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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.* 2006*a*; 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, 2006*b*; 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.* 2006*c*; 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

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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, 2006*b*). 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 anesthetised 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 titers 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 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

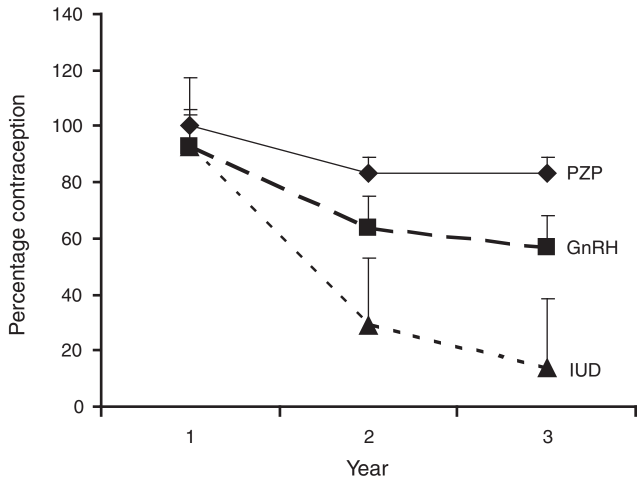

Fig. 1. Comparative rates of contraception for the three methods of contraception for each year of the four-year study. Rates of contraception for SpayVac-treated mares for Years 1, 2, 3 and 4 were 100% (12 of 12 mares), 83% (10 of 12), 83% (10 of 12) and 83% (10 of 12), respectively. Rates of contraception 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.

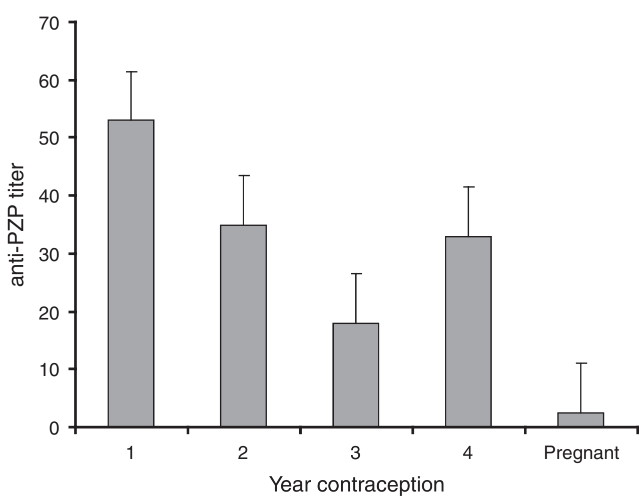

Fig. 2. Average anti-porcine zona pellucida (PZP) titres (±s.e.m.) for the autumn bleed for mares that were contracepted compared with titres of mares that became pregnant. The average titres for each of Years 1–4 were for the serum samples for the 10 mares that were infertile. The titre for the pregnant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

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 ± 1.4 ng mL^{-1}) than for pregnant IUD-treated mares (11.3 ± 2.7 ng mL^{-1}). The average serum oestradiol concentration of 62.5 ± 34.2 ρg 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 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 ultrasonagraphy 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. 3. Average anti-gonadotrophin-releasing hormone (GnRH) titres (±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.

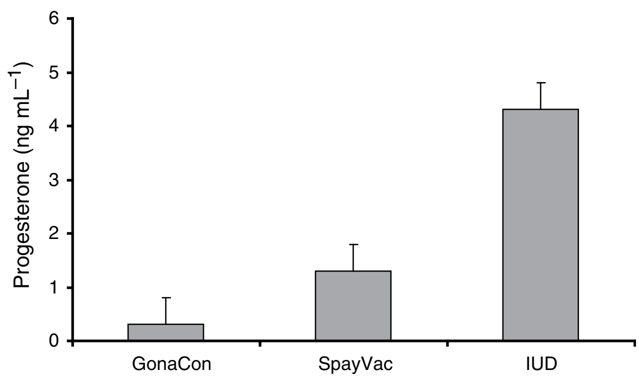

Fig. 4. Average progesterone values (ng mL^{-1}) (±s.e.m.) in non-pregnant GonaCon-treated mares ($n = 35$) and SpayVac-treated mares ($n = 46$) and non-pregnant intrauterine device (IUD)-treated mares ($n = 17$) for the autumn bleed for all years of the study. Values for GonaCon- and SpayVac-treated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

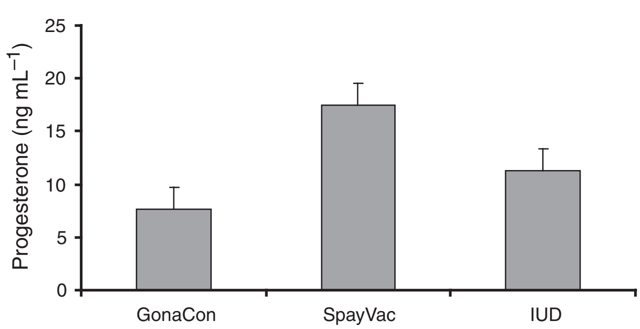

Fig. 5. Average serum progesterone (ng mL^{-1}) at the autumn bleed for pregnant GonaCon-treated-mares ($n = 8$), pregnant SpayVac-treated mares ($n = 2$) and pregnant intrauterine device (IUD)-treated mares ($n = 7$). Values were significantly different between IUD- and GonaCon-treated mares ($P < 0.025$); 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 ultrasonagraphy 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.

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

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.

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 $4\ \text{ng mL}^{-1}$ 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 $1.3\ \text{ng mL}^{-1}$ 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 GonCon 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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