0.5–1.0 ng/mL; Presence of luteal tissue: > 1.0 ng/mL.

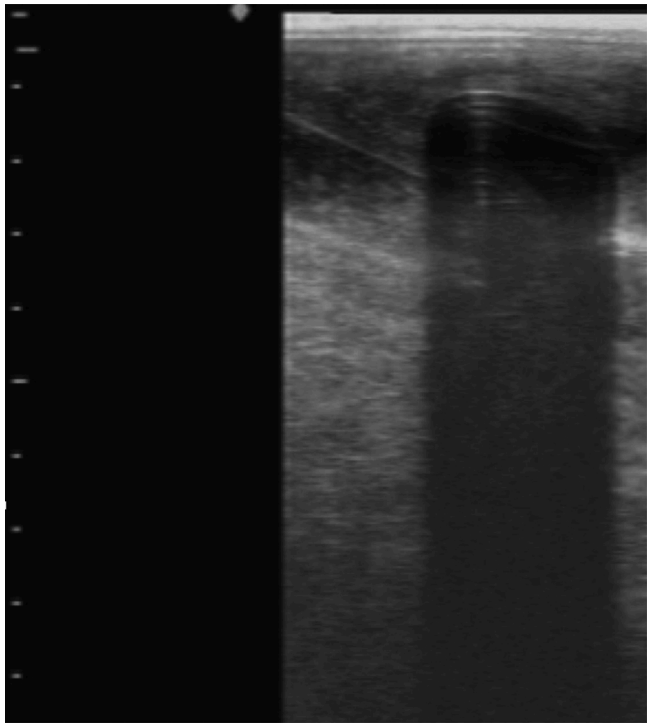

Fig. 3. Ultrasonic image of a self-assembled iUPOD in the uterus.
Note the intrauterine fluid in close proximity to the device.
Scale on the left is in increments of 10 mm.

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 (Figs. 1 and 3). The iUPOD (40 $\times$ 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)

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[a,b]
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.
[a] Pregnant in the post-removal period. Mares conceived within 2 cycles (30 days) post-iUPOD retrieval.
[b] Echogenic fluid.

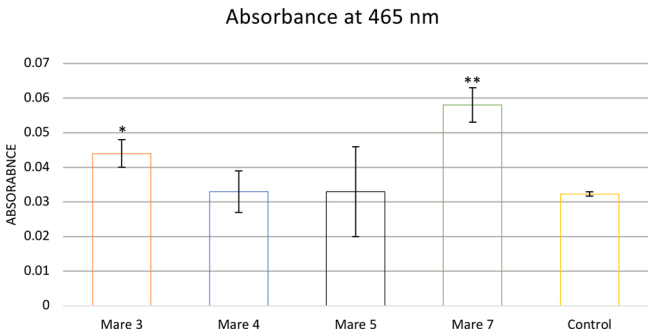

Fig. 4. Biofilm detected by the Crystal Violet Assay on iUPODs in four mares (Group B) with devices *in situ* for 120 days.
[a]Samples from all mares were evaluated in triplicate, except for one mare for which there was a duplicate evaluation.
[b]Mare with the most recorded matings $P < .05$*; $< .01$**.

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 a infrequent of ultrasonic exams (*i.e.*, every three weeks) it was apparent based on the presence and location of copora 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 ± 0.02 ng/mL) and three of eight mares were in diestrus (P_4 = 5.99 ± 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 Fig. 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 ± 0.0046**; 0.033 ± 0.0055); 0.033 ± 0.0132); 0.044 ± 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

Note: move this line up 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 47×49 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 $\geq$ 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 and Mikolajczyk, 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

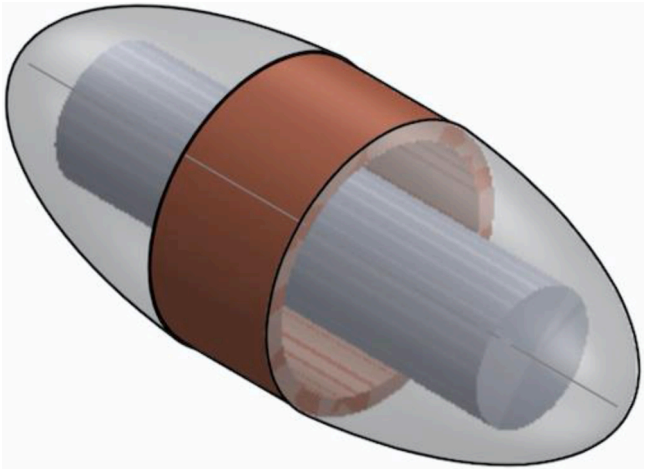

Fig. 5. A 2 + IUCD - Intrauterine copper Device prototype.
Copper self-assembling iUPOD unit could potentially be used to suppress reproduction in feral populations of equids.

concentrations at 0.1 to 0.5 ng/mL or 0.5–1.0 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 × 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 ± 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 3–5 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.

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References

Baker, D.L., Powers, J.G., Ransom, J.I., McCann, B.E., Oehler, M.W., Bruemmer, J.E., Galloway, N.L., Eckery, D.C., Nett, T.M., 2018. Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine (GonaCon-Equine) in free-ranging horses (Equus caballus): limitations and side effects. PLoS One 13. https://doi.org/10.1371/journal.pone.0201570 e0201570.

Daels, P.F., Hughes, J.P., 1995. Fertility control using intrauterine devices: an alternative for population control in wild horses. Theriogenology 44, 629–639.

Gradil, C., 2017. IUD modulation of the reproductive cycle. In: Proceedings of the 8th International Conf. Wild. Fert. Cont. May 29. Reno, NV, p. 44.

Gradil, C., Uricchio, C.K., Schwarz, A., 2019. Self-assembling intrauterine device (Upod) modulation of the reproductive cycle in mares. J. Eq. Vet. Sci. 83, 102690. https://doi.org/10.1016/j.jevs.2019.02.009.

Hobbs, R.J., Hinds, L.A., 2018. Could current fertility control methods be effective for landscape-scale management of populations of wild horses (Equus caballus) in Australia? Wild. Res. 45, 195–207. https://doi.org/10.1071/WR17136.

Joone, C.J., Gradil, C.M., Picard, J.A., Taylor, J.D., de Tonnerre, D., Cavalieri, J., 2021. The contraceptive efficacy of a self-assembling intra-uterine device in domestic mares. Aust. Vet. J. https://doi.org/10.1111/avj.13055.

Kadouri, D.E., Tran, A., 2013. Measurement of predation and biofilm formation under different ambient oxygen conditions using a simple gasbag-based system. Appl. Environ. Microbiol. 79, 5264–5271. https://doi.org/10.1128/AEM.01193-13.

Kane, A.J., 2018. A review of contemporary contraceptives and sterilization techniques for feral horses. Hum. Wild. Int. 12, 111–116. https://doi.org/10.26077/h8mj-q280. https://digitalcommons.usu.edu/hwi/vol12/iss1/12.

Kaneshiro, B., Aeby, T., 2010. Long-term safety, efficacy, and patient acceptability of the intrauterine copper T-380A contraceptive device. Int. J. W. H. 2, 211–220. PMCID: PMC2971735.

Katila, T., 2012. The equine cervix. Pferdeheilkunde 28, 35–38. https://www.pferdeheilkunde.de/10.21836/PEM20120108.

Killian, G., Miller, L.A., Diehl, N.K., Rhyan, J., Thain, D.S., 2006. Long-term efficacy of three contraceptive approaches for population control of wild horses. In: Proc. of the 21st Vert. Pest Conf. March 1-4. Visalia, CA, pp. 67–71.

Killian, G.J., Thain, D., Diehl, N.K., Rhyan, J.C., Miller, L.A., 2008. Four-year contraception rates of mares treated with single-injection porcine zona pellucida, GnRH vaccine, and intrauterine devices. Wild. Res. 35, 531–539.

Kirkpatrick, J.F., Lyda, R.O., Frank, K.M., 2011. Contraceptive vaccines for wildlife: a review. Am. J. Reprod. Immunol. 66, 40–50. https://doi.org/10.1111/j.1600-0897.2011.01003.x.

Klein, V., Müller, K., Schoon, H., Reilas, T., Rivera del Alamo, M., Katila, T., 2016. Effects of intrauterine devices in mares: a histomorphological and immunohistochemical evaluation of the endometrium. Reprod. Domest. Anim. 51, 98–104. https://doi.org/10.1111/rda.12651.

Lagree, K., Mon, H.H., Mitchell, A.P., Ducker, W.A., 2018. Impact of surface topography on biofilm formation by *Candida albicans*. PLoS One 13. https://doi.org/10.1371/journal.pone.0197925 e0197925.

LeBlanc, M.M., Neuwirth, L., Jones, L., Cage, C., Mauragis, D., 1998. Differences in uterine position of reproductively normal mares and those with delayed uterine clearance detected by scintigraphy. Theriogenology 50, 49–54. https://doi.org/10.1016/S0093-691X(98)00112-5. PMID: 10734473.

Merritt, J.H., Kadouri, D.E., O'Toole, G., 2005. Growing and analyzing static biofilms. Curr. Prot. Microbiol. https://doi.org/10.1002/9780471729259.mc01b01s00. Jul; 01: Unit -1B.1.

Munro, C., Stabenfeldt, G., 1984. Development of a microtiter plate enzyme immunoassay for the determination of progesterone. J. Endocrinol. 101, 41–49.

NICE Clinical Guidelines, No. 30, 2019. Long-acting Reversible contraception.London: National Institute for Health and Care Excellence (UK). https://www.ncbi.nlm.nih.gov/books/NBK553263/.

Nie, G.J., Johnson, K.E., Braden, T.D., Wenzel, J.G.W., 2003. Use of an intra-uterine glass ball protocol to extend luteal function in mares. J. Eq. Vet. Sci. 23, 266–273.

Ortiz, M.E., Croxatto, H.B., Bardin, C.W., 1996. Mechanisms of action of intrauterine devices. Obstet. Gynecol. Surv. 51, 42–51. https://doi.org/10.1097/00006254-199612000-00014. PMID: 8972502.

Portus, B.J., Reilas, T., Katila, T., 2005. Effect of seminal plasma on uterine inflammation, contractility and pregnancy rates in mares. Equine Vet. J. 37, 515–519.

Rivera del Alamo, M.M., Reilas, T., Kindahl, H., Katila, T., 2008. Mechanisms behind intrauterine device-induced luteal persistence in mares. Anim. Reprod. Sci. 107, 94–106.

Rivera del Alamo, M.M., Reilas, T., Palviainen, M., Katila, T., 2018. Annexin A1, a phospholipase A2 inhibitor, is induced by intrauterine plastic spheres. J. Eq. Vet. Sci. 66, 207.

Stanford, J.B., Mikolajczyk, R.T., 2002. Mechanisms of action of intrauterine devices: update and estimation of postfertilization effects. Am. J. Obst. Gynecol. 187, 1699–1708.

Taha, M., Kohnen, C., Mallya, S., Kou, Y., Zapata, A., Ramirez-Arcos, S., 2018. Comparative characterization of the biofilm-production abilities of Staphylococcus epidermidis isolated from human skin and platelet concentrates. J. Med. Microbiol. 67, 190–197.
Tryon, S., 2019. Long-Term Management Options for the Bureau of Land Management's Wild Horse and Burro Program. July 16. Wild Horses and Burros. https://www.doi.gov/ocl/wild-horses-and-burros-0.
United Nations, Department of Economic and Social Affairs, Population Division, 2019. Contraceptive Use by Method: Data Booklet (ST/ESA/SER.A/435).
Wu, J.P., Pickle, S., 2014. Extended use of the intrauterine device: a literature review and recommendations for clinical practice. Contracept 89, 495–503.