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# Hormone monitoring: An important tool for the breeding management of wildlife species

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## Summary

Hormone monitoring is a well established tool for evaluating endocrine activity of wildlife, and has substantially enhanced captive management and propagation of a number of endangered species. This review presents examples of a broad spectrum of uses for hormone monitoring in several high priority species: elephants, rhinoceroses and ursid and felid species. Depending on the species, different sample materials are commonly used to assess reproductive function. Blood samples provide the most immediate information on the endocrine status of an animal and yield results not only on steroid but also on protein hormones such as LH, FSH, inhibin, relaxin and prolactin. However, with the exception of elephants, most captive wildlife species suffer undue stress if blood samples are routinely taken. The development of non-invasive urine or faecal hormone monitoring techniques has been key to understanding the reproductive biology of such species. Today, impressive endocrine databases exist for many wildlife species, with the vast majority based on studies of hormones excreted in urine or faecal samples. Of interest is the high degree of variability in reproductive mechanisms observed not only between

## Zusammenfassung

**Die Bedeutung von Hormonbestimmungen für das Zuchtmanagement von Wildtieren in Menschenobhut**

Die Messung von Hormonen stellt ein etabliertes Instrumentarium zur Charakterisierung der endokrinen Aktivität von in Menschenobhut gehaltenen bedrohten Tierarten dar; diese Methoden haben wesentlich zur Verbesserung der Nachzuchterfolge dieser Arten beigetragen. Diese Übersichtsarbeit zeigt Beispiele für die umfangreichen Anwendungsmöglichkeiten von Hormonbestimmungen in mehreren gut untersuchten Arten von Wildtieren. Je nach Tierart werden unterschiedliche Probenmaterialien, wie Kot-, Harn- oder Blutproben, verwendet. Abgesehen von wenigen Ausnahmen (wie z.B. Elefanten) können bei der überwiegenden Anzahl von Wildtieren (z.B. Nashörnern, Bären, Katzenarten) Blutproben nicht routinemäßig und stressfrei entnommen werden. Deshalb bilden für die meisten Wildtierarten nicht-invasive endokrinologische Untersuchungen aus Kot- oder Harnproben die Grundlage für das Verständnis der Reproduktionsbiologie. Elefanten nehmen diesbezüglich eine Sonderstellung ein, da diese traditionellerweise intensiv trainiert werden und deshalb Blutproben routinemäßig gewonnen werden können. Deshalb zählen Elefanten, endokrinologisch betrachtet, zu den am besten untersuchten Wildtierarten. Blutproben bieten den Vorteil, dass sowohl Steroidhormone, als auch Proteohormone wie LH, FSH, Inhibin, Relaxin und Prolaktin untersucht werden können. Eine vergleichende Betrachtung der umfangreichen endokrinologischen Daten der genannten Tierarten zeigt eine sehr große Variabilität der reproduktiven Mechanismen zwischen den taxonomischen Gruppen. Darüber hinaus treten große individuelle Unterschiede innerhalb der untersuchten Spezies auf. Des Weiteren zeigt diese Review das enorme Potenzial dieser endokriner Untersuchungen in Bezug auf Grundlagen- und angewandter Forschung, sowie deren Anwendungsmöglichkeiten für *in situ* als auch für *ex situ* Untersuchungen bei Wildtieren.

taxonomic groups but even between individuals of a certain species. Endocrine monitoring has enormous basic and applied potential for wildlife conservation and has implications for how we manage wildlife species both *in situ* and *ex situ*.

**Abbreviations:** acCL = accessory corpora lutea; AI = artificial insemination; eCG = equine chorionic gonadotropin; ET = embryo transfer; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; hCG = human chorionic gonadotropin; IVF = *in vitro* fertilisation; LH = luteinizing hormone; ovCL = ovulatory corpora lutea; PGFM = prostaglandin F2alpha metabolite; TRH = thyrotropin-releasing hormone

## Introduction

There is a growing appreciation of how an understanding of basic reproductive processes can aid species management and conservation. Understanding basic endocrine function is key because nearly every aspect of reproduction is controlled by hormones and their analysis is the most precise method for monitoring the functional status of the reproductive axis. Currently, increasing emphasis is placed on improving breeding both through natural means as well as with assisted reproductive techniques, particularly artificial insemination (AI) (PUKAZHENTHI et al., 2006; ANDRABI and MAXWELL, 2007; WILDT et al., 2010; SARAGUSTY et al., 2011). Optimizing reproductive performance of populations in crisis, while preserving genetic diversity, requires a multi-disciplinary approach and profound knowledge of reproductive behaviour, endocrinology, spermatology, embryology and gamete cryopreservation. Such fundamental data are available for a number of high priority species, providing the foundation for addressing reproductive issues, including diagnosis and treatment of fertility problems (PUKAZHENTHI et al., 2006; WILDT et al., 2010).

Hormones are present and can be measured in a variety of biological products, including blood, saliva, urine, faeces, milk, feathers and hair (reviewed by HODGES et al., 2010). Transported in the blood from endocrine organs to target tissues, circulating blood hormone levels provide the most immediate information on the endocrine status of an animal. Important reproductive hormones that can be measured in blood samples include steroids, such as oestrogens, progestagens and androgens, and protein hormones, such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), inhibin, relaxin and prolactin. However, with the exception of elephants, and to a lesser extent dolphins (O'BRIEN and ROBECK, 2012) and tapirs (PUKAZHENTHI et al., 2013), most wildlife species suffer undue stress if blood is routinely sampled. For this reason, non-invasive approaches based on faecal and urine analyses have been developed and are commonly used in the majority of cases (SCHWARZENBERGER, 2007). Depending on the species, different sample materials can be used to assess reproductive function and the choice of what to collect depends on a range of factors, including the type of hormone to be measured, the information desired, the availability of appropriate assays and processing techniques and the practicality of sample collection, particularly when repeated sampling over extended periods is necessary.

There are, however, situations when blood sampling is appropriate, due to the lack of other suitable possibilities, if the analyte of choice is only present in blood products, or where husbandry practices and/or animal training are at a sufficient level that venipuncture represents little additional risk or stress. For example, the reproductive status of Asian and African elephants is routinely monitored by blood progestagen analysis and numerous studies have characterized the profiles of circulating pituitary, adrenal and ovarian hormones (reviewed by HODGES, 1998; BROWN, 2000, 2008; HILDEBRANDT et al., 2006, 2011; LUEDERS et al., 2012). The availability of blood samples has undoubtedly provided insights into protein hormone patterns (e.g., LH, FSH, inhibin, prolactin, relaxin) that otherwise would not have been possible because the hormones are not excreted in an immunogenic form into urine or faeces (BROWN et al., 2010). From a comparative standpoint, elephants are a model for what can be achieved in biological studies of wildlife species. Animal training practised at a very high level has supported the elucidation of endocrine patterns of steroid and protein hormones, which when used in combination with ultrasonography has been fundamental to the development of an AI technique that is reliable despite a particularly challenging reproductive tract anatomy (HILDEBRANDT et al., 2006, 2011; BROWN, 2008).

This review compares and contrasts the reproductive endocrinology of four high profile taxa – elephants, rhinoceroses, ursid species (especially the giant panda) and felids. Of interest is the high degree of variability observed not only between these taxonomic groups, but across species within each group. We also highlight how hormone monitoring has played a key part in breeding management of these species.

### Elephant reproductive biology

There are two species of elephants managed under human care, the Asian (*Elephas maximus*) and the African savannah (*Loxodonta africana*) elephant. The ability to collect serial blood samples combined with ultrasonographic examinations, and the widespread incorporation of elephant cows in reproductive monitoring programs, has produced an outstanding set of reproductive data and identified several unique endocrine features in female and in male elephants (BROWN, 2000, 2008; HILDEBRANDT et al., 2006, 2011; LUEDERS et al., 2012). These comprehensive studies were only possible because elephant cows

can easily be trained for blood draws and transrectal ultrasound assessments. In fact, our knowledge of elephant endocrinology has no match in any other zoo animal species, making the elephant a model for endocrine studies of other wildlife species held in captivity.

In the U.S., endocrine monitoring of zoo elephants based on weekly blood samples is routine and part of the regular animal management. But not all zoos can regularly collect blood, so methods for non-invasive reproductive monitoring through urinary and faecal analyses have also been developed (NIEMULLER et al., 1993; WASSER et al., 1996; HEISTERMANN et al., 1997; FIESS et al., 1999; GUAL-SILL et al., 1999; CZEKALA et al., 2003a; BROWN et al., 2010). In Europe, urine analysis is routinely used for oestrous cycle monitoring in about half the female elephants. Urine is collected by mid-stream catch from elephants trained to urinate on command, or of enclosure floors. In contrast, faecal endocrine monitoring is not routinely used for captive elephants but has become invaluable for endocrine studies of free-ranging African elephants (WITTEMYER et al., 2007; AHLERS et al., 2012; BENAVIDES VALADES et al., 2012).

Reproductive monitoring in elephants has revealed many features (Tab. 1) that to date have only been found in elephants. The elephant has the longest spontaneous oestrous cycle of any mammal studied to date, 13–17 weeks in duration with a 4–6 week follicular phase and an 8–10 week luteal phase (see reviews HODGES, 1998; BROWN, 2000, 2008; HILDEBRANDT et al., 2006, 2011). Studies have determined that the major circulating luteal steroid in both elephant species is not progesterone, as in other mammals, but 5$\alpha$-reduced pregnanes (e.g. 5$\alpha$-DHP). Nonetheless, due to varying antibody cross-reactivities with circulating pregnanes, 'progesterone' assays are routinely used for monitoring luteal activity in elephants (HEISTERMANN et al., 1997; HODGES et al., 1997; SCHWARZENBERGER et al., 1997).

From a comparative endocrinological view, the occurrence of a 'double LH surge' during the follicular phase is most interesting. The first LH surge occurs 10–20 days after the drop in progestagens to baseline values, with the second surge occurring 19–22 days later (KAPUSTIN et al., 1996; BROWN et al., 1999). Ovulation is induced by the second LH surge at the end of the follicular phase, although the two LH surges are qualitatively and quantitatively similar. Although the surges occur in both species, comparative analyses indicate that on average LH surge concentrations are higher in Asian (5–30 ng/ml) than in African (1.5–8 ng/ml) elephants (BROWN et al., 2004a). Two waves of oestrogen production precede each LH surge (TAYA et al., 1991; KAPUSTIN et al., 1996; BROWN et al., 1999; CZEKALA et al., 2003a).

The discovery of the double LH surge was significant from a management standpoint because the first LH surge is now used to time breeding (both natural and AI) to coincide with ovulation three weeks later (BROWN, 2000, 2008; BROWN et al., 2004; HILDEBRANDT et al., 2006, 2011; HERMES et al., 2007). A unique strategy for the development of an ovulatory follicle in relation to the double LH peak, and in combination with the formation of accessory corpora lutea (acCL), has been recently identified in elephants (LUEDERS et al., 2010, 2011). Two follicular waves occur during the follicular phase, correlated with the two oestradiol surges. Each follicular wave is terminated by an LH peak. After the first LH surge, larger follicles luteinize and develop acCLs; after the second LH peak, a single ovulatory CL (ovCL) is formed in addition to the acCLs. Interestingly, progestagens start rising only after the second LH peak, even though the luteinized follicles clearly form before ovulation (HERMES et al., 2000; LUEDERS et al., 2010, 2011).

In addition to the correlation of the double LH peak with the formation of acCL, the pattern of FSH and inhibin secretion in elephant cows has been described. Inhibin is produced by granulosa cells of ovarian follicles and its main function is to suppress FSH. High FSH concentrations at the end of the luteal phase appear important for recruiting and initiating the two successive waves of follicular development, but none of the follicles that develop before the first LH surge achieve FSH independence (BROWN et al., 2004; BROWN 2006). Serum levels of inhibin gradually increase from two weeks prior to the second LH surge and ovulation and continue to rise until six weeks afterwards (BROWN et al., 1991; LUEDERS et al., 2011; KAEWMANEE et al., 2011; YAMAMOTO et al., 2012). Inhibin levels do not return to baseline until about three weeks before the end of the luteal phase. The source of inhibin during the follicular phase is the granulosa cells of large preovulatory antral follicles that luteinize as a result of the first LH surge, whereas CLs represent a major source during the luteal phase (HILDEBRANDT et al., 2011; LEUDERS et al., 2011; KAEWMANEE et al., 2011).

In the elephant, pregnancy lasts 20–22 months and can be diagnosed on the basis of elevated progestagens beyond the normal luteal phase (see reviews BROWN, 2000, 2008; HILDEBRANDT et al., 2006, 2011). Progestagens during gestation are produced by the ovulatory CL, as well as acCL that are formed prior to ovulation in response to the first LH surge (ALLEN, 2006; LUEDERS et al., 2012). Concentrations plateau during the second half of gestation. Progestagen concentrations are higher in Asian elephants carrying male calves, although this foetal sex difference is not apparent in African elephants (MEYER et al., 2004). Foetal sex determination might be possible in Asian elephants about mid gestation by measuring levels of circulating maternal

testosterone (DUER et al., 2002), as concentrations are higher in females carrying male foetuses.

Prolactin immunoreactivity increases significantly (by 20–100 fold) after about six months of gestation in both elephant species (MCNEILLY et al., 1983; BROWN et al., 1995; BROWN and LEHNHARDT, 1997; HODGES, 1998; MEYER et al., 2004). This allows for pregnancy diagnosis based on single sample analysis (BROWN, 2000; HILDEBRANDT et al., 2006), although only in blood as prolactin is not excreted in urine (BROWN et al., 2010). Besides prolactin, immunoreactive relaxin in pregnant elephants has been measured. Relaxin increases by week 20 of pregnancy and reaches peak concentrations in the second trimester, followed by a slow decline beginning approximately 30 weeks before term. A smaller, secondary relaxin rise is observed during the final eight weeks preceding parturition (NIEMULLER et al., 1998; MEYER et al., 2004).

Much of the prolactin-like immunoactivity is probably due to a placental lactogen-like product, which is likely to be important for sustaining CL activity, stimulating foetal growth and preparing the mammary glands for lactation (MEYER et al., 2004; YAMAMOTO et al., 2010). The mechanism of two LH peaks in female elephants might have evolved to ensure sufficient luteal capacity for maintaining a pregnancy that lasts 22 months (LUEDERS et al., 2010). In addition, foetal gonadal progestagens may supplement significantly the progestagens secreted by the multiple CL of pregnancy. As in equids, elephant foetal gonads undergo a phase of marked growth and enlargement during the second half of gestation, although there are no data to support the existence of a placental gonadotropin-like factor (like eCG) in pregnant elephant cows (ALLEN at al., 2006).

There is a broad range of individual variation in gestation length, making individual predictions of parturition difficult (HILDEBRANDT et al., 2006). However, progestagens generally decrease to baseline levels two to five days before parturition, providing staff with sufficient time to prepare for the birth (BROWN, 2000; HILDEBRANDT et al., 2006).

Reproductive problems and advancing age have severely reduced the number of captive female elephants that are currently considered suitable breeders. Efforts to improve the population's longterm sustainability have centred on significantly increasing reproductive output and breeding all reproductively viable elephants. In the wild, the inter-calf interval is about 4–6 years and females can have calves into their 50's. In comparison, nulliparous females in captivity are considered post-reproductive after 35 years of age because of an increased risk of dystocia and stillbirths in females from about 24 years of age (HERMES et al., 2004; HILDEBRANDT et al., 2006, 2011).

A major reproductive problem, particularly in captive African elephants, is a high rate of ovarian acyclicity. In contrast, ovarian cycle problems are not generally a concern for captive Asian elephants because most are over 35 years of age. The most recent reproductive survey found that 11% of Asian and 46% of African elephant females do not exhibit normal ovarian cycles, although some elephants can alternate between cyclic and non-cyclic periods (DOW et al., 2011). The causes of acyclicity are not completely understood, although it is unlikely that any single factor is responsible for the rates of acyclicity found in captivity (BROWN, 2008).

One apparent cause of ovarian cycle problems in African elephants (but interestingly not in Asians) is linked to hyperprolactinaemia (BROWN et al., 2004a; YAMAMOTO et al., 2010; DOW et al., 2011, 2012). Hyperprolactinaemia is associated with infertility in human women and in domestic species (ZACUR, 1999; SERRI et al., 2003; FRASOR et al., 2003). Today, 71% of acyclic African elephant females have this condition (DOW et al., 2012). Whereas over two-thirds of noncycling African elephants exhibit elevated prolactin, the other third has low, baseline levels with no cyclic fluctuations. Prolactin appears to participate in normal follicular development because concentrations increase during the follicular phase of the oestrous cycle and reach maximum levels immediately preceding ovulation (BECHERT et al., 1999; BROWN et al., 2004; PRADO-OVIEDO et al., 2013). It is under inhibitory control by hypothalamic dopamine (FREEMAN et al., 2000) and cabergoline, a dopamine agonist, is an effective drug for the treatment of hyperprolactinaemia in women (SERRI et al., 2003). However, in a clinical trial in African elephants (1–2 mg twice weekly for 4–12 months, n=8) only one of the females resumed cycling (BALL and BROWN, unpubl. data). Perhaps increasing the dosage or treatment time might be more effective but this needs to be tested.

Social influences may also be related to prolactin secretion and ovarian activity, i.e. 'stress' and behavioural - dominance status (FREEMAN et al., 2004, 2009). In African elephants it is generally the more dominant elephants that are acyclic (FREEMAN et al., 2009). In the wild, the largest, oldest female in a herd is the matriarch and is crucial to elephant survival. In captivity, dominance is still important for maintaining social harmony even if there is no true matriarch. Stable dominance hierarchies help reduce aggression and promote reproduction by allowing females to conserve energy that would otherwise be spent on establishing or maintaining rank. A consequence of the stress response may be reduced dopamine secretion and thus reduced inhibition of prolactin, or an up-regulation of other prolactin releasing factors, such as TRH (FREEMAN et al., 2000). It is hypothesized that in zoos the dominant females may expend more energy on peacekeeping of the herd, rather than on reproduction (FREEMAN et al., 2009).

From a management perspective, endocrine monitoring has been key to understanding the reproductive status of captive elephants. Profiles can be generated using serum or plasma, urine, faeces and even saliva, although not all hormones are found in all media. In the past two decades, techniques such as using the 'double LH surge' to time AI or natural breeding and daily progestagen monitoring to estimate parturition have contributed significantly to the increased reproductive success of elephants (BROWN, 2000, 2008; HILDEBRANDT et al., 2006, 2011). Although there are still gaps in our information, we have a fairly broad understanding of elephant endocrinology. In comparison to other non-domestic species kept in zoos, elephants are certainly among the best studied species of wildlife.

**Rhinoceros reproductive biology**

A taxon for which non-invasive endocrine monitoring has been an essential supportive management tool is Rhinocerotidae. The four rhinoceros species in captivity include two African (the white rhinoceros, *Ceratotherium simum*, and the black rhinoceros, *Diceros bicornis*) and two Asian species (the Indian or greater one-horned rhinoceros, *Rhinoceros unicornis*, and the Sumatran rhinoceros, *Dicerorhinus sumatrensis*). As in other taxa, the reproductive physiology is different among the four species (ROTH 2006; HERMES et al., 2007). Differences are most obvious in oestrous cycle lengths. As reviewed by ROTH (2006) and HERMES et al. (2007), progesterone profiles measured in faeces or urine, and to a lesser extent in serum and saliva, identified oestrous cycles of different lengths: 21–25 days in the Sumatran (HEISTERMANN et al., 1998; ROTH et al., 2001), about 27 days in the black (SCHWARZENBERGER et al., 1993; BERKELEY et al., 1997; BROWN et al., 2001; LANCE et al., 2001; RADCLIFFE et al., 2001; GARNIER et al., 2002), 30–35 or 65–70 days in the white (HINDLE et al.,1992; RADCLIFFE et al., 1997; SCHWARZENBERGER et al., 1998; PATTON et al., 1999; BROWN et al., 2001) and 40–48 days in the Indian rhinoceros (KASSAM and LASLEY, 1981; KASMAN et al., 1986; SCHWARZENBERGER et al., 2000; GOMEZ et al., 2004; STOOPS et al. 2004). Individual differences in length of the cycle occur in every species but are most pronounced in the Indian rhinoceros (HERMES et al., 2007).

There also may be differences in the type of ovulation. Black, white and Indian rhinos are all spontaneous ovulators, whereas ovulation in the Sumatran rhinoceros has been described as being induced by mating (ROTH et al., 2001) although one individual Sumatran studied by HEISTERMANN et al. (1998) appeared to be a spontaneous ovulator.

The types of hormones excreted in the faeces of the four species are different. Progesterone metabolites containing a 20-oxo group are present in all four species, whereas 20$\alpha$-hydroxy groups have only been identified in black and Indian rhinoceroses (SCHWARZENBERGER et al., 1993, 2000; LANCE et al., 2001). From a practical standpoint, assays using antibodies produced for progesterone analysis, but with considerable cross-reactivities against 5-reduced pregnanes, can be used for reproductive monitoring in all species. In addition, assays using antibodies for measurement of 20$\alpha$-OH can also be used for reproductive monitoring in black and Indian rhinoceroses (SCHWARZENBERGER et al., 1993, 1996, 2000). The follicles of African rhino species do not seem to produce significant quantities of oestrogens and these hormones cannot be used as reliable indicators of follicular development. In contrast, the exceptionally large follicles (about 12–14 cm in diameter; STOOPS et al., 2004) in the Indian rhinoceros produce high amounts of androgens, which act as oestrogen precursors (SCHWARZENBERGER et al., 2000; GOMEZ et al., 2004). Consequently, oestrogens as well as androgens can be measured in urinary, faecal and saliva samples and used to assess follicular development (KASSAM and LASLEY, 1981; KASMAN et al., 1986; SCHWARZENBERGER et al., 2000; GOMEZ et al., 2004; STOOPS et al., 2004).

In all rhinoceros species, the length of pregnancy ranges from 15 to 16 months and pregnancy can be diagnosed by monitoring concentrations of progesterone metabolites in faecal, urinary, plasma or saliva samples. Systemic progesterone concentrations exceed luteal phase levels by 3–5 months after mating, when levels start to increase until mid-pregnancy. The source of increasing hormone levels is the placenta (or the foeto-placental unit) and monitoring progesterone metabolite concentrations is routinely used for diagnosing pregnancy in all species of rhinoceroses kept in captivity (for review see ROTH, 2006; HERMES et al., 2007; for species-specific publications see KASMAN et al., 1986; RAMSEY et al., 1987; SCHWARZENBERGER et al., 1993, 1996, 1998, 2000; CZEKALA and CALLISON, 1996; BERKELEY et al., 1997; RADCLIFFE et al., 1997, 2000; PATTON et al., 1999; BROWN et al., 2001; LANCE et al., 2001; ROTH et al., 2001; GARNIER et al., 2002; HILDEBRANDT et al., 2007).

An important issue from a management standpoint is aggression (sometimes rather severe) between males and non-oestrus females (for review see ROTH 2006; HERMES et al., 2007). Black, Indian and Sumatran rhinoceroses are usually kept solitary, except when females are in oestrus, sometimes making breeding management challenging. Breeding success for the most social of the species, the white rhinoceros, is also suboptimal, although males and females can be kept together in groups in captivity. Only about 20% of the captive population of white rhinoceroses in captivity breed successfully. The low breeding success of captive white rhinoceroses is in

sharp contrast to the population expansions observed in this species in South Africa (OWEN-SMITH, 1988).

As in elephants, substantial progress in understanding the problems of breeding white rhinoceroses in captivity has been achieved through the combination of long-term non-invasive faecal hormone monitoring and ultrasonographic examinations (RADCLIFFE et al., 1997; HERMES et al., 2004, 2006, 2007, 2012; HILDEBRANDT et al., 2007). The 'normal' oestrous cycle of the white rhinoceros is about 35 days in length, although cycles of 70 days in length, as well as missing ovarian activity ('flatliners'), or persistent luteal activity are commonly seen (SCHWARZENBERGER et al., 1998; RADCLIFFE et al., 1997; PATTON et al., 1999; BROWN et al., 2001). Ultrasonographic examinations of reproductive organs have in most cases been performed on sedated animals, and thus not on a daily basis. Nonetheless, because results from a large number of individuals are available, the effect of long non-reproductive periods on the genital health in captive female white rhinoceroses is rather well understood (HERMES et al., 2004, 2006).

The dominant problem of long non-fertile periods in the white rhinoceros is the development of progressive genital pathology over time. In a study of 54 female white rhinoceroses, 30 individuals were found to have developed reproductive tract pathologies such as cystic endometrial hyperplasia; leiomyomas of the cervix, uterus and ovary, adenoma, para-ovarian cysts and hydromucometra (HERMES et al., 2006). The stages and the severity of the lesions were found to be an age-related consequence of long non-reproductive periods (HERMES et al., 2006). The pathophysiological mechanism is that ovulatory-sized follicles do not ovulate but become atretic or form haemorrhagic luteinized follicles, as also reported for the other species (RADCLIFFE et al., 1997, 2001; ROTH et al., 2004; STOOPS et al., 2004). The situation is particularly well understood in the white rhinoceros, in which the endocrine active and persistent but irregular luteal activity not only paves the way for the reproductive tract pathologies but also depletes the ovarian stock of available follicles and thus lowers the reproductive potential, resulting in premature senescence (HERMES et al., 2004, 2006, 2007, 2012).

In order to prevent the development of ovarian and uterine pathologies, all efforts should be undertaken to breed female white rhinos before the age of ten years (VERSTEEGE, 2012). The recommendation is to keep white rhinoceroses in groups of one male and 2–4 females. This does not guarantee successful breeding, as breeding includes a strong mate-choice component. To stimulate breeding in non-reproducing white rhinos, it is recommended to change group composition. Suggestions include transfer of males or females between groups, or temporary separation of males from the herd of females for a few months. Anecdotal reports indicate that such changes in group composition result in pregnancies, but not in all individuals, underlining the strong mate-choice component in the species.

In some white rhinoceros cows, ovarian activity diminishes to flatliner status. In a recent study, protocols for inducing ovulation using the synthetic progestin chlormadinone acetate in combination with hCG or the GnRH analogue deslorelin were found to be highly effective at inducing ovulation in white rhinoceros females, even in cows with persistent luteal activity. However, in the majority of cases oestrus induction failed to induce a lasting effect and regular, spontaneous reproductive activity was not sustained (HERMES et al., 2012). Nonetheless, protocols for assisted reproductive technologies, such as ovarian super-stimulation, oocyte recovery and *in vitro* fertilization, have been preliminarily tested in black and white rhinoceroses (HERMES et al., 2009a).

Protocols for AI have been developed and successfully applied in some white rhino females, even using frozen and thawed semen (HILDEBRANDT et al., 2007; HERMES et al., 2009b). However, in contrast to AI in elephants, the success rate is low. There are several reasons why AI cannot be utilized to its full potential: 1) laboratory-based indicators of impending ovulation are not as clear as the double LH surge in elephants or the oestrogen peak in the giant panda; 2) because of the strong mate-choice component, oestrus behaviour is not always clearly recognisable; and 3) further complicating the situation, AI requires full anaesthesia of the cow and thus can only be performed once per oestrus period.

## Ursid reproductive biology

There are eight extant species of Ursidae: polar bear – *Ursus maritimus*, brown bear – *Ursus arctos*, American black bear – *Ursus americanus*, Asiatic black bear – *Ursus thibetanus*, sloth bear – *Ursus ursinus*, sun bear – *Helarctos malayanus*, giant panda – *Ailuropoda melanoleuca* and spectacled bear – *Tremarctos ornatus*. Comprehensive endocrine data are available for four species in the genus Ursus, for the giant panda and the sun bears. The sloth bear and the spectacled bear are relatively little-studied (DEHNHARD et al., 2006; SPADY et al., 2007).

The first endocrine studies in species of the genus Ursus were generated from combined results of single blood samples collected from anaesthetized free-ranging individual animals (MCMILLIN et al., 1976; FORESMAN and DANIEL, 1983; PALMER et al., 1988). These studies revealed long-term endocrine changes such as the annual testosterone rhythm in males and progesterone profiles in pregnant and non-pregnant female American black bears and polar bears. Continuing

endocrine studies of individual animals only became possible after the development of non-invasive hormone monitoring. The giant panda was one of the first species to be studied (BONNEY et al., 1982; KLEIMAN 1983; CHAUDHURI et al., 1988; MONFORT et al., 1989). Similarly, results of non-invasive endocrine studies have considerably advanced our knowledge of the reproductive physiology of the non-seasonally breeding sun bear (SCHWARZENBERGER et al., 2004; FREDERICK et al., 2010). Recently, longitudinal faecal hormone analysis has been described for monitoring reproductive activity of female Hokkaido brown bears (ISHIKAWA et al., 2003) and of polar bears (STOOPS et al., 2012).

The well characterized endocrine profile of the giant panda, as revealed by studies in the 1980's, has served as a model for other bear species. The monoovulatory physiology of the giant panda has been generalized to other ursine species (SPADY et al., 2007). However, as in the other taxa discussed in this review, the reproductive biology is fairly diverse among the different species of bears (GARSHELIS, 2004; SPADY et al., 2007). Birth and oestrus dates are seasonally restricted in seven of the eight bear species. Embryonic diapause occurs in these seasonally breeding species and leads to variable gestation length. The outlier is the sun bear, as females of both captive and wild populations exhibit regular oestrous cycles and give birth year-round (GARSHELIS, 2004; SCHWARZENBERGER et al., 2004; SPADY et al., 2007; FREDERICK et al., 2010).

Bears are often considered to be induced ovulators (SPADY et al., 2007), although at least for the non-seasonal sun bear this assumption cannot be generalized. Induced ovulation was definitively confirmed through endoscopic examinations of the corpus luteum in American black bears (BOONE et al., 2004) and by ovarioectomy in the Japanese subspecies of the Asiatic black bear (OKANO et al., 2006). Yet, as in other carnivores, especially in captivity and in proximity to males or females, bears occasionally exhibit spontaneous ovulation and pseudopregnancy, even without copulation (SATO et al., 2001; OKANO et al., 2006).

The mating systems of bears in the genus Ursus can be characterized as promiscuous. Bears in the wild have been observed to copulate numerous times and with numerous partners, presumably to induce ovulation. Males may mate with multiple females. Likewise, females frequently mate with more than one male (GARSHELIS, 2004; JOSHI et al., 2006; BELLEMAIN et al., 2006; SPADY et al., 2007; STEYAERT et al., 2012). Our understanding of the ovarian dynamics of bears owes more to observational studies of free-ranging individuals, partially fitted with telemetry devices and more recently in combination with genetic studies, than to endocrine studies of captive animals.

Brown bears in Scandinavia have been particularly intensively studied during the past decade (for details see STEYAERT et al., 2012). Brown bear females are seasonally polyoestrous, with a mating season of approximately 2.5 months from late spring to early summer (GARSHELIS, 2004; SPADY et al., 2007; STEYAERT et al., 2012). Following induced ovulation, each corpus luteum becomes dormant, allowing females to re-enter oestrus after conception. This facilitates fertilization by multiple males and because of embryonic diapause the cumulative embryos all implant at the same time and comprise a single litter. In the Scandinavian brown bear population, 51% of free-living females engage in more than one male-female association per breeding season (STEYAERT et al., 2012). Polyoestrous cycling enables the development of multiple paternity, i.e. offspring in Scandinavian brown bears were found to be sired by different fathers in 14.5 and 28% of the litters with >2 and >3 young, respectively (BELLEMAIN et al., 2006). Multiple paternity of litters has also been documented by genetic studies in wild American black bears (ONORATO et al., 2004) and wild polar bears (ZEYL et al., 2009). Furthermore, dual paternities have been observed in captive breeding programs of the giant panda (HUANG et al., 2012).

The highly endangered giant panda is amongst the best studied species of bear and represents a good example of how biological information generated through research and applied to captive management has benefited population expansion, especially in the Chinese breeding centres for the species (CZEKALA et al., 2003; WILDT et al., 2006; SWAISGOOD et al., 2010). Improvements in captive management focusing on behavioural well-being, nutrition and medical management (particularly neonatal care and successful hand-raising of abandoned cubs) have certainly been key to improving the success of captive breeding (WILDT et al., 2006). Nonetheless, most important for the considerable growths in captive populations during the past two decades has been research into the species' reproductive biology and the application of the knowledge to the reproductive management of the giant panda. Although most information on the reproductive physiology of pandas in the 1980's and 1990's stemmed from only a few captive animals in North America and Europe, research on these 'single animals' contributed significantly to our overall understanding of the species' biology (BONNEY et al., 1982; KLEIMAN 1983; CHAUDHURI et al., 1988; MONFORT et al., 1989). The transfer of urinary hormone-monitoring technology to the giant panda breeding centres in China began in the early 1990's and since then the populations have grown significantly (CZEKALA et al., 2003).

The giant panda is sexually receptive for only two to three days once per year. Oestrogen metabolites rise

gradually from baseline to peak excretion over one to two weeks and then decline precipitously at the presumed time of ovulation (CHAUDHURI et al., 1988; MONFORT et al., 1989; CZEKALA et al., 2003). The peak in this hormone is an excellent marker of impending ovulation. Nowadays, for most female giant pandas kept in captivity the timing of natural mating and AI is supported by non-invasive urinary oestrogen metabolite analysis, in combination with behavioural mate introduction protocols (LINDBURG et al., 2001; MCGEEHAN et al., 2002; CZEKALA et al., 2003; SWAISGOOD et al., 2003; ZHANG et al., 2004; STEINMAN et al., 2006; WILDT et al., 2006; HUANG et al., 2012). Additional techniques have been developed for the detection of oestrus in the giant panda, such as vaginal cytology and detection of LH in urine (DURRANT et al., 2003, 2006), or the measurement of the oestrogen peak in faecal samples (KERSEY et al., 2010; YU et al., 2011) but have not yet gained the importance of monitoring oestrogens in urine samples, mainly because urine analysis is rather easy to perform.

Most pregnancies in giant pandas result from natural mating, for which compatible female and male pairings are timed on the basis of oestrogen analysis (CZEKALA et al., 2003). However, many female giant pandas do not breed naturally and so AI is used. Both fresh and frozen thawed semen has been used, though AI is not always successful. In a recent study, HUANG et al. (2012) confirmed that the best timing for mating (and AI) can be found in relation to the urinary oestrogen surge. Most pregnancies (and cubs) were produced from mating within one day before or after this surge. As the first mating close to the oestrogen surge was natural copulation followed by AI on the next day, most cubs were sired by the first male mating. Twins occurred in about 60%, and dual paternities were observed in 25% of twin sets (HUANG et al., 2012).

Most of the breeding females in the Chinese breeding centres are managed for maximum breeding output to produce offspring from the same females at yearly intervals. This is achieved by early weaning and hand-raising of cubs (CZEKALA et al., 2003; WILDT et al., 2006). The practice of removing cubs from their dam within a year of birth interrupts lactational anoestrus and thus triggers follicle development. Nonetheless, the population increase has a downside as not all males are reproducing. Paternity analysis has shown that most offspring at the breeding centres are sired by only a few naturally breeding males, leading to genetic overrepresentation of certain individuals in captivity (CZEKALA et al., 2003; WILDT et al., 2006).

In a study in free-ranging giant pandas in the Qinling Mountains, China, reproductive parameters for the wild population fell within the range of values for captive pandas (ZHU et al., 2001). Copulation dates for the free-ranging females clustered within a 30-day period from early March. Despite the 30-day variability in mating dates, the dates of birth were tightly grouped within a 10-day period in the second half of August, resulting in an average gestation length of about 146 days (range 128–161 d). Comparable to species in the genus Ursus, gestation in the giant panda is characterized by delayed implantation and variability in the early phase of pregnancy results in the synchronization of births (SPADY et al., 2007).

The endocrine profile of urinary immune-reactive pregnanediol in pregnant giant pandas is characterized by a slight increase after the oestrogen peak. Concentrations are low during the period of delayed implantation (primary progesterone phase) and only increase for the period of about six weeks prior to parturition (secondary progesterone phase) (CHAUDHURI et al., 1988; MONFORT et al., 1989; CZEKALA et al., 2003; STEINMAN et al., 2006; SPADY et al., 2007; KERSEY et al., 2010). Because urinary progestagen profiles cannot be used to differentiate pregnant from pseudo-pregnant females, diagnosis of pregnancy based on endocrine profiles is not feasible. However, WILLIS et al. (2011) recently reported that the activity of the acute phase protein ceruloplasmin in the urine of giant pandas increases in response to pregnancy. Levels of active urinary ceruloplasmin were elevated from the first week of pregnancy until 20–24 days prior to parturition, while no increase was observed during the luteal phase in known pseudopregnancies (WILLIS et al., 2011).

## Felid reproductive biology

Studying the reproductive biology of female felid species has been possible largely through the development of non-invasive faecal steroid metabolite analysis, which is currently the method of choice for monitoring endocrine function in these species. Early radiometabolism studies in the domestic cat revealed that 85–95% of metabolites are excreted in faeces within 1–2 days (SHILLE et al., 1990; BROWN et al., 1994). Using faecal steroid analysis, it is now well recognized that a range of endocrine patterns exists among the Felidae, with many traits and mechanisms being uncommon, if not unique (for review see BROWN, 2006, 2011).

Reproductive ovarian steroid cycle patterns have been published for about half of the non-domestic species of felids. Oestrous cycles are quite variable among species and range from about one to four weeks, with the shortest found in cheetahs and the longest in lions and clouded leopards. In general, oestrus lasts about 3–10 days. Cats have historically been categorized as induced ovulators; i.e. as requiring mating to stimulate ovulation. However, felids exhibit a range of ovulatory patterns from almost exclusively induced to various combinations of

induced and spontaneous. The differences occur not only across species but also between individuals within a species. Indeed, spontaneous increases in progestagens after oestrogen surges (indicating spontaneous ovulation) are non-existent or rare in the tiger, puma, snow leopard, cheetah, tigrina, ocelot and lynx but occur, at least occasionally, in the lion, leopard, Pallas's cat and fishing cat and regularly in the clouded leopard, margay and domestic cat. Thus, within this taxon ovulatory mechanisms vary, regulated to a certain degree by species and likely due to specific responses to physical and/or psychosocial stimuli (for review see BROWN, 2006, 2011).

In several felid species, follicular activity is influenced by season. The domestic cat is seasonally polyoestrous under natural photoperiods but will cycle year round when housed in a 12–14 h light cycle (SHILLE et al., 1979). Reproduction is at least somewhat seasonal in many non-domestic felids such as the tiger, clouded leopard, Pallas's cat, lynx and snow leopard (see reviews BROWN, 2006, 2011). Ovarian activity in these species is reduced under decreasing photoperiod and resumes after exposure to increasing light (i.e. the species are long-day breeders). Clouded leopards have a short anoestrus period in the late Fall but if housed indoors with continuous exposure to 12 h of artificial light per day they will cycle year round (BROWN et al., 1995; WIELEBNOWSKI et al., 2002a). Pallas's cats are highly seasonal and females exhibit ovarian activity for only ~3 months of the year (Jan–Mar) (BROWN et al., 2002). However, sudden transitions to 'long days' can stimulate premature follicular steroidogenesis: the Pallas' cats that were exposed to a month-long light event in November exhibited an early increase in faecal oestrogens (BROWN et al., 2002).

Melatonin probably regulates photoperiod-induced seasonality in the cat and concentrations are highest during the dark phase (LEYVA et al., 1989). Follicular development and oestrous cyclicity has been effectively suppressed in domestic cats by oral melatonin administered several hours before lights-off during a stimulatory photoperiod; i.e. during a regimen that mimics short-day melatonin secretion (GRAHAM et al., 2004). In contrast, follicular activity in lions, leopards, bobcats, pumas, margays, ocelots, tigrinas, jaguars and fishing cats appears not to be significantly influenced by season (BROWN, 2006, 2011).

There are also variable periods of follicular inactivity not associated with season (e.g. in cheetah, ocelot, fishing cat, lion and tiger). In each of these species, cycles have been observed in every month of the year, but for many individuals not continuously (BROWN, 2006, 2011). In cheetahs, periods of ovarian acyclicity have been associated with social suppression. A study evaluating behaviour and faecal oestrogen patterns in paired cheetahs found that although serious altercations were rare, oestrogen levels were lower than in single females, with subordinate females being more suppressed than dominants. Separation of the pairs resulted in reinitiating normal ovarian steroidogenic activity (WIELEBNOWSKI et al., 2002b). The cheetah husbandry manual now recommends housing breeding females separately. Furthermore the breeding management of this species is based on transporting cheetah between zoos. Although a strong mate-choice component is included, follicular development is usually induced and successful breeding occurs within a few days of the arrival of a transferred animal in another facility.

During pregnant and nonpregnant luteal phases, the concentrations of circulating and excreted progestagens are quantitatively similar in non-domestic and domestic cats, with the length of the nonpregnant luteal phase being about one third to one half of the length of pregnancy (WILDT et al., 1988). Comparable results were found in other felid species (BROWN, 2006, 2011). Thus, from a reproductive management standpoint it is difficult to diagnose pregnancy based solely on the continued elevation of faecal progestagen concentrations past the normal length of a nonpregnant luteal phase.

Measurement of prolactin and relaxin can be used diagnostically after mid-gestation, but generally only if blood samples are collected (BANKS et al., 1983; STEWART and STABENFELDT, 1985). Because of the limited possibility for collecting blood samples in non-sedated wild felid species, prolactin and relaxin measurements have not been established on a routine basis, although in one preliminary study relaxin was detected in the urine 28 days after mating in domestic cats (DE HAAS VAN DORSSER et al., 2007).

A practical method for the non-invasive diagnosis of pregnancy was described recently by measuring urinary and faecal prostaglandin metabolites (PGFM). The levels of these hormones increased during the last trimester of pregnancy in seven of the eight main lineages of Felidae and thus represent suitable indicators for the diagnosis of pregnancy (FINKENWIRTH et al., 2010; DEHNHARD and JEWGENOW, 2013).

One of the most important uses of hormone monitoring is to assess ovarian responses to ovulation induction and assisted reproductive procedures. Successful ovarian stimulation for AI and IVF/ET has been achieved in at least one-third of all cat species, although results are mixed and pregnancy success rates are below 20% for most species (PELICAN et al., 2006; HOWARD and WILDT, 2009). The primary problem is the variable responses and sensitivities to gonadotrophin treatment, especially in species that exhibit spontaneous ovulations. Gonadotrophins, eCG and hCG, are typically used to stimulate follicular development and induce ovulation. Most females ovulate 37–42 hours after hCG and AI can

only succeed if inseminations are conducted post-ovulation due to the interfering effects of anaesthesia (HOWARD et al., 1992). Most nondomestic felids do not exhibit clear signs of behavioural oestrus, so ovulation induction and AI are conducted at random with respect to the stage of the ovarian cycle.

It is not possible to extrapolate gonadotrophin doses from one species to another based on body size only. For some species, despite decades of work, dose combinations that do not over- or understimulate the ovaries have not been identified (GRAHAM et al., 2006; PELICAN et al., 2006; HOWARD and WILDT, 2009). Furthermore, current gonadotropin regimens appear to perturb normal female reproductive function and reduce fertility through ovarian hyperstimulation, resulting in ancillary follicle development and concentrations of oestrogen that are several-fold higher than those observed during natural oestrus (SWANSON et al., 1997; ROTH et al., 1997; PUKAZHENTHI and WILDT, 2004; PELICAN et al., 2006).

One promising approach to develop better hormonal protocols that will result in more predictable ovarian responses in felids relies on the use of synthetic progestins or GnRH analogues to suppress ovarian function temporarily prior to gonadotrophin stimulation (HOWARD and WILDT, 2009). A suppressed, quiescent ovary might be more consistently responsive to eCG/hCG because there would be few active follicles and no luteal tissue producing endogenous steroids to disrupt exogenous gonadotropin action (PELICAN et al., 2006; STEWART et al., 2010). The results of these approaches have been reviewed by HOWARD and WILDT (2009). Presenting a proof of principle for more endangered lion species such as the Asian lion, such an approach was recently tested in four African lionesses. Non-surgical AI was performed after downregulation of ovarian activity with etonogestrel (Implanon®) and ovarian stimulation with porcine FSH and pLH, allowing the recovery of uterine-stage embryos for cryopreservation (GÖRITZ et al., 2012). Further studies are needed to determine whether such protocols can result in the production of live offspring in further felid species. No doubt, faecal steroid monitoring will be key to assessing the efficacy of these hormonal therapies and for optimizing assisted reproductive protocols on a species-by-species basis (HOWARD and WILDT (2009).

## Conclusions

Based on endocrine studies, largely using non-invasive hormone monitoring, it is clear that different taxa of wildlife express marked variations in reproductive mechanisms (Tab. 1), even between individuals of a species. Endocrine monitoring has enormous potential for application in wildlife conservation and has implications for how we manage wildlife species both *in situ* and *ex situ*. The ability to assess endocrine activity easily and safely has greatly improved our understanding of the factors affecting reproductive activity. For example, endocrine monitoring allows more effective determination of how social and environmental cues modulate reproductive success. Determining oestrous cycles, the type of ovulation (induced versus spontaneous) and the effects of seasonality as well as diagnosing pregnancy has proven vital in managing natural and assisted breeding efforts. There is little doubt that the eventual practical application of assisted reproductive technology will largely depend on the data generated through hormone monitoring. A high priority is the development of protocols for timing ovulation or for the induction of consistent responses to ovulation induction protocols, providing an optimal maternal environment for fertilization and embryo development. There is also a need to control the reproductive cycle, including down-regulating endogenous ovarian activity (i.e. contraception, or down-regulating endocrine function in the case of hormone-responsive reproductive tract pathologies). For all of these reasons, we strongly advocate the wide-scale application of endocrine monitoring as one of the most important tools available in the wildlife life sciences.

**Tab. 1:** Characteristic reproductive traits of the taxa described in this review

| Taxon | Most relevant material for routine endocrine monitoring | Distinguishing reproductive features | How endocrine monitoring is used to support breeding management |
|---|---|---|---|
| **Elephantidae**: Asian elephant (*Elephas maximus*) African savannah elephant (*Loxodonta africana*) | Blood (primarily in the U.S.)<br><br>Urine (primarily in Europe) | Longest mammalian oestrous cycle of four months, longest gestation of 20–22 months<br>Principal hormones in the blood are 5$\alpha$-reduced pregnanes, not progesterone<br>Multiple ovulatory and anovulatory CL are produced per cycle, though the species is monovulatory<br>Follicular phase consists of two LH surges, ~ three weeks apart<br>Higher percentage of noncycling females (often related to hyperprolactinaemia) in African than in Asian elephants<br>Blood samples are commonly available, so elephants are among the best studied wildlife species | Determine age at puberty in males and females, which occurs earlier in zoos than in the wild<br>Determine reproductively viable females using weekly or bi-weekly progestagen monitoring<br>Timing of breeding is largely based on monitoring LH in daily blood samples<br>Highly efficient AI protocols, despite difficult reproductive tract anatomy<br>Diagnosis of pregnancy based on longitudinal progestagens (blood or urine), or single sample prolactin or relaxin analysis (blood only)<br>Assess responses to treatments for ovarian cycle problems |
| **Rhinocerotidae:** White rhinoceros (*Ceratotherium simum*) Black rhinoceros (*Diceros bicornis*) Indian rhinoceros (*Rhinoceros unicornis*) Sumatran rhinoceros (*Dicerorhinus sumatrensis*) | Faeces | Extensive species differences, i.e. oestrous cycle length, steroid hormone metabolism<br>Possible induced ovulation in the Sumatran rhinoceros<br>Gestation length is ~16 months<br>Solitary species, except for the white rhinoceros<br>Challenge of breeding white rhinos because of ovarian cycle abnormalities and reproductive tract pathologies<br>Prolonged oestrous cycles, especially in white rhinoceroses (and to a lesser extent in black rhinoceroses) | Breeding management largely based on behavioural observations<br>Faecal endocrine monitoring routinely used to investigate reproduction and reproductive problems; monitoring often accomplished by rectal ultrasonographic examinations<br>Protocols established for AI and for inducing ovulation in white rhinoceroses<br>Limitations of AI largely due to difficulties in timing ovulation<br>Diagnosis of pregnancy based on longitudinal faecal progestagen analysis |
| **Ursidae**: Eight species | Urine | Extensive species differences, e.g. the giant panda is seasonally mono-ovulatory; bears in the genus *Ursus* are seasonally polyoestrous, whereas the sun bear is a non-seasonally breeding species<br>Embryonic diapause of variable length in the seven seasonal breeding species<br>Multiple paternity identified by genetic studies in wild brown bears, American black bears and polar bears; dual paternities observed in captive breeding programs of the giant panda<br>Twin pregnancies in the giant panda, although under natural conditions only one offspring is raised by the dam | Endocrine monitoring particularly important for breeding management of the giant panda<br>Giant panda breeding management (natural breeding and AI) largely based on urinary oestrogen monitoring to time ovulation<br>Ovarian steroid profiles indistinguishable between pregnant and pseudopregnant females<br>In the giant panda, urinary excretory pattern of the acute phase protein, ceruloplasmin, differs between pregnant and nonpregnant females |
| **Felidae:** 41 species | Faeces | Extensive species differences in oestrous cycle length, seasonality and spontaneous vs induced ovulation<br>Many females exhibit irregular or no ovarian cycle activity<br>Limited success of AI or IVF in many species | Determination of puberty and gonadal activity of males and females by faecal steroid analysis<br>Monitoring responses to management or husbandry changes in effort to stimulate reproductive activity<br>Difficulties in developing appropriate ovarian stimulation protocols – not possible to extrapolate gonadotrophin doses from one species to another based on body size only<br>Diagnosis of pregnancy possible by measuring prolactin or relaxin in blood samples and by measuring prostaglandins in urine or faeces |

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