
The Importance of the Application of Non-invasive Techniques in the Study of Wild Animal Reproduction

Abstract

Conservation of biodiversity is one of the major goals of the scientists in many countries. Brazil is one of them and is also well known as the world's largest reserve of biodiversity, which leads to a strategic position in this matter. In the last decade a number of scientific works were published with an increasing participation of Brazilian researchers. One of the keys, besides environmental protection, for the successful conservation of endangered species is reproduction; and the first step is to know how the species function. Traditionally the study of reproductive physiology in wild animals depends on some kind of restraining, either physical or chemical, which implies different levels of stress. Serial blood sampling necessary to obtain some hormonal profiles are also stressful, unless the animal was trained to accept that procedure, which is hard or even impossible to achieve with some wild species. For that reason some non-invasive techniques were developed, *i.e.* extraction and measurement of fecal and urinary hormonal metabolites, utilizing radio immune assay (RIA) or enzymatic immune assay (EIA). Different methods of extraction were tested in order to achieve lower costs and reliable results; and validation of the immune assays with different antibodies for a number of wild species were performed with the same purpose. These non-invasive methods for the study of reproductive physiology can be used in free-living and captive wild species as a very useful tool for conservation.

Key Words: non-invasive, fecal hormones, urinary hormones, radio immune assay, enzyme immune assay, wild animals.

Invited Mini-review

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The interest in the environment and its degradation has increased over the last two decades, prompting questions about the cost to humanity of such relentless decline. Nowadays there are myriad institutions – including non-governmental ones – that investigate the responsibility for political acts and decisions that result in damage to the environment.

Through various tactics and strategies, professionals with diverse backgrounds coalesce around relevant aspects such as the preservation of biodiversity (animal and vegetal). As a counterpoint to the huge financial interest behind decisions that promote extensive environmental impact in various ecosystems, experts and citizens have searched for responses and alternative ways to prevent the destruction of a genetic reserve of huge potential, not yet fully known, that could yield important answers to significant problems that afflict mankind.

Among institutions responsible for generating scientific information that assists in conservation of nature are the Zoo Parks, Universities, Botanic Gardens and Research Institutes. The synergetic work of such institutions has produced works of research throughout the planet that have resulted in success that is significant though modest compared to the gigantic scale of the problems.

Some specific examples are: the American bison (*Bison bison* Hamilton – Smith, 1827), at present out of extinction risk; the Siberian tiger (*Panthera tigris altaica* Oken, 1816); the sea otter (*Enhydra lutris* Fleming, 1822); the black-footed-ferret (*Mustela nigripes* Lineu, 1758) and the golden lion tamarin (*Leontopithecus rosalia* Lesson, 1840), the latter being the object of a successful international project that obtained success not only in the captive reproduction of the species but also its reintroduction into nature, a project that depended on the participation of Brazilian experts.

Not by chance, the examples mentioned above are also subjects of the combined work of several institutions such as zoos, universities and research institutes. In countries such as England, South Africa, Germany and mainly the United States, there are advanced research centers where studies of wild animals are developed in areas such as: immunology, pharmacology, physiology, pathology, infectious and parasitic illnesses, including zoonosis, organ transplants, molecular biology, endocrinology, behavior and artificial reproduction.

In this context we can notice the importance of this multidisciplinary work that fights simultaneously for the conservation of species “*in situ*”, with field work and “*ex situ*”, reproducing in captivity the species to be studied and reintroduced. The importance of such work does not reside only in the conservationist aspect but also, and not least importantly, relates to biomedical research aspects that involve problems related directly to human health. With this focus we can mention the types of research that are accomplished by using wild animals as a biological model, *i.e.*: the search for the cure or prevention of AIDS (acquired immune deficiency syndrome), as well as works regarding transplants, genetic diseases, the production of vaccines against malaria and many others.

These examples demonstrate that the extinction of any specie may represent a priceless loss to humanity, as it takes with it all its genetic information and biological characteristics that could be vital to the maintenance of biodiversity in addition to an unknown number of fundamental answers for the maintenance of life on this planet.

Wild animal reproduction in captivity is a fundamental tool that enables us to carry out research without withdrawing any exemplar from nature. In this context, zoos and conservationist nurseries assume a prominent role as they are recognized as genuine genetic banks of wild species and work with reproduction as a primary objective.

It is common knowledge that several Brazilian wild species belong to a list of animals threatened with extinction and that many of these present reproductive difficulties in captivity, despite appropriate handling and feeding. These conditions demand measures that aim to improve the reproductive performance of such animals.

With this purpose, we examine, in the context of the reproductive physiology of different species, ways of adapting artificial reproductive techniques to anatomical, physiological and handling characteristics for each of them (Short, 1968; Wildt & Bush, 1984; Holt & Moore, 1988; Durrant, 1990; Hearn, 1994).

Traditionally, the methods used in these studies are the non-invasive ones, which for the animal implies physical or chemical restraint, as well as the use of injections and multiple blood collection. In some cases such as in artificial insemination, electroejaculation or embryo transfers, such procedures are inevitable. In the case of hormonal studies, during recent years a strong trend arose that commends the adoption of non-invasive techniques that would avoid the restraint of the animal, for instance in serial blood collection. The stress generated by the restraining procedure, besides compromising animal health, would render multiple and consecutive collection unfeasible, although it would certainly increase the hormonal parameters to be measured by the action of augmented cortisol over the hypothalamus-hypophysis-gonad axis with a consequent effect on the level of sexual steroids and gonadotrofins (Line *et al.*, 1987; Sapolsky & Krey, 1988; Carlstead *et al.*, 1992; Mendoza *et al.*, 1994; Jurke *et al.*, 1997; Whitten *et al.*, 1998; Möstl & Palme, 2002).

Among these non-invasive techniques include the extraction and dosage of hormones or their metabolites from feces and urine, which can be collected daily or several times a day with no kind of physical or chemical restraint; moreover they can be collected from free-range individuals, which enables long term serial studies with no interference of stress effects.

Therefore, a number of authors have been studying reproductive endocrinology of different wild species through extraction and dosage of steroid metabolites from feces and urine (Kasman *et al.*, 1985; Chaudhuri *et al.*, 1988; Monfort *et al.*, 1990; Bamberg *et al.*, 1991; Gross *et al.*, 1991; Lasley & Kirkpatrick, 1991; Monfort *et al.*, 1991; Paul-Murphy *et al.*, 1991; Knox *et al.*, 1992; Brown *et al.*, 1993; Brown *et al.*, 1994; Brown *et al.*, 1995; Graham *et al.*, 1995; Papageorge *et al.*, 1995; Shaw *et al.*, 1995;

Stavisky *et al.*, 1995; Walzer & Schwarzenberger, 1995; Morais *et al.*, 1996; Berkeley *et al.*, 1997; Heistermann *et al.*, 1997; Radcliffe *et al.*, 1997; Robeck *et al.*, 1997; Garrott *et al.*, 1998). Non-human primates – as per their phylogenic proximity to mankind – are especially important as a research biological model for studies connected to physiology, immunology, pharmacology, endocrinology and reproduction. For this reason several papers involving reproduction studies in non-human primate species through non-invasive methods have been published (Shideler *et al.*, 1985; Wasser *et al.*, 1988; Ziegler *et al.*, 1989; Masters & Markham, 1991; French *et al.*, 1992; Shideler *et al.*, 1993; Wasser *et al.*, 1993; Asa *et al.*, 1994; Jurke *et al.*, 1994; Pryce *et al.*, 1994; Bellem *et al.*, 1995; Brockman *et al.*, 1995; Heistermann & Hodges, 1995; Heistermann *et al.*, 1995; Kuederling *et al.*, 1995; Pryce *et al.*, 1995; Oerke *et al.*, 1996; Ialeggio *et al.*, 1997; Strier & Ziegler, 1997; Ziegler *et al.*, 1997; Guimarães *et al.*, 2002).

Always seeking resource optimization and simplification of techniques without jeopardizing the quality of the results, recent works involving extraction and dosage of hormonal metabolites in neo-tropical primates showed that fecal progesterin (a group of progesterone metabolites) dosage is the most effective non-invasive method of studying the ovary cycle of these species (Strier & Ziegler, 1997; Guimarães *et al.*, 2002). This technique enabled the characterization of follicular and luteinizing phases, as well as permitted the determination of the peri-ovulation period and the estimated ovulation day (Strier & Ziegler, 1997; Guimarães *et al.*, 2002). Besides reflecting the variation of progesterone plasma levels in a few hours interval (Adlercreutz & Martin, 1980; Ribeiro, 1994), fecal progesterin dosages, since they present a similar molecular and antigen structure among the species, also possess the advantage of permitting the utilization of traditional laboratory methods of hormone dosage, such as the radio-immuno-assay, using anti-progesterone antibody produced in laboratory animals (Ribeiro, 1994; Guimarães *et al.*, 2002).

The methods of fecal progesterin extraction and dosage, initially complex and lengthy, demanded purification through chromatographic separation and analysis and also the identification and dosages of metabolites (Risler *et al.*, 1987; Wasser *et al.*, 1988). Due to the high cost and the complexity in executing such techniques, several researchers accomplished comparative studies between performance of sophisticated methods and simple techniques of reduced costs, producing results that allow the implementation of projects with a large number of samples at reduced budgets (Meirelles, 1993; Strier & Ziegler, 1994; Ziegler *et al.*, 1996; Strier & Ziegler, 1997).

Information obtained through non-invasive methods are of great utility to the planning of reproduction programs, such as the choosing of females with an adequate pattern of cycles to dictate the best moment for an artificial insemination or even optimizing parameters for insemination through natural breeding.

Another relevant aspect is the applicability of this method to field studies where it becomes possible to verify the reproductive condition or even the phase of the

ovary cycle in free-range females. These data are vital to the study of social structure, hierarchic interrelation, family and reproductive strategy which a specie adopts for the purpose of adaptation.

No less important than the aforementioned reasons, these techniques also are ethically justified. Increasingly, the conscientiousness of researchers must be applied to minimize the agony of animals used in experiments, seeking alternative methods that reduce the stress caused by contention and handling, avoiding for instance the fear and pain caused by many of the traditional invasive methods (Wolfensohn & Lloyd, 1998).

The Department of Animal Reproduction of Zootechnics and Medical Veterinary Faculty of the University of São Paulo, currently are developing a line of research in reproductive endocrinology, using non-invasive methods. This line of research is essentially composed of projects that utilize different techniques of extraction and dosages of sexual steroid hormones from fecal metabolites, with several approaches in different species such as: Amazonian manatee (*Trichechus ininguis*), jaguarundi (*Herpailurus yagouaroundi*), jaguar (*Panthera onca*), ocelot (*Leopardus pardalis*), brown capuchins (*Cebus apella*), black howler monkey (*Alouatta caraya*), blue-fronted parrot (*Amazona aestiva*), golden hamster (*Mesocricetus auratus*), Guinea pig (*Cavia porcellus*), maned wolf (*Chrisocyon brachyurus*), African lion (*Panthera leo*) and fallow deer (*Dama dama*).

From basic physiological information obtained through these non-invasive techniques, projects are being developed to study characteristics of the ovarian cycle, seasonal variation occurrences, ovulation detection, pregnancy diagnosis, parturition proximity, sex identification of monomorphic species, efficiency of contraceptive methods, purging methods and identification of different hormone metabolites, as well as the influence of animal well being on reproductive performance, stress effects and the interrelation between hormone levels and reproductive behavior patterns (behavioral endocrinology).

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