Chapter 199

Contraception

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Downregulation of fertility in the horse is a limited subject. Historically, equine fertility control has focused on castration of the stallion. Most often, this common procedure is carried out not only to limit reproduction but also to eliminate steroid production and accompanying untoward behavior. Beginning in the 1970s, interest in equine contraception increased, largely because of uncontrolled populations of free-ranging wild and feral equids, and the need for management of captive exotic equids in zoological parks.

Contraception of the stallion

Steroids

It has long been known that exogenous androgens can exert a downregulation of endogenous androgens and sperm production in the stallion.[1] Increased doses of exogenous androgens cause a significant reduction in spermatogenesis.[2] This phenomenon is caused by negative feedback on hypothalamic gonadotropin-releasing hormone (GnRH),[3] and subsequent downregulation of pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH),[4] and ultimately a decline in gonadotropin-dependent spermatogenesis.[5]

To achieve oligospermia to the degree necessary to reduce fertility, the androgens had to be given frequently. For example, testosterone propionate (200 μg/kg) had to be administered every other day for 88 days to cause significant depression in motile sperm.[6] These antifertility effects of androgen treatment were largely reversible.[7]

This fundamental biological strategy led to the first attempts at actual contraception aimed at free-ranging wild horses. Trials with domestic pony stallions examined the effects of six repeated monthly injections of testosterone propionate (TP) and 17-alpha-ethinylestradiol, 3-cyclopentyl ether (quinestrol), at doses of 1.7 g/100 kg. Both regimens resulted in significant degrees of oligospermia and decreased sperm motility.[8] The practicality for application in the field, however, was limited because of the need for repeated treatments. Thus, testosterone propionate was microencapsulated in a polymer of DL-lactide (mTP) (Southern Research Institute, Birmingham, AL), permitting a sustained release for up to 6 months after intramuscular injection. On contact with intercellular water, the lactide coating erodes and releases the active steroid inside. The lactide coating is converted to carbon dioxide and lactic acid.

Seven experimental and eight control stallions in Idaho were immobilized from a helicopter with 300 mg succinylcholine and received injections of 5.0, 7.5, or 10 g of mTP in the hip. Stallion libido and quantitative aspects of sexual behavior, based on elimination marking behavior,[9] were unaffected and breeding took place, but there was an 83% reduction in foal production compared with mares bred by control stallions. There were no differences in fertility rates between the three doses administered.[10]

There were concerns for the safety of stallions and the dangers and high costs associated with helicopter use and immobilizing drugs, and these led to a second field trial in which the microencapsulated TP was delivered remotely, by dart. Wild harem stallions on Assateague Island National Seashore, MD (ASIS), were given 3 g of microencapsulated TP administered with barbless darts, from the ground and without immobilization. The pharmacological success of the mTP was evident, with a 28.9% fertility rate for the mares accompanying the treated stallions and a 45% fertility rate among the mares accompanying untreated stallions. Unfortunately, the logistics of delivering 3.0-g microcapsules in four separate doses to each stallion was daunting and impractical for routine use.

Vasectomy has also been tested in order to limit population growth in wild horses. In an unpublished study, two wild stallions in the Pryor Mountain National Wild Horse Range were vasectomized,

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and for 2 years following the surgery, no foals appeared among accompanying mares (J.F. Kirkpatrick, unpublished data). Equally important, the stallions exhibited normal sexual behaviors, a requisite for maintaining harem groups. This experiment was later repeated with larger numbers of wild stallions in Nevada, and the results were much the same, except that it was shown that not all breeding is done by dominant harem stallions.[11]

Immunocontraception

Interest turned to immunological approaches to contraception in the stallion. The first approach tested used an anti-GnRH vaccine (Peptide Technology Ltd, Sydney, Australia).[12] Because of the molecule's homology across mammalian species, there were difficulties in producing a strongly immunogenic vaccine against GnRH. Further studies led to a conjugated form of GnRH,[13] in which the GnRH molecule was conjugated to ovalbumin. At doses of 200–400 mg there was a significant reduction in testicular dimensions, testosterone production, and daily sperm production for up to 30 weeks.

More recently, Janett *et al.*[14] injected eight adult stallions with three inoculations of 200 µg of a GnRH-protein conjugate (Equity; CSL Limited, Australia), over an 8-week period. Testosterone values, scrotal width, semen quality, and libido decreased significantly over the 8 months, but results varied among individual animals.

Contraception of the mare

Steroids

Logistical difficulties in treating stallions with steroids, concerns about steroid-related pathologies, and a general concern that the treatment of wild stallions would have serious genetic consequences for the gene flow in free-ranging herds turned the focus of contraception in horses to the mare. Based on experience with persistent corpora lutea[15] and data that indicated that plasma progesterone concentrations in excess of 0.5–1.0 ng/mL inhibited ovulation in mares,[16–18] attempts were made to administer contraceptive doses of progestins to wild horses.[19] Two-gram doses of northisterone (norethindrone), microencapsulated in a slow-release lactide matrix similar to that described above for testosterone propionate, were delivered remotely to six wild mares on Assateague Island. All six treated mares produced a foal one year later, a highly improbable event among Assateague mares, where normal foaling rates seldom exceed 35%.[20]

In a second steroid contraceptive experiment, groups of 30 captive wild mares in Nevada were each implanted with silastic rods containing various doses of estradiol (E, 4–12 g) and/or progesterone (P, 8–24 g) or no hormone.[21] Fewer mares receiving 8 g E, 12 g P plus 4 g E, or 8 g E plus 8 g P displayed behavioral estrus, but all animals displaying estrus, treated or control, ovulated. These data indicated a rapid decline in plasma steroid concentrations within 5 weeks of implantation and suggested increased metabolic clearance of the steroid. Because of the rapid decline in estradiol and progesterone concentrations, silastic implants containing the synthetic estrogen ethinylestradiol (EE2) or EE2 plus P were placed in captive wild horses.[22] Animals pregnant at the time of implantation delivered healthy foals, and contraceptive efficacy ranged from 86% to 100% through two breeding seasons. Endocrine studies of these mares suggested that contraception was affected by blocking ovulation and/or implantation. In a similar study, intraperitoneal implants of EE2 alone also resulted in contraceptive efficacy of 75% to 100% through two breeding seasons, and rates of EE2 decline in the plasma suggested a contraceptive life of 16, 26, and 48–60 months, for 1.5 g, 3.0 g, and 8.0 g of EE2, respectively.[23]

A third experiment aimed at using steroids to inhibit fertility in mares utilized estradiol and progesterone incorporated in biodegradable poly (DL-lactide) microspheres.[24] Mares were given 0.625 g progesterone plus 50 mg estradiol, 1.25 g progesterone plus 100 mg estradiol, or 1.875 g progesterone plus 150 mg estradiol. While the intervals between estrus and ovulation were lengthened by the various treatments, rates for embryo recovery (performed by uterine lavage) did not differ between groups.

Results achieved with estradiol, progesterone, and ethinylestradiol in mares highlight advantages and disadvantages of natural versus synthetic steroids for contraceptive purposes in the horse. Steroids native to the mare, such as estradiol and progesterone, are required in impractically large doses due to their rapid enzymatic degradation in vivo. The use of some long-acting synthetic steroids such as ethinylestradiol may delay metabolic degradation and permit more sustained contraception. But the potential risk, however small, of the passage of these synthetic steroids to humans or non-target wildlife has made acceptance by regulatory agencies [Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), or the Environmental Protection Agency (EPA)] unlikely.

Immunocontraception

Because of the difficulties with delivering large masses of microencapsulated steroids, dangers associated with capture and restraint of horses, surgical procedures associated with intraperitoneal implants, concern over long-term effects of steroid contraception, and passage of synthetic steroids through the food chain, attention turned to immunocontraception.

One immunologically based contraceptive strategy involves blocking the release of gonadotropin-releasing hormone (GnRH) or its attachment to the pituitary target cells responsible for the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), with subsequent abolition of trepic actions on the ovary or testis.[25] Immunization of domestic mares against GnRH blocked ovulation in three of five mares for 4 months.[26] Each mare was inoculated with 2.0 mg GnRH conjugated to human serum albumin emulsified in Freund's complete adjuvant (FCA). Analysis of plasma LH revealed a lack of pulsatile secretion, which was correlated to antibody titers. The high variability in the mare's response to the antigen and the subsequent variability in antibody titers suggested this approach was unreliable. When a dose of 200 or 400 mg of conjugated GnRH-ovalbumin was injected into four mares,[13] ovarian activity ceased 4 weeks after preliminary treatment and caused significantly decreased progesterone secretion for up to 20 weeks.

In another study,[27] Goodloe *et al.* immunized domestic mares with GnRH conjugated to keyhole limpet hemocyanin (KLH), using as an adjuvant either aluminum hydroxide (alum) or Triple Adjuvant (TA) consisting of monophosphoryl lipid A/trehalose 6,6-dimycolate/BCG cell wall skeleton. Those mares immunized with GnRH plus TA had higher antibody titers and significantly less ovarian follicular activity. The vaccine was field tested on 29 feral mares on Cumberland Island National Seashore, GA. The vaccine was freeze-dried, sequestered in a solid biodegradable 0.25-caliber bullet, and administered by an air-powered gun (Ballistivet, Inc., White Bear Lake, MN). After embedding in the muscle of the target mare, the compressed compound forming the biobullet degrades over 24 h, releasing the antigen. A total of 25 treated mares survived until the next foaling season and 17 (68%) produced foals, which was not significantly different from control foaling rates.

A more recent attempt to immunize wild horses against GnRH was carried out in Nevada.[28] Mares received either 1800 or 2800 μg GnRH vaccine (National Wildlife Research Center, Fort Collins, CO) with Adjuvac adjuvant, which is a dilution of a commercial Johne's disease vaccine (Fort Dodge, Ames, IA). Following a single breeding season, 0 of 18 mares, in both treatment groups, were pregnant on the basis of ultrasound evaluations. All GnRH-treated mares had low concentrations of serum estrogen and progesterone, which is consistent with the predicted actions of a GnRH vaccine. Imboden *et al.*[29] immunized domestic mares with a GnRH-protein conjugate and aqueous adjuvant (Improvac; CSL Limited, Australia). Each of nine mares received a primer and booster inoculation of 400 μg. Eight of nine mares had injection site reactions (including swelling, pain, stiffness, pyrexia, and apathy) but these subsided by 4–5 days post-injection. All test mares ceased ovarian cyclicity for 23–100 weeks, and reproductive behaviors were partially or completely inhibited in seven of nine mares. Botha *et al.*[30] applied the same vaccine in doses of 400 μg to 55 mares, 35 days apart, and 175 days later none of the treated mares remained in anestrus, while 50% of controls were pregnant. In a similar study, Elhay *et al.*[31] injected mares with the GnRH-protein conjugate Equity, at doses of 200 μg, and a saponin-based adjuvant. Inoculations were given at Day 0 and Day 28. Serum progesterone, estradiol, and estrus-related behavior all declined over a minimum period of 3 months. Robinson and McKinnon[32] reported on prolonged (2 years) failure to express recrudescence to cyclicity in a population of adult mares in horses treated with 200 μg of Equity for racing and presented later for breeding.

A second immunological approach to equine contraception is based on the identification of antibodies directed against the zona pellucida of the ovum in naturally infertile mares,[33] and immunological cross-reactivity of equine zona-positive antisera and porcine sperm binding.[34] Liu *et al.*[35] immunized 10 captive wild mares and four domestic mares with the protein equivalent of 2000 to 5000 porcine zonae pellucidae (PZP). Freund's complete adjuvant was used for the first inoculation and Freund's incomplete adjuvant (FIA) for the 3 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 had decreased.

A field test of the PZP vaccine was carried out on Assateague Island National Seashore (ASIS).[36] Twenty-six free-ranging wild mares were remotely inoculated with the protein equivalent of 5000 PZP (65 μg) and FCA in March 1988 by means of barbless darts. The mares all received a second inoculation, with FIA, 2 weeks later, and 18 of the 26 mares received a third inoculation with FIA a month later. No foals were produced among the treated mares a year later whereas 50% of the six sham-injected control mares produced foals. Of the 26 PZP-treated mares, 14 were pregnant at the time of inoculation and all 14 produced healthy foals; thus the PZP vaccine had no effect on pregnancies in progress or the health of the foals. Once antigen recognition had taken place, a single annual booster inoculation was sufficient to maintain contraceptive levels of antibodies,[37] and animals that did not receive booster inoculations returned to normal fertility.[35,37]

Studies of PZP contraception in wild mares examined the long-term effects on ovarian endocrine function and reversibility of contraceptive action, through the use of urinary and fecal steroid metabolite analysis.[38] These studies examined urinary estrone conjugates and immunoreactive pregnanediol glucuronide-like progesterone metabolites (iPDG) in mares treated from one through seven consecutive years with the PZP vaccine. Reversibility occurred in all durations of treatment except the 7-year group,[39] which failed to ovulate and remained infertile over a 12-year period. Prolonged treatment (3 years or more), however, did temporally depress urinary estrogens.[40,41] Continued multi-year application of the PZP vaccine to ASIS horses, for management purposes, resulted in zero population growth within 2 years, and a marked reduction in herd population.[42,43] Associated with these long-term treatments were decreased mortality rates, increased body condition scores, and increased longevity among treated mares.[44] Turner and Kirkpatrick[45] observed normal foal survival and no out-of-season births for these mares,[45] while Powell[46] reported absence of behavioral side-effects, and Powell and Monfort[47] found normal ovarian cyclicity.

Additional and successful contraceptive studies with PZP and other species of equids were mounted with feral burros,[48] Przewalski's horses,[49] and zebra.[50] The non-seasonal nature of the burros' and zebras' breeding season necessitated booster inoculations more frequent than annually.

Following the success of multi-injection field applications of PZP contraception in wild horses, modifications to the original protocol were tested toward maximizing vaccine (i) delivery efficiency, (ii) duration, and (iii) cost-effectiveness. Among these modifications was the substitution of Freund's modified adjuvant (Calbiochem Inc., La Jolla, CA) for FCA[51] with no loss of immunogenicity. Regarding vaccine delivery, Willis *et al.*[52] employed the biobullet technology described above. The PZP was lyophilized and incorporated into a biodegradable 25-caliber biobullet composed of calcium carbonate and hydroxypropylcellulose. Each bullet weighed 375 mg and contained 200 μg of PZP and was adjuvanted with 500 μg of synthetic trehalose dicorynomycolate (TDCM) glycolipid and squalene oil. Each of three domestic mares was given two inoculations. Serum antibody titers were detectable for 40 weeks, and two of the three mares remained infertile. However, use of the same approach with free-ranging horses in New Zealand yielded disappointing results. Each of 26 mares received 400 μg of PZP in biobullets, and 23 became pregnant following treatment.[53] The causes for the failure were multiple. The TDCM adjuvant was likely inadequate for mounting an adequate immune response to the PZP. Because an earlier study with captive New Zealand horses, using this adjuvant resulted in highly variable antibody titers at all doses.[54] Additionally, the limited range of the biobullets and the inability to determine if they had struck the intended target probably reduced effectiveness.

The difficulty in delivering PZP remotely, to hundreds or thousands of wild horses in the western USA, led to the study of one-inoculation, long-acting forms of the vaccine. In an initial trial, 132 adult wild mares in Nevada were captured and freeze branded.[55] Mares received one of the following treatments: (i) saline and adjuvant (FCA); (ii) two inoculations of 65 μg PZP, with FCA for the first inoculation and FIA for the second, 2 weeks later; or (iii) 65 μg of PZP + FCA along with 65-μg PZP boosters sequestered in controlled-release biodegradable lactide-glycolide microspheres, as described by Wang *et al.*[56] and prepared by the School of Pharmacy at the University of Iowa. The microspheres, designed to release across approximately 1 month, were suspended in the PZP/adjuvant emulsion for injection. Control mares had a 54.5% foaling rate a year later, the mares receiving the standard two-inoculation treatment had a 4.5% foaling rate, and the mares receiving the one-inoculation, microsphere-delivered PZP had a 20% foaling rate. In a larger-scale test of the microspheres,[57] 267 Nevada wild mares were captured, freeze branded, and treated with (i) the standard two-inoculation PZP treatment, with FCA/FIA adjuvants; or (ii) a single inoculation of 65 μg PZP + FCA + 60–80 μg PZP in microspheres with no adjuvant; or (iii) 65 μg PZP + FCA + 60–80 μg PZP in microspheres with a carbomer adjuvant (Carbopol 934; B.F. Goodrich, Cleveland, OH,). Control mares had a pregnancy rate of 62.5% a year later, while 12.8% of the two-inoculation group and 11.3% of the microsphere + carbomer group became pregnant.

Clearly the lactide-glycolide microsphere technology was effective in delivering a one-inoculation PZP contraceptive. However, difficulty in injecting this vaccine was encountered, because the suspended microspheres settle out over time in syringes and darts. This led to experiments with lactide-glycolide pellets, small enough to fit into a 14-gauge needle of a syringe or dart.[58] The pellet-containing PZP vaccine was originally tested in domestic mares and yielded anti-PZP titer patterns across 45 weeks, which were similar to those in mares given the standard two-inoculation PZP treatment.[57] The controlled-release pellet vaccine was then field tested as a one-inoculation, 1-year duration treatment on free-roaming wild mares in three different herds in Nevada. Fertility rates among untreated mares were 46–62% across herds, and among treated mares were 11–26% across herds. The lower fertility rates were associated with herds in which mares had been captured and hand-injected, while the higher fertility rates occurred in mares corralled and darted. As stated previously, the higher fertility rates with dart delivery likely reflected incomplete delivery of vaccine. In 2000, 96 adult wild mares in Nevada were captured and treated with a primary dose of 100 µg aqueous PZP + booster doses of PZP incorporated in controlled-release lactide-glycolide polymer pellets. While previous pellet-containing vaccine tests employed two pellets releasing at approximately 1 and 3 months, this study included a third pellet, releasing at approximately 12 months, to serve as an annual booster. Reproductive success from 2001 through 2004 was, respectively, 5.9%, 14.0%, 32.0%, and 47.5%, while untreated mares on the same range had a 53.8 ± 1.3% reproductive success rate.[58]

Finally, two attempts have been made to inhibit fertility in horses using intrauterine devices (IUDs). In the initial attempt, silastic O-ring-shaped IUDs (silastic 382; Dow Corning, Midland, TX) were placed in six mares that were then turned out with 12 non-treated mares and a fertile stallion.[59] None of the IUD-treated mares became pregnant, while all 12 non-treated mares became pregnant. Reversibility of contraception was 100% a year later, after the IUDs were removed. Mild endometritis occurred in most of the treated mares, but disappeared after removal of the IUDs. In a second trial,[28,60] copper-containing Tcu-380A IUDs (Ortho-McNeil, Raritan, NJ) were placed in 15 mares. Each IUD contained a total surface area of 380 mm^2 of copper. Although 3 of these 15 mares became pregnant, it appears that contraception failed in these animals because the IUDs were not retained. The major drawback with this approach, is the need to capture horses, and to know their pregnancy status before application.

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