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Endocrine Monitoring of Reproduction and Stress

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INTRODUCTION

Most wild mammals kept in captivity are managed intensively. Under such conditions, the ability to monitor reproductive status can greatly facilitate attempts to enhance breeding success for many species. More specifically, objective and reliable methods for monitoring key reproductive events, such as ovulation and pregnancy, not only find widespread application in the management of natural breeding, but also provide the basis for efforts designed to accelerate reproduction by assisted/artificial means.

Animal welfare is also a key management issue when maintaining wild mammals in a captive situation (see Kagan and Veasey, chap. 2, this volume). Avoidance of stress (and of situations and procedures likely to cause it) is therefore a very important aspect of the overall zoo management paradigm, but until recently, physiological measures by which stress in zoo animals can be assessed were difficult to obtain.

This chapter provides an overview of the available endocrine-based methodologies for monitoring reproduction and stress in captive wild mammals. Although coverage includes measurement of hormones in blood, we emphasize methods based on noninvasive sample collection. Thus, we provide a bibliography of selected studies describing the use of urinary and fecal hormone analysis for determination of reproductive status and stress in males and females across the main mammalian taxa. The database for this derives predominantly from studies carried out since the original edition of this volume was published in 1996.

ENDOCRINE METHODOLOGIES FOR ASSESSING PHYSIOLOGICAL STATUS

GENERAL CONSIDERATIONS

Hormone analysis is the most precise of the indirect methods for monitoring the functional status of the reproductive and stress axes. However, since correct interpretation of hormonal

data requires at least some knowledge of the physiology of the species in question, monitoring methods based on hormonal analysis first need to provide the basic physiological information (hormone metabolism, patterns of secretion and excretion) on which their subsequent application depends. Although certain basic commonalities exist among mammal species concerning the endocrinology of reproductive and adrenal function, marked differences in the nature, patterns, and levels of hormones secreted and/or excreted make extrapolation of results from one species to another difficult and potentially misleading.

Hormones are present and can be measured in various biological matrices, including blood, saliva, urine, and feces. The choice of which to use for analysis depends on a range of factors, including the type of information required, the assay techniques involved, species differences in steroid metabolism and route of excretion, and the practicality of sample collection, particularly when repeated sampling over extended periods is necessary. In general, the advantages of sample collection without the need for animal contact mean that noninvasive approaches based on urine and (more recently) fecal analysis are the preferred option in most situations.

HORMONE ASSAYS

Measurements of hormones and their metabolites are usually carried out by immunological procedures using hormone- or hormone-group-specific antibodies. Two main types of immunoassays are available: radioimmunoassays (RIA), which use radioactively labeled hormone as the competitive tracer in the quantification process, and enzymeimmunoassays (EIA), in which either enzyme- or biotin-labeled preparations are employed. Being nonisotopic, EIAs avoid the problems associated with use and disposal of radioactivity and are also less costly. Furthermore, the end point is a color change that is simple to quantify and relies on less expensive instrumentation. As such, EIAs are potentially more suitable for zoos

and other institutions where sophisticated laboratory facilities are not available.

Since all immunoassays are highly sensitive, assay performance has to be carefully assessed both during the initial setup phase and during routine use. There are 4 main criteria of validation: *sensitivity* (minimum amount of hormone that can be detected), *precision* (within- and between-assay repeatability), *accuracy* (ability to detect the correct amount of hormone in the sample), and *specificity*. The latter has 2 components: the degree of specificity of the antibody itself and the possible influence of interfering substances (matrix effects), which need to be controlled for and, if present, removed by incorporating additional sample purification steps or preparation of standards in hormone-stripped sample. Concerning antibody specificity, highly specific assays may be useful when the identity of the major metabolite is known and when species comparisons are of interest. Since, however, excreted samples (especially feces) can contain a large number of metabolites, specific measurement is often difficult to achieve and might be less useful in cases where the antibody detects only metabolites of low abundance. Group-specific assays utilize antibodies that cross-react with several metabolites of related structure. Since knowledge of the relative abundance of individual metabolites is not necessary, these assays have advantages in that they can usually be applied to a wider range of species (see Heistermann and Hodges 1995; Heistermann, Palme, and Ganswindt 2006; Schwarzenberger et al. 1996a; Wasser et al. 2000), thus helping to overcome the problems of species specificity in hormone metabolism. Care needs to be taken, however, in order to avoid problems arising from the comeasurement of structurally related but physiologically distinct substances, which can generate misleading results (e.g. comeasurement of androgens of adrenal and testicular origin in fecal assays).

One frequently asked question is whether commercial immunoassay kits developed for humans can be used in exotic species. The answer is not straightforward, because validity depends on species, hormone, and sample matrix. For example, kits effectively measure steroids in serum of many species, but usually not urine or feces. Kits for chorionic gonadotropin (hCG) and luteinizing hormone (hLH) work well with urine and/or serum in most great apes, but generally not other species. No commercial kit should be used without proper validation.

SAMPLE COLLECTION AND STORAGE

Blood. For measurement of most hormones, blood serum or plasma can be used. Steroids usually need to be extracted from the matrix before evaluation; however, many assay kits now are available that do not require sample preparation before assay. Care should be taken to avoid repeated freeze-thaw cycles, which can damage protein hormones.

Saliva. Many animals can be trained to provide saliva samples on demand using positive reinforcement and food rewards. With larger animals, it is possible to collect several milliliters directly into a container (e.g. Gomez et al. 2004), whereas in smaller animals, it may be necessary to obtain samples using cotton swabs or other absorbent materials. Offering chew

items to an animal is another way to collect saliva samples. Samples should be frozen after collection, and most assays require fairly extensive extraction procedures. Some commercial companies have developed assays specific for saliva to avoid problems associated with matrix effects.

Urine. Samples can be collected midstream (uncommon), from a container placed underneath a drain or channel in the floor of the enclosure, or by aspiration from the ground using a pipette or syringe. If possible, samples should be centrifuged to remove cellular or other debris. Volumes as small as 0.2 mL are sufficient for most assays, and it is generally not necessary to collect more than 1 mL. Samples should be stored frozen, preferably in 2 aliquots to avoid excessive freeze-thaw cycles and as a precaution against leakage.

Feces. Fecal samples are collected directly from the floor; a thumbnail-size amount generally is enough for analytical purposes. A larger aliquot may be needed for samples with a high proportion of fibrous material (e.g. rhinoceroses, *Ceratotherium*, *Diceros*, *Rhinoceros*, and *Dicerorhinus*; elephants, *Loxodonta* and *Elephas*; giant panda, *Ailuropoda melanoleuca*). Because steroids in feces can be unevenly distributed, samples should be homogenized using a gloved hand or improvised spatula before transfer to storage container (Brown et al. 1994b; Wasser et al. 1996; Millsbaugh and Washburn 2003). Fecal processing and storage methods can differentially affect steroid metabolite concentrations, with responses being species specific (e.g. Terio et al. 2002; Hunt and Wasser 2003; Galama, Graham, and Savage 2004; Millsbaugh and Washburn 2003). In this respect, storing fecal samples by simply freezing at -20°C is the most effective way of preserving steroid hormones for long periods of time and should therefore be preferred over storage of samples in alcoholic solvents. In fact, significant alterations in fecal steroid concentrations can occur during long-term storage in ethanol, even when samples are frozen (Khan et al. 2002; Hunt and Wasser 2003). Feces imported into some countries may require special treatment in order to kill pathogens (e.g. autoclave, formalin, acetic acid, ethanol, sodium hydroxide), and this could potentially influence steroid levels (Millsbaugh et al. 2003).

For both urine and feces, it is essential to collect only samples of known origin and to avoid cross-contamination (feces with urine and vice versa) as well as contamination with water and any form of detergent. However, as long as urine samples are not overly dilute, indexing by creatinine should account for fluid differences. For example, steroids in feral horses were measured using urine-soaked snow (Kirkpatrick, Shideler, and Turner 1990).

Since diurnal patterns of secretion are particularly pronounced for some hormones (e.g. testosterone and glucocorticoids), time of sample collection is a variable that needs to be controlled. Although the magnitude of diurnal changes is most evident in blood and urine, they can still also be noticeable in the feces of certain small-bodied species (e.g. callitrichids: Sousa and Ziegler 1998; rodents: Cavigelli et al. 2005) in which fecal passage rate is relatively high. Thus, wherever possible, samples should be collected at roughly the same time each day.

Hodges and Heistermann (2003) deal with other practi-

cal aspects concerning the use of urinary and fecal assays to generate hormonal data for monitoring physiological function (e.g. sampling frequency, sample preparation, interpretation of results, etc.).

MEASUREMENT OF HORMONES IN DIFFERENT BIOLOGICAL MATRICES

BLOOD

Measurement of hormones in blood is still probably the most informative and widely used approach to monitoring physiological function in laboratory and domestic animals. The advantages include fewer problems associated with sample preparation (e.g. less need for complicated extractions and hydrolysis), no need for indexing concentrations, real-time reflection of hormonal status (little or no time lag), and the possibility of monitoring short-term endocrine changes. In most zoo animals, however, difficulties associated with capture or restraint necessary for the collection of blood samples makes this procedure impractical for routine (i.e. repeated and/or regular) application. There are, nevertheless, situations in which blood sampling is justifiable, because either suitable alternatives are lacking or husbandry practices and/or degree of animal training are of sufficient level that venipuncture represents little additional risk or stress. For example, in North America the reproductive status of Asian and African elephants is routinely monitored by blood progesterone analyses (Brown 2000), and numerous studies have characterized circulating pituitary, adrenal, and ovarian hormone profiles in these species (Kapustin et al. 1996; Carden et al. 1998; Brown 2000; Brown, Wemmer, and Lehnhardt 1995; Brown et al. 1999; Brown, Walker, and Moeller 2004). Longitudinal blood sampling also has been used to monitor steroid and protein hormones during the estrous cycle and pregnancy in a number of wildlife species, including rhinoceroses (Berkeley et al. 1997; Roth et al. 2001, 2004), Baird's tapir, *Tapirus bairdii* (see Brown et al. 1994a), beluga whales, *Delphinapterus leucas* (see Robeck et al. 2005a), mithuns, *Bos frontalis* (see Mondal, Rajkhowa, and Prakash 2005), yaks, *Bos grunniens* (see Sarkar and Prakash 2006), buffalo, *Bubalus bubalis* (see Mondal and Prakash 2004), camelids (see Bravo et al. 1991, 1996), and felids (see Brown 2006 for a review). Moreover, blood sampling is often conducted as part of the validation procedure for noninvasive monitoring techniques in order to demonstrate good correspondence between circulating and excreted hormone profiles (e.g. Brown et al. 1995; Berkeley et al. 1997; Heistermann, Trohorsch, and Hodges 1997; Goymann et al. 1999; Walker, Waddell, and Goodrowe 2002).

SALIVA

Minute quantities of steroids are also present in saliva and can be measured using highly sensitive immunoassay procedures. Hormones enter saliva by passive diffusion, so concentrations are not affected by salivary flow rate (e.g. Riad-Fahmy et al. 1982). Salivary steroid concentrations usually are significantly lower than circulating levels, because only the unbound fraction is present. While the collection of saliva can, under certain circumstances, be called a noninvasive procedure,

and as such has proved useful for monitoring physiological status in women, domestic livestock, and dogs (e.g. Negrão et al. 2004; Queyras and Carosi 2004), there have only been a few studies in which salivary hormone analyses have been used in exotic species. Most of these have involved analysis of salivary cortisol to assess adrenal activity in relation to stress (e.g. Ohl, Kirschbaum, and Fuchs 1999; Lutz et al. 2000; Cross and Rogers 2004), although monitoring reproductive steroids has been reported in the rhinoceros (Czekala and Callison 1996; Gomez et al. 2004). Other studies, however, have reported limited usefulness of salivary analyses for assessing reproductive function, with poor correlations observed between circulating and secreted concentrations (Atkinson et al. 1999; Fenske 1996). In a study on the Indian rhinoceros, *Rhinoceros unicornis*, several estrogen and progesterone RIAs and EIAs gave poor results, whereas commercial assay kits designed specifically for human saliva were effective (Gomez et al. 2004). Thus, the inability to detect biologically relevant immunoactivity in saliva may be due to assay matrix effects. One recent study reported the successful use of liquid chromatography-mass spectrometry to measure salivary testosterone in the bottlenose dolphin, *Tursiops truncatus* (Hogg, Vickers, and Rogers 2005).

URINE

The primary motivation for the development of urinary hormone assay methodology was the growing awareness (and demand) in the early 1980s for more scientific input into zoo animal management, and the establishment of efficient, coordinated breeding programs for targeted species. Urinary hormone analysis was seen as the most likely alternative to preexisting invasive procedures required for blood sampling. As a result of a large number of studies carried out in the early to mid-1980s (see Hodges 1985; Lasley 1985; Heistermann, Möstl, and Hodges 1995 for references), there were major advances in urine hormone analysis methodology, in terms of ease of performance, sensitivity, and reliability. The ensuing methods and their subsequent application have generated an enormous comparative database on reproductive and, more recently, stress physiology in wild mammals and other vertebrate taxa.

Because most urine samples are either single voidings or incomplete 24-hour collections, creatinine determination is used to compensate for differences in urine concentration and volume. Despite certain limitations involved in the use of creatinine measurements, there is a good correlation between the hormone/creatinine index and 24-hour excretion rates (e.g. Hodges and Eastman 1984), and the method has successfully generated hormone profiles in diverse species.

Most steroids in urine are present in the conjugated form, either as sulphate or glucuronide residues. Early analyses of steroids in urine involved the laborious process of hydrolysis and solvent extraction before assay; however, the subsequent introduction of nonextraction assays allowing direct measurement of steroid conjugates has greatly simplified procedures for most species (e.g. Shideler et al. 1983; Lasley et al. 1985; Hodges and Green 1989; Heistermann and Hodges 1995). By avoiding the need for hydrolysis, a process that itself can be inefficient, direct assays for steroid con-

jugates have the additional advantage of often generating a more informative hormone profile than previously possible with extraction methods (e.g. Shideler et al. 1983; Lasley and Kirkpatrick 1991).

Depending on clearance rate (which can vary according to both hormone and species) and frequency of sampling, there is a certain time lag between any given secretory event (and resulting change in circulating hormone level) and its detection in urine. The lag time from steroid production/secretion to appearance in excreted urine can be as short as 2 hours (e.g. Bahr et al. 2000), but is generally within the range 6 to 14 hours (Czekala et al. 1992; Brown, Wemmer, and Lehnhardt 1995; Monfort et al. 1995, 1997; Busso et al. 2005). Thus, in practical terms, changes in the pattern of urinary hormone excretion usually reflect physiological events that happened several hours earlier, and this has to be taken into account when interpreting urinary hormone profiles.

Although most urinary-based assays are directed toward the measurement of steroid hormones, gonadotrophic hormones (pituitary LH, FSH, and, in some species, chorionic gonadotrophins) are also excreted into the urine. Since the structure of all such peptide hormones (beta subunit) is species specific, care needs to be taken when selecting antibodies for heterologous assays, as is usually the case in studies of exotic species. One particularly versatile monoclonal antibody against bovine LH has been shown to demonstrate good cross-reactivity with LH from diverse mammalian species as well as with hCG and eCG (Matteri et al. 1987), and has been used to characterize urinary LH profiles during the ovarian cycle in a number of wildlife species, including primates (Ziegler, Matteri, and Wegner 1993; Shimizu et al. 2003a), marine mammals (Robeck et al. 2004, 2005b), and rhinoceroses (Stoops, Pairan, and Roth 2004). Urinary prolactin (Ziegler et al. 2000a; Soltis, Wegner, and Newman 2005), chorionic gonadotrophin (Munro et al. 1997; Shimizu et al. 2003a; Tardif et al. 2005), and FSH (Shimizu et al. 2003a; Shimizu 2005) also have been measured in several nondomestic mammalian species. One important caveat is that identification of protein hormones in circulation does not mean that they will necessarily be measurable in urine; they may be structurally altered before excretion or not excreted in significant amounts.

FECES

In addition to urinary excretion, large amounts of steroids are excreted into feces. In fact, in several mammalian species (e.g. many of the Felidae—Shille et al. 1990; Brown et al. 1994a; Graham and Brown 1996), fecal excretion predominates. Radiometabolism studies in particular have yielded important data on the relative importance of the urinary and fecal pathways of steroid excretion. From these studies, it is clear that major differences exist, not only between species, but also between hormones within the same species. Thus, among primates for example, the squirrel monkey, *Saimiri sciureus*, excretes both estrogen and progesterone metabolites mainly (~70%) via the fecal route (Moorman et al. 2002), whereas the cotton-top tamarin, *Saguinus oedipus*, also a New World monkey species, eliminates estrogens almost exclusively (~92%) via the urinary pathway (Ziegler et al. 1989) but progestagens (~95%) into feces (ibid.). Similarly, both

the Sumatran rhinoceros, *Dicerorhinus sumatrensis* (see Heistermann et al. 1998), and African elephant, *Loxodonta africana* (see Wasser et al. 1996), excrete estrogens primarily into urine and progestagens predominantly into feces.

One of the main advantages of fecal analysis is the relative ease of collection of fecal samples from animals living in group situations or under natural conditions. Generally, it is not necessary to separate animals; thus, caretakers can avoid physical disruption of the group and maintain social context. In most situations, fecal sampling also provides the only feasible option for longitudinal studies in the wild (although there are examples where urine collection has been successful), thus explaining the increasing interest in fecal assay methodologies over the last 5 to 10 years.

Unlike urinary analysis, where direct (nonextraction) assays are the norm, measurement of steroids in feces requires an extraction step before assay. There are numerous described extraction procedures, the choice being partially dependent on the hormone being measured, the method of sample storage used, or personal preference (Heistermann, Tari, and Hodges 1993; Shideler et al. 1994; Schwarzenberger et al. 1996b; Palme and Möstl 1997; Whitten et al. 1998; Moreira et al. 2001). In general, extraction with organic solvents (ethanol, methanol) containing 5 to 20% water results in good steroid recoveries. In most species, steroids are excreted in the free (unconjugated) form into the feces. Species of some taxa, however, such as felids (Brown et al. 1994b, 1995) or some callitrichids (Ziegler et al. 1996), do excrete hormones into feces predominantly as conjugates, which often can be measured using antibodies that directly quantify conjugates or cross-react with them. However, there are situations where hydrolysis following fecal extraction can improve results (ibid.).

Fecal samples vary considerably in consistency and water content, and this needs to be controlled, generally by expressing hormone levels per unit weight (gram) using either the wet weight of the portion of fresh material to be extracted or the weight of the dry powder after lyophilization (e.g. Hodges and Heistermann 2003). Gut passage time adds to clearance rate, which markedly increases the lag time (delay between hormone secretion and excretion) when comparing fecal and urinary measurements. Time lags associated with fecal analysis are longer and more variable (e.g. 6 to >48 hours), both between and within species. Additionally, a variety of factors, including diet, health status, and stress level, can affect gut passage times. Radiometabolism studies indicate that in most large-bodied mammals for which data are available, steroids are excreted in feces 24 to 48 hours after their appearance in circulation (see Schwarzenberger et al. 1996a for review), although shorter times of <20 hours have been described for sheep (Palme et al. 1996), the domestic cat (Brown et al. 1994a), and the small-bodied common marmoset, *Callithrix jacchus* (4 to 8 hours: Bahr et al. 2000; Möhle et al. 2002). Knowledge of the time lag for the hormone and species in question is therefore important in order to interpret correctly the changes in fecal hormone levels in relation to physiological events.

An additional biological validation step is generally advisable in order to demonstrate that excreted hormonal measures (particularly those in feces) accurately reflect physi-

ological events. For assessing ovarian activity, it is useful to demonstrate a predicted rise and fall in metabolite concentrations coincident with behavioral measures of estrus, time of ovulation, or onset of pregnancy. Alternatively, a cause-and-effect relationship between physiological changes (and resultant secretory events) and excretion of hormone metabolites can be demonstrated by administration of pharmacological agents known to stimulate hormonal production (e.g. gonadotrophin-releasing hormone [GnRH] or adrenocorticotrophic hormone [ACTH]).

NONINVASIVE REPRODUCTIVE ASSESSMENT IN FEMALES

For many years, particularly in the 1980s and early 1990s, urinary hormone analysis was the predominant method for

monitoring reproductive function in exotic mammals. The method was applied extensively across all major mammalian taxonomic groups and, apart from its immediate practical value in the zoo-management context, yielded a tremendous amount of basic information on comparative aspects of female reproductive cycles (see table 33.1 for examples).

Although urinary hormone monitoring is quick and inexpensive, urine samples can be difficult to collect. Due to the relative ease of collection of fecal material, zoo professionals now prefer analyses of estrogen and progesterone metabolites excreted in feces to assess female reproductive function in most mammalian taxa (table 33.2), even though there are certain disadvantages in terms of increased labor and cost of processing samples.

Tables 33.1 and 33.2 mainly summarize studies carried out in the captive environment (with the exception of pri-

TABLE 33.1. Selected studies in which urinary hormone analysis has yielded information on endocrine profiles in exotic mammals helpful in monitoring ovarian function and pregnancy

Species	Ovarian cycle/ovulation	Pregnancy
Primates		
Lemuridae		
Red-bellied lemur, <i>Eulemur rubriventer</i>		Gerber, Moisson, and Heistermann 2004
Blue-eyed black lemur, <i>Eulemur flavifrons</i>		Gerber, Moisson, and Heistermann 2004
Northern bamboo lemur, <i>Hapalemur occidentalis</i>		Gerber, Moisson, and Heistermann 2004
Callitrichidae		
Common marmoset, <i>Callithrix jacchus</i>	Nivergelt and Pryce 1996	Nivergelt and Pryce 1996
Wied's black-tufted-ear marmoset, <i>Callithrix kuhlii</i>	French et al. 1996	French et al. 1996
Pygmy marmoset, <i>Cebuella pygmaea</i>	Carlson, Ziegler, and Snowdon 1997	
Saddle-back tamarin, <i>Saguinus fuscicollis</i>	Heistermann and Hodges 1995	Heistermann and Hodges 1995
Geoffroy's tamarin, <i>Saguinus geoffroyi</i>	Kuhar et al. 2003	Kuhar et al. 2003
Golden lion tamarin, <i>Leontopithecus rosalia</i>	Monfort, Bush, and Wildt 1996	
Golden-headed lion tamarin, <i>Leontopithecus chrysomelas</i>	De Vleeschouwer, Heistermann, and Van Elsacker 2000; French et al. 2002	
Goeldi's monkey, <i>Callimico goeldii</i>	Pryce, Schwarzenberger, and Doebeli 1994	Jurke et al. 1994
Cebidae		
Capuchin monkey, <i>Cebus apella</i>	Carosi, Heistermann, and Visalberghi 1999	
Pitheciidae		
White-faced saki, <i>Pithecia pithecia</i>	Shideler et al. 1994; Savage et al. 1995	Shideler et al. 1994; Savage et al. 1995
Titi monkey, <i>Callicebus moloch</i>	Valleggia et al. 1999	Valleggia et al. 1999
Atelidae		
Muriqui, <i>Brachyteles arachnoides</i>	Ziegler et al. 1997	
Red howler monkey, <i>Alouatta seniculus</i>	Herrick et al. 2000	Herrick et al. 2000
Black-handed spider monkey, <i>Ateles geoffroyi</i>	Campbell et al. 2001	Campbell et al. 2001
Cercopitheciinae		
Tonkean macaque, <i>Macaca tonkeana</i>	Thierry et al. 1996; Aujard et al. 1998	Thierry et al. 1996
Japanese macaque, <i>Macaca fuscata</i>	Fujita et al. 2001	
Rhesus monkey, <i>Macaca mulatta</i>	Gilardi et al. 1997	
Long-tailed macaque, <i>Macaca fascicularis</i>	Shideler et al. 1993a; Shimizu et al. 2003a, b	Shideler et al. 1993a
Baboon, <i>Papio ssp.</i>		French et al. 2004
Owl-faced guenon, <i>Cercopithecus hamlyni</i>	Ialleggio et al. 1997	
Colobinae		
Hanuman langur, <i>Semnopithecus entellus</i>	Heistermann, Finke, and Hodges 1995	
Yunnan snub-nosed monkey, <i>Rhinopithecus bieti</i>	He et al. 2001	He et al. 2001
Black and white colobus, <i>Colobus guereza</i>	Harris and Monfort 2003	
Hylobatidae		
White-handed gibbon, <i>Hylobates lar</i>	Nadler, Dahl, and Collins 1993	

(continued)

TABLE 33.1. continued

Species	Ovarian cycle/ovulation	Pregnancy
Hominidae		
Common chimpanzee, <i>Pan troglodytes</i>	Deschner et al. 2003; Shimizu et al. 2003a	Shimizu et al. 2003a
Bonobo, <i>Pan paniscus</i>	Heistermann, Palme, and Ganswindt 1996; Jurke et al. 2000	Heistermann, Palme, and Ganswindt 1996
Lowland gorilla, <i>Gorilla gorilla</i>	Bellem, Monfort, and Goodrowe 1995	Bellem, Monfort, and Goodrowe 1995
Pongidae		
Orang utan, <i>Pongo pygmaeus</i>	Asa et al. 1994; Shimizu et al. 2003b	
Perissodactyla		
Rhinocerotidae		
White rhinoceros, <i>Ceratotherium simum</i>	Hindle, Möstl, and Hodges 1992	
Black rhinoceros, <i>Diceros bicornis</i>	Hindle, Möstl, and Hodges 1992	
Indian rhinoceros, <i>Rhinoceros unicornis</i>	Stoops, Pairan, and Roth 2004	
Sumatran rhinoceros, <i>Dicerorhinus sumatrensis</i>	Heistermann et al. 1998	
Equidae		
Grevy's zebra, <i>Equus grevyi</i>	Asa et al. 2001	Ramsay et al. 1994
Grant's zebra, <i>Equus burchelli</i>		Ramsay et al. 1994
Hartman's mountain zebra, <i>Equus zebra</i>		Ramsay et al. 1994
Przewalski's horse, <i>Equus przewalskii</i>		Ramsay et al. 1994
Tapiridae		
Tapir, <i>Tapirus spp.</i>		Ramsay et al. 1994
Proboscidea		
African elephant, <i>Loxodonta africana</i>	Heistermann, Trohorsch, and Hodges 1997; Fiess, Heistermann, and Hodges 1999	Fiess, Heistermann, and Hodges 1999
Asian elephant, <i>Elephas maximus</i>	Niemüller, Shaw, and Hodges 1993; Czekala et al. 2003b	Niemüller, Shaw, and Hodges 1993; Brown and Lehnhardt 1995
Artiodactyla		
Bovidae		
Bison, <i>Bison bison</i>	Kirkpatrick, Bancroft, and Kincy 1992	Kirkpatrick, Bancroft, and Kincy 1992
Dall's sheep, <i>Ovis dalli</i>	Goodrowe et al. 1996	Goodrowe et al. 1996
Dik-dik, <i>Madoqua guentheri</i>	Robeck et al. 1997	Robeck et al. 1997
Camelidae		
Llama, <i>Lama glama</i>	Bravo et al. 1993	Bravo et al. 1996
Alpaca, <i>Lama pacos</i>	Bravo et al. 1993	Bravo et al. 1996
Cervidae		
Eld's deer, <i>Rucervus eldii</i>	Monfort, Arthur, and Wildt 1990; Hosack et al. 1997	Monfort, Arthur, and Wildt 1990
Moose, <i>Alces alces</i>	Monfort, Brown, and Wildt 1993	Monfort, Brown, and Wildt 1993
Père David's deer, <i>Elaphurus davidianus</i>	Monfort, Martinet, and Wildt 1991	Monfort, Martinet, and Wildt 1991
Giraffidae		
Okapi, <i>Okapia johnstoni</i>	Schwarzenberger et al. 1999	Schwarzenberger et al. 1999
Edentata		
Myrmecophagidae		
Tamandua, <i>Tamandua tetradactyla</i>	Hay et al. 2000	
Carnivora		
Canidae		
African wild dog, <i>Lycaon pictus</i>	Monfort et al. 1997	Monfort et al. 1997
Herpestidae		
Mongoose, <i>Helogale parvula</i>	Creel et al. 1992, 1995	Creel et al. 1992, 1995
Ursidae		
Giant panda, <i>Ailuropoda melanoleuca</i>	Monfort et al. 1989; Czekala et al. 2003a; Steinman et al. 2006	Monfort et al. 1989; Steinman et al. 2006
Cetacea		
Delphinidae		
Bottle-nose dolphin, <i>Tursiops truncatus</i>	Robeck et al. 2005b	
Killer whale, <i>Orcinus orca</i>	Robeck et al. 2004	
Rodentia		
Mouse, <i>Mus musculus</i>	deCatanzaro et al. 2003, 2004; Muir et al. 2001	deCatanzaro et al. 2003, 2004

TABLE 33.2. Selected studies in which fecal hormone analysis has yielded information on endocrine profiles in exotic mammals helpful in monitoring ovarian function and pregnancy

Species	Ovarian cycle/ovulation	Pregnancy
Primates		
Lemuridae		
Mongoose lemur, <i>Eulemur mongoz</i>	Curtis et al. 2000	Curtis et al. 2000
Red-fronted lemur, <i>Eulemur rufus</i>	Ostner and Heistermann 2003	Ostner and Heistermann 2003
Indriidae		
Verreaux's sifaka, <i>Propithecus verreauxi</i>	Brockman et al. 1995; Brockman and Whitten 1996	Brockman et al. 1995; Brockman and Whitten 1996
Lorisidae		
Pygmy loris, <i>Nycticebus pygmaeus</i>	Jurke, Czekala, and Fitch-Snyder 1997	Jurke, Czekala, and Fitch-Snyder 1997
Callithrichidae		
Common marmoset, <i>Callithrix jacchus</i>	Ziegler et al. 1996	
Cotton-top tamarin, <i>Saguinus oedipus</i>	Ziegler et al. 1996	
Goeldi's monkey, <i>Callimico goeldii</i>	Pryce, Schwarzenberger, and Doebeli 1994	
Golden lion tamarin, <i>Leontopithecus rosalia</i>	French et al. 2003	French et al. 2003
Cebidae		
Capuchin monkey, <i>Cebus apella</i>	Carosi, Heistermann, and Visalberghi 1999	
Squirrel monkey, <i>Saimiri sciureus</i>	Moorman et al. 2002	Moorman et al. 2002
Pitheciidae		
White-faced saki, <i>Pithecia pithecia</i>	Shideler et al. 1994	Shideler et al. 1994
Atelidae		
Black-handed spider monkey, <i>Ateles geoffroyi</i>	Campbell et al. 2001; Campbell 2004	Campbell et al. 2001
Muriqui, <i>Brachyteles arachnoides</i>	Ziegler et al. 1997; Strier and Ziegler 1997	Strier and Ziegler 1997
Cercopithecinae		
Long-tailed macaque, <i>Macaca fascicularis</i>	Shideler et al. 1993b; Engelhardt et al. 2004	Shideler et al. 1993b
Japanese macaque, <i>Macaca fuscata</i>	Fujita et al. 2001	
Lion-tailed macaque, <i>Macaca silenus</i>	Heistermann et al. 2001	
Sooty mangabey, <i>Cercocebus atys atys</i>	Whitten and Russell 1996	
Yellow baboon, <i>Papio cynocephalus</i>		Wasser 1996
Colobinae		
Hanuman langur, <i>Semnopithecus entellus</i>	Heistermann, Finke, and Hodges 1995; Ziegler et al. 2000b	Ziegler et al. 2000b
Douc langur, <i>Pygathrix nemaeus</i>	Heistermann, Ademmer, and Kaumanns 2004	
Hylobatidae		
White-handed gibbon, <i>Hylobates lar</i>	Barelli et al., 2007	
Hominidae		
Common chimpanzee, <i>Pan troglodytes</i>	Emery and Whitten 2003	
Bonobo, <i>Pan paniscus</i>	Heistermann et al. 1996; Jurke et al. 2000	Heistermann et al. 1996
Lowland gorilla, <i>Gorilla gorilla</i>	Miyamoto et al. 2001; Atsalis et al. 2004	
Perissodactyla		
Rhinocerotidae		
White rhinoceros, <i>Ceratotherium simum</i>	Schwarzenberger et al. 1998b; Brown et al. 2001	Patton et al. 1999
Black rhinoceros, <i>Diceros bicornis</i>	Berkeley et al. 1997; Brown et al. 2001	Schwarzenberger et al. 1996b; Brown et al. 2001
Indian rhinoceros, <i>Rhinoceros unicornis</i>	Schwarzenberger et al. 2000	Schwarzenberger et al. 2000
Sumatran rhinoceros, <i>Dicerorhinus sumatrensis</i>	Heistermann et al. 1998; Roth et al. 2001	Roth et al. 2001
Equidae		
Grevy's zebra, <i>Equus grevyi</i>	Asa et al. 2001	Asa et al. 2001
Chapman's zebra, <i>Equus burchelli antiquorum</i>		Skolimowska et al. 2004b
Przewalski mare, <i>Equus przewalskii</i>	Scheibe et al. 1999	
Domestic horse, <i>Equus caballus</i>	Barkhuff et al. 1993	Palme et al. 2001; Skolimowska, Janowski, and Golonka 2004a
Proboscidea		
African elephant, <i>Loxodonta africana</i>	Wasser et al. 1996; Fiess et al. 1999	Fiess, Heistermann, and Hodges 1999
Artiodactyla		
Bovidae		
Bison, <i>Bison bison</i>	Kirkpatrick, Bancroft, and Kincy 1992; Matsuda et al. 1996	Kirkpatrick, Bancroft, and Kincy 1992
Bighorn sheep, <i>Ovis canadensis</i>		Borjesson et al. 1996; Schoenecker, Lyda, and Kirkpatrick 2004

(continued)

TABLE 33.2. continued

Species	Ovarian cycle/ovulation	Pregnancy
Mhorr gazelle, <i>Nanger dama mhorh</i>	Pickard et al. 2001	Pickard et al. 2001
Sable antelope, <i>Hippotragus niger</i>	Thompson, Mashburn, and Monfort 1998; Thompson and Monfort 1999	
Scimitar-horned oryx, <i>Oryx dammah</i>	Morrow and Monfort 1998; Morrow et al. 1999; Shaw et al. 1995	
Camelidae		
Vicuña, <i>Vicugna vicugna</i>	Schwarzenberger, Speckbacher, and Bamberg 1995	
Cervidae		
Moose, <i>Alces alces</i>	Schwartz et al. 1995	Schwartz et al. 1995
Père David's deer, <i>Elaphurus davidianus</i>	Li et al. 2001	Li et al. 2001
Pudu, <i>Pudu puda</i>	Blanvillain et al. 1997	
Sika deer, <i>Cervus nippon</i>	Hamasaki et al. 2001	Hamasaki et al. 2001
Giraffidae		
Giraffe, <i>Giraffa camelopardalis</i>	del Castillo et al. 2005	del Castillo et al. 2005; Dumonceaux, Bauman, and Camilo 2006
Okapi, <i>Okapia johnstoni</i>	Schwarzenberger et al. 1993, 1999	Schwarzenberger et al. 1993, 1999
Hippopotamidae		
Hippopotamus, <i>Hippopotamus amphibius</i>	Graham et al. 2002	Graham et al. 2002
Edentata		
Myrmecophagidae		
Giant anteater, <i>Myrmecophaga tridactyla</i>	Patzl et al. 1998	Patzl et al. 1998
Carnivora		
Canidae		
Blue fox, <i>Vulpes lagopus</i>	Sanson, Brown, and Farstad 2005	Sanson, Brown, and Farstad 2005
Fennec fox, <i>Vulpes zerda</i>	Valdespino, Asa, and Bauman 2002	Valdespino, Asa, and Bauman 2002
Maned wolf, <i>Chrysocyon brachyurus</i>	Velloso et al. 1998	Velloso et al. 1998
Red wolf, <i>Canis rufus</i>	Walker, Waddell, and Goodrowe 2002	Walker, Waddell, and Goodrowe 2002
African wild dog, <i>Lycaon pictus</i>	Monfort et al. 1997	Monfort et al. 1997
Felidae		
Cheetah, <i>Acinonyx jubatus</i>	Czekala et al. 1994; Brown et al. 1996b	Czekala et al. 1994; Brown et al. 1996b
Clouded leopard, <i>Neofelis nebulosa</i>	Brown et al. 1995b	Brown et al. 1995b
Ocelot, <i>Leopardus pardalis</i>	Moreira et al. 2001	
Pallas' cat, <i>Felis manul</i>	Brown et al. 2002	Brown et al. 2002
Tiger, <i>Panthera tigris</i>	Graham et al. 1995	Graham et al. 1995
Mustelidae		
Black-footed ferret, <i>Mustela nigripes</i>	Brown 1997; Young, Brown, and Goodrowe 2001	Brown 1997; Young, Brown, and Goodrowe 2001
Otter, <i>Enhydra lutris</i>	Larson, Casson, and Wasser 2003; Da Silva and Larson 2005	Larson, Casson, and Wasser 2003; Da Silva and Larson 2005
Herpestidae		
Meerkat, <i>Suricata suricatta</i>	Moss, Clutton-Brock, and Monfort 2001	Moss, Clutton-Brock, and Monfort 2001
Ursidae		
Sun bear, <i>Helarctos malayanus</i>	Schwarzenberger et al. 2004	Schwarzenberger et al. 2004
Hokkaido brown bear, <i>Ursus arctos lasiotus</i>	Ishikawa et al. 2002	
Giant panda, <i>Ailuropoda melanoleuca</i>	Steinman et al. 2006	Steinman et al. 2006
Ailuridae		
Red panda, <i>Ailurus fulgens</i>	MacDonald, Northrop, and Czekala 2005	Spanner, Stone, and Schultz 1997; MacDonald, Northrop, and Czekala 2005
Rodentia		
Erethizontidae		
Porcupine, <i>Erethizon dorsata</i>	Bodgan and Monfort 2001	Bodgan and Monfort 2001
Muridae		
Mouse, <i>Mus musculus</i>	deCatanzaro et al. 2004; Muir et al. 2001	
Cetacea		
Right whale, <i>Eubalaena glacialis</i>		Rolland et al. 2005

mates), but both urine (under certain circumstances) and feces can generate much useful data on hormonal status of animals in the wild; e.g. urinary hormone analysis was useful for monitoring female reproductive status in free-ranging vervet monkeys, *Chlorocebus pygerythrus* (see Andelman et al. 1985), and chimpanzees, *Pan troglodytes* (see Deschner et al. 2003). Fecal hormone changes during the estrous cycle and pregnancy have been measured in free-ranging African wild dogs, *Lycaon pictus* (see Creel et al. 1997), meerkat, *Suricata suricatta* (see Moss et al. 2001), bison, *Bison bison* (Kirkpatrick et al. 2001), and black rhinoceros, *Diceros bicornis* (see Garnier et al. 1998). Fecal steroid measures have provided single-sample pregnancy diagnosis in a variety of ungulates, such as bighorn sheep, *Ovis canadensis* (see Schoenecker, Lyda, and Kirkpatrick 2004), elk, *Cervus elephas* (see Stoops et al. 1999; Garrott et al. 1998), and moose, *Alces alces* (see Berger et al. 1999). Monfort (2003) provides a review of urinary and fecal studies on free-ranging wildlife.

NONINVASIVE REPRODUCTIVE ASSESSMENT IN MALES

Determining testicular endocrine activity in male mammals is an important step in the assessment of male reproductive function and fertility (see Spindler and Wildt, chap. 32, this volume). The secretion of testosterone (the major androgen secreted by the testis) is highly pulsatile; thus, circulating testosterone concentrations can vary markedly within hours or even minutes, making interpretation of endocrine condition based on single (or infrequent) samples difficult. The noninvasive approach, based on the analysis of the breakdown products of testosterone excreted in urine and feces, is therefore useful, not only in providing a more integrated picture (measures represent cumulative secretion over a number of hours), but also when longitudinal information on male testicular endocrine activity is desirable. To date, however, there is limited information on the metabolism of testosterone, its route of excretion, and the nature of the metabolites excreted (e.g. cats: Brown, Terio, and Graham 1996; primates: Möhle et al. 2002; Hagey and Czekala 2003; African elephant: Ganswindt et al. 2002, 2003). These studies have shown that testosterone metabolism is highly complex and often species specific, resulting in excretion of a number of metabolites, with native testosterone usually being quantitatively of minor importance (and virtually absent in feces of several species). There can even be considerable variation in excreted androgen steroid metabolite forms among closely related species (e.g. Hagey and Czekala 2003); thus, validation of any urinary and fecal androgen measurement as an index of testicular activity is essential before being used to assess male reproductive condition. In this respect, comeasurement of metabolites derived from androgens of extratesticular (e.g. adrenal) origin, such as dehydroepiandrosterone (DHEA), is a potential problem when using fecal measurements for assessing male gonadal status in primates (Möhle et al. 2002).

Although still relatively limited (in comparison to studies in females), the use of noninvasive endocrine methodologies for assessing male gonadal function has shown a marked increase over the last few years (table 33.3), largely due to improvements in the reliability of the laboratory methods. Many of the studies of primate species listed in table 33.3 were car-

ried out in the wild, while most nonprimate studies were of captive animals. The vast majority of nonprimate studies used fecal material to measure androgens. It is not clear why there is such a paucity of urinary data in nonprimate species, but for felids it is known that nearly all androgen metabolites are excreted in feces (Brown, Terio, and Graham 1996).

NONINVASIVE ASSESSMENT OF STRESS

Since most (although not all) types of stressors induce an increased release of the stress hormones cortisol or corticosterone from the adrenal gland, glucocorticoid output is commonly used as a physiological (endocrine) measure of stress. Although blood glucocorticoid concentrations are an accepted indicator, the invasive nature of blood sampling (itself capable of eliciting a stress response) limits the application of this approach in wild animals.

Comparative information on the metabolism and route of excretion of glucocorticoids is limited (see Palme et al. 2005 for review). Nevertheless, the measurement of native cortisol excreted into urine has been used to monitor stress physiology in a variety of captive mammals (see table 33.4). Measurement of glucocorticoid metabolites in feces, however, is less straightforward. Although the use of this approach has increased recently (table 33.4), there are a number of confounding factors relating to both methodology and interpretation of data that continue to limit its utility. For example, since native glucocorticoids seem to be virtually absent from feces in most species, the use of standard cortisol or corticosterone assays is generally not appropriate for measuring fecal glucocorticoid output (although they have been successfully used in some species; see Wasser et al. 2000; Heistermann et al. 2006). Group-specific assays, capable of measuring a range of fecal glucocorticoid metabolites, are generally more suitable, in that they are more likely to detect at least some of the more abundant metabolites present and also have greater potential for cross-species application (e.g. Palme et al. 2005; Heistermann et al. 2006). However, when using these assays, it is difficult to know for any given species which and how many metabolites are being recognized and what their relative abundance is. Also, it has been shown that the group-specific assays have the potential to cross-react with structurally related testosterone metabolites (domestic dog: Schatz and Palme 2001; African elephant: Ganswindt et al. 2003; chimpanzee: Heistermann, Palme, and Ganswindt 2006), which can confound the actual glucocorticoid measurement and generate misleading results (e.g. measurement of glucocorticoid output during musth in African elephants: Ganswindt et al. 2003). Furthermore, a host of biological factors, such as seasonal changes in glucocorticoid excretion, reproductive and body condition, sex, age, social status, and diet, can all influence glucocorticoid levels, requiring the exercise of additional caution when interpreting fecal glucocorticoid measurements for the purposes of assessing stress (von der Ohe and Servheen 2002; Touma and Palme 2005; Millsbaugh and Washburn 2003).

Additionally, not every type of stressor is mediated via increased activity of the hypothalamo-pituitary-adrenal (HPA) axis, which would result in elevations in glucocorticoid output. Negative findings with respect to glucocorticoid assess-

TABLE 33.3. Selected studies in which urinary and fecal hormone analysis has yielded endocrine information in exotic mammals helpful in the assessment of male reproductive activity

Species	Urinary analysis	Fecal analysis
Primates		
Indriidae		
Verreaux's sifaka, <i>Propithecus verreauxi</i>		Brockman et al. 1998; Kraus, Heistermann, and Kappeler 1999
Lemuridae		
Red-fronted lemur, <i>Eulemur rufus</i>		Ostner, Kappeler, and Heistermann 2002
Ring-tailed lemur, <i>Lemur catta</i>		Cavigelli and Pereira 2000; Von Engelhardt, Kappeler, and Heistermann 2000; Gould and Ziegler 2007
Callithrichidae		
Common marmoset, <i>Callithrix jacchus</i>	Möhle et al. 2002	Möhle et al. 2002; Castro and Sousa 2005
Wied's black-tufted-ear marmoset, <i>Callithrix kuhlii</i>	Nunes et al. 2002; Ross, French, and Patera 2004	
Golden lion tamarin, <i>Leontopithecus rosalia</i>		Bales et al. 2006
Cotton-top tamarin, <i>Saguinus oedipus</i>	Ziegler et al. 2000c	
Cebidae		
Capuchin monkey, <i>Cebus nigritus</i>		Lynch, Ziegler, and Strier 2002
Atelidae		
Black howler monkey, <i>Alouatta caraya</i>		Morland et al. 2001
Mantled howler monkey, <i>Alouatta palliata</i>		Cristóbal-Azkarate et al. 2006
Cercopithecinae		
Japanese macaque, <i>Macaca fuscata</i>		Barrett et al. 2002
Long-tailed macaque, <i>Macaca fascicularis</i>	Möhle et al. 2002	Möhle et al. 2002
Chacma baboon, <i>Papio ursinus</i>		Beehner et al. 2006; Bergman et al. 2006
Pongidae		
Orangutan, <i>Pongo pygmaeus</i>	Maggioncalda, Sapolsky, and Czekala 1999	
Hominidae		
Common chimpanzee, <i>Pan troglodytes</i>	Möhle et al. 2002; Muller and Wrangham 2004	Möhle et al. 2002
Bonobo, <i>Pan paniscus</i>	Sannen et al. 2003; Dittami et al. 2007	
Lowland gorilla, <i>Gorilla gorilla</i>	Stoinski et al. 2002	
Mountain gorilla, <i>Gorilla beringei</i>	Robbins and Czekala 1997	
Perissodactyla		
Rhinocerotidae		
White rhinoceros, <i>Ceratotherium simum</i>		Brown et al. 2001; Kretzschmar, Ganslosser, and Dehnhard 2004
Equidae		
Grevy's zebra, <i>Equus grevyi</i>	Chaudhuri and Ginsberg 1990	
Plains zebra, <i>Equus burchelli</i>	Chaudhuri and Ginsberg 1990	
Proboscidea		
African elephant, <i>Loxodonta africana</i>	Ganswindt et al. 2002	Ganswindt et al. 2002; Ganswindt, Heistermann, and Hodges 2005
Artiodactyla		
Bovidae		
Bighorn sheep, <i>Ovis canadensis</i>		Pelletier, Bauman, and Festa-Bianchet 2003
Bison, <i>Bison bison</i>		Mooring et al. 2004
Cervidae		
Eld's deer, <i>Rucervus eldii</i>	Monfort et al. 1995	
Fringe-eared oryx, <i>Oryx beisa callotis</i>		Patton et al. 2001
Pampas deer, <i>Ozotoceros bezoarticus</i>		Pereira, Duarte, and Negrão 2005
Père David's deer, <i>Elaphurus davidianus</i>		Li et al. 2001
Sika deer, <i>Cervus nippon</i>		Hamasaki et al. 2001
Carnivora		
Canidae		
Maned wolf, <i>Chrysocyon brachyurus</i>		Velloso et al. 1998
Red wolf, <i>Canis rufus</i>		Walker, Waddell, and Goodrowe 2002
African wild dog, <i>Lycaon pictus</i>		Monfort et al. 1997

(continued)

TABLE 33.3. continued

Species	Urinary analysis	Fecal analysis
Felidae		
Jaguar, <i>Panthera onca</i>		Morato et al. 2004a, 2004b
Ocelot, <i>Leopardus pardalis</i>		Morais et al. 2002
Pallas' cat, <i>Felis manul</i>		Brown, Terio, and Graham 1996a, 2002
Eurasian lynx, <i>Lynx lynx</i>		Jewgenow et al. 2006
Iberian lynx, <i>Lynx pardinus</i>		Jewgenow et al. 2006
Hyenidae		
Spotted hyena, <i>Crocuta crocuta</i>		Dloniak et al. 2004
Mustelidae		
Black-footed ferret, <i>Mustela nigripes</i>		
Herpestidae		
Meerkat, <i>Suricata suricatta</i>		Moss, Clutton-Brock, and Monfort 2001
Ursidae		
Hokkaido brown bear, <i>Ursus arctos lasiotus</i>		Ishikawa et al. 2002
Malayan sun bear, <i>Helarctos malayanus</i>		Hesterman, Wasser, and Cochrem 2005
Ailuridae		
Red panda, <i>Ailurus fulgens</i>		Spanner et al. 1997
Rodentia		
Chinchillidae		
Chinchilla, <i>Chinchilla lanigera</i>	Busso et al. 2005	Busso et al. 2005
Muridae		
Blind mole rat, <i>Spalax ehrenbergi</i>	Gotterich et al. 2000	
Mouse, <i>Mus musculus</i>	Muir et al. 2001	Muir et al. 2001
Mongolian gerbil, <i>Meriones unguiculatus</i>		Yamaguchi et al. 2005

TABLE 33.4. Selected studies in which urinary and fecal hormone analysis has yielded endocrine information in exotic mammals helpful in the assessment of adrenal activity

Species	Urinary analysis	Fecal analysis
Primates		
Lemuridae		
Ring-tailed lemur, <i>Lemur catta</i>		Cavigelli 1999
Red-fronted lemur, <i>Eulemur rufus</i>		Ostner, Kappeler, and Heistermann 2007
Indriidae		
Verreaux's sifaka, <i>Propithecus verreauxi</i>		Fichtel et al. 2007
Callitrichidae		
Common marmoset, <i>Callithrix jacchus</i>	Torii et al. 1998; Bahr et al. 2000	Heistermann, Palme, and Ganswindt 2006
Wied's black-tufted-ear marmoset, <i>Callithrix kuhlii</i>	Smith and French 1997	
Bearded emperor tamarin, <i>Saguinus imperator subgriseus</i>	McCallister, Smith, and Elwood 2004	
Golden lion tamarin, <i>Leontopithecus rosalia</i>		Bales et al. 2006
Cotton-top tamarin, <i>Saguinus oedipus</i>	Ziegler, Scheffler, and Snowdon 1995	
Goeldi's monkey, <i>Callimico goeldii</i>	Jurke et al. 1995; Dettling et al. 1998	
Cebidae		
Squirrel monkey, <i>Saimiri sciureus</i>	Soltis, Wegner, and Newman 2003	
Capuchin monkey, <i>Cebus apella</i>		Boinski et al. 1999; Lynch, Ziegler, and Strier 2002
Atelidae		
Spider monkey, <i>Ateles fusciceps rufiventris</i>	Davis, Schaffner, and Smith 2005	
Cercopithecinae		
Long-tailed macaque, <i>Macaca fascicularis</i>	Crockett et al. 1993	Wasser et al. 2000; Heistermann, Palme, and Ganswindt 2006
Pig-tailed macaque, <i>Macaca nemestrina</i>	Crockett, Shimoji, and Bowden 2000	
Lion-tailed macaque, <i>Macaca silenus</i>	Clarke, Czekala, and Lindburg 1995	
Barbary macaque, <i>Macaca sylvanus</i>		Heistermann et al. 2006
Baboon, <i>Papio</i> spp.	French et al. 2004	Wasser et al. 2000; Beehner and Whitten 2004
Douc langur, <i>Pygathrix nemaeus</i>		Heistermann, Ademmer, and Kaumanns 2004

(continued)

TABLE 33.4. continued

Species	Urinary analysis	Fecal analysis
Pongidae		
Orang utan, <i>Pongo pygmaeus</i>	Maggioncalda, Sapolsky, and Czekala 1999	
Hominidae		
Common chimpanzee, <i>Pan troglodytes</i>	Bahr et al. 2000; Muller and Wrangham 2004	Whitten et al. 1998; Heistermann, Palme, and Ganswindt 2006; Reimers, Schwarzenberger, and Preuschoft 2007
Lowland gorilla, <i>Gorilla gorilla</i>	Bahr et al. 1998; Stoinski et al. 2002	Heistermann, Palme, and Ganswindt 2006
Mountain gorilla, <i>Gorilla beringei</i>	Robbins and Czekala 1997	
Perissodactyla		
Rhinocerotidae		
White rhinoceros, <i>Ceratotherium simum</i>		Wasser et al. 2000; Turner, Tolson, and Hamad 2002
Black rhinoceros, <i>Diceros bicornis</i>		Brown et al. 2001; Turner, Tolson, and Hamad 2002
Equidae		
Domestic horse, <i>Equus caballus</i>		Möstl et al. 1999; Merl et al. 2000
Proboscidea		
African elephant, <i>Loxodonta africana</i>	Brown, Wemmer, and Lehnhardt 1995a; Ganswindt et al. 2003	Ganswindt et al. 2003; Ganswindt, Heistermann, and Hodges 2005
Asian elephant, <i>Elephas maximus</i>	Brown, Wemmer, and Lehnhardt 1995a	
Artiodactyla		
Bovidae		
Gerenuk, <i>Litocranius walleri</i>		Wasser et al. 2000
Scimitar-horned oryx, <i>Oryx dammah</i>		Wasser et al. 2000
Cervidae		
Eld's deer, <i>Rucervus eldii</i>	Monfort, Brown, and Wildt 1993	
Elk, <i>Cervus elaphus</i>		Millsbaugh et al. 2001; Creel et al. 2002
Mule deer, <i>Odocoileus hemionus</i>	Saltz and White 1991	
Red deer, <i>Cervus elaphus</i>		Huber, Palme, and Arnold 2003
Roe deer, <i>Capreolus capreolus</i>		Dehnhard et al. 2001
Giraffidae		
Okapi, <i>Okapia johnstoni</i>		Schwarzenberger et al. 1998a
Carnivora		
Canidae		
Wild dog, <i>Lycaon pictus</i>		Monfort et al. 1998
Wolf, <i>Canis lupus</i>		Creel et al. 2002; Sands and Creel 2004
Felidae		
Cheetah, <i>Acinonyx jubatus</i>		Terio, Citino, and Brown 1999; Jurke et al. 1997
Clouded leopard, <i>Neofelis nebulosa</i>		Wielebnowski et al. 2002; Young et al. 2004
Jaguar, <i>Panthera onca</i>		Morato et al. 2004a
Leopard cat, <i>Felis bengalensis</i>	Carlstead et al. 1992; Carlstead, Brown, and Seidensticker 1993	
Herpestidae		
Mongoose, <i>Helogale parvula</i>	Creel et al. 1992; Creel, Creel, and Monfort 1996	
Hyenidae		
Spotted hyena, <i>Crocuta crocuta</i>		Goymann et al. 1999
Mustelidae		
Black-footed ferret, <i>Mustela nigripes</i>		Young, Brown, and Goodrowe 2001; Young et al. 2004
Domestic ferret, <i>Mustela putorius</i>	Schoemaker et al. 2004	
Ursidae		
Giant panda, <i>Ailuropoda melanoleuca</i>	Owen et al. 2004; 2005	
Grizzly bear, <i>Ursus arctos horribilis</i>		Hunt and Wasser 2003
Lagomorpha		
Leporidae		
European rabbit, <i>Oryctolagus cuniculus</i>		Cabezas et al. 2007
European hare, <i>Lepus europaeus</i>	Teskey-Gerstl et al. 2000	Teskey-Gerstl et al. 2000
Rodentia		
Chinchillidae		
Chinchilla, <i>Chinchilla lanigera</i>	Ponzio et al. 2004	Ponzio et al. 2004
Muridae		
Mouse, <i>Mus musculus</i>	Touma et al. 2003	Touma et al. 2003
Rat, <i>Rattus norvegicus</i>	Eriksson et al. 2004; Brennan et al. 2000	Eriksson et al. 2004; Cavigelli et al. 2005
Red-backed voles, <i>Myodes gapperi</i>		Harper and Austad 2000

ment (i.e. no elevation in glucocorticoid levels in response to stressful conditions) thus do not necessarily indicate that an animal is not under stress or not negatively affected by the situation under study. Thus, measurement of the pituitary hormone prolactin (measurable in urine but not feces) may provide useful complementary information on stress status in mammals. Although primarily involved in the initiation and maintenance of lactation in mammals, prolactin often increases in response to stress (e.g. Eberhart, Keverne, and Meller 1983; Maggioncalda et al. 2002).

Catecholamines are also released in response to environmental and psychosocial stress and are measurable in plasma and urine (Dantzer and Mormede 1983; Dimsdale and Ziegler 1991; Hjemdahl 1993; Hay et al. 2000). When samples are properly collected, analyzed, and interpreted, catecholamine data can provide valuable information on sympathoadrenal activity; however, analytical problems are common. Other methods of studying HPA function involve assessing changes in pituitary-adrenocortical reactivity by using adrenocorticotrophic hormone (ACTH) and corticotrophin releasing hormone (CRH) challenges and dexamethasone suppression tests (Hay et al. 2000). We do not know whether any of these techniques have been validated for wildlife species. However, given the growing concerns over maintaining animals in captivity, zoo professionals need more comprehensive protocols, involving a combination of behavioral as well as physiological measures, to evaluate stress objectively.

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