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Contraception of Wild and Feral Equids

Jay F. Kirkpatrick

J.W. Turner Jr.

I.K.M. Liu

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Abstract: Fertility control in wild horses has been attempted with both stallions and mares. Nonreversible surgical sterilization by means of vasectomy has been successful in inhibiting reproduction in wild horses in Montana and Nevada. Administration of a microencapsulated form of testosterone to wild stallions reduced sperm counts and motility and foal counts. In a third approach, intraperitoneal Silastic™ implants containing ethinylesdtradiol and progesterone blocked ovulation in wild mares for up to 3 years.

The first immunological fertility control of free-ranging wildlife was accomplished with wild horses. Initial experiments demonstrated that immunization with porcine zonae pellucidae was capable of causing contraception in domestic mares. Later, contraception was achieved with the vaccine in free-ranging horses. That study demonstrated that the vaccine (1) could be delivered remotely via darts, (2) was safe to administer to pregnant animals, and (3) did not alter social behavior. A followup study revealed that a single annual booster inoculation would extend the contraceptive effects for a second year, and the vaccine's effects are reversible after short-term use.

After 6 years of treating 52 different mares with porcine zonae pellucidae, contraceptive efficacy exceeded 95 percent. In more recent studies, investigators are studying the effects of long-term treatment (4 to 7 consecutive years) upon ovarian function. The porcine zona pellucida (PZP) vaccine has also proved to be effective in contracepting free-roaming feral donkeys in Virgin Islands National Park, captive Przewalski's horses, and onagers. Tests are currently under way on 150 feral horses in Nevada for the purpose of developing a one-inoculation form of the PZP vaccine that will deliver from 1 to 3 years of contraceptive protection. An initial field test of this vaccine indicated a high degree of success with a single inoculation over a single year, and a field test of a second-generation of microcapsules also indicated a high degree of contraceptive efficacy over a single year.

Keywords: equids, immunocontraception, porcine zonae pellucida, wildlife contraception

Introduction

Over the past 20 years, advances in wildlife contraception have been driven largely by concern over increases in wild horse *(Equus caballus)* populations. Prior to 1971 in North America, wild horse populations were controlled by the removal of horses from rangeland and their sale for various commercial purposes. The passage of the Wild Horse and Burro Act (P.L. 92–195) in 1971 by the U.S. Congress provided almost complete protection to wild and feral equids on public lands. At the time of the passage of this law, there were an estimated 17,000 wild horses on U.S. public lands. In an attempt to provide some form of population control, the U.S. Department of the Interior's Bureau of Land Management, the agency responsible for management of wild horses in the United States, initiated the Adopt-a-Horse program. Horses were gathered and adopted by people interested in acquiring a wild horse. However, the high cost of this program, its inability to remove sufficiently large numbers of horses, and increased reproductive success by the horses remaining on rangelands led to steady population increases between 1970 and 1980. By 1980, the estimated number of wild horses on poublic lands had increased to somewhere between 60,000 and 80,000. Clearly, alternative control methods were necessary.

Chemically or immunologically induced inhibition of fertility in equids has been little studied. Historically, equine fertility control has focused on castration of the domestic stallion. Most often, this common procedure is carried out not only to prevent reproduction but also to reduce testicular androgen production and accompanying aggressive behavior. Recently, interest in contraception of the equids has increased, largely because of uncontrolled populations of free-roaming wild horses and feral burros *(E. asinus).*

Contraception of the Stallion

Initial attempts at chemical contraception of wild horses focused on the stallion and attempted to exploit the haremlike social structure common to the majority of wild equids. To test the concept, two adult wild stallions inhabiting the Pryor Mountain Wild Horse Refuge in Montana were vasectomized and permitted to return to their bands. After 2 years, no foals appeared among the mares accompanying the vasectomized stallions (Kirkpatrick 1982). When this experiment was later repeated with larger numbers of wild stallions in Nevada, the results indicated that dominant stallions were responsible for the vast majority of successful breedings (Eagle et al. 1993).

Several potential contraceptive compounds, including testosterone cypionate, testosterone propionate, quinestrol (17α–ethinylestradiol 3–cyclopentyl ether), 17β estradiol, and α–chlorohydrin (3–chloro–1,2–propanediol) were tested in domestic pony stallions. The α–chlorohydrin led to neurological disorders, so tests were discontinued. Repeated intramuscular injections of the two androgens and quinestrol (1.7 g per 100 kg of body weight, monthly × 6 months) resulted in significant oligospermia and a significant reduction in sperm motility, but Silastic implants contraining estradiol failed to achieve significant reductions in sperm counts, probably because of poor release rates (Kirkpatrick 1982, Turner and Kirkpatrick 1982).

A microencapsulated form of testosterone propionate (mTP) was selected for field tests of contraceptive efficacy in wild horses in Challis, ID. The microencapsulation polymer (D,L–lactide) coating (Southern Research Institute, Birmingham, AL) permitted a sustained release for up to 6 months after intramuscular (i.m.) injection. On contact with intercellular water, the lactide coating erodes and releases the active steroid. The coating is converted to carbon dioxide and lactic acid.

Fifteen wild stallions—seven experimental subjects and eight controls—were located by helicopter and darted with approximately 300 mg succinylcholine. After immobilization, stallions received i.m. injections of 5, 7.5, or 10 g mTP in the hip. Stallion libido and quantitative aspects of sexual behavior, based on elimination marking behavior (Turner et al. 1981), were unaffected, and breeding took place. There was an 83-percent reduction in foal production compared with mares bred by control stallions (2 *v.* 13 foals, respectively), with no differences between fertility and the doses of mTP administered (Kirkpatrick et al. 1982, Turner and Kirkpatrick 1982).

Concerns for the safety of the stallions, dangers of immobilization, and high cost of opioid immobilizing drugs (approximately $50/dose of etorphine and reversal agent) led to an attempt to deliver 3 g mTP remotely to wild harem stallions on Assateague Island National Seashore, by means of barbless darts. In this study, the stallions were located and darted from the ground without capture or immobilization. The pharmacologic success of mTP contraception was evident; there was a 28.9-percent fertility rate for the mares accompanying treated stallions and an approximate 45-percent fertility rate among control mares. However, the difficulties of delivering 3 g mTP in four separate doses to each stallion were discouraging (Kirkpatrick and Turner 1987, Turner and Kirkpatrick 1991).

Contraception in the Mare

Steroids

The difficulty of darting a stallion up to four times plus concerns over band infidelity by mares turned the focus of wild horse contraception to the mare. Attempts were made to administer contraceptive doses of progestins to wild mares. Based on experience with persistent corpora lutea (Stabenfeldt et al. 1974) and data which indicated that plasma progesterone concentrations in excess of 0.5 to 1 ng/mL inhibited ovulation in the mare (Squires et al. 1974, Noden et al. 1978, Palmer and Jousset 1975), microencapsulated northisterone (norethindrone, mNET) was administered remotely, with barbless darts, to six wild mares on Assateague Island (Kirkpatrick and Turner 1987, Turner and Kirkpatrick 1991). This particular progestin, which had been used successfully to inhibit fertility in women, was given at a dose of approximately 2 g, in microcapsules similar to those used in previous studies with testosterone propionate. All six mares receiving the mNET produced a foal a year later—a highly improbable event among Assateague mares, where annual foaling rates seldom exceed 55 percent (Keiper and Houpt 1984).

In another experiment, groups of 30 captive wild mares in Nevada were each implanted with Silastic rods containing 8 g estradiol (E), 24 g progesterone (P), 8 g E plus 8 g P, 4 g E plus 12 g P, 12 g E plus 12 g P, or no hormone (Vevea et al. 1987, Plotka et al. 1988). Fewer mares receiving 8 g E, 4 g E plus 12 g P, or 8 g E plus 8 g P displayed estrus, but all animals displaying estrus—treated or control—ovulated. These data indicated a rapid decline in plasma steroid concentrations within 5 weeks of receiving steroid implants and suggested increased metabolic clearance of the steroid, an event that would also explain the failure of the mNET treatments described above. Because of the rapid decline in E and P concentrations, Silastic implants containing the synthetic estrogen ethinylestradiol (EE2) or EE2 plus P were placed in captive wild mares (Plotka et al. 1989). Animals pregnant at the time of implantation delivered healthy foals. Contraceptive efficacy ranged from 88 percent to 100 percent through two breeding seasons and was approximately 75 percent for three seasons. Endocrine studies of these mares suggested that contraception occurred by blocking ovulation and/or implantation.

In a similar study, intraperitoneal implants of 1.5, 3, or 8 g EE2 alone also resulted in contraceptive efficacy of 75 percent to 100 percent through two breeding seasons. Rates of EE2 decline in the plasma suggested a contraceptive life of 16, 26, and 48–60 months for the 1.5-g, 3-g, and 8-g doses, respectively (Plotka and Vevea 1990, Eagle et al. 1992).

Results achieved with estradiol, progesterone, and ethinylestradiol in mares brought to focus advantages and disadvantages of natural versus synthetic steroids for contraception in equids. Steroids native to the mare, such as estradiol and progesterone, are recognized by the mare's metabolic enzymes and degraded so rapidly that contraceptive doses must be so large that they are difficult or impossible to administer. The use of some synthetic steroids, such as ethinylestradiol, may delay metabolic degradation and permit sustained contraceptive effects and provide useful fertility inhibition in certain instances. Any risk, however small, of the passage of these synthetic steroids to humans or other wildlife may make registration by regulatory agencies such as the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), or the Environmental Protection Agency (EPA) unlikely or difficult.

Immunocontraception

Attention has turned to immunocontraception because of (1) the difficulties associated with the delivery of large doses of microencapsulated steroids, (2) dangers associated with capture and restraint of horses, (3) surgical procedures associated with intraperitoneal implants, (4) concern over long-term effects of steroidal contraception, and (5) passage of synthetic steroids through the food chain. One immunologically based contraceptive strategy involves blocking the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, thereby preventing pituitary secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and their subsequent tropic actions on the ovary or testes (Schanbacher 1984). Immunization of domestic mares against GnRH blocked ovulation in three of five mares for 4 months (Safir et al. 1987). Each mare was inoculated with 2 mg of GnRH conjugated to human serum albumin emulsified in Freund's Complete Adjuvant (FCA). Analysis of plasma LH revealed a lack of pulsatile secretion that was correlated with antibody titers. The high variability in the mares' response to the antigen and the subsequent variabilities in antibody titers suggested that this approach was unreliable.

In a study to immunize captive wild mares with GnRH conjugated to ketolymphohemocyanin (KLH), either aluminum hydroxide (alum) or monophosphoryl lipid A/trehalose 6,6–dimycolate/BCG cell wall skeleton (triple adjuvant, TA) was used as the adjuvant (Goodloe et al. 1988). Mares immunized with GnRH plus TA had higher antibody titers and significantly less ovarian follicular activity. The vaccine was field-tested in 29 wild mares on Cumberland Island National Seashore, in Georgia. The vaccine was freeze dried and administered as a solid biodegradable 0.25-caliber bullet by means of an air-powered gun. After imbedding in the tissue of the target mare, the compressed compound forming the biobullet degrades over 24 hours, releasing the antigen. A total of 25 treated mares survived (four died from natural causes) and 17 (68 percent) produced foals, which was not significantly different from foaling rates of control mares.

Immunization against GnRH has also been attempted in the stallion (Dowsett et al. 1991). Four weanling colts were passively immunized, either intramuscularly or subcutaneously, with an anti-GnRH antibody (Peptide Technology, Ltd., Sydney, Australia) and given booster inoculations approximately 75 days later. Colts immunized intramuscularly maintained plasma testosterone concentrations of < 0.15 ng/mL (equivalent to concentrations in geldings) for 5 months following the booster inoculations. Thereafter, testosterone concentrations rose to control levels for yearlings. Colts immunized subcutaneously had slightly increased plasma testosterone concentrations (up to 0.37 ng/mL) between the two immunizations but decreased concentrations similar to those seen in the intramuscularly injected group after the second inoculation. Antibody titers were generally higher in the colts immunized intramuscularly, although sexual development was effectively delayed for 12 months in both groups of colts.

A second immunocontraception strategy for equids was based on the identification of antibodies directed against the zona pellucida of the ovum in naturally infertile mares (Liu and Shivers 1982) and immunological cross-reactivity of equine zona-positive antisera and porcine sperm binding (Shivers and Liu 1982). Liu et al. (1989) immunized 10 captive wild mares and 4 domestic mares with the protein equivalent of 2,000 to 5,000 porcine zonae pellucidae. FCA was used for the first inoculation and Freund's Incomplete Adjuvant (FIA) for the three monthly booster inoculations that followed. Of the 14 treated mares, 13 failed to conceive during the first year. The four domestic mares all conceived during the second year after antibody titers decreased.

A field test of the PZP vaccine was carried out on Assateague Island National Seashore (Kirkpatrick et al. 1990). For the test, 26 wild mares were remotely inoculated with approximately 5,000 porcine zonae pellucidae (65 μg protein) and FCA in March 1988 by means of barbless darts. The mares received a second inoculation with PZP + FIA 2 weeks later, and 18 of the mares received a third inoculation with that combination 1 month later. No foals were produced by the treated mares, whereas 50 percent of the 6 sham-injected mares (controls) produced foals, and 45 percent of 11 untreated mares produced foals. Post-treatment foaling rate for the PZP-treated mares was significantly different ($P < 0.002$) than that for the 2 pretreatment years, for sham-treated control mares and for untreated mares. Of the 26 PZP-treated mares, 14 were pregnant at the time of inoculation, and all 14 produced healthy foals 2–3 months following PZP treatment. Thus, the PZP vaccine had no effect on pregnancies in progress or the health of the foals born. Social behaviors and organization were also unaffected by treatment. Once antigen recognition had taken place, a single annual booster inoculation was sufficient to maintain contraception.

In March 1989, 14 of the previously treated mares were given a single booster inoculation. A year later, only one foal was produced. The 12 mares immunized in 1988 but not given a booster in 1989 produced 5 foals, demonstrating for a second time the reversibility of the vaccine's contraceptive effects (Kirkpatrick et al. 1991a). After 6 consecutive years of booster inoculations among the Assateague mares and 104 mare-years (the number of mares treated × the number of years of treatment), four foals have been produced, for an effectiveness of 96 percent. Over the 6-year period, the difference between foaling rates among treated and untreated mares was highly significant ($P < 0.001$).

Field trials were initiated in 1992 with feral donkeys in Virgin Islands National Park. Sixteen adult female donkeys received an initial inoculation of 65 μg of porcine zonae pellucidae + FCA, a second identical inoculation 3 weeks later, and a third 10–12 months later. Eleven untreated adult females were used as controls. Results were not calculated until 12 months after the initial inoculation in order to allow for any pregnancies already in progress at the time of inoculations. Based on observed foals and on fecal steroid analysis (Kirkpatrick et al. 1991b and c), of the treated females, only 1 of 16 (6.2 percent) produced a foal or was pregnant, while among the controls, 6 of

11 (54.5 percent) produced foals or were pregnant. The difference between foaling and pregnancy rates between the two groups was highly significant ($P < 0.01$).

Large-scale field trials with wild horses were initiated in December 1992 in Nevada. Slightly more than 500 wild horses were captured by driving them by helicopter into a trap and portable corrals. Adult mares (n = 131) between the ages of 4 and 12 were included in the study. All mares were freeze-branded with numbers for later identification. One group received two inoculations of PZP about 3 weeks apart with FCA used for the first inoculation and FIA for the second. A second group received only a single inoculation + FCA. A third group received a single inoculation of porcine zonae pellucidae + FCA that contained another 65 µg of pellucidae in lactide–glycolide microspheres. These microspheres were designed to release the antigen over a 4- to 6-week period.

During September 1993, fecal samples were collected from 78 treated or control animals and analyzed for steroid hormones and steroid hormone metabolites, which signal pregnancy (Kirkpatrick et al. 1991b and c). Of 27 untreated mares, 14 (52 percent) were pregnant. Of 17 sham-injected controls, 9 of 17 (53 percent) were pregnant. None of 14 mares receiving 2 inoculations of porcine zonae pellucidae were pregnant. Of 20 mares receiving only a single inoculation of raw pellucidae, 6 (30 percent) were pregnant. No samples were recovered from mares receiving the porcine zonae pellucidae in microspheres.

During June 1994, after aerial foal counts, a total of 65 experimental mares were observed, and foaling results were in general agreement with pregnancy data. From 32 mares that received 2 inoculations, only a single foal was observed. Of 16 sham-treated mares, 10 had foals (63 percent), and of 7 mares receiving only a single inoculation, 3 had foals (43 percent). Of 10 mares receiving a single inoculation of the microencapsulated porcine zonae pellucidae, none had foals. These data suggested a contraceptive effect of a single inoculation with microencapsulated porcine zonae pellucidae that lasted 8–9 months following treatment.

The PZP vaccine has also been tested in captive exotic equids, including 23 Przewalski's horse *(Equus przewalskii)*, 1 onager *(E. hemionus)*, and 26 zebra *(E. zebra grevyi)* (Kirkpatrick et al. 1992b, 1993). Thus far, results are available only for the Przewalski's horses and the onager, and the vaccine has been 100-percent effective in preventing pregnancies in these species (Kirkpatrick et al. 1993). It is better than 80-percent effective in zebras.

Two important issues are raised with regard to PZP immunocontraception of equids. The first is the possibility of long-term effects of the vaccine on ovarian function. PZP-induced contraception is thought to be due to a block to fertilization (Liu et al. 1989). At least two of the major glycopreoteins of the noncellular zona pellucida, ZP3 (Florman and Wassarman 1985) and ZP4 (Hasagawa et al. 1992), are necessary components of the receptor molecule for sperm surface molecules. The role of the ZP3 receptor in the horse has been confirmed in vitro as a zona pellucida-induced acrosome reaction with horse sperm (Arns et al. 1991). The initial study of PZP immunization of horses (Liu et al. 1989) and field tests with the Assateague wild horses (Kirkpatrick et al. 1990, 1991a) demonstrated that the contraceptive effects of PZP immunization were reversible after 1 year of treatment. Plasma progesterone concentrations during the year of treatment indicated a luteal phase and therefore evidence of ovulation. However, the long-term effects of continuous PZP immunization have not been described in either the horse or other species. Reversibility of the contraceptive effect has been demonstrated in several species but only after short-term application of the vaccine (Gulyas et al. 1983, Sacco et al. 1987, Liu et al. 1989).

In the dog and the rabbit, PZP immunization appeared to be very damaging to ovarian follicles and often led to cessation of ovarian function, with accompanying anovulation and depression of estrogen concentrations (Wood et al. 1981, Mahi–Brown et al. 1985). Unusually large doses (5,000 µg) caused anovulation in the baboon (Dunbar et al. 1989). These data suggested that the antibody response of the treated animal attacks not only the zona pellucida of the mature ovum but oocytes and possibly other

Table 1. Antibody titers in response to porcine zonae pellucidae in domestic mares after one inoculation using porcine zonae pellucidae plus PZP-containing microspheres or two inoculations of porcine zonae pellucidae given 3 weeks apart

		Dates of inoculation					
Treatment	**Horse**	**3/13**	**4/2**	**4/16**	**5/7**	**6/18**	**10/14**
		Antibody titers (% of positive reference serum)					
FCA–PZP bolus & PZP microspheres	Aldo	5	99	114	131	106	79
	Mia	4	70	107	123	82	28
	Imp	6	33	70	95	98	131
	Lab	5	38	76	120	114	39
	Dee	5	56	108	106	99	26
FCA–PZP bolus + FIA–PZP bolus 3 weeks later	Ant	4	90	130	131	152	115
	Con	6	66	145	135	144	58
	Len	8	58	118	123	91	35
	Rou	3	73	116	133	123	79
	Sou	4	67	147	126	136	94

FCA = Freund's Complete adjuvant. FIA = Freund's Incomplete adjuvant.

ovarian tissue with resulting changes in estradiol and progesterone secretion. These effects have not been demonstrated in the horse after short-term use, but there is evidence that these same effects may appear after long-term use.

After 3 consecutive years of PZP treatment, three of seven Assateague mares showed decreased urinary estrogen concentrations and no evidence of ovulation (Kirkpatrick et al. 1992a), and after 6 consecutive years of ovulation, five mares showed no evidence of ovulation. However, three of these five mares showed recurring rhythmic estrogen peaks, suggestive of developing follicular waves, and two of these five mares demonstrated classical estrous behavioral concurrent with an estrogen peak and permitted breeding (Kirkpatrick, unpubl. data). Another mare demonstrated a luteal-phase urinary progesterone metabolite pattern after 5 consecutive years of treatment and 1 year off, suggesting that normal ovarian function returned. The next 3–5 years will provide critical data regarding the effects of the PZP vaccine on ovarian function and reversibility after long-term vaccination with porcine zonae pellucidae.

The second critical issue is related to the number of initial inoculations necessary for contraception. While it has been clearly established that two inoculations, given 3–6 weeks apart, will provide contraception for about a year, the need to give two inoculations decreases the usefulness of the vaccine for wild and feral equids. Thus, the single most important direction for future research is the development of a one-inoculation form of the vaccine which provides at least a full year, and ideally 2–3 years, of contraception protection.

In an initial attempt to develop a one-inoculation PZP vaccine, the PZP antigen was incorporated into nontoxic, biodegradable, 50-µ, homogenous lactide–glycolide microspheres. Upon i.m. injection, the lactide–glycolide material erodes, releasing the anitgen over predetermined periods of time (Eldridge et al. 1989). The carrier itself is metabolized to lactic acid and carbon dioxide.

Five domestic mares were inoculated with an initial bolus of 65 µg of porcine zonae pellucidae + FCA plus another 65 µg of pellucidae contained in microspheres. Antibody titers were compared to titers in mares inoculated with two doses of pellucidae (65 µg PZP/FCA + 65 µg PZP/FIA) given 3 weeks apart. Contraceptive antibody titers lasted for approximately 200 days with the one-inoculation preparation (table 1).

This same preparation was administered to 14 wild mares on Assateague Island. One dart failed to inject. That mare, plus only one other mare, produced foals following treatment. The differences were significant ($P < 0.05$) comparing either 2 foals/14 mares or 1 foal/13 mares (to account for the failed dart) with untreated control mares. Similar research is currently under way to produce a one-inoculation vaccine using microcapsules. The microcapsules are made from the same lactide–glycolide material, but after injection they release the PZP antigen in pulses rather than continuously.

Conclusions

PZP-induced contraception of the mare may be useful to prevent unwanted pregnancies among wild and feral equids. In the case of captive exotic equids, such as the Przewalski's horse and the zebra, contraception may be useful to prevent the expression of undesirable genetic traits ("floppy mane" or the fox allele, for example) or merely to prevent the production of unwanted surplus animals without the need to remove animals and disrupt well-defined social groups. Anti-GnRH vaccines may be useful for the same purpose in the stallion, or simply to reduce aggression among stallion groups. Finally, contraception may represent a publicly acceptable approach to the management of wild and feral horses inhabiting public lands.

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