PHARMACOLOGIC MANIPULATION OF THE REPRODUCTIVE CYCLE

CHAPTER 40

CONTRACEPTION

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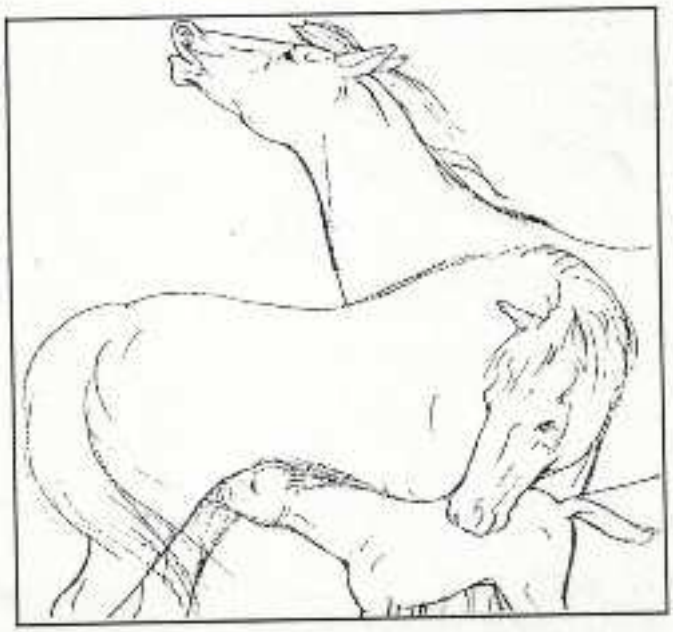

Down regulation of fertility in the horse is a limited subject. Historically, equine fertility control has focused on castration of the stallion. Most often, this common procedure is carried out not only to limit reproduction but also to eliminate androgen production and accompanying aggressive behavior. Recently, interest in contraception of the horse has increased, largely because of uncontrolled populations of free-roaming feral horses.

CONTRACEPTION OF THE STALLION

Initial attempts at chemical contraception of feral horses focused on the stallion and attempted to exploit the harem-like social structure common to most equids. To test the concept, Kirkpatrick and Turner vasectomized two mature feral stallions inhabiting the Pryor Mountain National Wild Horse Range, in Montana, and studied their bands for the following two years. No foals appeared among accompanying mares, and stallions exhibited normal sexual behaviors. This experiment was later repeated with a larger number of feral stallions in Nevada and the results were much the same (Cheryl Asa, St. Louis Zoo, personal communication).

Several potential contraceptive compounds, including testosterone cypionate, testosterone propionate, quinestrol (17α-ethinylestradiol 3-cyclopentyl ether), estradiol-17β, and α-chlorohydrin (3-chloro-1,2-propanediol) have been tested in domestic pony stallions. The α-chlorohydrin led to neurological disorders and tests were discontinued. Repeated intramuscular injections of the two androgens and quinestrol (1.7 g/100 kg, monthly × 6) resulted in significant oligospermia and impairment of sperm motility, but Silastic implants containing estradiol failed to achieve significant reductions in sperm numbers, probably because of poor release rates.[1,2]

A microencapsulated form of testosterone propionate (mTP) was selected for field tests of contraceptive effectiveness in feral horses in Challis, Idaho. The microencapsulation polymer (D,L-lactide) coating (Southern Research Institute, Birmingham, AL) permitted a sustained release, after intramuscular injection, for up to 6 months. On contact with intercellular water, the lactide coating erodes and releases the active steroid inside. The actual coating is converted to carbon dioxide and lactic acid. A total of 7 experimental and 8 control stallions were located by helicopter and darted in December with approximately 300 mg succinylcholine and after immobilization stallions received injections of 5.0, 7.5, or 10 g IM mTP in the hip. Stallion libido and quantitative aspects of sexual behavior, based on elimination marking behavior,[3] were unaffected and breeding took place, but there was an 83% reduction in foal production compared with mares bred by control stallions (2 foals vs. 13, respectively), with no differences between fertility and the doses of mTP administered.[2,4]

Concerns for safety of stallions, dangers of immobilization, and high cost of immobilizers (approximately $50 per dose of etorphine and reversal agent for an equid) led to an attempt to deliver 3.0 g mTP remotely,

to feral 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, with a 28.9% fertility rate for the mares accompanying treated stallions and an approximate 45% fertility rate among control mares, but the logistics of delivering 3.0 g microcapsules in four separate doses to each stallion was discouraging.[5]

CONTRACEPTION OF THE MARE

STEROIDS

Difficulty of darting a stallion up to four times, plus concerns over band infidelity by mares, turned the focus of contraception in horses to the mare. Based on experience with persistent corpora lutea[6] and data which indicated that plasma progesterone concentrations in excess of 0.5 to 1.0 ng/mL inhibited ovulation in mares,[7–9] attempts were made to administer contraceptive doses of progestins to feral horses. Kirkpatrick and Turner, using remotely delivered darts, administered the microencapsulated synthetic progestin norethisterone (norethindrone, mNET) to six feral mares on Assateague Island.[5] This progestin, which has been used so successfully to inhibit fertility in women was given in a dose of approximately 2.0 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%.[10]

In another experiment, groups of 30 captive feral mares in Nevada were each implanted with Silastic rods containing 8.0 g estradiol (E), 24 g progesterone (P), 8 g E plus 8 g P, 4 g E plus 12 g P, 12gE plus 12 g P, or no hormone.[11] Fewer mares receiving 8 g E, 12 g P plus 4 g E, 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 3 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 (EE_2) or EE_2 plus P were placed in captive feral mares.[12] Animals pregnant at time of implantation delivered healthy foals, and contraceptive efficacy ranged from 88 to 100% through two breeding seasons and was approximately 75% for three seasons. Endocrine studies of these mares suggested that contraception was affected by blocking ovulation and/or implantation. In a similar study, intraperitoneal implants of 1.5, 3.0, or 8.0 g EE_2 alone also resulted in contraceptive efficacy of 75 to 100% through two breeding seasons, and rates of EE_2 decline in the plasma suggest a contraceptive life of 16, 26, and 48 to 60 months for the 1.5 g, 3.0 g, and 8.0 g, respectively.[13]

Results achieved with estradiol, progesterone, and ethinylestradiol in mares bring to focus 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 recognized by the mares' 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 down regulation in certain instances. Any risk, however small, of the passage of these synthetic steroids to humans or wildlife may make registration by regulatory agencies such as the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), or the Environmental Protection Agency (EPA) unlikely.

IMMUNOCONTRACEPTION

Because of difficulties associated 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 has turned to immunocontraception. One immunologically based contraceptive strategy involves blocking the release of gonadotropin-releasing hormone (GnRH), thereby preventing pituitary secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and their subsequent tropic actions on the ovary or testes.[14] Immunization of domestic mares against GnRH blocked ovulation in three of five mares for 4 months.[15] 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 mares' response to the antigen and the subsequent variabilities in antibody titers suggested this approach was unreliable.

In a study to immunize captive 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.[16] 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. The vaccine was freeze-dried and administered as a solid biodegradable 0.25-caliber bullet by means of an air-powered gun (Ballistivet, Inc., White Bear Lake, MN). After imbedding in the tissue of the target mare, the compressed compound forming the biobullet degrades over 24 h, releasing the antigen. A total of 25 treated mares survived and 17 (68%) produced foals, which was not significantly different from control foaling rates.

Immunization against GnRH has also been attempted in the stallion.[17] Four weanling colts were passively im-

munized, 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 inoculation after which testosterone concentrations rose to control levels for yearlings. Colts immunized subcutaneously had small increases in plasma testosterone concentrations, up to 0.37 ng/mL, between the two immunizations but decreases similar to those seen in the intramuscular 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 horses is based on the identification of antibodies directed against the zona pellucida of the ovum in naturally infertile mares[18] and immunological cross-reactivity of equine zona-positive antisera and porcine sperm binding.[19] Liu et al. immunized 10 captive feral mares and 4 domestic mares with the protein equivalent of 2000 to 5000 porcine zonae pellucidae (PZP).[20] Freund's complete adjuvant was used for the first inoculation and Freund's incomplete adjuvant (FIA) for the three monthly booster inoculations which followed. Of the 14 treated mares 13 failed to conceive during the first year. The 4 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.[21] For the test, 26 feral mares were remotely inoculated with approximately 5000 PZP (65 μg protein) and FCA in March 1988 by means of barbless darts. The mares received a second inoculation, with FIA, 2 weeks later, and some mares received a third inoculation with FIA 1 month later. Only a single foal was produced by the treated mares whereas 50% of the 6 sham-injected controls produced foals, and 45% of 11 untreated 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. Once antigen recognition has taken place, a single annual booster inoculation is sufficient to maintain contraceptive levels of antibodies,[22] and animals that do not receive booster inoculations return to normal fertility.[20,22] Trials are currently under way with PZP contraception in feral burros inhabiting the Virgin Islands National Park and captive Przewalski's horses in the Cologne Zoo.

The mechanism of action of PZP-induced contraception in mares is thought to be a block to fertilization.[20] One of the three major proteins of the noncellular zona pellucida ZP3 is the receptor molecule for sperm surface molecules.[23] The role of the ZP3 receptor in the horse has been confirmed in vitro as a zona pellucida–induced acrosome reaction with horse sperm.[24]

Despite return of normal fertility among PZP-treated mares, the long-term effects of continuous PZP immunocontraception have not been described. In the domestic rabbit,[25] the domestic dog,[26] and the baboon[27] evidence suggests the antibody response of the treated animal attacks not only the zona pellucida of the mature ovum but oocytes and other ovarian tissues, with resulting changes in estradiol and progesterone secretion. These effects have not been demonstrated thus far in the horse. Plasma progesterone concentrations from PZP-treated mares indicated that ovulations and luteal formation occurred during the period of infertility, and no evidence of histologic changes in the ovary was found.[20] Work in progress indicates that three consecutive years of treatment of feral mares have no effect on sexual behavior or ovarian endocrine profiles.

PZP-induced contraception in the mare may be useful to prevent untimely or undesirable breedings and anti-GnRH contraception may be useful for the same purpose in the stallion and at the same time eliminate the aggressive behaviors associated with intact stallions. In the case of captive exotic equids, such as Przewalski's horse and zebras, contraception may be useful to prevent the expression of undesirable genetic traits ("floppy mane," for example) or merely to prevent the production of surplus animals, without the need to remove animals and disrupt the well-defined equid social structure. Finally, contraception may represent a publicly acceptable approach to the management of feral horses and burros inhabiting public lands.

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