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REVERSIBLE CONTRACEPTION IN NONDOMESTIC ANIMALS

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Abstract: Reversible fertility control is a potentially powerful management tool for both captive exotic and free-roaming wildlife. This approach to wildlife management represents an effective and humane alternative to current control methods, which are largely lethal. A number of new contraceptive technologies, designed primarily for use in humans or domestic animals, can be applied to wildlife populations. Reversible contraceptive agents examined here include nonhormonal compounds, nonsteroidal hormones, steroid hormones, barrier methods, and immunocontraception. A brief history of their use in wildlife is examined, along with directions for future research and the benefits of wildlife contraception.

Key words: Contraception, fertility control, immunocontraception, wildlife, zoo.

INTRODUCTION

The focus of conservation biology historically has been to increase the number and diversity of wildlife, including both captive exotic and free-roaming species. However, there are cases where too much of a good thing can lead to harmful consequences. Captive breeding programs and technologies involving exotic species have become very efficient, and zoological parks often face problems associated with surplus animals. Finding socially and biologically acceptable solutions for the use of these excess animals has been difficult. In the case of free-roaming wildlife, populations historically have been kept within the limits of food supplies and habitats through controlled hunting, poisoning, trapping, and relocation. Some of these methods have been ineffective and all are presently controversial, with an unknown future. Consequently, uncontrolled population increases among some species are occurring throughout the world.

For almost 20 yr, reproduction in captive exotic animals has been regulated in a number of species by means of reversible contraception. However, the concept, particularly for free-roaming species, has become a serious focus recently for zoo veterinarians and wildlife mangers. To evaluate various wildlife contraceptives, the characteristics of the ideal wildlife fertility control agent should first be identified.[36,112] These characteristics include 1) a high degree of effectiveness; 2) a lack of toxicity and harmful side effects, particularly to pregnant animals; 3) reversibility and a flexible duration of action, to preserve the reproductive and genetic integrity of the target animals;.4) low cost; 5) minimal or no effect on social organization or behavior; 6) remote delivery, preferably with a single administration; and 7) inability of the contraceptive agent to be passed from the treated animal to predators, scavengers, or humans through the food chain. These characteristics have been formed primarily with free-roaming wildlife in mind, whereas the contraceptive management of captive animals permits far greater latitude when selecting the ideal agent.

This review describes reversible forms of contraception that have been used in captive exotic animals, free-roaming wildlife, or closely related species. Experimental approaches and advantages and disadvantages of each form of contraceptive are discussed. The contraceptive approaches discussed are nonhormonal chemical agents; hormonal agents, including both steroid and nonster-

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oid hormones; reversible barriers; and immunocontraception.

NONHORMONAL CHEMICALS

The contraceptive efficacy of clomiphene isomers (Merrell Dow Pharmaceuticals, Cincinnati, Ohio 45215, USA) has been investigated in captive red foxes (*Vulpes vulpes*).[20] Weekly 300-mg doses of one isomer (1-[p-beta-diethylamino-ethoxyphenyl]-1,2-diphenyl-2-chloroethylene) given during the breeding season did not interfere with estrous behavior or subsequent mating but did prevent pregnancies in all vixens receiving the drug. These data suggest that clomiphene impairs fertilization; however, neither the mechanism of action nor the reversibility of the approach was investigated.

Nonhormonal contraceptives have been used most frequently in birds. Arasan® (DuPont Co., Wilmington, Delaware 19880, USA) (a common fungicide and seed disinfectant) and the anticholesterol agent SC-12937 (22,25-diacholesterol dihydrochloride) have both caused reversible inhibition of ovulation in pigeons (*Columbia livia*).[25] In later studies, pigeons were fed SC-12937 in wheat (0.1% by weight).[121] Each pigeon consumed about 0.3 g of the drug over an 11–16-day period. In rural areas and small towns, reproductive inhibition among treated pigeons ranged from 89 to 100% for 3–7 mo; whereas in large cities, reproductive inhibition fell to 10%, primarily because of the large number of birds. Other investigators[63] found that SC-12937 inhibited meiosis in the pigeon testis and that the drug interfered with Sertoli cell function and produced atrophy of Leydig cells, with subsequent loss of sexual behaviors. In another study, clutch size in pigeons was reduced significantly;[109] however, SC-12937 also caused a debilitating illness. Although the toxic effects of SC-12937 were clearly demonstrated in several other studies,[6,76,95] the drug was later registered by the USDA and produced commercially as Ornitrol® (G. D. Searle and Co., Chicago, Illinois 60680, USA). Its use for contraception in birds remains illegal in the U.K.

Another compound, triethylene melamine (TEM), reduced testis weights and decreased spermatogenesis in starlings (*Sternus vulgaris*).[22,23] TEM also inhibited reproduction in breeding populations of red-winged blackbirds (*Agelaius phoeniceus*)[116] and yellow-throated sparrows (*Petronia xanthocollis*).[9] Testis weights were reduced in TEM-treated birds and meiosis was inhibited, but the drug was toxic and some study birds died. The compound was field tested on Kent Island, Maryland (USA), and fertility was clearly reduced among red-winged blackbirds, but mortality was not studied.[73]

The antifertility agent thiotepa (tris [1-azirdinyl] phosphine sulfide) (supplied by Dr. A. B. Borkovec, USDA, Beltsville, Maryland 20705, USA) acts by interfering with nucleic acid synthesis. The compound was fed to blackbirds in doses of 2.5–3.6 mg/kg and triggered a reduction of 90% in testis weights and of ≥50% in hatching rates.[85,86] Despite this success, the drug was toxic at higher dose levels with an LD_{50} of 6.2 mg/kg. Attention then returned to SC-12937, which was fed to house sparrows (*Passer domesticus*) for 2 mo at a dose of 0.1% in canary seed.[73] In a colony of 11 males and 12 females, hatching success was 0% among treated birds vs. 64% in controls. Hatching success reached pretreatment levels among treated birds 4–5 wk after withdrawal of the drug. Two treated birds died during the study, but the cause of death was not determined. Red-winged blackbirds were fed cracked corn with 0.1% active drug.[52–55] Although reproductive effects were similar to those seen in treated pigeons, these birds also lost weight and showed the same debilitating effects seen in previous studies.

It has been known for almost 50 yr that certain plants contain substances that act as reproductive inhibitors, yet few investigators have attempted to use these naturally occurring substances to control reproduction in wildlife. A recent review[29] listed 50

plant families, genera, and species that have documented antifertility effects in male and female mammals. Use of some of these plants, particularly in managing reproduction in herbivores, deserves further consideration. For example, an ethanolic extract of western gromwell (*Lithospermum ruderale*) root was fed to mice, decreasing litter production by 50% when consumed 10 days before and 10 days during breeding activity.[21] Ethanolic extracts of bitter cherry (*Prunus emarginata*) and trumpet honeysuckle (*Lonicera ciliosa*) were fed to mice as 1–2% of their diet, and again litter production was reduced by about 50%.[5] All three of these plants were used by native Americans in the northeastern U.S. as contraceptives. Seven ethanolic fractions were extracted from winter wheat sprouts and fed to mountain voles (*Microtus montanus*).[8] Five of the fractions caused significant reductions in uterine weights, and two fractions, *p*-coumaric acid (PCA) and 4-hydroxy-3-methoxystyrene (4-VG), caused significant reductions in litters. An extract from the root of thunder god vine (*Tripterygium wilfordii*), which is available commercially for the treatment of arthritis, has been shown recently to have powerful antifertility effects in male rats and humans, lowering sperm counts and decreasing sperm motility.[38]

STEROIDAL HORMONAL AGENTS

Canidae

The concept of steroid hormonal contraception was originally directed at human fertility control[30] and until recently has been the most popular contraceptive approach for wildlife.[49] The use of contraceptive steroids in canids began with the discovery that mismated domestic bitches would resorb early embryos after administration of diethylstilbestrol (DES).[46] Red foxes given 50-mg doses of DES orally from 9 days before mating to 10 days postmating became infertile.[50] The DES apparently acted by causing implantation failure or early embryonic resorption. The same dose of DES had no clear effects on male foxes. One hundred-milligram doses of DES administered to red fox vixens in meat baits on the day of mating or 10 days thereafter prevented implantation.[50] However, if the DES was administered in tallow rather than meat, it lost its contraceptive efficacy, suggesting that the choice of bait is important in the delivery of synthetic steroids to carnivores.

In an attempt to inhibit reproduction in both red and gray fox (*Urocyon cinereoargenteus*) in Virginia (USA), ground beef baits were loaded with 50 mg of DES.[18] Data subsequently obtained from trapped foxes demonstrated that fertility was significantly reduced only in the gray fox vixens, probably because the baits were taken more readily by gray foxes than by red foxes.

A synthetic estrogen related to DES, mestranol (MES; 17-δ-ethinyl-3-methoxyestra-1,3,5 [10]-trien-17β-ol) (Sigma Chemical Co., St. Louis, Missouri 63178, USA), is also an effective reproductive inhibitor in foxes.[18] No foxes fed MES for 5 days after mating produced pups. Although most first feedings were readily accepted, the vixens only nibbled at subsequent drugged feedings, indicating that MES could not be disguised for prolonged administration to foxes. This bait acceptance problem with MES surfaced again in studies with rodents. In this same study, chlormadinone acetate (6-chloro-6-17-acetoxyprogesterone) administered orally every 4, 7, or 10 days prevented estrus in most vixens until the feeding regimen was halted.

In one study,[20] an attempt was made to inhibit male fertility. Spermatogenesis was inhibited by feeding males a mixture of DES and chlormadinone acetate in meat baits at weekly intervals for 4 wk, beginning in early December. Five weeks after the cessation of drug administration, spermatozoa were present in the seminiferous tubules but not in the epididymides. No significant impairment of spermatogenesis was observed with either of these compounds alone at the same dosages.

Attempts were also made to control re-

production in female coyotes (*Canis latrans*) with DES. Initial tests with captive animals showed that DES given just before or after mating inhibited implantation, just as it had in foxes.[4] Tallow baits filled with 100 mg of DES were air dropped in sections of New Mexico (USA).[72] Female coyotes were caught several weeks later in the drop area, and approximately 75% showed implantation failure or signs of fetal resorption. There were, however, limitations on the effectiveness of DES baits for coyotes. First, DES ingestion had to be timed relatively precisely with the animal's reproductive cycle for success. This problem was compounded by the fact that coyotes did not take nonliving baits with any regularity during the breeding season. Second, the absorption of DES in tallow baits was inefficient, as was seen for foxes in a later study.[30] Finally, coyotes that did take the DES-loaded baits remained sexually active longer than usual and were apparently reproducing later in the season.[4,59] In contrast, field tests of the effectiveness of 50 mg of DES in sugar-coated ground-fat baits carried out on red foxes in North Dakota (USA) showed that the average litter size was reduced from 4.6 (control foxes) to 3.0 for foxes taking at least one bait.[3]

After these attempts at contracepting foxes and coyotes, research on contraception in canids was redirected toward domestic dogs. Silicone rubber implants containing testosterone and androstenedione were placed s.c. in female beagles.[99] The bitches were kept in a constant state of anestrus for 420–840 days, and when the implants were removed, normal fertility was restored. Estrus was clearly suppressed; however, this study did not explain the mechanism of steroid action. Unfortunately, in addition to producing antifertility, the androgenic steroids caused both physical and behavioral masculinization of these bitches.

At about the same time, the effectiveness of an oral progestin, medroxyprogesterone acetate (MPA) (Depo-Provera,® Upjohn, Kalamazoo, Michigan 49001, USA), was tested on captive female red foxes.[104] Each fox received 25 mg of MPA® for 4 or 8 days during the breeding season, which resulted in a significantly reduced number of litters produced. Shortly thereafter, other investigators explored the use of oral progestins for controlling fertility in canids. When melengestrol acetate (MGA) (17δ-acetoxy-6-methyl-16-methylene-4,6,pregnandiene-3,20-dione), an oral progestin closely related to MPA, was given to domestic bitches in doses of ≥200 μg/day, estrus was completely inhibited for a treatment period of 243 days.[102] The positive results obtained with MGA led to the first progestin approved for commercial use in dogs. This compound, megestrol acetate (MA) (Ovaban,® Schering Corp., Kenilworth, New Jersey 07033, USA), is a highly reliable and effective oral contraceptive in the bitch, with few side effects.[122] In The Netherlands, another synthetic progestin, proligesterone (14δ-17δ-propylidene-dioxy-progesterone), was tested in dogs and suppressed estrus in 97% of the bitches fed the compound.[117]

Attention then returned to androgenic steroids. When given orally to bitches for 240 days, the synthetic androgen mibolerone (17β-hydroxy-7δ, 17-dimethylester-4-ene-3-one) suppressed estrus and probably ovulation for the entire test period without the objectionable masculinization seen earlier with testosterone.[103] Nine years later, this same drug was given to five captive female timber wolves (*Canis lupis*) in doses of 6 μg/kg/day for 2–22 mo,[33] resulting in complete suppression of estrus. The only discernable side effect was increased aggression directed toward cage mates. During this same period, the Carnation Research Laboratories (Los Angeles, California 90036, USA) conducted extensive experiments with mibolerone. In one of the larger tests, 600 privately owned dogs in eight states were placed on oral mibolerone. Fertility inhibition was almost 100%, and no untoward side effects were noted (L. Miller, Carnation Research Laboratories, pers. comm.). Ultimately, the U.S. Food and Drug

Administration (FDA) licensed the steroid for prescription only by veterinarians, because of human consumption of dog food. Effective contraception requires 30 μg/day, a condition acceptable for zoo animals but unfeasible for most wild populations.

Felidae

As early as 1963 it was demonstrated that synthetic progestins could suppress estrus in felids. Megestrol acetate administered to domestic cats[14] and feral cat populations in the U.K. successfully suppressed estrus. In a field test in Scotland,[89] an initial dose of 5.0 mg in meat baits, followed by 2.5 mg/wk, was given to 15 female cats, only four of which had litters. Two years later, the experiment was repeated in England with feral cats.[72] The drug is available commercially in Europe under the name Ovarid® (Glaxo Drugs, Fort Lauderdale, Florida 33309, USA). Medroxyprogesterone acetate has also been used in Denmark for controlling feral cats since 1971. It was administered weekly in doses of 2.5–5.0 mg to 504 female cats. Of these, only 20 (4.0%) became pregnant and approximately 1.0% showed signs of mammary tumors or pyometra.

Injectable MPA (1.0 g initially, followed by 0.5 g 3 mo later) or MGA in Silastic® (Dow Corning, Hemlock, Michigan 48626, USA) implants (500 mg) has been used to achieve long-term suppression of estrus in captive African lions (*Panthera leo*), tigers (*Neofelis tigris*), leopards (*Panthera pardus*), and jaguars (*Panthera onca*).[97] When the implants were removed, fertility was restored. Similar Silastic implants containing MGA have been placed in free-roaming lions in Etosha National Park, Namibia, with the same positive results.[79] Social hierarchy was studied among the treated lions, and no significant effects on behavior were observed. Oral mibolerone has been administered to captive jaguars, leopards (*Panthera* sp.), and lions,[33] with actual doses ranging from 6 to 19 μg/day. Suppression of estrus was successful in all three species; however, side effects included refusal to eat at high dose levels and masculinization in some of the lions. One female lion grew a mane.

The use of progestins for fertility control of exotic felids should be carried out with caution. Case reports suggest a number of adverse clinical findings in captive exotic felids treated with contraceptive progestins.[15,32,61,88] The various conditions associated with progestin use include diabetes mellitus in a jaguar, weight gain with cutaneous atrophy and adrenocortical suppression in servals (*Felis serval*), increased aggressiveness in a Bengal tiger and a snow leopard (*Panthera uncia*), implant rejection in ocelots (*Felis pardalis*), and various mammary gland and uterine abnormalities in leopards, tigers, and jaguars. Although no cause-and-effect relationship has been established between progestin therapy and these abnormalities in exotic felids, there are many similar examples in the domestic cat.[19,28,39,40,43,120]

Ungulates

Population increases in hoofstock (including cervids, caprids, and equids), due to the efficiency with which these animals reproduce in captivity, cause substantial problems for zoos because of the large amount of space required for exhibits. Wild and feral populations of hoofstock living on refuges or enjoying legislated protection often increase at alarming rates and threaten their own habitat. As early as 1968, attempts were made to regulate reproduction in Yellowstone National Park's wapiti (*Cervus elaphus*) herd by administering i.m. doses of 75–200 mg of DES to 36 pregnant animals between December and March.[35] Pregnancy was terminated in 30% of the treated animals. An alternative approach was tried with female white-tailed deer (*Odocoileus virginianus*); they were fed DES before and during pregnancy.[36,37] In both cases, with doses of 50–100 mg/day, there was significant fetal loss. The i.m. approach was more effective but was impractical in animals as secretive as white-tailed deer.

One year later, both DES and MGA were implanted in white-tailed does during pregnancy.[1] The Silastic implants contained 0, 50, 100, or 150 mg of MGA or 75 mg of DES. The calculated hormone release ranged from 0.02 to 12.58 mg/day. The efficacy of the implants was clearly superior to that of injected steroids; pregnancy rates were significantly reduced among does treated with either hormone. The DES implants were effective before or after conception, whereas the MGA implants were effective only if given before conception. In an attempt to regulate reproduction in white-tailed deer in Mammoth Cave National Park, Kentucky (USA), DES was administered orally in an encapsulated form to does.[67] Doses of 500–1,000 mg encapsulated in modified gelatin interrupted pregnancy in a high percentage of cases. However, poor acceptance by the does, the need for very high doses, and postabortion pregnancies indicated that the approach was not practical. Two synthetic progestins, MGA and DRC-6246 (17δ-allyl-17β-hydroxy-3-oxoestra-4,9,11-triene) were also tested.[68] One group of does was given 50 mg/day of MGA and another group was given 1.0 g/day of DRC 6246 during the breeding season. Although does accepted both compounds without reservation, neither one prevented or delayed conception, possibly because too little of these steroids had accumulated in the body fat to bring about prolonged action.[70] Subdermal implants containing DES and DRC-6246 were placed in 23 sexually active mature female deer.[69,70] Average daily release rates for these steroids were 193 μg of DES and 93 μg of DRC-6246. Nine of the experimental does died from stress related to handling; none of the remaining experimental does became pregnant. Although the experiment demonstrated that fertility could be inhibited pharmacologically, the impracticality of capturing the animals and the resulting stress were distinct shortcomings to this approach.

In another study, 0.6–1.0 mg of MGA was fed to does daily during the breeding season.[71] Reproduction was completely inhibited, there were no untoward side effects, and fertility was later restored. To be effective, however, the drug had to be given daily, a requirement that is difficult to meet in wild populations. In more recent studies, homogenous Silastic implants containing either MGA or levonorgestrel were placed s.c. in white-tailed does.[82] Levonorgestrel was ineffective in preventing pregnancies. The MGA implants were effective if implanted prior to conception, but the use of implants in pregnant does prevented normal parturition and were thus contraindicated for use in pregnant deer.

In an attempt to control a deer population on Angel Island near San Francisco, California (USA), females were captured and given subdermal Silastic implants containing MGA.[38] The deer receiving the implants did not reproduce, but only about one-third of the population could be captured and treated. In another similar experiment, subdermal Silastic implants containing 800 mg of MGA were placed in 25 feral goats (*Capra hircus*) in Texas (USA) (U. S. Seal, pers. comm.). The 25 experimental nannies plus another 25 control females were placed with five billies; after 2 yr, no kids were produced by the treated goats.

Interest in contraception of hoofstock has been sparked by the dramatic increases in feral horse (*Equus caballus*) populations in the USA during the past 15 yr. Early studies[51,111] reported successful inhibition of reproduction in feral horses by lowering sperm counts and reducing sperm motility in stallions. A microencapsulated form of testosterone propionate (MTP) was injected into 10 immobilized stallions at doses of 3.0–10 g several months before the April–June breeding season in Challis, Idaho (USA). The polymer (poly-DL-lactide) coating (Southern Research Institute, Birmingham, Alabama 35255, USA) permitted a sustained release for up to 6 mo. The stallions' behavior was unaffected and breeding took place, but there was an 83% reduction in foal production among mares bred by the

treated stallions. Repeated injections of testosterone cypionate and single injections of quinestrol (17α-ethinylestradiol-3-cyclopentylether) can also cause oligospermia in stallions.[91] Remote delivery of MTP to uncaptured stallions, at doses of 3.0 g of active drug, reduced foaling rates by about 45% in field tests on Assateague Island, Maryland (USA), but required four darts per animal to reach a minimally effective dose.[90,113]

Concern over harem infidelity by mares and the potential ineffectiveness of treating stallions led to two different attempts to reduce fertility in mares using steroids. Subcutaneous implants containing various doses of estradiol (E_2) and progesterone (P_4) were placed in captive feral mares.[81] Temporary decreases in behavioral estrus occurred among the treated mares, but levels of the implanted steroids decreased and behavioral estrus reappeared among these mares by 5 wk after treatment. The temporary nature of this treatment may have been due to increased clearance rates of these steroids after administration. In contrast, ethinylestradiol (EE_2) homogenous Silastic implants (1.5–8.0 g) placed i.p. in captive feral mares were effective in suppressing estrus and ovulation through two breeding seasons.[83]

In another study,[30] a microencapsulated synthetic progestin, northisterone, was administered to six feral mares on Assateague Island using remotely delivered darts. This progestin, which has been used very successfully to inhibit fertility in women, was given in doses of 2.0 g. All six mares receiving the progestin foaled 1 yr later, a highly improbable event among Assateague mares, which suggests that fertility was actually enhanced among treated mares and supports the hypothesis that progestin administration to mares increases progestin metabolic clearance rates.

Rodentia

The concept of chemical fertility control for managing pest rodent populations was originally put forth in 1967.[44] Baits containing 0.05% mestranol were fed to wild rats (*Rattus norvegicus*), resulting in an immediate and significant reduction in pregnancies.[44] However, poor acceptance of the steroid-treated bait gave short-lived results. This line of research was expanded to include voles (*Microtus* spp.).[45,106] Again, rats did not accept mestranol baits well, and doses had to be reduced to 0.005% before voles would accept the baits regularly. Reproduction was inhibited in the voles, and pups receiving mestranol through the mother's milk never developed sexually, becoming irreversibly sterile.

Quinestrol was then investigated in rodents.[113,74] This compound was more effective than mestranol for antiovarian treatment in rodents. Doses as low as 10 μg completely inhibited reproduction, but once again poor bait acceptance in rats made the approach impractical. More recently, reproduction was successfully inhibited in black-tailed prairie dogs (*Cynomys ludovicianus*) in Wind Cave National Park, South Dakota (USA), by feeding them DES-treated oats (0.11% active ingredient).[34] Reproductive inhibition was almost 100% effective, and there was total reversibility when the drug treatment was withdrawn.

Other mammals

Two common small mammals, the skunk (*Mephitis mephitis*) and the raccoon (*Procyon lotor*), that are implicated in diseases such as Lyme disease and rabies and that adapt well to urban areas have not often been the target of fertility control. In one study attempting to contracept skunks, DES-loaded baits were unable to significantly reduce reproduction.[105] In a more recent study,[10] a single Norplant® rod (Wyeth-Ayerst, Philadelphia, Pennsylvania 19101, USA) implanted in wild and captive female skunks was 100% effective in inhibiting fertility over a 3-yr period. Each Norplant rod contains 36 mg of the synthetic progestin levonorgestrel and can be placed without surgery using a 10-g trocar.

The use of contraception in captive exotic mammal in North American zoos has been

reviewed recently.[81] A wide variety of primates and carnivores have been contracepted with several steroids and delivery systems designed for humans, including oral norgestrel, oral norethindrone (norethisterone)/ethinyl estradiol, oral MGA, oral mibolerone, implants (levonorgestrel), injectable MPA, and MGA Silastic implants. Success has varied with the species, the drug dose, and the techniques of application, illustrating the need for careful evaluation of any steroid contraceptive method in wildlife.

Aves

Steroid contraception has also been applied to birds. Oral delivery of MPA caused reversible inhibition of ovulation in pigeons.[26] When mestranol was incorporated into grit and fed to pigeons at approximately 183 μg/day,[107,108] fertility was reduced by 26–67%, and there was no evidence of debilitating toxicity. Even F_1 males hatched from treated adults had significantly depressed fertility. Similar results were obtained feeding mestranol-loaded grit to laboratory quail (*Coturnix coturnix*).[121]

NONSTEROIDAL HORMONAL AGENTS

The primary target for nonsteroid hormonal contraception has been hypothalamic peptide-releasing hormones. Fertility in male dogs was successfully suppressed with repeated injections of a gonadotropin-releasing hormone (GnRH) agonist.[118] The agonist (D-Nal[2]^6-GnRH) was administered i.m. in doses of 10 μg/kg; testis volume, ejaculate volume, sperm count, and sperm motility all declined for as long as 172 days. Libido was depressed but could be restored with testosterone implants. Estrus in dogs was also successfully suppressed with nafarelin ([6-D-(2-napthyl)-alanine] GnRH), a potent GnRH agonist analog.[119] Using an s.c. osmotic pump, 2, 8, or 32 μg were delivered daily and completely suppressed estrus for up to 18 mo. Although successful in captive domestic dogs, this approach has little usefulness in wild canid populations. Nevertheless, the approach may be useful with zoo animals or larger free-roaming species where capture is not a problem. In other studies[6,12] in which male rhesus monkeys (*Macaca mulatta*) and female macaques (*Macaca* sp.) were given GnRH agonists, testicular function and ovarian function were significantly depressed, but sexual behavior was also inhibited.

BARRIER METHODS

A mechanical birth control device has been tested in white-tailed deer.[66] The device, designed to prevent intromission and ejaculation by the male, was manufactured from Silastic and anchored in the vagina of the doe. The device failed to prevent pregnancy, and this approach was abandoned with deer. John Hughes and Peter Dahls (pers. comm.) at the University of California at Davis have developed an equine intrauterine device (IUD). They placed IUD's into six fertile mares and turned the mares out with a fertile stallion during the breeding season. The stallion stayed with the mares, and all were serviced numerous times during estrus. None of the IUD mares conceived, whereas all six control mares conceived and carried foals to term. One year later, the IUD's were removed and the six previously treated mares conceived during their first estrus.

Among captive exotic species, silicone vas plugs are currently being tested in a number of felids and primates.[84] These plugs are inserted into the vas deferentia surgically and prevent the transport of sperm. The results of these tests are not yet available.

IMMUNOCONTRACEPTION

Immunocontraception attempts to stimulate the target animal's immune system to interfere with some critical reproductive event. Upon an injection of an antigen into the target animal, antibodies are produced

against some molecule requisite to successful reproduction. Immunocontraception can be aimed at specific molecules in the reproductive process, including hypothalamic peptide-releasing hormones, pituitary gonadotropic hormones, or sperm or egg receptor molecules necessary for fertilization. Preovulatory or prespermatogenic mechanisms, the process of fertilization, or the fertilized ovum or embryo can also be targeted. Direct immunological intervention of reproductive steroids with steroid–protein ligands is not biologically sound because almost all somatic cells contain cytosol receptors for steroids, and broad biological consequences can result.

In an attempt to immunize dogs against their own luteinizing hormone (LH), injections of human chorionic gonadotropin (hCG) were administered.[2] This experiment failed because canine LH did not cross-react with anti-hCG antibodies. One year later some success was reported in passively immunizing dogs against LH using antibodies that were considerably more specific for canine forms of the hormone.[30]

An immunological approach to fertility control was also attempted in cats.[17] Feline ovaries were homogenized and used to raise rabbit antibodies against the protein fractions. The antibodies, when administered to pregnant cats, caused some fetal resorption, but the results were generally discouraging. Once again, as in dogs, nonspecificity of the antibody appeared to be the problem. In a different immunological approach, male dogs were immunized against their own GnRH with GnRH conjugated to human serum globulin.[38] Plasma testosterone, LH, and sperm counts were all depressed. Unfortunately, this approach of immunizing the male against GnRH results in the loss of libido and requires the addition of testosterone in some form to maintain normal male behavior.[36,53] Among hoofstock, female sheep have been immunized with an LH–ovalbumin conjugate, resulting in the elimination of estrus and almost 100% success in preventing pregnancy.[50] Domestic cows[1] and swine[18] have been actively immunized against GnRH with a resulting suppression of ovarian activity.

In immunologic interruption of fertilization, an antigen would cause the female to produce antibodies against sperm. This antigen would meet most of the criteria for the ideal wildlife contraceptive agent. For many years, progress in this direction was impeded because most sperm antigens (molecules residing in the sperm cell membrane or intracellular enzymes) were not specific to sperm. This meant that the female would raise antibodies that would attack not only sperm but also the kidney, liver, blood cells, and a variety of other tissues. In the last decade, sperm antigens have been identified that are increasingly more specific to sperm alone.[31,71,94] Since 1987, two different sperm antigens have been identified that appear to be absolutely sperm specific and may be ideal contraceptives. Both antigens consist of a sperm cell membrane protein necessary for the recognition and attachment of the egg. One of these, PH-20, was identified in hamster sperm but has not yet been found in other animals.[87] The other, now known as SP-10, has been located on sperm from baboons (*Papio* sp.), monkeys (*M. mulatta* and *M. fascicularis*), and humans.[41,42] Both are injected into the female, who then produces antibodies against sperm, thereby preventing fertilization without interfering with hormones or behavior. The SP-10 antigen is so promising that it has been designated for priority research support by the World Health Organization. Neither of the sperm antigens have been tested on any wildlife species, but SP-10 could currently be applied to some captive primates.

A second strategy for inhibiting fertilization is by raising antibodies against the ovum protein receptor for sperm. The mammalian egg is surrounded by a noncellular membrane known as the zona pellucida (ZP). The ZP is made up of three glycoproteins, one of which, ZP3, is the sperm receptor.[31] Injections of raw ZP protein or ZP3 cause the female to raise antibodies against the

sperm receptor on the ovum. Thus far, porcine ZP (PZP) has been an effective inhibitor of fertilization in humans and a variety of nonhuman primates,[29,92,93] dogs,[94] rabbits,[124] horses,[47] and deer.[114]

The most common route of delivery of PZP is i.m. injection. This is not a problem in most captive animals that can be readily handled. In free-roaming wildlife and some captive exotic species, however, handling is not feasible, and remote delivery of the antigen is important. In a field test of this concept, remote delivery of PZP, using barbless darts fired from a capture gun, has been used to successfully contracept free-roaming feral mares on Assateague Island National Seashore.[47] In the initial study, mares received either two or three inoculations over a 6-wk period, after which there were no pregnancies among treated mares over the course of the following year. The results of that study indicated that PZP immunization of feral horses 1) can be carried out remotely, 2) can be applied to pregnant mares without interfering with pregnancies already in progress or with the health of the foals, 3) will not affect social behaviors, and 4) is reversible. In a follow-up study, a single annual inoculation was effective in preventing pregnancy during two subsequent years, indicating that once antigen recognition has occurred, a single annual booster inoculation can raise antibody titers to contraceptive levels[48]

This same PZP antigen has proven effective in inhibiting fertility among white-tailed deer over a 2-yr period.[114] Trials currently in progress with captive exotic species indicate PZP antigen is an effective contraceptive agent in sika (*Cervus nippon*), sambar (*Cervus unicolor*), and axis (*Axis axis*) deer at the Bronx Zoo (New York, USA) and in Przewalski's horses (*Equus przewalski*) at the Cologne Zoo (Germany). Trials with PZP immunization are also in progress with an orangutan (*Pongo pygmaeus*) at the Toledo Zoo (Ohio, USA), patas monkeys (*Erythrocebus patas*) at the Calgary Zoo (Alberta, Canada), West Caucasian tur (*Capra ibex*) at the Metro Zoo, Toronto (Ontario, Canada), and muntjac (*Muntiacus reevesi*) at the Bronx Zoo.

The requisite of multiple inoculations (two inoculations appear to be required) for contraceptive efficacy represents a major disadvantage of the PZP antigen, at least for its application to free-roaming wildlife. Microencapsulation, however, may allow conversion of the PZP antigen to a single-dose vaccine. This process, which provides a sustained release of drug, has been used successfully with contraceptive steroids in humans[100] and feral horses[51] and with other forms of antigenic protein.[23] Preliminary studies using an osmotic minipump indicate that continuous release of the PZP antigen causes contraceptive titers of antibodies in mares. Incorporation of the PZP antigen into continuous-release microspheres is also under investigation.

RECENT ADVANCES AND DIRECTIONS FOR FUTURE RESEARCH

In the 1960's, when interest in chemical fertility control of free-roaming and captive species began, the number of available contraceptive chemicals was relatively small and delivery systems were limited. Today, 25–30 different commercially available injectable or oral contraceptive steroids are available and several long-acting progestins and androgens show great promise. For example, a single s.c. injection of Danazol® (Winthrop Laboratories, New York, New York 10016, USA) (a synthetic steroid used in the treatment of endometriosis) causes prolonged suppression of menstrual cycles in macaques (*M. fascicularis*).[18] Other advances include 1) the development of biodegradable encapsulation and micronization processes to permit long-term sustained release of injectable contraceptive agents,[100] 2) the improvement in absorption of oral progestins,[101] and 3) recent FDA approval and commercial availability of Norplant implants. These implants offer long-term (up

to 5 yr) contraception, with nonsurgical placement, and the fewest side effects of any contraceptive progestin available.

Steroid use, however, must be applied judiciously. Among some captive species, particularly felids, the increasing incidence of steroid-related pathologies suggest the need for alternative approaches. Also, if zoos are to display behaviorally normal specimens in the course of their educational missions, long-term application of steroids might not be wise. Among free-roaming wildlife, steroids usually require capture of the target animal or, at the very least, the remote injection of unrealistically large quantities to achieve contraception. Also, the issue of steroid passage through the food chain has not been resolved.

Beyond steroids, entirely new approaches to fertility reduction in wild species are emerging. Synthetic analogues of GnRH are effective in reducing fertility in both males and females, but behavioral changes are likely unless these analogs are accompanied by exogenous steroids. The ability to interfere with fertilization without any changes in steroid-dependent social or sexual behavior comes closer to meeting the criteria for the ideal wildlife contraceptive. Additional information is needed on the long-term effects of immunocontraception on ovarian function, on adjuvants that do not cause ulceration or tuberculosis-positive tests, and on sustained-release systems for antigens for one-inoculation immunization. All three of these areas are currently under investigation.

Virus-vectored immunocontraception is already under investigation.[15] This methodology would deliver nonpathogenic vaccinia virus, specific for the target species and containing the gene for the PZP antigen, to a particular wildlife species. After taking up residence in the host species, the virus would express the gene for the PZP antigen, thereby immunizing the animal. The targets for this research are rabbits and foxes in Australia.

There is some concern that long-term use of PZP might cause a depletion of oocytes, so alternative immunologic approaches to inhibiting fertility in the female are under investigation. Anticumulus oophorus antibodies have been generated in rabbits for passive immunization of female mice, resulting in reversible infertility.[16] Infertility is caused by disrupting the communication between cumulus cells and the developing oocyte. This same principle might be applied to other species.

Another area of research is enhanced bait acceptance by small mammals. Control of fertility in rodents, certain canids, and other small mammals is possible if resistance to treated bait can be overcome. Encapsulating steroids in a disguised and acceptable medium may be feasible. For example, microencapsulated 5-mg doses of steroid were palatable to human patients,[15] whereas previously the drug had been unacceptable in uncoated 200-μg doses.

The effects of fertility control agents on the length of the target species' breeding season must be determined. Polyestrus seasonal breeders that remain infertile throughout the spring and early summer breeding season might merely delay the reproductive season. A population whose reproductive activity normally concludes in summer but is delayed until fall would produce young that probably would not survive the winter. Such a situation would be inhumane and unacceptable.

An important issue facing the subject of wildlife contraception is whether the solution is worse than the problem. The biosystem within which each target species dwells is dynamic and flexible within certain limits. In most cases, those limits have not been delineated. Work should be performed on the principle of minimal perturbance, which means conducting extensive preliminary studies to establish normal reproductive and behavioral patterns followed by long-term studies to assess treatment effects. Such studies must encompass complete pharmacology of the agents, catabolic pathways, and possible biological activity of the ex-

creted metabolites that may be ingested by other species in the habitat."

The extent to which drugs interfere with libido and sociosexual behavior is also important. In any wildlife fertility control program, it is probably not wise to attempt zero population growth. If males are the target and only a percentage will be made infertile, then the contraceptive agent must not interfere with libido or other behaviors that contribute to their success in mating. Another important consideration is the long-term effects upon social organization. Any potential contraceptive agent must be screened for direct and indirect behavioral effects. This issue is as important for captive exotic animals as for free-roaming wildlife.

Sociopolitical aspects of wildlife contraception should also be researched. The lessons learned over the past decades of human contraceptive research must not be lost to those who wish to apply this technology to captive and free-roaming wildlife. Regardless of the safety or efficacy of a chemical fertility control agent, there are important public issues to be considered and political barriers that must be overcome if widespread success is to be achieved. Wildlife, captive or free-roaming, and its preservation and management is often a highly charged emotional issue. Methods for educating the public about fertility control in wild species should be investigated. Guidelines for the ethical use of wildlife contraception should also be developed. As technology advances, the ability to manipulate animal populations will increase proportionately. The use of wildlife contraception must take into account the well-being of the target species as well as the needs of people.

Chemical fertility control is a flexible management tool, permitting a large variety of manipulations. Where contraceptive agents can be delivered remotely to large animals, particular herds, bands or even individuals can be singled out. For example, the immunocontraception of Przewalski's horses at Cologne is aimed at keeping animals with genetic anomalies, such as fox allele or floppy mane, out of the reproductive pool without interfering with social structure. Reversibility of drug action adds even more management flexibility. A large and unanticipated winter kill, for example, can be offset by withdrawing the treatment for one or more breeding seasons, or endangered captive species can be temporarily removed from breeding populations but permitted to breed again in later years.

There are important biological reasons why fertility control is an attractive wildlife management tool. Removing an animal from the population by lethal means is permanent; the genes are lost from the pool forever. Reversible contraception used with a sound management plan can help maintain the gene pool, which is extremely important for rare captive species and in places where dwindling habitat results in localized overpopulations of endangered species.

Another advantage is the ability to concentrate on target species without serious damage to other animals. Delivering bait to a particular species without having other animals ingest the drug is probably impossible. However, through careful manipulation of drug types and dosages, effects upon nontarget species can be minimized. Timing is also important; the peak breeding season for one species often differs significantly from that of another, which may permit bait delivery without interfering with nontarget species. Red foxes, for example, breed from December to March, whereas skunks do not begin to breed until March. Thus, a bait-delivered fertility control program could be timed for skunks to avoid interference with fox reproduction.

Finally, chemical fertility control affords an approach that, when properly used, will not influence the social structure of the animal populations involved. Because sexually mature individuals are neither removed nor harassed, the hierarchy of the population is not altered unwittingly by human intervention.

Much of the future of wildlife contraception will be influenced by public opinion.

federal research-sponsoring agencies, wildlife managers, and zoo administrators. The public must be educated regarding the nature of the subject, chances for success, benefits, and consequences of not pursuing this goal. The guidelines for research funding among federal, state, and private research-sponsoring agencies must be broadened to include wildlife fertility control, and those in charge of zoos and wildlife management at all levels must be willing to allocate resources to these new approaches.

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