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"IN MY EXPERIENCE . . ."

NEW DEVELOPMENTS IN FERAL HORSE CONTRACEPTION AND THEIR POTENTIAL APPLICATION TO WILDLIFE

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The concept of sex steroids as contraceptives is not new and was originally directed toward fertility control in humans (Pincus et al. 1958). In the 1960's the development of extended-action steroids as contraceptives was explored (reviewed, Beck et al. 1980), and application of this technology to captive exotic animals was pioneered in the 1970's (Seal et al. 1976). In the light of rapidly increasing problems of wildlife overpopulation, and continued advances in contraceptive technologies, new approaches to fertility control in wild, free-roaming animal populations are now being examined (Kirkpatrick and Turner 1985, 1991). Because of local overpopulations of free-roaming feral horses (*Equus caballus*) in some areas of North America and the highly publicized nature of management efforts to control these populations, the feral horse has been the focus of a number of contraception studies in free-roaming populations (Kirkpatrick et al. 1982, Turner and Kirkpatrick 1982, Plotka and Vevea 1990).

This article examines approaches to fertility control in feral horses, including currently available and experimental antifertility agents and delivery systems, and their potential for adaptation to various free-roaming species, particularly ungulates. As a first step, it is useful to list the characteristics of the ideal wildlife fertility control agent. First, it has to provide a high degree of effectiveness across a given breeding season. Second, it has to be free of harmful side effects to the animals receiving it, including pregnant animals. Third, the ideal contraceptive should be reversible. The genetic pool in each population exists in the dynamic state, with each reproductively active animal having the potential to influence the pool. Impact on the process of natural selection will be minimized when a fertility control program is reversible. There are also important social and political reasons demanding reversibility of wildlife contraception (Kirkpatrick and Turner 1985). Fourth, the ideal agent will be relatively inexpensive. However, no fertility control program can compete on a cost-effectiveness basis with a management method such as hunting, where the public not only provides the manpower but also provides revenue. Fifth, the ideal agent should have a flexible duration of action, so that a single treatment can act for a predetermined period or number of breeding seasons. Sixth, the agent should have minimal to no effect on social organization or behavior. Finally, the ideal agent should be capable of being delivered remotely. The capture or immobilization of large numbers of animals, regardless of the skill of the management team, may lead to injuries, mortality, and monetary expense that will ultimately be unacceptable (Turner and Kirkpatrick 1986).

FERAL HORSE CONTRACEPTION

Contraception in feral horses has focused on steroids and vaccines, with delivery methods including surgical implants or intramuscular (i.m.) injection in immobilized animals and i.m. injection by remote-delivery projectile. However, the subject is not a simple one. A number of variations on these basic approaches have been explored, and an awareness of attendant advantages and disadvantages has emerged during the course of several feral horse studies.

One of the first issues faced in the feral horse studies was which sex should be targeted for fertility inhibition. Although ovulation inhibition in domestic mares by pharmacological doses of progestins had been demonstrated by Loy and Swan (1966), and confirmed by subsequent studies (Ginther 1979), the social structure of feral horse herds made males seem a preferable target (Turner and Kirkpatrick 1986). In data from 14 of 16 herds surveyed, dominant stallions controlled and bred harems of several (range 2–24, average 5) females, preventing males from outside the harem and subordinate males from breeding (Kirkpatrick and Turner 1986). Because sexual behavior and harem maintenance behavior were regulated by testosterone (Turner and Kirkpatrick 1982), we reasoned that any agent that could satisfy the basic characteristics of the ideal contraceptive while permitting maintenance of normal testosterone levels would be a promising candidate.

In a screening study 4 potential antifertility agents were evaluated in 24 pony stallions (Turner and Kirkpatrick 1982). The agents were α-chlorohydrin (nonsteroidal), 2 long-acting formulations of testosterone (testosterone cypionate [TC] and microencapsulated testosterone propionate [MTP]) and the potent, long-acting synthetic estrogen Quinestrol (17α-ethinylestradiol 3-cyclopentyl ether). The α-chlorohydrin was unacceptable because of neurotoxic side effects. The Quinestrol and both androgens were effective. We chose to use androgens, which had less potential for contamination of the environment.

Within 6–8 weeks after treatment initiation with i.m. injection of MTP (2.6 g/100 kg) or TC (1.7 g/100 kg, monthly 6×), significant decreases from control values occurred in sperm number and sperm motility, while libido scores (based on vulval sniffing, flehmen, erection, and mounting) did not change. These effects persisted for approximately 6 months (treatment phase). In the recovery phase, the affected parameters had returned to control values. No side effects were observed. In this preliminary study, the treatment decreased sperm production but did not compromise the normal sexual behavior of the male. Presumably a harem stallion given this treatment in the field would maintain his harem while being infertile.

MTP was chosen for a field trial on the basis of its more extended action in a single injection. The MTP, prepared by Southern Research Institute (Birmingham, Ala.), consisted of microdroplets of testosterone propionate coated with a nontoxic biodegradable polymer of varying thickness. The basic principle is that a thick coating biodegrades more slowly than a thin coating. By varying the thickness of the coating, it is possible to achieve delay times for MTP release ranging from several days to more than 6 months. By including a range of coating thicknesses in a single injection, hormone presence in the blood can be continuous throughout the release period. Both release rate and duration depend on the chemical characteristics of the agent which is microencapsulated. The current technology has been refined to potentially provide steroid preparations with up to an 18-month release period capability (T. Tice, Southern Research Institute, Birmingham, Ala., pers. commun.).

In an initial field study, 10 harem stallions in the Challis Horse Range in Central Idaho were immobilized from a helicopter and injected directly with MTP several months prior to the 1980 breeding season (Kirkpatrick et al 1982). Pretreatment foaling, in 1980, was similar in control and treated bands. In the summer of 1981, 83% fewer foals were produced among mares in the harems of treated stallions. In 1982 the foal counts in the treated bands had returned to pretreatment (1980) levels. Sexual behavior was evaluated from 1980 to 1982 using standard male parameters of mounting, intromission, and ejaculation. A sociosexual scent marking behavior, exhibited by males (Turner et al. 1981), was used as an index of harem maintenance behavior. There were no differences in stallion behavioral parameters between treated and control animals in the years monitored (1980–1982), with the exception that mating behavior continued further into the summer in treated bands. This probably reflected continued estrus cycling in the mares due to infertile matings (C. Asa, St. Louis Zoo, St. Louis, Mo., pers. commun.).

On the basis of these data, we concluded that a single injection of MTP given several months prior to the breeding season significantly decreased the fertility relative to untreated controls for a single breeding season, did not interfere with stallion behavior, and permitted a return to normal fertility in the breeding season of the following year.

Despite the encouraging outcome of this study, we found the method for delivery of the drug to be unacceptable. Factors such as the cost (approximately $50.00 per dose of etorphine and reversal agent for an equid), the immobilization-treatment-recovery time, and the danger to the animals made immobilization undesirable. We therefore focused on a method for remote delivery of the drug without the intermediate immobilization step (Harder and Peterle 1974), by loading the antifertility agent into a dart to permit administering the MTP directly.

In a trial to establish the feasibility of this approach, 15 feral horse bands in a 64-km² area of the Challis Horse Range were located from helicopter. After harem stallions were identified by observing characteristic movement patterns in the band response to the helicopter, remote delivery capability was demonstrated by firing a paint ball from a paint gun (Nelson Paint Company, Iron Mountain, Mich.). Thirteen of the 15 stallions were hit on target in the first pass, and the remaining 2 stallions were marked on a second pass with an average elapsed time of 5.25 minutes from locating a band to hitting the target. Most of this time was used in approach, descent, and maneuvering the horses into a safe path of movement. Usually less than 15 seconds elapsed from the beginning of close pursuit to firing.

A second issue which emerged from the field test was whether to treat males or females. The vast majority of feral horse herds have a single dominant male breeding the harem (Keiper and Houpt 1984), and treating males would be more cost and time efficient. However, it appeared that treatment could be delivered to several horses with relative ease and speed after a band of horses was within firing range. This potentially lessened the time advantage of treating males. Because helicopter time would be the major cost in treatment, the cost advantage may also be minimal. Pursuing remote delivery for female fertility control also offered the potential to increase population management flexibility and permit the possible application of this technology to nonharem species.

Between February 1986 and August 1987 on Assateague Island National Seashore (Maryland), we attempted to determine the antifertility effectiveness of MTP delivered remotely to stallions and of microencapsulated norethisterone (MNET) delivered remotely to mares. MNET is a potent synthetic progestin which has been shown to be a safe and effective extended-action antifertility agent in primates (Beck et al. 1980), acting primarily by blocking ovulation. The microencapsulated form of MNET, prepared by Southern Research Institute (Birmingham, Ala.), was designed to release over a 6-month period from the time of administration (March 1986) through the entire breeding season.

In the male study 4 harem stallions, each with a harem of proven fertility, were treated by remote delivery in February–March 1986. The 14 mares of proven fertility which were associated with the 4 treated stallions exhibited a fertility rate of 28.9% during the foaling season of 1987 (Kirkpatrick and Turner 1987). The foaling rate for a control population of 15 fertile mares for the 1987 season was 45.4%, and the foaling rate for the experimental mares for the previous 5 years ranged from 42% to 50%.

In the MNET study the drug was administered in February–March 1986 by remote delivery to 6 mares of proven fertility. No inhibition of fertility was observed (Kirkpatrick and Turner 1987). The study data did not permit determination of whether the failure was due to the agent, the dose, or the mode of delivery. It appears that the method of delivery was not the cause of failure, because the remote delivery method did work for males. Although progestin-mediated contraception has proven effective in some other species (reviewed, Kirkpatrick and Turner 1985), it may be that protestins are simply ineffective as contraceptive agents in feral mares. Plotka et al. (1988) were unsuccessful in suppressing estrus for longer than 5 weeks in captive feral males with Silastic® implants containing large amounts (24 g) of progesterone.

Valuable information in these Assateague Island studies was derived from technical problems associated with the remote administration of microencapsulated steroid. First, treatment administration occurred during several very cold days (−10 C), such that the increased viscosity of the carboxymethyl cellulose used to suspend the microcapsules sometimes interfered with rapid injection. It was thus necessary to keep the carrier warm prior to delivery. Second, the suspension of microcapsules tended to settle out and clump in the dart if not delivered within 10 minutes of initial mixing. Third, delivery of nonimmobilizing drugs (i.e., no handling of animal) necessitated barbless or micro-barbed darts which would ultimately fall out. Thus, velocity and trajectory had to be regulated carefully to ensure injection without rebound. Fourth, while it was possible to remotely deliver the effective amount of microencapsulated steroid with multiple injections in these studies, this would be unacceptable for routine use. The volume : dose ratio must be reduced sufficiently to permit administration of the complete dosage in a single dart.

If the problem of drug volume can be overcome, there is another remote delivery method which may be promising. B. Goodloe, R. J. Warren, and D. C. Sharp ("Sterilization of feral horses by immunization against LHRH," presentation, Wildl. Dis. Assoc. Conf., Univ. Ga., 7–11 Aug 1988) have successfully delivered antifertility agents to feral horses in a biodegradable bullet fired from a CO_2-powered rifle (Ballistivet, Inc., Minneapolis, Minn.). The hollow 0.25 caliber bullet is made of a compressed food-grade material. Once the bullet is lodged, biodegradation occurs over 24 hours, and the agent is freed for action. Maximum deliverable volume is 0.3 cc.

While the remote delivery dart or bullet methods cannot presently be easily used to administer steroids due to excessive volumes required for available steroids, they may be useful with water-soluble agents which can be delivered at high concentration, lyophilized, or in low volume. Most of the water-soluble reversible contraceptive agents currently being studied are vaccines.

Immunoantifertility

Immunoantifertility currently appears to be 1 of the most promising areas of contraceptive technology. The general principle is that antibodies are raised in the individual against some structural or functional protein or peptide involved in the reproductive process. The presence of the antibodies hinders or prevents some aspect of the reproductive process. Successful immunocontraception has been achieved by raising antibodies against (1) gonadotropin releasing hormone (GnRH) in both sexes, (2) spermatozoa, and (3) ovarian zona pellucida. The latter has received the most extensive investigation.

GnRH is a hypothalamic peptide which regulates pituitary gonadotropin release. The gonadotropins, follicle stimulating hormone, and luteinizing hormone (LH), in turn regulate aspects of gonadal function, including gamete production. Thus, reproduction may be inhibited by immunizing an individual against self GnRH, which makes the GnRH unavailable for biological actions. Anti-GnRH has been used successfully to reduce fertility in several species, including pigs (*Sus scrofa*) (Esbenshade and Britt 1985), rats (*Rattus norvegicus*) (Ladd et al. 1988, Ladd et al. 1989), and rabbits (*Oryctolagus cuniculus*) (Ladd et al. 1988). In a study of domestic ewes (*Ovis aries*), Roberts and Reeves (1988) reported that immunization against either LH or a combination of estradiol-ovalbumin and testosterone-ovalbumin resulted in marked reduction of lambing relative to albumin-immunized controls.

Two contraceptive studies using anti-GnRH in the horse have been reported. In 1 study a conjugated form of GnRH was used successfully to raise antibodies in captive feral mares, but contraceptive results were poor (R. Goodloe, R. J. Warren, and D. C. Sharp, 1988, unpubl. presentation). Using a similar approach, Dowsett et al. (1990) suppressed GnRH in colts and reduced testosterone concentrations for up to 20 weeks post-immunization, suggesting contraceptive potential in males.

One major drawback of using antibodies against GnRH, LH, or sex steroids as a means of inducing infertility is that gonadal steroid production or steroid bioavailability will be decreased by these manipulations. Thus, steroid replacement will be required to ensure integrity of both reproductive and social behavior of the population involved.

Immunization of individuals against gametes or gamete proteins has the distinct advantage of avoiding steroid/behavioral effects, and this approach currently appears to be promising for immunocontraception. Antibodies to spermatozoa have been causatively implicated in human infertility (Menge 1980, Bronson et al. 1984). Spermatozoal or testicular extracts used to immunize individuals of several species have been shown to decrease fertility via both pre- and post-fertilization effects (Carron et al. 1988, Edwards 1964, Menge and Naz 1988). Antibodies raised against a recently isolated sperm-specific glycoprotein antigen found in the sperm cell plasma membrane (Naz et al. 1986) have been shown *in vivo* and *in vitro* to inhibit aspects of fertilization in several species (Naz 1988, Menge and Naz 1988, Herr et al. 1990).

In the female, active immunization of several species with porcine zona pellucida (PZP) has been associated with reduced fertility (reviewed, Henderson et al. 1987, Shivers and Liu 1982), and antizona antibodies have blocked *in vitro* fertilization in humans (Sacco et al. 1981). To date, reported side effects of PZP immunization have included some alteration in ovarian follicular growth and function in rabbits (Skinner et al. 1984), monkeys (*Saimiri* sp.) (Sacco et al. 1983), dogs (*Canis familiaris*) (Mahi-Brown et al. 1985), and baboons (*Papio* sp.) (Dunbar et al. 1989), with potential irreversibility reported for dogs. It may be possible to avoid the potential side effect problems of PZP antibodies by using cumulus oophorus matrix antibodies, which are unlikely to react with younger follicles. Rabbit oophorus matrix has been shown to effectively inhibit human fertilization *in vitro* (Tesarik 1989).

It should be noted that many of the initial PZP studies utilized high antigen concentrations for immunization. At lower concentrations side effects may be minimal to nonexistent. This appears to have been the case for PZP immunocontraception in the mare. In a recent study with captive feral and domestic mares, Liu et al. (1989) successfully produced reversible immunoinfertility by immunization of mares with PZP. Pregnancy was prevented for approximately 8 months in 14 of 15 mares. When antibody titers had decreased to lower levels in 4 monitored mares, they conceived normally.

In a subsequent study of free-roaming feral mares on Assateague Island, Kirkpatrick et al. (1990) determined the effectiveness of remote delivery PZP immunocontraception. Between February and April of 1988, 26 Assateague mares of proven fertility received 2 or 3 inoculations (1 ml each) with PZP vaccine in adjuvant. The treatments were administered remotely via dart rifle as described for the other Assateague studies. Only 1 foal was born to the 26 treated mares, and among untreated control mares there was a 50% pregnancy/foaling rate. Regarding reversibility, 14 of the nonpregnant, PZP-treated mares were given a remotely delivered PZP booster inoculation in February or March 1989. Pregnancy determinations based on urinary steroids (Kirkpatrick et al. 1988) were made in samples collected in the fall of 1989. Results revealed only a 7.2% pregnancy rate in these mares, as compared to a 41.6% pregnancy rate among the 12 PZP-immunized mares which did not receive a booster inoculation (Kirkpatrick et al. 1991). Of 16 mares of similar age never treated with PZP, 43.7% were pregnant in the fall of 1989. These findings demonstrate the reversibility of treatment and the effectiveness of an immunization booster.

POTENTIAL FOR WILDLIFE CONTRACEPTION

From the standpoint of both antifertility agents and delivery techniques, a fair armamentarium for feral horse contraception already exists. The potential for broadened application of contraceptive technology to other wildlife populations has not yet been explored, although contraceptive efficacy has been reported for a number of domestic and captive exotic species (Kirkpatrick and Turner 1985). By carefully assessing the reproductive patterns, behavior, habits, and environment of a given free-roaming species, it may be possible to adapt existing contraceptive technology to assist in the management of some species. In this regard a brief discussion of advantages and disadvantages of currently available technology (Table 1) may be useful.

Major contraceptive agents and procedures have already been presented. However, to summarize, the agents are primarily natural and synthetic sex steroids and immunotropic protein and peptide antigens. The steroids are able to act over extended time periods via structural modifications to the molecule, microencapsulation, or gradual release from Silastic® polymer rods. Although steroids have the advantages of being well researched, biologically active in most vertebrates, and often active orally, they also have several serious disadvantages. Among captive feral mares, placement of Silastic® rod implants containing estradiol and progesterone (Vevea et al. 1987, Plotka et al. 1988) met with limited success in controlling fertility. Although Plotka and Vevea (1990) reported successful inhibition of fertility in captive feral mares given Silastic® rod implants containing ethinylestradiol, the use of such synthetic steroids, which often exhibit poor biodegradability, raises the issue of possible consumption by nontarget species, including humans. This circumstance makes acceptance for registration with regulatory agencies such as the FDA, USDA, and the EPA unlikely.

The use of natural steroids, which are rapidly metabolized, may minimize the biodegradability issue. However, the dosages of these steroids must be relatively large in order to inhibit fertility. This may limit the administration of the agents to surgical implants, which necessitates the undesirable circumstances of capturing and handling the target animals. Thus the potential seems low for the use of steroids for contraception use in free-roaming

Table 1. Current wildlife contraceptive delivery systems, route of administration, agents, and characteristics of potential target species of their use.

Delivery system	Route (IM vs. oral)	Agent: Type[a]	Agent: Format[b]	Target animal: Size (large or small)	Target animal: Style (secretive or exposed)	Target animal: Habitat (closed or open)
Capture and chute	IM	S, N, I	SI, E, ILA, V	L	E	C, O
Live trap and restraint	IM	S, N, I	SI, E, ILA, V	L, S	SE	C, O
Immobilizer	IM	S, N, I	SI, E, ILA, V	L	E	O
Remote delivery	IM	S, N, I	SI, E, ILA, V	L	E	O
Bait or food	O	S, N	E, ILA[c]	L, S	SE, E	C, O

[a] Agent type: Steroid (S), Non-steroid chemical (N), Immunological (I).
[b] Agent format: Subdermal implants (SI), Encapsulation (E), Intrinsic long-action (ILA), Vaccination (V).
[c] Steroids only.

wildlife. One exception to this may lie in the use of long-term, nonsurgical subcutaneous (sc) implants of steroids in certain smaller mammals which can easily be live trapped. In a recent study Bickle et al. (1991) successfully inhibited fertility (no litters in 23 treated females) in free-roaming ($n = 4$) and captive ($n = 19$) female skunks (*Mephitis mephitis*) given subcutaneous implants of levonorgestrel (Norplant®), a progestational steroid. The implant was a 2.5 × 30 mm flexible rod inserted sc into the neck via trocar. While the possibility of consumption by nontarget species remains, this issue may be minimized, for example, when treatment is applied to an urban population of skunks, in which predation and scavenging are minimal.

The immunological approach to wildlife contraception appears promising on the basis of the feral equid data demonstrating a high degree of effectiveness and reversibility. Immunocontraceptives have the advantage of high potency for low volume delivery. In addition, they do not have potential for contaminating the environment and do not have behavioral effects. Potential disadvantages also must be considered. For example, immunocontraceptives are not active orally without modification, may require more than 1 inoculation for the initial immunization, and may be variably effective across species. However, with the use of biodegradable polymer coatings it may be possible to provide oral delivery of active vaccine (Saffran et al. 1990). Furthermore, microencapsulation, which permits timed-release of the agent, can potentially eliminate the need for multiple inoculation. The potential side effects of long-term use are unknown, with the extreme possibilities including permanent infertility or escape from the antifertility effect over a period of years.

Assuming the availability of a viable wildlife contraceptive agent, a method of delivery must be chosen. The 2 pathways routinely used for getting chemicals into an animal are oral and intramuscular. In some respects the delivery aspect of wildlife contraception is the most variable and difficult to accomplish because of the wide variety of species and habitats. Put simply, however, the possibilities are "hands-on" or "hands-off." The "hands-on" methods include round-up and capture, live trapping, or chemical immobilizer. Once captured or trapped the animal can be darted, hand-injected, or implanted with the antifertility agent. The viability of the "hands-on" approach will depend on the size, accessibility, and numbers of the targeted species. For example, skunks can readily and safely be live-trapped, and it may be feasible to treat sufficient numbers to eventually limit their population in a given urban area. Access to species such as large ungulates can often be accomplished with chemical immobilizer, and treatment can be made in the field at the immobilization location. However, the already discussed disadvantages of immobilizer in terms of cost and danger to the animal may well outweigh the on-site "hands-on" advantage, particularly when dealing with large numbers of animals.

In contrast, the "hands-off" methods provide an on-site remote delivery methodology without capture, live trapping, or immobilization. Remote delivery methods include the use of baits and the use of a gun which fires a dart or plastic bullet containing the antifertility agent. The earliest wildlife contraception efforts used baits (Marsh and Howard 1969), and this approach remains potentially viable for many species, especially for small mammals and birds (Kirkpatrick and Turner 1985). Two common disadvantages of baits are the lack of target specificity and poor bait acceptance (Harder and Peterle 1974).

These disadvantages are not shared by remote delivery methods using a projectile fired from a gun. Despite its limited usage to date for contraception, this form of remote delivery has several distinct advantages over "hands-on" methods. Perhaps most importantly it reduces the incidence of harassment, injury, and death in the target animals. In our experience with feral horses remote delivery darting has proven to be a far more cost-efficient and time-efficient method than capture and handling, requiring fewer personnel and equipment by eliminating the capture-immobilization step.

However, there will undoubtedly be circumstances where the delivery method used is dictated by situation, animal characteristics, and habitat. For example, darting of animals from a blind at waterholes may be useful in arid areas, and firing darts from a helicopter may be effective for some large ungulates in open or semi-open terrain. Live trapping and injection or baits may be preferable for some smaller species inhabiting burrows or dense underbrush.

SUMMARY

During the past decade the problem of overpopulation of many wildlife species on preserves of limited area and in urban parks has reached crisis proportions despite existing management efforts. The development of contraceptive technology for free-roaming wildlife may become essential. Using research studies of contraception in free-roaming feral horses as a contextual framework for critical analysis, an evaluation of the potential for wildlife contraception is presented. Topics include species-specific requirements regarding choice of sex, type of agent, and method of delivery. Agent types include steroidal, nonsteroidal, and immunocontraceptives. Considerations of delivery include release characteristics of the agents and capture versus remote delivery. In the continuing development of contraceptive technology for wildlife it is important to address, in addition to efficacy, issues of environmental and animal safety, reversibility, and cost effectiveness.

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