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The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species

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During the past two decades, techniques for faecal steroid analysis have been developed and have been used for research with mammalian, bird and, to a lesser extent, reptile, amphibian and fish species. Various techniques for the analysis of reproductive (oestrogen, androgen, progestagen) and adrenocortical (glucocorticoids) steroid hormones have been established and have been applied to a wide range of research questions studying captive and free-ranging wildlife, as well as domestic and laboratory species. Because of species-specific differences in steroid metabolism in even closely related species, careful validation of assay methods is necessary in order to generate meaningful and accurate results. For future research and management of free-ranging and captive wildlife, the great potential of non-invasive endocrine monitoring will be utilized more than ever. In light of this, captive wildlife species are ideal research subjects, as longitudinal sample collection is possible and studies connecting physiology, endocrinology, reproduction and stress with various social and/or environmental factors can be carried out and can be analysed to determine how they impact animal health.

Key-words: adrenal steroid hormones; assay validation; biodiversity conservation; excreted steroids; faecal steroid analysis; non-invasive; reproductive steroid hormones; steroid metabolism.

INTRODUCTION

During the past two decades, techniques for faecal steroid analysis (oestrogen, androgen, progestagen and glucocorticoid metabolites) have been developed and have been used for research with mammals (for references, see Table 1), birds (i.e. Baltic *et al.*, 2005; Goymann, 2005; Möstl *et al.*, 2005; Palme *et al.*, 2005; Wasser & Hunt, 2005; Szoke *et al.*, 2006) and, to a lesser extent, reptile (i.e. Atkins *et al.*, 2002; Rittenhouse *et al.*, 2005), amphibian (Szymanski *et al.*, 2006) and fish (i.e. Turner *et al.*, 2003; Ellis *et al.*, 2004; Wysocki *et al.*, 2006) species. Techniques have been applied to a wide range of research questions studying captive and free-ranging wildlife, as well as domestic and laboratory species. These techniques are now widely accepted and several reviews focusing on steroid metabolism and on the validation of faecal steroid assays have been published recently (Schwarzenberger *et al.*, 1996, 1997; Whitten *et al.*, 1998; Möstl & Palme, 2002; von der Ohe & Servheen, 2002; Monfort, 2003; Wielebnowski, 2003; Buchanan & Goldsmith, 2004; Graham, 2004; Millspaugh & Washburn, 2004; Möstl *et al.*, 2005; Palme, 2005; Palme *et al.*, 2005; Touma & Palme, 2005; Ziegler & Wittwer, 2005; Keay *et al.*, 2006; Lane, 2006). In addition, reviews emphasizing the importance of faecal steroid analysis for the elucidation of endocrine physiology in certain taxa (carnivores: Young, K. M. *et al.*, 2004; Brown, 2006; primates: Shimizu, 2005; rhinoceros: Roth, 2006), and as an important tool for the management and conservation of wildlife (Pukazhenthi & Wildt, 2004; Cockrem, 2005; Andrabi & Maxwell, 2007), or as reliable indicators of stress (Wasser *et al.*, 2000; Möstl & Palme, 2002; Millspaugh & Washburn, 2004; Queyras & Carosi, 2004; Touma & Palme, 2005; Keay *et al.*, 2006; Lane, 2006) have been published.

The aim of this review is briefly to summarize studies using faecal steroid analysis in captive and free-ranging mammalian wildlife species. The focus is on recently published literature summarizing reproductive (oestrogens, androgens and progestagens)

TAXA/SPECIES	APPLICATION (WHAT HAS BEEN STUDIED?)	CAPTIVE/ FREE-RANGING (WILD), ♂/♀	HORMONES DETERMINED IN FAECES	REFERENCES
PRIMATES				
HOMINIDAE				
Chimpanzee *Pan troglodytes*	oestrous cycle, sexual swelling	captive, ♀	E, P	Emery & Whitten (2003)
	dominance rank, challenge hypothesis	wild, ♂	A	Muehlenbein *et al.* (2004)
	immune endocrine interactions, intestinal parasites	wild, ♂ & ♀	A, GC	Muehlenbein (2006)
	social rehabilitation of former laboratory chimpanzees, animal welfare	captive, ♂	GC	Reimers *et al.* (2007)
Lowland gorilla *Gorilla gorilla*	reproductive senescence; oestrous cycle, sexual behaviour	captive, ♀	E, P	Atsalis *et al.* (2004)
HYLOBATIDAE				
White-handed gibbon *Hylobates lar*	oestrous cycle, sexual swelling, ovulation, paternity confusion	wild, ♀	E, P	Barelli *et al.* (2007)
OLD WORLD MONKEYS				
CERCOPITHECIDAE				
Baboon *Papio cynocephalus*	peripartum physiology (life-history stages)	wild, ♀	E, A, P, GC	Altmann *et al.* (2004)
	sexual maturation (infancy, puberty); oestrous cycle, sexual swelling and consortship	wild, ♂ & ♀	E, A, P, GC	Gesquiere *et al.* (2005, 2007)
	pregnancy, foetal loss	wild, ♀	E, P, GC	Beehner *et al.* (2006)
Chacma baboon *Papio hamadryas ursinus*	seasonal reproduction and environmental stressors	wild, ♂ & ♀	GC	Weingrill *et al.* (2004)
	♂ hierarchy, immigrant ♂, aggression, stress	wild, ♂	GC	Bergman *et al.* (2005)
Hanuman langur *Semnopithecus entellus*	oestrous cycle, concealed ovulation, paternity confusion	wild, ♀	P	Heistermann *et al.* (2001)
Japanese macaque *Macaca fuscata*	oestrous cycle, conception, paired urine and faecal samples	captive & wild, ♀	E, P	Fujita *et al.* (2001)
	social rank, aggression, stress	wild, ♂	A, GC	Barrett *et al.* (2002)
	peripartum physiology (maternal style)	captive, ♀	E, P	Bardi *et al.* (2003)
	reproductive behaviour	semi-wild, ♀	E, P	O'Neill *et al.* (2004)
Long-tailed macaque *Macaca fascicularis*	oestrous cycle, sexual swelling, post-conception mating, paternity confusion	wild, ♀	E, P	Engelhardt *et al.* (2007)

Table 1. Continued

TAXA/SPECIES	APPLICATION (WHAT HAS BEEN STUDIED?)	CAPTIVE/ FREE-RANGING (WILD), ♂/♀	HORMONES DETERMINED IN FAECES	REFERENCES
Red colobus *Piliocolobus badius*	food availability, parasite infections, adrenal activity	wild, ♂ & ♀	GC	Chapman *et al.* (2006)
Red-shanked douc langur *Pygathrix nemaeus*	oestrous cycle, change in group composition	captive, ♀	E, P, GC	Heistermann *et al.* (2004)
Rhesus macaque *Macaca mulatta*	peripartum physiology (maternal style)	captive, ♀	E, P	Bardi *et al.* (2003)
Stump-tailed macaque *Macaca arctoides*	♂ reproductive behaviour in response to vaginal secretions	captive, ♀	E	Cerda-Molina *et al.* (2006)
NEW WORLD MONKEYS				
ATELIDAE				
Black-handed spider monkey *Ateles geoffroyi*	oestrous cycles, foetal loss; paired urine and faecal samples	captive & wild, ♀	E, P	Campbell *et al.* (2001)
	artificial insemination, vaginal cytology	captive, ♀	E, P	Hernández-López *et al.* (2007)
Mantled howler monkey *Alouatta palliata*	challenge hypothesis, aggression, mating system	wild, ♂	A	Cristobal-Azkarate *et al.* (2006)
Muriqui monkey *Brachyteles arachnoides*	seasonality, reproductive behaviour, oestrous cycle	wild, ♂ & ♀	E, A, P, GC	Strier *et al.* (2003)
CEBIDAE				
Common marmoset *Callithrix jacchus*	reproductive suppression, parent–daughter relations, oestrous cycle	captive, ♀	E, P, GC	Ziegler & Sousa (2002)
Golden lion tamarin *Leontopithecus rosalia*	social rank, challenge hypothesis	wild, ♂	A, GC	Bales *et al.* (2006)
Tufted capuchin *Cebus apella nigritus*	seasonality, reproductive behaviour, aggression	wild, ♂	A, GC	Lynch *et al.* (2002)
White-faced capuchin *Cebus capucinus*	reproductive behaviour, oestrous cycles, concealed ovulation, paternity confusion	wild, ♀	E, P	Carnegie *et al.* (2005)
LEMURIFORMES				
INDRIIDAE				
Verreaux's sifaka *Propithecus verreauxi*	social rank, sexual behaviour, seasonality	wild, ♂	A	Brockman *et al.* (2001)

LEMURIDAE				
Red-fronted lemurs *Eulemur fulvus rufus*	seasonality, aggression, challenge hypothesis pregnancy, foetal sex determination	wild, ♂ & ♀	E, A, P	Ostner *et al*. (2002), Ostner & Heistermann (2003)
Ring-tailed lemurs *Lemur catta*	social rank, sexual behaviour, aggression, seasonality	wild, ♂ & ♀	GC	Cavigelli *et al*. (2003), Gould *et al*. (2005)
	adrenal activity as predictor of mortality	wild, ♂ & ♀	GC	Pride (2005)
MARSUPIALS				
DASYURIDAE				
Chuditch *Dasyurus geoffroii*	oestrous cycle, vaginal cytology	captive, ♀	E	Stead-Richardson *et al*. (2001)
MACROPODIDAE				
Tammar wallaby *Macropus eugenii*	animal well-being, immune function, paired plasma and faecal samples	captive, ♀	GC	McKenzie & Deane (2005)
PETAURIDAE				
Squirrel glider *Petaurus norfolcensis*	oestrous cycle, pregnancy, seasonality, urinary cytology	captive, ♀	E, P	Woodd *et al*. (2006)
TACHYGLOSSIDAE				
Short-beaked echidna *Tachyglossus aculeatus*	sex identification, oestradiol:androgen ratio; hCG stimulation, seasonality, sperm production	captive, ♂ & ♀	E, A	Oates *et al*. (2002), Johnston *et al*. (2007)
TARSIPEDIDAE				
Honey possum *Tarsipes rostratus*	radiometabolism study, oestrous cycle, embryonic diapause, seasonality, reproductive stress	captive, ♂ & ♀	E, P, GC	Bradshaw *et al*. (2004), Oates *et al*. (2004, 2007)
VOMBATIDAE				
Common wombat *Vombatus ursinus* Southern hairy-nosed wombat *Lasiorhinus latifrons*	oestrous cycle, seasonality, sexual behaviour, paired blood and faecal samples	captive, ♀	P	Paris *et al*. (2002)
PILOSA				
BRADYPODIDAE				
Three-toed sloth *Bradypus variegatus*	oestrous cycle, pregnancy, paired blood–faecal samples	captive, ♀	E, P	Mühlbauer *et al*. (2006)
MYRMECOPHAGIDAE				
Giant anteater *Myrmecophaga tridactyla*	oestrous cycle, pregnancy, post-partum period, aging ♀	captive, ♀	E, P	Patzl *et al*. (1998)

Table 1. Continued

TAXA/SPECIES	APPLICATION (WHAT HAS BEEN STUDIED?)	CAPTIVE/ FREE-RANGING (WILD), ♂/♀	HORMONES DETERMINED IN FAECES	REFERENCES
RODENTIA				
CHINCHILLIDAE				
Chinchilla *Chinchilla lanigera*	radiometabolism study, pregnancy, seasonality	captive, ♀	E, P	Busso *et al*. (2007)
CRICETIDAE				
Syrian hamster *Mesocricetus auratus*	oestrous cycle, superovulation	captive, ♀	E, P	Chelini *et al*. (2005)
ERETHIZONTIDAE				
Common porcupine *Erethizon dorsatum*	oestrous cycle, pregnancy, pseudopregnancy	captive, ♀	E, P	Bogdan & Monfort (2001)
MURIDAE				
Midday gerbil *Meriones meridianus*	population density, habitat type and ecological indices, adrenal activity	wild, ♂ & ♀	GC	Kuznetsov *et al*. (2004)
SCIURIDAE				
Cape ground squirrel *Xerus inauris*	pregnancy, assay validation	captive, ♀	E, P	Pettitt *et al*. (2007)
LAGOMORPHA				
LEPORIDAE				
European rabbit *Oryctolagus cuniculus*	physiological stress response, odour and presence of a predator, ACTH challenge	captive, ♂ & ♀	GC	Monclus *et al*. (2006), Cabezas *et al*. (2007)
	long-term stress and fitness, body condition, survival probability of wild animals put into captivity	wild, ♂ & ♀		
SIRENIA				
DUGONGIDAE				
Dugong *Dugong dugon*	assessment of reproductive status	wild, ♂ & ♀	E, A	Lanyon *et al*. (2005)
CETACEA				
BALAENIDAE				
North Atlantic right whale *Eubalaena glacialis*	adrenal activity, reproductive rank	wild, ♂ & ♀	E, P, A, GC	Rolland *et al*. (2005), Hunt *et al*. (2006)

PROBOSCIDEA				
ELEPHANTIDAE				
African elephant *Loxodonta africana*	oestrous cycle, pregnancy; paired faecal and urine samples	captive, ♀	E, P	Fiess *et al*. (1999)
	musth, intensity, duration, behaviour, sexual activity	captive & wild, ♂	A, GC	Ganswindt, Heistermann & Hodges (2005), Ganswindt, Rasmussen *et al*. (2005)
	ecological conditions (vegetation) and reproduction	wild, ♀	P	Wittemyer *et al*. (2007)
PERISSODACTYLA				
EQUIDAE				
Grevy's zebra *Equus grevyi*	oestrous cycle, pregnancy, abortions	captive, ♀	E, P	Asa *et al*. (2001)
Przewalski's horse *Equus ferus przewalskii*	oestrous cycle, pregnancy, sexual behaviour	semi-wild, ♀	P	Scheibe *et al*. (1999)
RHINOCEROTIDAE				
Black rhinoceros *Diceros bicornis*	oestrous cycle, pregnancy, comparative study plasma and faeces, regumate treatment to prevent abortion	captive, ♀	E,P	Berkeley *et al*. (1997)
	ultrasonography, oestrous cycles, pregnancy	captive, ♀	P	Radcliffe *et al*. (2001)
	identification of faecal steroid metabolites, pregnancy, gas-liquid chromatography-mass spectrometry	captive, ♀	P	Lance *et al*. (2001)
	oestrous cycle, seasonality, foetal loss, behavioural observation	wild, ♀	P	Garnier *et al*. (2002)
White rhinoceros *Ceratotherium simum*	comparative study; oestrous cycle, pregnancy, testicular, adrenal activity	captive, ♂ & ♀	E, P, A, GC	Brown *et al*. (2001)
Black rhinoceros *Diceros bicornis*	comparative study; adrenal activity, environmental factors, matched plasma, urine and faeces	captive, ♂ & ♀	GC	Turner *et al*. (2002)
	comparative study; adrenal activity, behaviour, environmental factors, reproduction	captive, ♀	GC	Carlstead & Brown (2005)
White rhinoceros *Ceratotherium simum*	pregnancy diagnosis, embryonic loss, reproductive failure, infertility, genital health, ultrasonography	captive, ♀	P	Radcliffe *et al*. (1997), Hermes *et al*. (2006)
	reproductive behaviour, territoriality, environmental factors, GnRH application	wild, ♂	A	Rachlow *et al*. (1998), Kretzschmar *et al*. (2004)
	oestrous cycle, pregnancy, parturition, reproductive behaviour	captive, ♀	P	Schwarzenberger *et al*. (1998), Patton *et al*. (1999)
	pregnancy, parturition, vaginal electrical impedance	captive, ♀	E, P, GC	Bowers *et al*. (2005)

Table 1. Continued

TAXA/SPECIES	APPLICATION (WHAT HAS BEEN STUDIED?)	CAPTIVE/ FREE-RANGING (WILD), ♂/♀	HORMONES DETERMINED IN FAECES	REFERENCES
Indian rhinoceros *Rhinoceros unicornis*	oestrous cycle, pregnancy, post-partum period	captive, ♀	E, A, P	Schwarzenberger *et al*. (2000)
Sumatran rhinoceros *Dicerorhinus sumatrensis*	oestrous cycle, pregnancy, paired blood and faecal samples, ultrasonographic monitoring, regumate treatment to prevent abortion	captive, ♀	P	Roth *et al*. (2001, 2004)
ARTIODACTYLA				
BOVIDAE				
Arabian oryx *Oryx leucoryx*	oestrous cycle, pregnancy, post-partum period	captive, & wild, ♀	E, P	Ostrowski *et al*. (2005)
Bighorn sheep *Ovis canadensis*	social rank, reproductive behaviour	wild, ♂	A	Pelletier *et al*. (2003)
	pregnancy diagnosis, radiocollared animals	wild, ♀	E, P	Schoenecker *et al*. (2004)
Bison *Bison bison*	rut, dominance, stress, mating success, reproductive behaviour	semi-wild, ♂	A, GC	Mooring *et al*. (2004, 2006)
	seasonality, oestrous cycle, post-partum cycle, pregnancy, reproductive behaviour	semi-wild, ♀	P	Vervaecke & Schwarzenberger (2006)
Buffalo *Bubalus bubalis*	ovulation induction, oestrous synchronization, norgestomet implant,	captive, ♀	P	Hattab *et al*. (2000)
Chamois *Rupicapra rupicapra*	gastrointestinal and lung helminths, ♂-biased parasitism, endocrine-immune interaction	wild, ♂ & ♀	E, A, GC	Hoby *et al*. (2006)
Fringe-eared oryx or Gemsbok *Oryx gazella callotis*	aggression control, bachelor herd, behavioural observation	captive, ♂	A	Patton *et al*. (2001)
Gerenuk *Litocranius walleri walleri*	oestrous cycle, seasonality, oestrous synchronization, artificial insemination, pregnancy	captive & wild, ♂ & ♀	A, P	Penfold *et al*. (2005)
Mhorr gazelle *Gazella dama mhorr*	oestrous cycle, pregnancy, oestrous synchronization	captive, ♀	P	Pickard *et al*. (2001)
Rocky mountain goat *Ovis canadensis canadensis*	faecal parasite loads, seasonality, radiotelemetry	wild, ♂ & ♀	GC	Goldstein *et al*. (2005)

Rocky mountain goat *Oreamnos americanus*	reproductive physiology, seasonality, pregnancy	captive, ♀	P	Kallert *et al*. (2002)
Sable antelope *Hippotragus niger*	oestrous cycle, seasonality, oestrous synchronization	captive, ♀	E, P	Thompson & Monfort (1999), Thompson *et al*. (1998)
Scimitar-horned oryx *Oryx dammah*	oestrous cycle, seasonality, pregnancy, oestrous synchronization, artificial insemination, ultrasonography	captive, ♀	E, P	Morrow *et al*. (1999, 2000)
CERVIDAE				
Brown brocket deer *Mazama gouazoubira*	oestrous cycle, pregnancy, oestrous behaviour	captive, ♀	P	Pereira, Polegato *et al*. (2006)
Chinese water deer *Hydropotes inermis*	oestrous cycle, seasonality, reproductive behaviour	captive, ♂ & ♀	P, A	Mauget *et al*. (2007)
Elk *Cervus elaphus canadensis*	pregnancy, fecundity free-ranging herd, nutrition associated abortion, body condition	wild, ♀	E, P	Stoops *et al*. (1999), Cook *et al*. (2002)
Red deer *Cervus elaphus*	seasonal variation; faecal samples were partly genotyped for individual and sex identification	captive, ♂ & ♀	GC	Huber *et al*. (2003)
Pampas deer *Ozotoceros bezoarticus bezoarticus*	seasonality, reproductive behaviour, antler cycle, human disturbance	wild, ♂	A, GC	Pereira *et al*. (2005), Pereira, Barbanti Duarte & Negrao (2006)
Pere David's deer *Elaphurus davidianus*	♂ and ♀ reproductive behaviour	wild, ♂ and ♀	E, A, P	Li *et al*. (2001
Reindeer *Rangifer tarandus*	welfare and adrenal activity, comparison of GC in blood, saliva, urine, faeces with IgA	captive, ♀	GC	Rehbinder & Hau (2006)
Sika deer *Cervus nippon yesoensis*	seasonality, oestrous cycle, conception	captive, ♀	P	Matsuura *et al*. (2004)
Southern pudu *Pudu puda*	oestrous cycle, pregnancy, seasonality	captive, ♀	P	Blanvillain *et al*. (1997)
White-tailed deer *Odocoileus virginianus*	oestrous cycle, pregnancy, assay validation through application of oestradiol and progesterone by blow gun	captive, ♀	E, P	Kapke *et al*. (1999)
	faecal-steroid monitoring of immunocontraception	wild, ♀	P	Walter *et al*. (2002)

Table 1. Continued

TAXA/SPECIES	APPLICATION (WHAT HAS BEEN STUDIED?)	CAPTIVE/ FREE-RANGING (WILD), ♂/♀	HORMONES DETERMINED IN FAECES	REFERENCES
GIRAFFIDAE				
Giraffe *Giraffa camelopardalis*	oestrous cycle, post-partum period, pregnancy, behaviour; monitoring contraception (GnRH agonist implant)	captive & semi-wild, ♀	P	Del Castillo *et al.* (2005), Dumonceaux *et al.* (2006), Patton *et al.* (2006)
Okapi *Okapia johnstoni*	reproductive physiology, oestrous cycle, pregnancy, post partum period, abortion, regumate treatment to prevent abortion	captive, ♀	P	Schwarzenberger *et al.* (1999)
HIPPOPOTAMIDAE				
Hippopotamus *Hippopotamus amphibius*	oestrous cycle, pregnancy, puberty, lactational anoestrous, miscarriage	captive, ♀	P	Graham *et al.* (2002), Wheaton *et al.* (2006)
SUIDAE				
Babirusa *Babyrousa babyrussa*	oestrous cycle, pregnancy, seasonality, comparative study on reproduction in suidae	captive, ♀	E, A, P	Berger *et al.* (2006)
Common warthog *Phacochoerus africanus*				
Red river hog *Potamochoerus porcus*				
CARNIVORA				
AILURIDAE				
Red panda *Ailures fulgens fulgens*	seasonality, oestrous cycle, induced ovulation, pregnancy diagnosis, pseudopregnancy, captive management (group size)	captive, ♀	E, P, A	Spanner *et al.* (1997), MacDonald *et al.* (2005), Wei *et al.* (2005)
CANIDAE				
African wild dog *Lycaon pictus*	social rank, reproductive suppression, social stress	wild, ♂ & ♀	E, P, A, GC	Creel *et al.* (1997)
Bush dog *Speothos venaticus*	oestrous cycle, seasonality, ♂ effect	captive, ♂ & ♀	E, P, A	DeMatteo *et al.* (2006)
Fennec fox *Vulpes zerda*	oestrous cycle, vulval swelling, blood and faeces	captive, ♀	E, P	Valdespino *et al.* (2002)
Gray wolf *Canis lupus*	oestrous induction; GnRH implant (deslorelin)	captive, ♀	E, P	Asa *et al.* (2006)
	social rank, reproductive suppression, social stress	wild, ♂ & ♀	GC	Sands & Creel (2004)

Maned wolf *Chrysocyon brachyurus*	seasonality, oestrous cycle, pregnancy, pseudopregnacy, infusion of radiolabelled testosterone	captive, ♂ & ♀	E, P, A	Velloso *et al.* (1998)
Red wolf *Canis rufus*	seasonality, oestrous cycle, pregnancy, pseudopregnacy, blood samples; longitudinal study of adrenal activity	captive, ♂ & ♀	E, P, A, GC	Walker *et al.* (2002), Young, K. M. *et al.* (2004)
FELIDAE				
Cheetah *Acinonyx jubatus*	adrenal activity, chronic stress, adrenal gland morphology; social management, movement between facilities	captive & wild, ♂ & ♀	E, A, GC	Wielebnowski, Ziegler *et al.* (2002), Terio *et al.* (2004), Wells *et al.* (2004), Young, K. M. *et al.* (2004)
Clouded leopard *Neofelis nebulosa*	oestrous cycle, pregnancy, seasonality, ovulation induction	captive, ♀	E, P	Brown *et al.* (1995)
	environmental stressor related to captive management; ACTH challenge	captive, ♂ & ♀	GC	Wielebnowski, Fletchall *et al.* (2002), Young, K. M. *et al.* (2004)
	ovulation induction, GnRH agonist and gonadotrophin injection, artificial insemination	captive, ♀	E, P	Pelican *et al.* (2006)
Eurasian lynx *Lynx lynx*	seasonality, ultrasonography, electroejaculation, spermatogenesis	captive, ♂	A	Göritz *et al.* (2006)
Jaguar *Panthera onca*	restraint and electroejaculation	captive, ♂	GC	Morato *et al.* (2004)
Pallas' cat *Otocolobus manul*	oestrous cycle, pregnancy, seasonality, ovulation induction, artificial insemination	captive, ♂ & ♀	E, P, A	Brown *et al.* (2002)
Tiger *Panthera tigris*	ovulation induction, different doses of gonadotrophins, artificial insemination	captive, ♂	E, P	Graham *et al.* (2006)
HERPESTIDAE				
Meerkats *Suricata suricatta*	oestrous cycles, pregnancy, post-partum period; social rank, cooperative breeding, stress, faecal and urine samples	wild, ♂ & ♀	E, P, A, GC	Moss *et al.* (2001), Young, K. M. *et al.* (2004), Young, A. J. *et al.* (2006)
HYAENIDAE				
Spotted hyena *Crocuta crocuta*	social rank, stress	wild, ♂ & ♀	GC	Goymann *et al.* (2001, 2003)
	social rank, pregnancy, aggression, rank-related maternal effects	wild, ♀	A	Dloniak *et al.* (2006)

Table 1. Continued

TAXA/SPECIES	APPLICATION (WHAT HAS BEEN STUDIED?)	CAPTIVE/ FREE-RANGING (WILD), ♂/♀	HORMONES DETERMINED IN FAECES	REFERENCES
MUSTELIDAE				
Black-footed ferret *Mustela nigripes*	seasonality, oestrous cycles, pregnancy, pseudopregnancy, vaginal lavage, vulva size, litter size correlation with faecal oestradiol; adrenal activity, infusion of radiolabelled cortisol and corticosterone, ACTH challenge	captive, ♂ & ♀	E, P, GC	Young, K. M. *et al.* (2001, 2004)
European badger *Meles meles*	behavioural and physiological response to restraint, stress	wild, ♂ & ♀	GC	Schütz *et al.* (2006)
Eurasian otter *Lutra lutra*	population structure, faecal DNA and steroids	wild, ♂ & ♀	P, A	Kalz *et al.* (2006)
Sea otter *Enhydra lutris*	reproductive physiology, oestrous cycle, pregnancy	captive, ♀	E, P	Larson *et al.* (2003)
OTARIIDAE				
Steller sea lion *Eumetopias jubatus*	wildlife rehabilitation, stress	captive, ♀	GC	Petrauskas *et al.* (2006)
URSIDAE				
Brown bear *Ursus arctos* Giant panda *Ailuropoda melanoleuca* Spectacled bear *Tremarctos ornatus*	seasonal reproduction, pheromones, comparative study, faecal and urine samples	captive, ♀	E, P, A	Dehnhard *et al.* (2006)
Malayan sun bear *Helarctos malayanus*	oestrous cycle, pregnancy, seasonality, aging ♀, immuno-contraception	captive, ♀	E, P, A	Schwarzenberger *et al.* (2004)

Table 1. Faecal steroid metabolites have been analysed in a variety of mammalian species; owing to space limitations the enclosed table does not include all available literature. Further tables including references on faecal steroid analysis have been published in previous reviews: for example, studies on faecal steroid monitoring in felids have been summarized by Brown (2006) and studies on rhinoceros have been summarized by Roth (2006). Studies on the application of radioactively labelled steroids and on the validation of faecal glucocorticoid metabolites are only partly included in this table, as these studies have been summarized in recent reviews by Palme *et al*. (2005), Touma & Palme (2005) and Keay *et al*. (2006). Reproductive hormones (E, oestrogens; A, androgens; P, progestagens); adrenal hormones (GC, glucocorticoids). DNA, deoxyribonucleic acid.

and adrenal (glucocorticoid) steroid metabolites. Additional references on glucocorticoids not included in this review can be found in the reviews by Möstl & Palme (2002), Palme *et al*. (2005), Touma & Palme (2005) and Keay *et al*.(2006).

METHODOLOGICAL ISSUES IN APPLYING FAECAL STEROID ASSAYS

In comparison with the more traditional analysis of steroid hormones in blood, faecal steroid analysis has several advantages. The most obvious is that the technique is non-invasive to the investigated subjects and thus does not introduce variables that may alter the results. Consequently, in many studies in which either blood sampling on a regular basis was difficult or not possible, such as in research on wildlife, very stress-prone species or animals of small size, faecal samples have become a substitute for analysing hormones in the serum or plasma. Faecal steroid analysis has been applied on species of all sizes, ranging from animals as small as mice to elephants or whales (for references, see Table 1 and Touma & Palme, 2005). Faecal steroid analysis can be applied in longitudinal studies and in conjunction with other parameters, such as behaviour or reproduction, can give an accurate insight into the endocrine physiology of a species. Animals in captivity are ideal research subjects because regular sample collection is possible and, thus, techniques can be validated easily. Once established, non-invasive endocrine analysis can be used as a tool to assist in the husbandry of animals in captivity and to investigate social and ecological effects of animals in the wild (Schwarzenberger *et al*., 1996, 1997; Whitten *et al*., 1998; Möstl & Palme, 2002; von der Ohe & Servheen, 2002; Monfort, 2003; Graham, 2004; Millspaugh & Washburn, 2004; Möstl *et al*., 2005; Touma & Palme, 2005; Ziegler & Wittwer, 2005; Keay *et al*., 2006; Lane, 2006).

Studies in free-ranging species are often confronted with difficulties in locating samples, usually involving observation of known individuals and collecting samples upon defecation. However, non-invasive faecal steroid analysis also offers the opportunity to study free-ranging animals for which direct observation of defecation is difficult or impossible. Difficulties with locating samples in some studies have been recently overcome by training dogs for scat detection (Wasser *et al*., 2004), and this has even been used in open water for detecting samples from free-ranging North Atlantic right whales *Eubalaena glacialis* (Rolland *et al*., 2005; Hunt *et al*., 2006). An even more sophisticated approach would be the combination of faecal steroid analysis with molecular genetic techniques for identifying individual animals from faecal deoxyribonucleic acid (DNA). However, the technical difficulties inherent in the analysis of low quantities of DNA and considerable variability in the results generally tend to limit the efficiency of this approach (Broquet *et al*., 2007). Therefore, the combination of non-invasive faecal DNA and steroid analysis in not yet common practice. Nonetheless, the potential of this auspicious approach has been demonstrated recently; for example, in a study on seasonal faecal glucocorticoid excretion in Red deer *Cervus elaphus* (Huber *et al*., 2003) and in a study on the population structure of European otters *Lutra lutra* (Kalz *et al*., 2006).

Like all laboratory-based methods, assay validation is most important for obtaining useful and accurate results. However, the particularity with faecal steroid analysis is that the parent hormones progesterone, testosterone, cortisol or corticosterone are not (or only barely if at all) present in the faeces. Consequently, it is inaccurate to speak of faecal-progesterone or faecal-cortisol analysis, although this designation is common practice in a considerable proportion of the published literature.

Proper faecal steroid assay validation is all related to steroid metabolism (Palme *et al*., 1996, 2005; Schwarzenberger *et al*., 1996, 1997; Möstl *et al*., 2005; Palme, 2005). Steroids are metabolized by the liver before excretion via urine or bile into the faeces. During the intestinal passage, steroid metabolites can be re-absorbed into the enterohepatic circulation. The intestinal passage causes a lag-time between the circulation of steroids in plasma and their appearance in the faeces; this delay correlates with the time for the intestinal passage of bile to the rectum (Palme *et al.*, 1996, 2005; Schwarzenberger *et al.*, 1996, 1997; Möstl *et al.*, 2005; Palme, 2005). Owing to the intestinal passage, steroid metabolites in the faeces represent pooled endocrine activity over the previous several hours. Thus, faecal steroid analysis presents a more dampened hormone profile over time with less interference from daily rhythm and acute stress (Schwarzenberger *et al.*, 1996, 1997; Whitten *et al.*, 1998; Möstl & Palme, 2002; von der Ohe & Servheen, 2002; Monfort, 2003; Graham, 2004; Millspaugh & Washburn, 2004; Möstl *et al.*, 2005; Palme, 2005; Palme *et al.*, 2005; Touma & Palme, 2005; Ziegler & Wittwer, 2005; Keay *et al.*, 2006; Lane, 2006).

A particular disadvantage of faecal steroid analysis is the presence of a vast number of different faecal metabolites existing in even closely related species. For the development of techniques for faecal steroid analysis, experiments on the metabolism of radioactively labelled steroids have provided a valuable insight into the metabolism and the excretion of hormone metabolites via faeces and urine. The route of excretion varies considerably among species, and between steroids within the same species. Radiometabolism studies affirmed that oestrogens in the form of oestradiol and/or oestrone are present in faecal samples and as such can easily be determined by using specific assay or a total oestrogen assay. In contrast, testosterone, progesterone and, especially, cortisol/corticosterone are heavily metabolized and the original hormone is barely present in the faeces. Therefore, specific assays (i.e. those typically used for the analysis of hormones in blood samples) are often of limited value (Palme *et al.*, 1996; Schwarzenberger *et al.*, 1996, 1997; Graham *et al.*, 2001; Möstl *et al.*, 2005; Palme, 2005).

For the development of species-specific assays, the determination of the major faecal hormone metabolites via the analysis of infused radioactively labelled steroids (Palme *et al.*, 1996, 2005; Schwarzenberger *et al.*, 1997; Möstl *et al.*, 2005; Palme, 2005) or the identification of faecal steroid metabolites via gas chromatography/mass spectrometry (Lance *et al.*, 2001) would be analytical possibilities. However, these valuable approaches cannot be applied to all wildlife species and in fact, owing to possible health hazards related to the use of radioactively labelled steroids, radiometabolism studies have mainly been carried out with animals in research facilities (Palme, 2005), rather than animals in zoos. Therefore, the best alternative approach is to test several assays with high cross-reactions against a certain group of steroids; that is, group-specific assays (Palme *et al.*, 1996; Schwarzenberger *et al.*, 1996, 1997; Möstl *et al.*, 2005; Palme, 2005). Because of species-specific differences in hormone metabolite excretion, validation and testing of several such assays should be carried out separately for even closely related species (Palme *et al.*, 1996; Schwarzenberger *et al.*, 1996, 1997; Graham *et al.*, 2001; Young, K. M. *et al.*, 2004; Möstl *et al.*, 2005; Palme, 2005; Berger *et al.*, 2006; Heistermann *et al.*, 2006).

An example of how different the reproductive and endocrine physiology of even closely related species can be, is demonstrated by endocrine studies in the family Rhinocerotidae. Through the use of faecal steroid analysis (additional hormones in urine and saliva have been investigated), endocrine profiles of four of the five extant species have been analysed. None of the four rhinoceros species (White rhinoceros *Ceratotherium simum*, Black rhinoceros *Diceros bicornis*, Indian or Greater one-horned rhinoceros *Rhinoceros unicornis* and Sumatran rhinoceros *Dicerorhinus sumatrensis*) exhibit reproductive cycles of similar length. Furthermore, faecal steroid metabolites excreted in these species vary considerably, underlining the necessity to develop endocrine tests for each species separately (for references, see Table 1, and Roth, 2006).

Assays that have cross-reactivities with a broad range of pregnanediones and hydroxylated pregnanes have been used successfully to quantify progesterone metabolites in the faeces of a wide range of species including a variety of carnivores (Brown, 2006) and artiodactyl species (Schwarzenberger *et al.*, 1996, 1997; Graham *et al.*, 2001). In contrast. the metabolism of glucocorticoids is more complex and a larger number of assays is used for the analysis of faecal glucocorticoid metabolites (Wasser *et al.*, 2000; Möstl & Palme, 2002; Young, K. M. *et al.*, 2004; Möstl *et al.*, 2005; Palme, 2005; Heistermann et al., 2006; Keay *et al.*, 2006). Currently, as researchers worldwide are applying different techniques, results between studies are only comparable in their physiologic outcome, but usually not in absolute metabolite concentrations.

Although it is strongly suggested that antibodies with known high cross-reactivities for steroid metabolites be used, even commercially available antibodies advertised as hormone specific have been used with success for faecal steroid analysis in several studies. The reason is that these antibodies obviously recognize and bind metabolites, although these cross-reactivities are unknown, because testing is typically carried out only for the better-known hormones and in some instances a few metabolites found in blood. Therefore, biological validation, such as day(s) of mating or parturition, or comparison of stressed versus non-stressed animals, in relation to sustained changes in faecal steroid concentration is of utmost importance. However, while it may not be as critical that the exact steroid metabolites are identified, assays developed for use with faecal steroid analysis usually show a stronger differentiation between pregnant and non-pregnant (Schwarzenberger *et al.*, 1996, 1997), or stressed versus non-stressed animals (Heistermann *et al.*, 2006).

In addition to metabolism, and hence the use of an appropriate immunoassay with adequate cross-reactivities, other concerns in faecal steroid analysis are extraction techniques, storage and stability of faecal metabolites, determination of faecal immuno-reactive steroid metabolites by high-performance liquid chromatography, gut transit time, diurnal and seasonal variations, as well as gender and diet (Schwarzenberger *et al.*, 1996, 1997; Khan *et al.*, 2002; Möstl & Palme, 2002; von der Ohe & Servheen, 2002; Millspaugh & Washburn, 2004; Möstl *et al.*, 2005; Palme, 2005; Palme *et al.*, 2005; Touma & Palme, 2005; Ziegler & Wittwer, 2005; Keay *et al.*, 2006; Lane, 2006). A general recipe from these studies is to attempt to collect samples at the same time of the day and to freeze samples immediately upon collection. If freezing is not possible, storage in ethanol in order to prevent microbial degradation is the method of choice.

Seasonal variation in faecal steroid analysis is of concern, especially when studying free-ranging wildlife living in a highly seasonal habitat. Of particular importance seems to be the question as to whether food availability, which determines the amount of faeces produced and which is lower during severe winter or drought, would result in higher faecal steroid concentrations. However, experiments on the faecal output of progesterone metabolites in ovariectomized domestic cows supplemented with progesterone-releasing intravaginal devices and with different levels of feed intake (Rabiee *et al.*, 2001) did not demonstrate such a relationship. Although feed intake did influence plasma progesterone concentrations, faecal progesterone metabolites were not affected by the level of feed intake or faecal output, but the daily excretion rate of faecal metabolites was associated with the volume of faeces. Comparable results have been found in an Elk (*C. elaphus*, Cook *et al.*, 2002) on a restricted diet. Derived from this experiment, it is likely that seasonal variation in faecal cortisol metabolites, as described in, for example, Red deer *C. elaphus* (Huber *et al.*, 2003), Chacma baboons *Papio hamadryas* (Weingrill *et al.*, 2004) and Chamois *Rupicapra rupicapra* (Hoby *et al.*, 2006), is influenced by an endogenous rhythm.

APPLICATION OF FAECAL STEROID ANALYSIS FOR STUDYING WILDLIFE SPECIES

Validation in reproductive studies is rather straightforward as steroid metabolites fluctuate with reproductive events; for example, the oestrous cycle, pregnancy, puberty or seasonality. In recent years, a great many studies using faecal steroid analysis have been conducted on ♀ and ♂ reproductive physiology. Wildlife taxa that are most extensively studied are primates, carnivores, rhinoceros and artiodactyla (Table 1). Traditionally, more focus has been placed on the study of reproductive hormones in ♀♀, but studies on the reproductive physiology in the ♂ are increasing. Studies on the reproductive physiology include, for example, characterization of oestrous-cycle length, spontaneous versus induced ovulation, pregnancy, lactational anovulation, age at the onset of puberty and seasonal patterns of reproduction. Faecal steroid analysis has been used as a tool to enhance the management of wildlife species, both in captivity and in the wild. Important issues are the diagnosis of pregnancy, assessing the presence or absence of ovarian cyclicity, control for treatment of infertility and the assessment of various fertility-control techniques; for example, ultrasonographic monitoring of ovarian events and foetal gestational parameters, contraceptive treatments, oestrous synchronization and endocrine responses to ovulation-induction protocols (Table 1).

The validation of techniques in studying stress responses is more difficult and usually involves questions such as is an animal stressed or non-stressed and does this present as acute or chronic stress, positive or negative stress (distress) (Whitten *et al.*, 1998; Möstl & Palme, 2002; von der Ohe & Servheen, 2002; Wielebnowski, 2003; Millspaugh & Washburn, 2004; Touma & Palme, 2005; Keay *et al.*, 2006; Lane, 2006). Consequently, as compared with reproductive studies interpreting results from glucocorticoid studies is more difficult and more focus should be placed on proper validation of faecal steroid assays. Besides using known stressors (such as transport or confinement) for physiological validation, several studies (especially in wildlife species kept in captivity) included pharmacological validation experiments. Possibilities include the determination of the excretory fate of radioactively labelled steroids and the application of adrenocorticotropic hormone challenge and dexamethasone suppression tests (Möstl & Palme, 2002; Palme *et al.*, 2005; Touma & Palme, 2005). In conjunction with proper validation, the analysis of glucocorticoid metabolites is a good indicator of adrenal activity and it has been used to quantify certain stress responses. Results were analysed in relation to behaviour, social status, dominance rank, territoriality, reproduction, environmental factors, population density, disturbance, as an indicator of well-being and welfare, pain, the presence of predators, parasites and as a predictor of mortality (Table 1).

In conclusion, faecal steroid analysis of reproductive and adrenocortical steroid hormones has become an established and widely accepted technique for the analysis of captive and free-ranging wildlife species. Because of species-specific differences in steroid metabolism in even closely related species, careful validation of assay methods is necessary in order to generate meaningful and accurate results. In light of this, captive wildlife species are ideal research subjects, as longitudinal sample collection is possible and studies connecting physiology, endocrinology, reproduction and stress with various social and/or environmental factors can be carried out and used to determine how they impact animal health. For the future management of wildlife populations, these techniques will be important research tools and their importance for studying free-ranging animals within their natural habitat will increase further.

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