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Evaluation of Three Contraceptive Approaches for Population Control of Wild Horses

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ABSTRACT: Overpopulation of feral horses in several western states is an unquestioned problem. Current management strategies of removal and adoption are expensive, logistically challenging, and ineffective as a means of population control. We are testing three long-acting contraceptive approaches on feral Nevada mares. Modified reversible immunocontraceptive vaccines for gonadotropin releasing hormone (GnRH) and porcine zona pellucida (PZP) (SpayVac), and intrauterine contraceptive devices (IUDs), are being evaluated to determine: 1) their safety and efficacy for preventing pregnancy for multiple years, 2) whether the effects are reversible, and 3) whether there are notable contraindications. Preliminary data after 1 year suggest that IUD- and PZP-treated mares continue to exhibit breeding and estrus, while GnRH-treated mares are less likely to cycle. All mares in the GnRH and PZP treatments were infertile for the breeding season. Eighty percent of the IUD-treated mares were infertile; those mares that became pregnant likely failed to retain the IUD. A notable contraindication was that uterine edema normally observed in mares in the follicular phase of the estrous cycle was commonly observed in PZP-treated mares. Because administration of each contraceptive approach is different, and each has different effects and expected duration, one approach or a combination of approaches may be best suited for specific field applications. Subsequent years of this study should establish the efficacy and safety of one or more long-acting contraceptive approaches for feral horse population control.

KEY WORDS: contraception, feral horse, fertility control, GnRH vaccine, intrauterine device, SpayVac-PZP vaccine

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INTRODUCTION

Overpopulation of feral horses in several western states is an unquestioned problem. In Nevada, feral horses under state control number about 1,200 in an area of 360,000 acres. This represents 2 - 2.5× the number of horses suitable for that area. Feral horse populations grow at a rate of 15 - 20% a year while their range continues to shrink. Current management strategies of removal and adoption are expensive, logistically challenging, and have not been an effective means of population control. Conflicting interests associated with increased movement of people into feral horse ranges, sympathy to maintain feral 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 feral horse overpopulation.

Reduction of free-ranging horse numbers by limiting fertility holds great promise for humane and effective population control. Ideally, contraception of feral horses should be safe and potentially reversible, effective for several years, and have minimal effect on reproductive or harem maintenance behavior. However, fertility control by the porcine zona pellucida (PZP) vaccine in current use has not shown consistent effectiveness for more than 1 - 2 years (Turner et al. 2002). Annual vaccination involves much expense, manpower, and horse handling to maintain infertility and increases risks of injury to both the animals and human handlers. Use of long-acting contraceptive approaches on feral horses would achieve effective population reduction along with reduced costs and risks associated with frequent animal handling. Unfortunately, no proven options exist for long-term contraception of horses.

Immunocontraceptive vaccines are based on the principle that an animal's immune system can be used to prevent reproduction. The immunogens of these vaccines stimulate antibody production against native gamete proteins, reproductive hormones or proteins involved in early gestation. Vaccinated animals with sufficient titers form antibody-antigen complexes that interfere with or block an essential step in the reproductive process.

The most widely used and successful zona pellucida vaccines are derived from ovarian extracts of porcine zona pellucida (PZP). PZP is a complex of 4 large glycoproteins and is recognized as a foreign protein when injected into a non-porcine host. Because there is considerable amino acid sequence conservation among species for the zona pellucida, the immune response in PZP-treated animals includes antibodies that cross-react with native zona pellucida.

Injectable PZP vaccines have been used widely to induce infertility in a variety of species including deer (Turner et al. 1997), elephants (Fayer-Hosken et al. 1999), dogs (Mahi-Brown et al. 1989), baboons (Dunbar 1989), seals (Brown et al. 1997), burros, and horses (Liu et al. 1989, Kirkpatrick et al. 1996, Turner et al. 2002). Typically with a primary and booster vaccination, infertility can be achieved in 80-90% of animals for 1 year and further sustained by annual vaccination. Kirkpatrick, Turner, and colleagues have used the PZP vaccine effectively to manage reproduction in selected feral horse populations by annual vaccination (Turner et al. 2002). Their pioneering work has documented the apparent safety and reversibility of the vaccine and some physiological and behavioral responses of the mare (Kirkpatrick et al. 1996, Turner et al. 2002). These include repeated estrous cycles during the breeding season and improved body condition and increased longevity (Tuner and Kirkpatrick 2002). However, long-term treatment with PZP vaccine given annually is associated with some ovulation failure and depressed urinary estrogens (Kirkpatrick et al. 1995).

Gonadotropin releasing hormone (GnRH) stimulates the anterior pituitary gland to produce and release FSH and LH. These hormones in turn regulate gamete and hormone production by the gonads. By blocking GnRH, in either males or females, production of sex hormones and gametes ceases and treated animals enter a non-breeding condition similar to that of anestrous in seasonal breeders. Use of GnRH vaccine as a fertility blocking agent has been studied in cattle (Adams and Adams 1992), horses (Rabb et al. 1990), swine (Meloen et al. 1994), and rams (Brown et al. 1994). These studies have been of short duration, although we recently completed a long-term study using the GnRH vaccine on white-tailed deer establishing its efficacy, safety, and reversibility (Miller et al. 2000).

Extensive data for women have demonstrated that IUDs are safe and effective for long-term contraception (Fortney et al. 1999). According to "Population Reports" (Vol. XXIII, #5, December 1995), in studies evaluating the copper-containing TCu-380A IUD, fewer than 1 in 100 women became pregnant after 1 year, and 2.1 in 100 women became pregnant after 10 years of continuous use. Fertility was restored following IUD removal. IUDs have also been shown to block fertility in ewes (Hawk et al. 1974, French 1976), and cows and heifers (Hawk et al. 1968, Turin et al. 1997, Fordyce et al. 2001) in short-term studies. The mechanism of IUD-induced infertility is speculative (Ortiz et al. 1996). Humoral factors may be released that interfere with signaling between the ovary and the uterus, blocking fertilization and early embryo development. We are aware of only one report of IUD use in mares (Daels and Hughes 1995). Six mares implanted with a silastic ring IUD remained infertile for a season, but after IUD removal all had foals the next season. Mild chronic endometritis was observed in the treated mares, but no permanent changes in the endometrium remained after IUD removal.

Based on published studies and ongoing studies we have with deer, swine, and ponies, we believe that 3 long-acting contraceptive approaches warrant study in feral horses. These are the SpayVac PZP vaccine, a GnRH vaccine developed at the National Wildlife Research Center, and the 380 Copper "T" human intrauterine device.

The objectives of this study are to evaluate these approaches in Nevada mares for multiple-year contraceptive efficacy, whether the contraceptive effects are reversible, and whether notable contraindications are associated with the treatments. This paper reports the results from the first year of the study.

MATERIALS AND METHODS

Animals

The Nevada Department of Agriculture provided 53 feral mares and 3 stallions for the project. The horses were gathered from state lands and maintained at the State of Nevada Penitentiary, Carson City facility. Mares and stallions were dewormed 3× and vaccinated annually for eastern and western encephalitis, West Nile virus, influenza, equine rhinopneumonitis, and tetanus. Nevada mares typically weigh between 225-360 kg.

To handle feral mares for jugular blood sampling and vaccinations, they were run into a hydraulic chute and haltered. Contraceptive vaccines were given intramuscularly in the left lateral neck. For pregnancy evaluations by ultrasound or palpation, or IUD placement, the mares were chemically restrained, released from the chute, and legs secured. Chemical restraint providing 20 - 30 minutes of anesthesia involved one or a combination of sedative and anesthetic agents. Initially, a combination of detomidine hydrochloride (0.02 - 0.04 mg/kg) and xylazine hydrochloride 0.8 mg/kg and acepromazine maleate (0.02 - 0.04 mg/kg) was given intravenously. After 5 - 7 minutes to allow for maximum sedation, mares were then given intravenously a combination of xylazine hydrochloride (1.2 mg/kg) and tiletamine HCL (1mg/kg) and zolazepam HCL (1mg/kg).

Treatments

Mares were randomly assigned to the following groups: untreated controls (n = 8), single shot of 400μg SpayVac PZP (n = 12), single-shot 1,800μg GnRH vaccine (n = 15), 2,800μg GnRH vaccine (n = 3), and copper-containing IUDs (n = 15). Doses of the single-shot SpayVac PZP vaccine (400μg) were kindly provided by its developer, Dr. Robert Brown (Brown et al. 1997). The single shot GnRH-AdjuVac vaccine was developed at and provided by the NWRC. Both vaccines were used in combination with AdjuVac adjuvant (Miller et al. 2003). Human Cu 380 T intrauterine devices were purchased from Family Planning Sales Limited, Littlemore, Oxford, UK.

Because of the logistics of acquiring all of the mares for this study, the treatments were administered over a period of one year as the mares became available. The treatments were selected based on preliminary studies and our prior experience with them. We have determined that the single shot PZP (SpayVac) vaccine used in this study is very effective for contraception of white-tailed deer for 4 years, while others have reported long-term contraception of fallow deer (Fraker et al. 2002). We have also determined the GnRH vaccine to be effective in deer and

swine for 2 to 3 years in ongoing studies. In preliminary studies at Penn State University, we evaluated 3 different copper IUDs in pony mares. Based upon ease of placement, retention in the uterus, lack of apparent uterine inflammatory response, and pregnancy prevention (3 of 4 mares) for 24 months, the human copper "T" IUD was selected for this trial.

Nevada mares were routinely observed for breeding activity by staff and prisoner caretakers, and they were checked 2 times yearly by ultrasonographic monitoring for pregnancy, IUD retention and uterine inflammation, and blood sampled. Blood samples were assayed for estradiol, progesterone, and antibody titers to the contraceptive vaccines. Contraindications evaluated included general health and body condition, and uterine edema, which may be associated with hormonal changes or presence of IUDs.

RESULTS AND DISCUSSION

Most Nevada wild horses maintained at the Carson City facility were possible to work with and collect data. However, on some dates we were unable to collect data from certain individuals, because of inadequate anesthesia and/or safety concerns.

After one breeding season involving study mares being with a proven stallion for at least 60 days, none of the mares in the PZP-SpayVac (0/12 mares) or GnRH (0/18) vaccine groups were pregnant as evaluated by ultrasonography (Figure 1). For the IUD mares, 20% became pregnant (3/15). This compares with 75% of the control mares (6/8) becoming pregnant. These results clearly indicate that both contraceptive vaccines were highly effective in preventing pregnancy. Although somewhat less effective, the IUD treatment was also considered successful in preventing pregnancy in a high percentage of mares. Based on our inability to observe the IUD in pregnant mares, a tentative conclusion is that the pregnant individuals in the IUD treatment failed to retain their IUDs. This may have occurred because the human 380 copper "T" used in this study is quite small (about 1" long) and may have been more easily expelled from the mare uterus than the human uterus for which it was designed. We are currently testing larger copper-containing IUDs to determine if retention is improved.

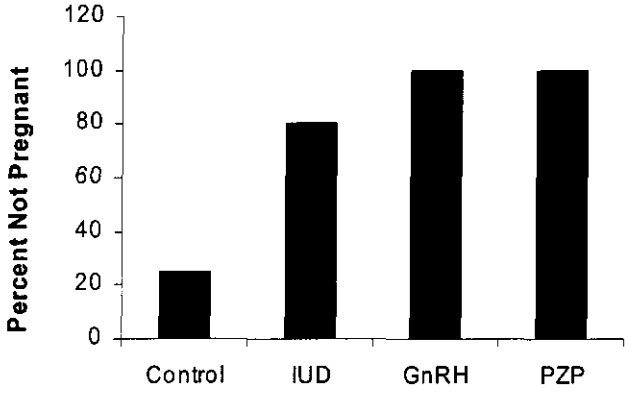

Figure 1. Percentage of mares not pregnant for each treatment group, based on ultrasonography.

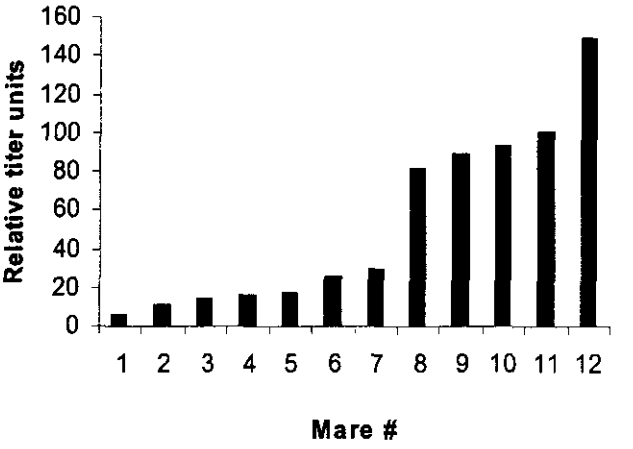

Figure 2. Relative antibody titers of individual mares (# 1-12) generated in response to the 400μg SpayVac-PZP vaccine, 7 months post-immunization.

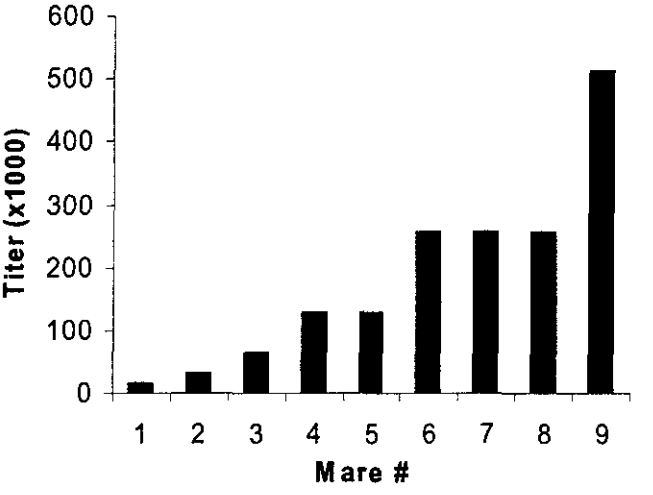

Figure 3a. Antibody titers of individual mares (#1-9) generated in response to the 1800μg GnRH vaccine, 7 months post-immunization.

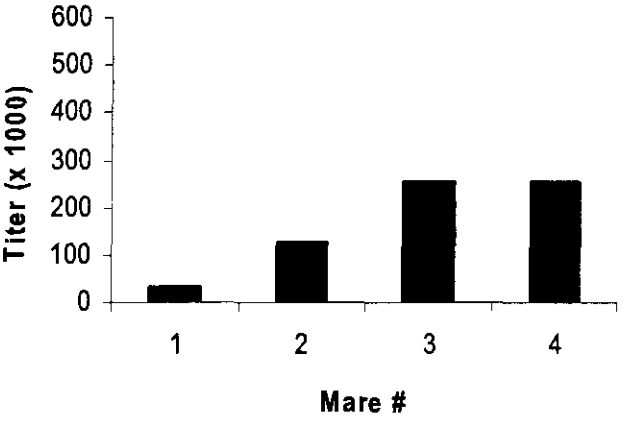

Figure 3b. Antibody titers of individual mares (#1-4) generated in response to the 2800μg GnRH vaccine, 7 months post-immunization.

Individual antibody titers for the SpayVac-PZP vaccine indicate a wide range of titers among individuals receiving the single 400μg dose (Figure 2). Seven of the individuals had titers one-third or less than the 5 mares with the highest titers. Nevertheless, all of the titers were adequate to impart contraception for the breeding season. For the GnRH mares receiving the 1,800μg dose, a range of titer responses was also evident (Figure 3a) but somewhat less diverse than that seen for the PZP mares (Figure 2.) The three mares receiving the 2,800μg did not produce titers that were greater than mares receiving the lower dose (Figure 3b), and all GnRH vaccine-treated mares were infertile. Future years of study on all vaccinated mares may provide evidence that higher titers are associated with a longer lasting contraceptive effect, but for now this remains speculative.

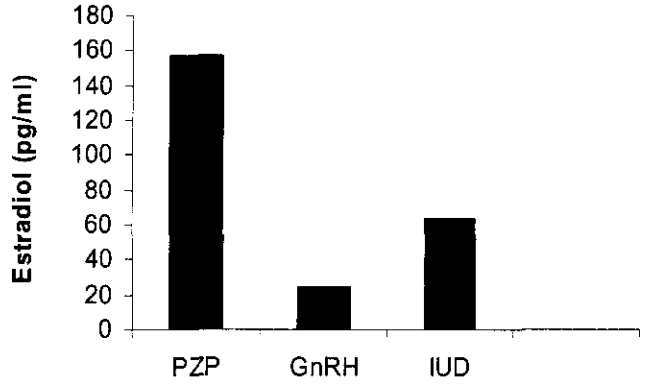

Figure 4a. Serum estradiol concentrations determined in November 2003 when mares were evaluated by ultrasonography for pregnancy.

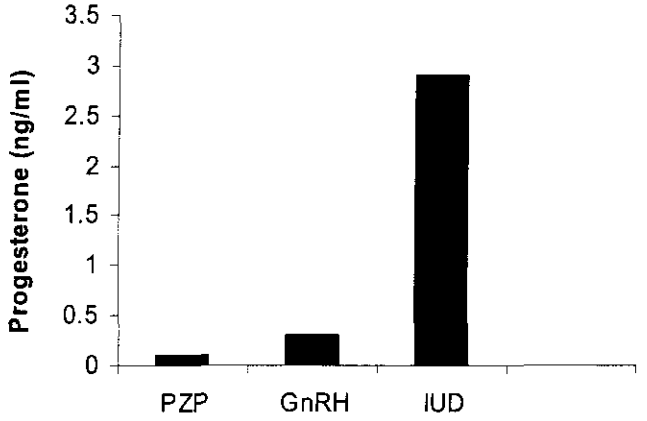

Figure 4b. Serum progesterone concentrations determined in November 2003 when mares were evaluated by ultrasonography for pregnancy.

An intriguing aspect of this study is related to the average estradiol (Figure 4a) and progesterone (Figure 4b) serum concentrations determined for each of the treatment groups. Average values determined from a single sampling of a population can provide some insights into whether animals within the population are experiencing estrous cycles. The typical mare estrous cycle is 22 days long (Ginther 1992). During 4 - 6 days of the cycle mares are in the follicular phase, during which estrogen secretions associated with follicular development increase and behavioral estrus occurs. During the remaining 16 - 18 days of the cycle, progesterone secretions are predominant. Therefore, for a randomly-sampled population of cycling mares, one would predict serum progesterone to be elevated and estradiol to be lower in 75 - 80% of the mares. However, for the PZP-treated mares, average serum estrogen was elevated in the majority of mares (Figure 4a) and serum progesterone was negligible (Figure 4b). These observations suggest that PZP-treated mares may not cycle normally and may fail to develop a functional, progesterone-secreting corpus luteum. An alternative explanation may be that the estrous cycles of the mares in the PZP group were synchronized and in estrus at the time of sampling, although there are no data to support this conclusion. In contrast to the PZP-treated mares, GnRH-treated mares had low serum concentrations of both estrogen and progesterone (Figure 4a and 4b), which is in accord with the predicted effect of the vaccine to immunologically castrate treated individuals (Miller et al. 2003).

Mares with IUDs had elevated average serum progesterone and somewhat elevated serum estrogen (Figure 4a. and 4b.), suggesting that the majority of mares within the IUD treatment are cycling. Unfortunately, we do not have average serum estrogen and progesterone concentrations for a group of control mares that were cycling and not pregnant to use for comparison. Seventy-five percent of the control mares in this study were pregnant.

There was no indication of adverse treatment effects on body condition and general health of the mares on the study. Ultrasonography of the uterus did not reveal evidence of uterine infection or fluid accumulation associated with the presence of the IUD. Uterine edema, typical of mares in estrus, was evident in ultrasonograms (Table 1). For cycling mares in a typical population, we would predict that ~25% of the females to be in the follicular phase based on an average cycle length of 22 days, and 4 - 6 of those days in the follicular phase. It is notable that we observed uterine edema in ~25% the mares in the IUD and GnRH treatments, as predicted. However, 82% of the PZP-treated mares showed evidence of uterine edema (Table 1). These findings support the serum steroid observations showing estrogen as the dominant serum hormone in PZP-treated mares (Figure 4a).

In conclusion, results of the first year of study indicated that both vaccines and the IUD contraceptive approaches are successful in preventing pregnancy in a high percentage of mares. Establishing the long-term efficacy and potential contraindications of these approaches, to be determined in future years of study, will be critical to establishing the utility of these approaches for population control of wild horses.

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

Treatment	Total # Ultrasounds	Predicted Tracts in Estrus (25%)	Observed Tracts Edema	Percent with Edema
PZP	11	2.8	9	82
GnRH	13	3.3	3	23
IUD	10	2.5	2	20

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