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An Alternative to Invasive Monitoring of Reproductive Function in Captive and Free-Ranging Wildlife

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The authors present an alternative to live capture of wildlife with resulting stress for both animal and human. They detail a non-invasive way of studying wildlife reproductive function in both captive and uncaptured free-ranging wildlife by means of urinary and fecal gathering and study. This alternative is based on the measurement of urinary and fecal steroids or their metabolites. A case is made for this method of study producing less biased and more scientific results, as well as being less expensive and safer for humans and animals.

KEY WORD INDEX:
humane, non-invasive, wildlife, research, animal reproduction

The effective management of captive and uncaptured free-ranging wildlife populations requires an understanding of the biology of each species and in particular their reproductive physiology. Of the more than 4000 mammalian species, the complete reproductive endocrine profile has been characterized for fewer than 50 species (Lasley & Kirkpatrick, 1991). Most of these 50 species include laboratory animals derived from species managed genetically for hundreds or even thousands of years. Consequently, we have little knowledge of the reproductive biology of most wildlife in their native habitats. These gaps in our knowledge have left us with a poor understanding of the mammalian reproductive response to environmental cues, stress, population density changes, and genetic shifts in populations.

The immediate and long-range management plans for a population of animals, captive or free-ranging, are largely dictated by the current reproductive potential of individual animals. In wildlife, the age at which sexual maturity is reached, pregnancy rates, and the ability to carry pregnancies to term are all reflections of a population's general health and stability. Until recently the measurement of these parameters of reproductive performance required capture of animals and the assessment of reproductive hormones circulating in the blood. The live capture of free-roaming wildlife for purposes of reproductive studies engages a variety of shortcomings and dangers to both animal and man (Lasley & Kirkpatrick, 1991) and vary in cost, difficulty, and risk, depending upon the species. Small mammals and birds can be live-trapped and released without irreversible harm, but this approach can confound a study of reproductive physiology by causing considerable stress. In studies of population densities and reproductive physiology in rodents, the subject populations are captured and handled to obtain blood samples. The biases produced by trapping, the inadvertent spread of pheromones by investigators, and the negative physiologic consequences of extended trap confinement on reproduction, recruitment, dispersal, and general demography have been well documented (Madison & McShea, 1987). Many of the same problems, when applied to primate studies, are exacerbated by the behavioral alterations common to captive populations (Sapolsky, 1983). Behavioral observations have been made and the endocrine status of the animals inferred (Dunbar, 1982), but never confirmed.

Large free-roaming species pose even greater problems. Reproductive studies of moose (*Alces alces*), caribou (*Rangifer tarandus*), feral horses (*Equus caballus*), or bison (*Bison bison*) traditionally require immobilization and/or capture, events associated with tremendous animal stress and sometimes significant mortality (Seal et al., 1985; Valkenburg et al., 1983), disruption of reproductive events (Ballard & Tobey, 1981; Larsen & Gauthier, 1989), and/or confounded endocrine status (Kirkpatrick et al., 1979). Currently, grizzly bears in Montana suffer a 4% research-related mortality rate (John Varley, Chief of Research, Yellowstone National Park, personal communication). The cost of safe immobilization often exceeds $50/animal and, depending upon the species, capture can be hazardous to the investigator. In his recently published book, **Mammalian Reproductive Biology**, Frank Bronson (1989) states, "Also important, but largely missing, are good hormonal data obtained from wild populations. How does one collect such data given . . . the interfering stress of capture? Some truly imaginative thinking will be required to solve this problem."

An alternative approach to the study of ovarian function in captive and free roaming wildlife is based on the measurement of urinary and fecal steroids or their metabolites. The technology which permits remote monitoring of reproductive endocrinology was originally developed to evaluate the reproductive status of captive exotic species (Loskutoff et al., 1983; Lasley, 1985), and its application has now been extended to human medicine as well. The questions that arise in wildlife biology are not altogether different from those encountered in modern human epidemiological studies. Not only is reproduction a vital health issue in its own right, but it serves as a sensitive indicator of the impact of environmental stressors. Physical, nutritional, chemical and even emotional or psychological stresses can have profound influences on reproductive capacity. These perturbations in the hypothalamic-pituitary-gonadal axis are often revealed before the more specific consequences of stress are recognized.

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MONITORING REPRODUCTION IN CAPTIVE EXOTIC SPECIES

Hormone metabolite monitoring was originally developed at the San Diego Zoo. Initial work was directed at developing methods for predicting sex of monomorphic birds, a procedure which previously required stressful handling of the bird or even minor surgery. The first step was to obtain a useful biological sample without handling and restraining the bird, and thereby avoid unecessary stress. It had long been known that free steroids, such as estrogen and testosterone, could be measured in urine. With this in mind, sex determination in monomorphic birds such as the Hispaniolan parrot (*Amazonia ventralis*) was accomplished by measuring free fecal steroids (bird feces is actually a mixture of urine and feces) and examining the estrogen:androgen ratios (Bercovitz et al., 1978). This eliminated the need for surgical procedures, or for invasive drawing of blood for genetic tests.

Other work, occurring largely during the late 1970s and early 1980s at the San Diego Zoo, was directed at detecting pregnancy and ovulation in captive exotic mammals. Even though free steroids, such as progesterone and estrogen, could be measured in urine, they are by their chemical nature not water-soluble, and as such their concentrations provided only crude estimates of blood hormones. A major breakthrough came when the major metabolites of these reproductive hormones were identified and assay systems were developed to measure these metabolites. Most of the early mammalian research was accomplished by measuring urinary concentrations of just two groups of metabolites of the two female reproductive hormones, estradiol and progesterone. These metabolites were estrone conjugates (E_1C) and pregnanediol-3-glucuronide (PdG). Estrone conjugates include estrone sulfate and estrone glucuronide, which collectively reflect plasma estrogens, whereas urinary PdG reflects a poorly defined group of progesterone metabolites. Urinary E_1C and/or PdG was used to characterize the estrous cycles, reproductive seasonality, and breeding potential of a variety of captive exotic species, including the okapi (*Okapia johnstoni*) (Loskutoff et al., 1982), giraffe (*Giraffa camelopardalis*) (Loskutoff et al., 1986), Indian rhinoceros (*Rhinoceros unicornis*) (Kasman et al., 1986), Asian elephant (*Elephas maximus*) (Ramsay et al., 1981), lion-tailed macaque (*Macaca silenus*) (Shideler et al., 1985), Goeldi's monkey (*Callimico goeldii*) (Carroll et al., 1989), gorilla (*Gorilla gorilla*) (Czekala et al., 1983; Mitchell et al., 1982a,b), and ruffed lemur (*Lemur variegatus*) (Shideler et al., 1983), to mention only a few. In these examples, urinary E_1C concentrations were useful for identifying preovulatory estrogen surges, whereas urinary PdG concentrations provided proof of ovulation and the establishment of luteal phases.

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Early pregnancy detection is also very important in the management of zoo animals, and the measurement of certain urinary hormone metabolites can provide a useful picture of fetal health or impending fetal demise (Kasman et al., 1988). In many species, the fetoplacental unit produces large quantities of estrogens, and urinary E_1C concentrations rise dramatically at some point in pregnancy. The measurement of urinary E_1C has been used successfully to diagnose pregnancy in species as diverse as the gorilla and orangutan (*Pongo pygmaeus*) (Czekala et al., 1983), the ruffed lemur (Shideler et al., 1983), Eld's deer (*Cervus eldi thamin*) (Monfort et al., 1990), tapirs (*Tapirus terrestris* and *T. indicus*) (Kasman et al., 1985), Przewalksii's horses (*Equus przewalskii*) and Hartmann's zebra (*E. zebra*) (Czekala et al., 1990). In other species, including the baboon (*Papio anubis*) (Hodges et al., 1986), and Indian rhinoceros (Kasman et al., 1986), pregnancy can be predicted by the rise in urinary PdG concentrations above luteal phase concentrations. Even the subtle differences between a true pregnancy and a pseudopregnancy, as in the case of the giant panda (*Ailuropoda melanoleuca*), can be detected by monitoring urinary PdG and E_1C concentrations (Bretzfelder, 1989).

Fecal steroid metabolites have also been used to monitor reproduction in zoo animals. Pregnancy was diagnosed in the red buffalo (*Syncerus caffer nanus*), yak (*Bos mutus*), Grevyi's zebra (*Equus grevyi*), Nubian ibex (*Capra ibex nubiana*), and hippopotamus (*Hippopotamus amphibius*) by measuring total fecal estrogens (Safer-Hermann et al., 1987). Cyclic changes of estradiol-17β and progesterone also were detectable in feces collected during the menstrual cycle of the pigtailed macaque (*M. nemestrina*) and baboon (*P. cynocephalus*) (Wasser et al., 1988).

These urinary and fecal steroid analysis methods have increased our knowledge of reproduction in captive exotic species, and facilitated the captive breeding of many endangered species. More to the point, this technology has done so without imposing unnecessary stresses upon these captive species.

APPLICATIONS IN FREE-RANGING WILDLIFE

Reproductive function has been monitored by means of urinary and fecal steroid analysis in a large variety of captive exotic species for a number of years, but application to true free-ranging wildlife is a relatively recent event. The rationale for taking a remote approach included management policies which prohibited capture of animals, costs of capture, dangers of injury or mortality to animal or investigator, and the need for frequent sampling. Such evaluations of reproductive function from the excreta have not been limited to mammalian species and one of the first applications was with avian species. Adaptations of the measurement of gonadal steroid metabolites in the mixed urine and feces of captive monomorphic birds was exploited as a non-capture method to evaluate the effects of environmental factors on reproduction in seabirds. The study of oil pollution-induced toxicity among seabirds (Fry & Lowenstein, 1985) was accomplished by collecting droppings from Cassin's auklet (*Ptychoramphus aleuticus*) and measuring testosterone, estradiol, and progesterone during the breeding season. Similar applications to free-ranging mammals have occurred only recently, although the rationale is identical (i.e., to eliminate the need for capture or restraint which carries the risk of injury and which may involve significant expense and may have profound negative effects on the parameters of interest). Kirkpatrick et al. (1988) detected early pregnancy in feral mares in Montana by collecting soil which had been moistened by recent urination. The samples, collected opportunistically, were returned to the laboratory for E_1C and creatinine measurement in soil extracts. It had previously been demonstrated that the rapid rise in E_1C was the early response of the ovary to equine gonadotropin (Daels et al., 1990). A sustained production of E_1C is indicative of an intact feto-placental unit and a surviving pregnancy (Kasman et al., 1988; Evans et al., 1984). Twelve of 15 feral mares assumed to be pregnant based on E_1C values of 1.0 µg/mg creatinine (Cr) or greater produced foals six to eight months later. In contrast, the mares which appeared not to be pregnant had E_1C concentrations of less than 1.0 µg/mg Cr and produced no foals the next summer. These data provided unique information regarding fecundity and fetal loss rates which might not have been gathered by any other strategy. Data gathered to date indicate that concurrent pregnancy and lactation are not serious contributing factors to fetal loss in feral horses.

A similar study design has been used in at least three other experiments dealing with free-roaming ungulates. In the first experiment, the measurement of urinary E_1C has been used to determine pregnancy rates among feral mares on Assateague Island National Seashore, MD, to assess the efficacy of population control by means of immunocontraception. Since capture and restraint is prohibited with these animals, both the treatments and the end-point assessments required remote methods. In this case induced infertility and the ultimate outcome of contraception was predicted eight months in advance of the foaling season, by collecting and evaluating urine samples (Kirkpatrick et al., 1990a).

In a second experiment, the phenomenon of forced copulation and induced abortion in feral horses, reported by Berger (1983) was reinvestigated using the more precise pregnancy detection methods afforded by urinary E_1C evaluation. Mares which were pregnant in August and which were subsequently taken over by new harem

URINE-SOAKED SAND BEING COLLECTED FROM FERAL HORSES ON ASSATEAGUE ISLAND NATIONAL SEASHORE. THE URINE IS REMOVED FROM THE SAND BY HAND CENTRIFUGATION IN A PLASTIC BAG AND LATER ANALYZED FOR REPRODUCTIVE STEROID METABOLITES.

stallions in October produced foals and demonstrated an absence of induced abortion. In a third example, alleged fetal loss associated with brucellosis infections was evaluated in free-ranging bison in Yellowstone National Park. Early pregnancy detection in the fall months, detected by sustained elevations of urinary PdG and total fecal estrogens, was compared to calving rates in the spring and indicated that there was little if any fetal loss among these bison. A number of ongoing studies, including the evaluation of fertility in wild mountain gorillas (*Gorilla gorilla beringei*) (Nancy Czekala, San Diego Zoo, pers. comm.), are taking advantage of this simple approach.

Pregnancy testing requires only a single urine sample, but the ovarian endocrine changes associated with the estrous cycle are dynamic and require frequent sampling in order to visualize the sequential events. The same non-capture approaches used for pregnancy detection can be used for monitoring cyclic ovarian function in free-ranging wildlife. In a recent study of the long-term effects of immunocontraception on the ovarian function of feral horses, it was necessary to examine cyclic ovarian endocrine profiles from treated and non-treated mares (Kirkpatrick et al., 1992a). Urine samples were collected every two or three days from free-roaming treated and control mares, and analyzed for E_1C and a group of non-specific progesterone metabolites (referred to as iPdG). Cyclic changes in blood estrogens are acurately reflected in urinary E_1C concentrations (Daels et al., 1991). In the Equidae, PdG is not a metabolite of progesterone as it is in most of the Artiodactyla. Instead, progesterone is metabolized to at least three and perhaps more uncharacterized metabolites, all of which are more polar than PdG (Kirkpatrick et al., 1990c). Figures 1A and 1B illustrate a non-conceptive and a conceptive ovulatory cycle in uncaptured control feral mares.

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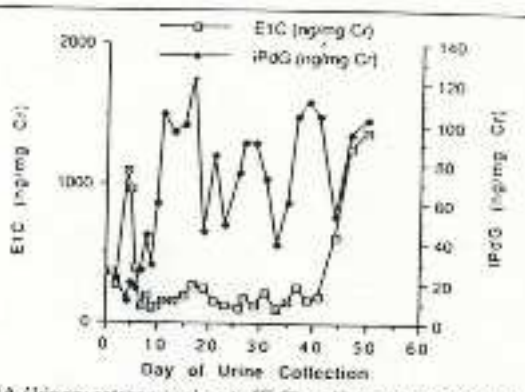


Figure 1A. Urinary estrone conjugate (E_1C) and immunoreactive pregnanediol-3-glucuronide-like (iPdG) concentrations in a feral mare from samples collected over a 51-day period during the breeding season. The hormone metabolite profile reveals a conceptive ovulatory cycle, characterized by a preovulatory E_1C peak, the luteal rise in iPdG following ovulation, and a marked increase in E_1C at about the 35th day of pregnancy (from Lasley and Kirkpatrick, 1991).

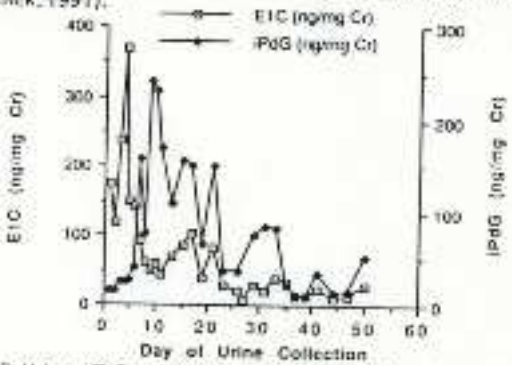


Figure 1B. Urinary E_1C and iPdG concentrations in a feral mare from samples collected over a 51-day period during the breeding season. The hormone metabolite profile reveals a nonconceptive ovulatory cycle, characterized by a preovulatory E_1C peak occurring coincidentally with an iPdG nadir, followed by a luteal phase iPdG profile (from Lasley and Kirkpatrick, 1991).

In both cases observable estrous behavior was coincidental with the preovulatory E_1C peak and the iPdG nadir. Ovulation was signalled by the dramatic rise in iPdG following the E_1C peak. The mare demonstrating the conceptive ovulatory cycle signalled a pregnancy by the dramatic elevation of E_1C at precisely 35 days post-ovulation, as previously demonstrated in domestic mares (Evans et al., 1984; Kasman et al., 1988). Figure 1C illustrates the urinary E_1C and iPdG patterns for a mare treated with an immunocontraceptive vaccine for three consecutive years. There is no evidence of a luteal phase and E_1C concentrations are significantly depressed, suggesting some form of ovarian follicular dysfunction. Equally significant is the fact that all these data were collected from uncaptured animals.

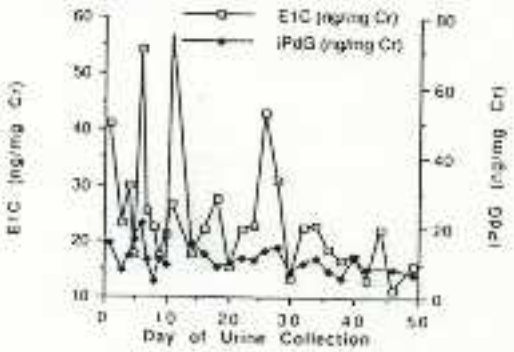
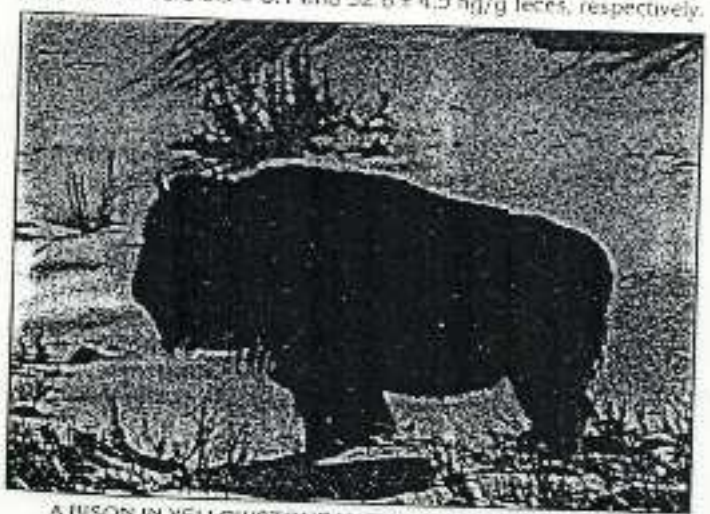


Figure 1C. Urinary E_1C and iPdG concentrations during a 51 day period during the breeding season, in a feral mare treated for three consecutive years with a contraceptive vaccine. There is no evidence of ovulation based on the iPdG patterns and E_1C is significantly depressed (from Kirkpatrick et al., 1992b).

The evaluation of urinary ovarian steroid metabolites in free-ranging wildlife provides valuable information, but urine collection can be time-consuming or in some cases dangerous. The use of fecal steroid evaluation has proven useful with a number of domestic species (Schwarzenberger et al., 1990) and can provide the field biologist with an alternative and less labor-intensive strategy for monitoring ovarian reproductive events. Pregnancy diagnosis has been accomplished in bison by the measurement of total fecal estrogens. This approach is based on the fecal excretion of large quantities of 17-estradiol, a biologically inactive metabolite of 17-estradiol (Safer-Hermann et al., 1987). We utilized a commercially available antibody (OP-11, Pantex Corp., Santa Monica, CA) which measured several estrogens and had a 30% cross-reactivity with 17-estradiol. The Equidae do not produce significant quantities of 17-estradiol, but sufficient other estrogens are excreted in the feces to provide a simple and accurate approach to pregnancy detection in feral horses (Kirkpatrick et al., 1990b). Mean fecal total estrogen concentrations for pregnant mares was 3.18 ± 0.70 ng/g feces vs. 0.55 ± 0.08 ng/g feces for non-pregnant mares. This same approach has been used to detect pregnancy among feral horses on Sable Island, Canada, in order to provide age-specific fetal loss data (Lucas et al., 1991). These two studies provided important information which showed that young feral mares have a high rate of fetal loss.

Conjugated steroid metabolites such as E_1C and PdG are often hydrolized in the gut, resorbed and recirculated in the blood. In most species, however, a substantial quantity is lost in the feces. The fecal component of steroid excretion differs from that of urine in at least two important aspects. First, the fecal excretion rate is usually slower than that of urine. Frequently a two or three day lag can occur between the detection of an event in serum versus the time it is detectable in feces. Second, the fecal metabolites are usually represented by a high percentage of unconjugated forms compared to urine. Neither of these differences detract from this approach, because one or two days are not critical for retrospective detection of reproductive events, and many of the same assays which detect conjugated product can be used to measure the unconjugated portion of the metabolite. This strategy was applied to feral horses successfully and the evaluation of simple fecal water extracts for E_1C and iPdG yields accurate predictors of pregnancy (Kirkpatrick et al., 1991a). Fecal E_1C and iPdG concentrations for seven pregnant mares were 4.2 ± 0.8 ng/g and 1,411 ± 569.6 ng/g feces, respectively. Fecal E_1C and iPdG concentrations for 33 non-pregnant mares were 0.5 ± 0.1 and 32.8 ± 4.5 ng/g feces, respectively.



A BISON IN YELLOWSTONE NATIONAL PARK, FROM WHICH FECAL OR URINE SAMPLES ARE COLLECTED. THESE SAMPLES ARE LATER ANALYZED FOR REPRODUCTIVE STEROID METABOLITES IN ORDER TO UNDERSTAND HOW BISON SELF-REGULATE THEIR REPRODUCTIVE POTENTIAL.

The North American bison is a large intractable animal which poses a significant hazard to investigators. It is probably for this reason that the bison estrous cycle had never been characterized endocrinologically. Urinary PdG concentrations were measured in 28 free-roaming sexually mature bison cows during the August rutting season.

Twenty of these cows were already pregnant at the start of collection, on August 1, but eight cows demonstrated complete non-conceptive estrous cycles. Collectively, the length of the bison estrous cycle, based on luteal phase PdG, was 23.12 ± days (Figure 2). Seven of these cows became pregnant during a subsequent estrous cycle (confirmed by parturition dates). Thus it was demonstrated for the first time that bison will exhibit more than a single ovulation in a season (Kirkpatrick et al., 1991b).

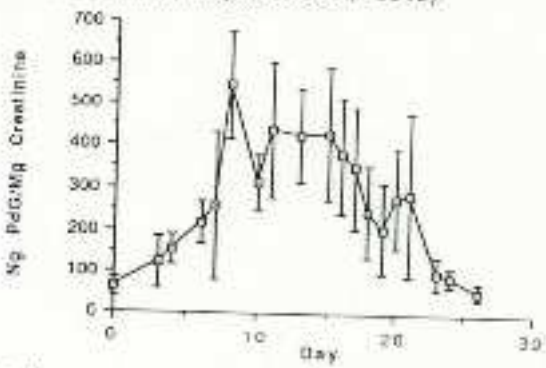


Figure 2. Mean urinary pregnanediol-3-glucuronide (PdG) for eight bison cows collected at a minimum of once every 3 days between 31 July and 30 August. Day 0 represents alignment of the PdG nadir for each cow (from Kirkpatrick et al., 1991b).

These same techniques are currently being applied to a long-term study of reproductive self-regulation among the bison of Yellowstone National Park. Early in the study it was apparent that very few lactating cows displayed behavioral estrus or were tended by a bull. When ovulation rates were measured, determined by urinary PdG and fecal progesterone, the results indicated that relatively few bison cows with calves were experiencing a physiological estrus or ovulating. Pregnancy rates for the same bison, determined from samples collected in November and measured for total estrogens, indicate that among all pregnancies only about 15% were among cows with calves. This in turn suggests that bison cows with calves are experiencing a post-partum anovulatory interval related to lactation, which might suggest some energy deficiency in these lactating animals. These data are the first of their kind regarding reproduction of the Yellowstone bison, and were possible only by means of non-capture techniques (Kirkpatrick et al., 1992b).

Most published reports dealing with remote monitoring of reproductive function reflect applications to domestic species or animals which are closely related to well-defined domestic species. There are indications that this will soon change, as a number of reports indicate that non-capture methods are being developed for a wide range of wildlife species. Pregnancy has been diagnosed in hunter-killed caribou (*Rangifer tarandus*) (Messier et al., 1990), and cyclic progesterone changes in captive muskoxen (*Ovibos moschatus*) (Desaulniers et al., 1989) have been characterized by means of fecal estrogens and progesterone. The reproductive cycle has been characterized in the captive steppe polecat (*Mustela eversmanni*) (Mead et al., 1990) by means of urinary progesterone and in the captive endangered black-footed ferret (*Mustela nigripes*) (Gross et al., 1990) by means of fecal progesterone.

Free-roaming wildlife species can provide a broad spectrum of valuable information with which to enhance our understanding of comparative reproduction and the reproductive strategies animals assume in natural settings. The use of urinary and fecal steroid analysis to monitor reproductive events can provide safe, accurate,

and non stressful methodologies for these types of studies. Additional research will soon provide even newer methods, such as field pregnancy tests and assays for new steroid metabolites. The application of this technology will, however, depend upon the willingness to use it by scientists and wildlife managers.

"These urinary and fecal steroid analysis methods have increased our knowledge of reproduction in captive exotic species . . . without imposing unnecessary stresses upon these captive species."

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Dr. Jay Kirkpatrick earned his Ph.D. in reproductive physiology from Cornell University in 1971. He holds academic positions as Associate Professor in the Department of Reproduction, School of Veterinary Medicine, University of California at Davis, and in the Department of Biological Sciences at Eastern Montana College in Billings. He is the Senior Staff Scientist with the Deaconess Research Institute in Billings, Montana.

Dr. Kirkpatrick is a consultant for the Humane Society of the United States and has served on the National Animal Damage Control Advisory Committee for the Secretary of Agriculture. He is a member of the Equid Taxonomic Advisory Committee for the AAZPA and is a Director for the organization In Defense of Endangered Species.

For the past twenty years, Dr. Kirkpatrick has carried out research on fertility control for wildlife. His purpose was to develop non-lethal and humane methods of controlling wildlife populations in urban areas and refuges. He also developed non-capture methods for studying reproduction in free-roaming wildlife. Dr. Kirkpatrick is probably best known for his contraceptive research with the feral horses of Assateague Island, and for the study of reproduction in the bison of Yellowstone National Park.

(photo credit: Karen Allen, AAZPA)

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Dr. Lasley earned his Ph.D. in endocrinology from the University of California at Davis in 1975. From 1975 to 1986, Dr. Lasley was the endocrinologist for the San Diego Zoo, where he pioneered the development of urinary assays for assessing ovarian function in captive exotic species. As a result of his work there, which led to increased success with captive breeding programs with a number of endangered species, he was awarded the Rolex Award for Enterprise in 1978.

In 1986, Dr. Lasley moved back to the University of California at Davis, where he holds academic appointments in the Department of Reproduction in the School of Veterinary Medicine, the Institute for Toxicology and Environmental Health, and the School of Medicine. In these capacities, he has adapted his urinary hormone assays to the study of human and domestic animal reproduction.

Drs. Lasley and Kirkpatrick began their collaboration in 1985, which led to the use of urinary and fecal steroid analysis in free-roaming wildlife species.