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The management of wildlife populations has reached a point where a number of forces are requiring change in our approaches to old problems (Gavin 1989; Wagner 1989; Yahner 1990). Nowhere is this more true than on public lands, and in particular, because of their unique mission, in our national parks (Houston 1971). New technologies, developed in disciplines seemingly far removed from conservation biology, are now available with which to answer old questions with new methods of inquiry. Two of these technologies include wildlife contraception and non-capture physiological studies which are based on urinary and fecal hormone analysis. This paper will describe some recent fertility control research and non-capture methods of studying physiology which have been successfully applied to wildlife in national parks of the U.S.

Dramatic increases in certain wildlife populations are a real or perceived problem today in many areas of the world. In some cases, such as wildlife refuges and national parks, the protection afforded to animals by laws and regulations, and the absence of sufficiently large numbers of predators, have resulted in populations of wildlife that occasionally exceed the habitats' biological carrying capacities. In other cases, increasing urbanization and changing public values and attitudes regarding traditional lethal wildlife control methods have led to large increases in some species of wildlife in urban and suburban America (Kirkpatrick and Turner 1985).

The species which have increased in number on public lands are generally large ungulates, such as elk (*Cervus elaphas*), bison (*Bison bison*), feral horses (*Equus caballus*), or feral donkeys (*Equus asinus*), which have no serious predators. In urban areas, the species which have increased dramatically are characterized by great adaptability to the human presence, and include such animals as white-tailed deer (*Odocoileus virginianus*), skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), and even beaver (*Castor canadensis*). In these latter cases, urbanization, and protection from intentional lethal controls, has been coupled with human-made increases in food and shelter, and the results are rapid population increases.

The population increases are often only imaginary problems, perceived by human populations that are often intolerant of wildlife and the competition it provides for something of value to humans, e.g., ornamental plants, flowers, vegetable gardens, park trees, access to back country, or conflict over range land that could be occupied by cattle or sheep. In some cases, the problems are real, and include animal-borne diseases such as rabies, or Lyme disease, or threats to human health through vehicle-animal collisions. In other cases the perceived or real problems include degradation of habitat or deteriorating health of the animals themselves, as a result of the population increases, or the mere existence of non-native species.

The decline of traditional lethal methods of wildlife management is a relatively recent event. Historically, population increases among wild and feral species have been controlled through hunting, trapping, poisoning, and occasionally relocation. Controlled hunting, although successful in certain instances, is coming under increased public scrutiny. Trapping, particularly with leg-hold devices, is extremely unpopular among growing segments of society, and legislation against steel traps has been passed or is pending in many states, provinces, and even countries, and even where still tolerated, trapping is relatively ineffective as a population control technique because of declining fur prices. Live-trapping and relocation of overpopulated species is expensive and works only where sufficient habitat exists. Poisoning animals is distasteful, often dangerous to humans, and notoriously non-specific. The shortcomings of poisoning are multiple and serious. First, the target animals are destroyed in less-than-humane fashion, healthy animals along with diseased ones. Second, population reduction is only

temporary, and each new breeding season results in new increases. Third, the poison kills non-target species (Kirkpatrick and Turner 1985).

An alternative to the control of animal populations through mortality control is the concept of fertility control. Until relatively recently, the concept was confined mostly to captive animals and was largely untested in wild populations, and skeptics considered the approach bizarre. However, the technology associated with chemical or immunological control in humans is impressive, and its application to domestic, wild, or feral species is fundamentally sound. The history of fertility-control research in animals has been extensively reviewed (Kirkpatrick and Turner 1985, 1991a).

No single animal has been more responsible for the resurgent interest in wildlife contraception than the feral horse. Attempts at contraception in the horse have occupied researchers since 1972 (Kirkpatrick et al. 1982; Kirkpatrick and Turner 1987; Turner and Kirkpatrick 1982, 1991; Plotka et al. 1988; Plotka and Vevea 1990; Eagle et al. 1992). The first successful attempts at contracepting free-roaming feral horses involved capture and the administration of large doses of long-acting testosterone (Kirkpatrick et al. 1982; Turner and Kirkpatrick 1982) to feral stallions in Idaho. The injected hormone reduced sperm counts in the stallions, without altering their behaviors, and, while the results were encouraging, and mares bred by treated stallions had 83 percent fewer foals, the capture of the animals and the resulting stress, the cost of the drugs, and the amount of drug that had to be administered to each animal made the process logistically difficult. In 1987, the U.S. National Park Service embarked upon a research program designed to humanely reduce reproductive rates among the feral horses of As-

sateague Island National Seashore, off the coast of Maryland. The author and two colleagues, Irwin K. M. Liu, of the University of California-Davis, and John W. Turner, of the Medical College of Ohio, turned to the future of modern contraceptive technology—immunocontraception.

Basically, immunocontraception is a method which stimulates the immune system of the target animal to produce antibodies that interfere with some fundamental event in the reproductive process, i.e., ovulation, sperm production, fertilization, implantation. Initially, Liu's laboratory prepared an experimental vaccine made from the protein membrane which surrounds the pig egg, known as the zona pellucida. The vaccine, now known as porcine zona pellucida, or PZP, was first injected into 14 captive mares in California and it caused infertility in 13 of them. The PZP injections caused the mares to produce antibodies against the pig protein. However, these antibodies also attached to the zonae pellucidae of the mares' own eggs, thereby preventing recognition and attachment by sperm and, therefore, fertilization (Liu et al. 1989).

The next task was to discover if the PZP vaccine could be administered to free-roaming feral mares remotely, without capturing or handling them. It was important to determine if the vaccine was safe to give to pregnant animals, whether its effects were reversible and whether the normal social behavior of the horses would be altered in any significant way. The vaccination program began in February 1988. Twenty-six Assateague mares known to be fertile were identified and each one was given two or three inoculations of PZP, by means of a self-injecting, barbless darts fired from a capture gun. The results were excellent. About 60 percent of the mares were pregnant at the time of inoculation, and they all delivered healthy foals in the spring of 1988, following

the inoculations. However, a year later, in 1989, contraception was 100 percent effective and not a single foal was produced by the treated mares. The social behavior of the treated mares was unaffected by the vaccine; females mated but did not get pregnant (Kirkpatrick et al. 1990a).

In 1989, the effectiveness of a single annual booster inoculation was tested. However, before this could be accomplished, the contraceptive effectiveness of the 1988 inoculations had to be known, yet the foaling season was still several months away. These were wild creatures and capture was not permitted. How does one pregnancy-test an uncaptured feral mare? To accomplish this seemingly impossible task, the investigators turned to some established zoo technology. During the 1980s, B. L. Lasley, then at the San Diego Zoo, and now at the University of California-Davis, developed a number of urinary endocrine tests for pregnancy and to monitor ovarian function in captive exotic species (Lasley and Kirkpatrick 1991). In October 1988, several methods of extracting urine from the island's sand and marshes were devised, after witnessing a mare urinating. Next, Lasley's tests were applied to the samples from the treated and control animals. The results indicated 100 percent success in inhibiting fertility and foal counts in August 1989 confirmed these results. Thus, the research team knew which mares were pregnant 7-8 months before the mares foaled, and not a single animal was handled.

During February 1989, armed with the newfound knowledge of pregnancy (or, rather, non-pregnancy) rates, the scientists split the original test group by administering single-dose booster inoculations to 14 of the 26 mares. Only one booster-treated mare produced a foal in 1990. The 12 mares that did not get booster inoculations pro-

duced foals at their normal, pre-treatment rates. This confirmed the reversibility of the vaccine, at least after short-term application (Kirkpatrick et al. 1991a). In March 1993, the Assateague horses received their sixth consecutive annual PZP contraceptive treatment. This research is now supported by the National Institutes of Health and The Humane Society of the U.S., as well as the USNPS, and it is focusing upon long-term effects upon ovarian function, and the development of a one-inoculation, multiple-year vaccination. The work on Assateague Island has resulted in only a single foal in 60 mare-years, among treated animals, instead of the predicted 30 foals. This contraceptive effectiveness, coupled with the vaccine's safety, has prompted park officials to begin developing a comprehensive management plan which utilizes contraception but which has a minimal effect upon the composition of the herd. If one accepts the use of darts, it appears that a humane solution to the management of the Assateague horses is at hand.

Increasing interest in controlling urban white-tailed deer, in settings where hunting is not legal, wise, or safe, led to the research team to turn its attention to these prolific animals. With the financial support of the PNC Corporation, The Ontario Department of Natural Resources, and The Humane Society of the U.S., the PZP vaccine was next tested in 7 captive white-tailed deer. Each treated doe was given two inoculations of the PZP vaccine, remotely, by means of a blow gun, in September 1989. None of the 7 treated does produced fawns a year later, while 6 of 7 control does produced fawns. These captive deer were given booster inoculations in the fall of 1990, and after three years of treatment not a single fawn was born (Turner et al. 1992).

In addition to feral horses and captive white-tailed deer, the PZP

vaccine has been used to inoculate and inhibit fertility in feral donkeys inhabiting Virgin Islands National Park, and numerous zoo animals. The latter experiments are designed to prevent unwanted reproduction among captive exotic species and to provide some relief for the large and growing "surplus" animal problem of zoos. At the same time the captive exotic species provide opportunities to test the contraceptive vaccine on species for which there may be an application in the wild. Thus far, with the financial support of individual zoos, the American Association of Zoological Parks and Aquariums, and The Humane Society of the U.S., the PZP vaccine has been demonstrated to be effective in Przewalski horses (*Equus przewalski*), banteng (*Bos javanicus*), sika deer (*Cervus nippon taiwanus*), axis deer (*Axis axis*), sambar deer (*Cervus unicornis*), muntjac deer (*Muntiacus reevesi*), Himalayan tahr (*Hemitragus jemlahicus*), and West Caucasian tur (*Capra ibex*) (Kirkpatrick et al. 1992a). Experiments are currently underway with addax (*Addax nasomaculatus*), llama (*Llama glama*), giraffe (*Giraffa camelopardalis*), blackbuck (*Antelope cervicapra*), wolf (*Canis lupus*), African lion (*Panthera leo*), tiger (*Neofelis tigris*), river hippopotamus (*Hippopotamus amphibius*), pygmy hippo (*Hippopotamus choeropsis*), and North American elk (*Cervus elephas*).

To evaluate various wildlife contraceptives, the characteristics of the ideal wildlife fertility control agent was described by several investigators (Seal 1991; Turner and Kirkpatrick 1986). These characteristics include (1) a high degree of effectiveness, (2) a lack of toxicity and harmful side effects, (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

ity of the contraceptive agent to be passed from the treated animal to predators or scavengers, or humans through the food chain (Kirkpatrick and Turner 1991). The PZP vaccine met, for the most part, these characteristics, with the exception that during the initial year of treatment, the vaccine had to be delivered in two inoculations, about one month apart. This one shortcoming was a major problem when considering the use of the vaccine in secretive or elusive animals such as free-roaming feral horses or white-tailed deer. Consequently, attention has turned to the development of a one-inoculation, multiple-year form of the vaccine, with the financial aid of the Geraldine R. Dodge Foundation, The Eppley Foundation for Research, the Morris County (New Jersey) Parks Commission and The Humane Society of the U.S.

Technology already existed that might be used to produce a form of the PZP vaccine that would result in a slow, continuous release after injection. This technology had been developed for the delivery of human vaccines (Eldridge et al. 1989; O'Hagan et al. 1991) and its application to the PZP vaccine seemed reasonable. The initial attempt at producing a one-inoculation PZP vaccine focused upon creating lactide microspheres, which contained the PZP antigen and which might release the antigen slowly, over the course of several months. A homogeneous mixture of the PZP antigen and D,L-lactide was made and the material was formed into small ($\approx 50 \mu$) spheres. Upon injection into the muscle of the target animal and contact with tissue fluids, the microspheres begin to degrade, releasing the antigen slowly. The D,L-lactide is metabolized to water, CO_2 , and lactic acid, all three of which are normal products of metabolism in mammals.

Controlled experiments with the first prototype of this form of the vaccine, using domestic mares, re-

vealed no difference between a single inoculation of the raw vaccine and the microspheres. It was thought that the size of the microspheres permitted release of the antigen too fast and thereby prevented both a prolonged release and a prolonged contraceptive effect. As a result of these experiments, a second generation of microspheres was developed and tested in December 1992, in experiments described below. However, the tests with the domestic mares provided another unanticipated and positive result. It was discovered that a single inoculation of the raw vaccine, in a thick emulsion with an oil-based adjuvant, provided contraceptive antibody titers for about 200 days. This unanticipated discovery suggested that a single inoculation might prevent pregnancy in those animals that were vaccinated immediately prior to their breeding season, and for which the breeding season did not last longer than 200 days.

This hypothesis was immediately tested in feral horses and white-tailed deer. Fourteen previously untreated feral mares on Assateague Island National Seashore were given a single inoculation of the raw vaccine in March 1992. On Assateague, mares normally begin to ovulate and breed in April and complete breeding activity by August, a period of 150 days. Eleven of these treated mares were pregnancy tested in October 1992, and only a single animal was pregnant, indicating that the one-inoculation was effective. In September 1992, a field test of the remote delivery of PZP to white-tailed deer was conducted at the National Zoo's Conservation and Research Center, in Front Royal, Virginia. Ten does were given two inoculations, 10 were given a single inoculation, and 10 were given sham injections. The results of this study will not be available until June 1993, but behavioral observations of mating behavior, through early March 1993, suggest

that the does receiving the one- and two-inoculation treatments are not pregnant. Equally important, the data suggest that non-pregnant does will not continue having estrous cycles beyond January, or at least the males will pay no attention to them after January (W. McShea, Smithsonian Institution, pers. comm.).

The final field study, as of March 1993, involved feral horses in Nevada. In early December 1992, with the financial support of the U. S. Department of the Interior, and the Bureau of Land Management, 500 feral horses were captured in eastern Nevada, and 131 mares between the ages of 5-12 were inoculated with one of three forms of the PZP vaccine, or with injections which contained no vaccine. One group received two inoculations of raw vaccine, given about one month apart. A second group received a single inoculation of the raw vaccine, and a third group received a single inoculation of the second generation of microspheres, which are thought to be a slower-releasing form of the vaccine than the first generation described above. The results of these experiments will be available in late 1993.

At the current time, still another approach to the one-inoculation, multiple-year PZP vaccine is being investigated. In this process, the PZP antigen is *encapsulated* with the biodegradable lactide material, rather than being incorporated into a homogeneous mixture, as with the microspheres (Eldridge et al 1989). The antigen is coated with the lactide material, and the thickness of the coating determines the time of release. After injection and exposure to tissue fluids, the coating begins to erode, and at some point the antigen is released. The microencapsules represent a type of injectable Contact[®] cold pill, and result in pulsed releases rather than continuous releases, as in the case of microspheres. The first prototype of

the microcapsules will be available by late summer 1993.

Research directed at the humane control of smaller wildlife species which have adapted to urban areas extremely well is also promising. One such animal is the common skunk. These highly adaptable animals have colonized urban areas, but as populations grow, the threat of rabies accompanies the population growth. Historically skunks have been destroyed by shooting trapping, or poisoning. The irony is that virtually all the skunks that are killed are healthy skunks. Furthermore, removal of the skunks only creates habitat vacuums, which draw skunks in from surrounding areas. Thus, programs of killing are forced to go on forever. A strategy was developed to permit a core population to exist but to contracept it. In this way the animals would defend their territories, prevent immigration of new skunks into the area, and not produce six or seven new skunks per female annually. To accomplish this, females were live-trapped, lightly anesthetized with ketamine, and a small contraceptive rod was implanted under their skin. These contraceptive rods, recently approved for use in humans by the FDA and known commercially as Norplant[®], are only 30 mm long and about the thickness of a drink stir-rod. The single rod was placed just under the skin without surgery, by pushing them through a large hypodermic needle, and the small puncture wound was dusted with a topical antibiotic. The entire process took only minutes and virtually anyone can be trained to carry out the simple procedure. Each of the treated and control skunks was given an ear tag and fitted with a radio-collar and released. The following year, four of the treated and six of the control skunks were located and captured. None of the treated skunks and all six of the control skunks had litters. Following this

successful pilot experiment, 20 captive skunks were given a single implant, and three years later, not a single litter has been born to the treated animals (Bickle et al. 1991). It is the ultimate goal of this line of research to train animal control personnel to control skunk populations in this way. Just consider that every ten skunks thus treated translate into 70 new skunks that never appear, and, best of all, no skunks have been killed. Similar experiments are already underway with captive raccoons.

Tests are also being conducted with urban beaver in Denver, Colorado. In this case, the beaver have moved into waterways within the city and its suburbs and created dams and destroyed trees within greenbelts and parks. During 1992, six female beaver were live-trapped, anesthetized, and given one to three Norplant® rods, and fitted with radio transmitters. Results of these experiments will be available in June 1993. The U. S. Forest Service has already expressed interest in using this method to control beaver in national forests (Sherri Tippi, Wildlife 2000, pers. comm.).

With each advance in wildlife contraception, however, comes greater threats of abuse of this technology. Should feral horses be contracepted just to provide more grass for cattle and sheep? Should predator populations be reduced in order to produce more game animals for hunters? Should contraception ever be used in an endangered species, such as the elephant? If so, under what conditions? Who should make the decisions about the use of contraceptive technology on wildlife? What criteria should be used? What are the allowable limits of stress to which animals should be subjected in order to apply wildlife contraception? Such questions must be answered before fertility control becomes a common wildlife management tool. There is a multitude of

ethical and moral questions to consider if we are to solve wildlife problems rather than make them worse. Such questions have already been posed and the remaining step is to develop responsible and ethical guidelines for wildlife contraception.

Another technological advance is aiding in wildlife research, particularly in national parks, where the capture and handling of animals properly comes under intense public scrutiny. The urinary and fecal hormone analysis technology used so successfully by zoos, for the purpose of understanding reproductive biology in captive exotic species, has now been applied to numerous studies in U.S. national parks. Indeed, the application of urinary and fecal hormone analysis has been pioneered within the national parks. The first attempt at measuring pregnancy in uncaptured horses was accomplished with feral horses, on the Pryor Mountain National Wild Horse Refuge in Montana, a portion of which includes Bighorn Canyon National Recreation Area. The object of this initial study with free-ranging wildlife was to understand if fetal loss played an important role in reproductive physiology of these unique and valuable horses (Kirkpatrick et al. 1988). Twenty-five feral mares were identified and urine samples were collected from each in August 1985. The mares were observed until they urinated and the urine was aspirated directly from the ground, or centrifuged from the urine-soaked soil. The samples were analyzed for estrone conjugates (E₁C), which are significantly elevated in mares during pregnancy. During the summer of 1986, the mares were located and observed for foals and the results indicated extreme accuracy in the diagnosis of pregnancy in this non-invasive way. These same techniques were applied to the horses of Assateague Island National Seashore during the contraceptive studies mentioned above,

in order to determine pregnancy rates months in advance of the foaling season and thereby design new experiments with more efficiency. These techniques are also being used to study the long-term effects of the PZP vaccine upon ovarian function in the Assateague horses. Urine samples are collected from treated and untreated mares on an every-other-day basis, between May 1 and June 30, during the period of peak breeding activity. The samples are analyzed for E₁C and urinary progesterone metabolites (iPdG) (Kirkpatrick et al. 1990c), and the patterns reveal even the most subtle changes in ovarian endocrine function, yet again, no animals are handled (Kirkpatrick et al. 1992b).

Another important advance developed at Assateague Island national Seashore, was the ability to diagnose pregnancy by means of fecal hormone analysis. As little as a half-gram of fresh mare feces can be analyzed for E₁C, iPdG or total estrogens, providing accuracy approaching 100 percent (Kirkpatrick et al. 1990b, 1991b). Using a combination of urinary and fecal steroid analysis, studies have been carried out which show that mares taken over by new stallions are not induced to abort, as had been previously reported (Kirkpatrick and Turner 1991a), and that unlike the management of the Assateague horses, the infamous roundup and sale of foals from Chincoteague National Wildlife Refuge causes a two-fold increase in foal production among those animals (Kirkpatrick and Turner 1991b). These studies were accomplished without capture or handling of the horses, under the scrutiny of many thousands of visitors and without any complaints. These pregnancy diagnosis techniques are currently being used to monitor pregnancy rates among PZP-treated feral donkeys in Virgin Islands National Park. Most recently, research on Assateague Is-

land National Seashore has led to the development of non-instrumented field tests for pregnancy (Kirkpatrick et al. 1993a).

One of the most exciting applications of urinary and fecal steroid analysis to wildlife research is occurring in Yellowstone National Park. Under the sponsorship of the National Science Foundation, remote monitoring of ovulation, pregnancy, and fetal loss has been accomplished with the Yellowstone bison, in an attempt to understand the mechanisms of reproductive self-regulation. After three years of study, it has been demonstrated that fetal loss is almost non-existent among the Yellowstone bison, that the unusually low fecundity of the Yellowstone bison is the result of ovulation failure among lactating cows, and that ovulation is rare among cows younger than four years (Kirkpatrick et al. 1993b). The information regarding the low incidence of fetal loss had other implications and cast serious doubt on the extent of brucellosis among these animals, a fact that was subsequently validated by examination of 220 animals destroyed by the state of Montana outside the park in 1992. In still another study within Yellowstone, pregnancy diagnosis in elk is being accomplished by means of fecal steroid analysis (R. A. Garrott, University of Wisconsin, pers. comm.).

Modern technologies of wildlife contraception and non-capture physiological studies will not completely eliminate the need for lethal controls, or the occasional immobilization and capture of animals. However, the state-of-the-art of wildlife contraceptive technology has already reached a point where it can be applied to certain populations of wild and feral species within national parks and other public lands, and there is much valuable information that can be collected from wildlife without capture, through

urinary and fecal hormone metabolite analysis. Wherever possible, the first choice for controlling animal populations or studying reproduction or diagnosing reproductive and endocrine status in free-ranging wildlife in our national parks should be these non-capture, non-lethal techniques. These approaches ben-

efit the animals studied and improve the overall quality of science. Additional technical advances soon will expand the capabilities of contraception and non-capture research, but the application of this technology for conservation biology will depend upon the willingness of researchers to routinely use these new methods.

ACKNOWLEDGMENTS

Many individuals and organizations are responsible for the research reported herein. Those deserving acknowledgment and who have not already been acknowledged in the paper include, Bruce Rogers, Gordon Olson, and John Karish of the USNPS; Allen T. Rutberg and John W. Grandy of The Humane Society of the U.S.; U.S. Senator Harry Reid; Ron Keiper and Rick Naugle of Pennsylvania State University; Lydia Kolter and Waltraut Zimmermann of the Cologne (Germany) Zoo; Paul Calle of the Bronx Zoo; Karen Goodrowe of the Toronto Metropolitan Zoo; S.E. Shideler, M. Bernoco, and C. Munro of the University of California-Davis; and Karen Allen of the American Association of Zoological Parks and more recently Conservation International. The author gratefully acknowledges the Deaconess Research Institute and The Humane Society of the U. S. for financial support for the preparation of this manuscript.

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