Theriogenology Wild 3 (2023) 100056

Contents lists available at ScienceDirect

Theriogenology Wild

journal homepage: www.journals.elsevier.com/theriogenology-wild

Dehydroepiandrosterone and dehydroepiandrosterone-sulfate: Biomarkers of pregnancy and of fetal health

Emilee N. Hart [a,*], Gessiane Pereira da Silva [b], Rafaela S.C. Takeshita [a]

[a] Department of Anthropology, Kent State University, Kent, OH, USA
[b] Universidade Federal Rural da Amazônia (UFRA), Programa de Pós-graduação em Saúde e Produção Animal na Amazônia (PPGSPAA), Belém, PA, Brazil

ARTICLE INFO

Keywords:
Gestation monitoring
Dehydroepiandrosterone-sulfate
Wildlife reproduction

ABSTRACT

Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are important estrogen precursors, secreted mainly by the adrenal cortex. At late gestation, both DHEA and DHEAS (DHEA(S)) are produced at high concentrations in some species due to the developing fetal adrenal gland. Failure in DHEAS increase during late gestation can indicate fetal death, which prompts its use as a biomarker of pregnancy and fetal health in wildlife. Here, we review the most common non-invasive biomarkers of reproduction in wildlife, the molecular mechanisms of DHEAS synthesis and action during gestation, in addition to the advantages and limitations of incorporating DHEA(S) in these studies. Using previously published data, we tested the specificity and sensitivity of fecal DHEAS as a predictor of successful gestation in four captive primate species (orangutans (*Pongo pygmaeus*), siamangs (*Symphalangus syndactylus*), Japanese macaques (*Macaca fuscata*), and howler monkeys (*Alouatta caraya*)). Using data from non-pregnant/non-lactating females, we set a threshold on fecal DHEAS levels for detecting successful pregnancy per species, controlling for age and housing condition (social vs single). We found that DHEAS had 100% specificity for all species (non-pregnant samples were below the threshold for pregnancy), and 100% sensitivity for Japanese macaques housed individually, and for orangutan and siamangs (all samples from successful pregnancies were above the threshold, and all samples from stillbirth were below the threshold). However, the sensitivity was 80% in howler monkeys and 50% in Japanese macaques housed socially. Our preliminary results indicate that, while DHEAS is a promising biomarker of fetal health, it is limited to late gestation and to some species. We suggest increasing the sample size to calculate the pregnancy threshold per species and to test multiple samples from the same individual when using this method.

1. Introduction

Pregnancy monitoring by ultrasound, laboratory, or physical exams are common practices in humans and domestic animals [1,2]. However, while these techniques can be easily performed in domestic species, their application to wild animal monitoring requires animal training, restraining, or anesthesia, which may cause stress and increase the risks of spontaneous abortion [3].

As an alternative to those methods, hormone metabolites measured non-invasively have been employed to monitor reproductive status, gestation, and fetal development in wild species [4–6]. The most common hormones used for this purpose are progesterone [7,8] and estrogens [6,9], given that they increase progressively during pregnancy, and can be easily measured in feces [5,9,10], urine [7], and hair [8].

However, the outcome of pregnancy may be difficult to predict by measuring estrogens and progestogens alone. Although early pregnancy loss results in a drastic reduction in these hormones within a few weeks in humans [11,12] and in other animals [5,13–15], perinatal loss or stillbirth conditions not caused by low progesterone levels may be masked. Two studies in bottlenose dolphins (*Tursiops truncates*) reported overall lower progesterone levels in perinatal loss compared with normal pregnancies, but some of the samples from the perinatal loss condition were above or within the expected range in normal pregnancies and above pre-pregnancy levels [15,16]. Similarly, one study in Japanese macaques (*Macaca fuscata*) reported that progesterone and estrogen levels in two stillbirth cases were above non-pregnant levels even at late gestation, [5], suggesting that single data points of these hormones are not reliable as markers of fetal health without extensive efforts to establish baseline levels for successful gestation in each species.

* Correspondence to: 750 Hilltop Drive, 226 Lowry Hall, Kent, OH 44242, USA.
E-mail address: ehart14@kent.edu (E.N. Hart).

https://doi.org/10.1016/j.therwi.2023.100056
Received 7 March 2023; Received in revised form 15 August 2023; Accepted 31 August 2023
Available online 1 September 2023

In this context, fetal-derived hormones might be better predictors of fetal health and can help us distinguish between prenatal and postnatal death, which is important for studies on fertility and mortality rates in wild animals where daily tracking of the entire population is not viable. Some of those fetal-derived hormones are dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), which are steroids produced by the fetal adrenal gland during late gestation in humans and other primates [5,10,17,18]. These steroids are transferred to the mother via the placenta, where they are converted to estrogens, which play essential roles in gestation maintenance and in regulating the mechanism of parturition [17–19].

For these reasons, the measurement of DHEA(S) during gestation is a potential tool to monitor fetal health in wild animals, especially in nonhuman primates where the production of this steroid is more abundant [20]. However, there are species-specific differences in the regulatory mechanisms and steroid metabolic pathways involved in the secretion of these hormones that must be considered and described prior to the establishment of DHEAS as a biomarker of fetal health. Here, we review the current biomarkers used for gestation monitoring in wild animals, the importance of DHEA(S) for gestation and fetal development, and the advantages and pitfalls of using DHEA(S) metabolites for monitoring wildlife reproduction.

2. Noninvasive monitoring of wildlife reproduction

Monitoring wildlife reproduction and gestation can contribute to population dynamics as it can be used to estimate population trends and ascertain potential environmental effects on population density. This data can assist with population management and can help us evaluate the efficacy of conservation efforts for species with decreasing population sizes. However, traditional methods for gestation monitoring such as ultrasound can be hazardous to the subjects and to the researchers due to the need to capture and anesthetize the animals [21–25]. For this reason, non-invasive sample collection has been beneficial in longitudinal studies of placental mammals with a relatively long gestational period, such as felids, ungulates, cetaceans, and primates [5,26–28].

The most commonly used method of non-invasive monitoring of gestation in wildlife is through hormone analysis of sex steroids. This is because the key gonadal steroids involved in gestation, estrogens and progestins, conserve the same molecular structure throughout mammalian species [29]. The metabolites of these hormones are concentrated in the urine and feces after clearing from the gut, allowing for the measurement of these hormones in the excreta through commercial immunoassay kits [29]. However, fecal and urine samples are susceptible to degradation as a result of delayed collection time and bacterial enzymes [30]. Moreover, these samples contain hormonal metabolites that may cross-react with the target hormone in certain immunoassays. Therefore, proper validation of the sampling protocol and of the immunoassay for each species must be followed when measuring hormones non-invasively [31]. Considering these factors, the similarity of the target steroid hormones and accessibility of samples from wildlife has allowed for the standardization and frequency of hormone analyses across, but not limited to, several taxonomic groups discussed here.

2.1. Carnivora

Noninvasive endocrine analysis is helpful in carnivore reproductive monitoring as longitudinal assessments could identify periods of estrus (elevated estradiol) and periods of diestrus (elevated progesterone) [32–34] without the risk of anesthesia or stress interrupting ovarian activity [35]. For example, fecal hormone analysis is the most common method for monitoring estrous cycle in felids, because fecal estrogen metabolites (FEM) and fecal progesterone metabolites (FPM) fluctuate less than circulating hormone levels throughout the day [31]. However, there is a 24-hour delay between the rise in circulating hormone levels and metabolite concentrations which can impact detection of estrus and misdiagnose pregnancy, as most felid species have short periods of fecundity [31,32]. Additionally, in carnivores such as the giant panda (*Ailuropoda melanoleuca*) [36], the red panda (*Ailurus fulgens*) [37], and wolverines (*Gulo gulo*) [38], there is a delayed spike in progesterone after mating, signaling a delayed implantation, or embryonic diapause, which results in a wide range of gestation length. For this reason, when using endocrine analyses for pregnancy diagnosis in carnivores, longitudinal sample collection and observation is necessary to avoid false positive or negative diagnoses [39].

Early pregnancy detection in carnivores is also hindered by prolonged luteal phases and pseudopregnancies. In carnivore species with a prolonged luteal phase, these progesterone concentrations are similar to those in early pregnancy [32,39] thus making early detection more difficult. However, in Formosan black bears (*Ursus thibetanus formosanus*), early pregnancy detection is possible as FEM and FPM are significantly different from nonpregnant females within the first two months of gestation, though this difference was not consistently observed yearly pregnancies with the same females [40], which makes hormones unreliable for diagnosing pregnancies in these species. Similarly, pseudopregnancies in carnivores are difficult to detect by endocrine and behavioral analyses alone due to their similar hormone patterns to pregnant females after observing mating behavior. In these nonpregnant females, the corpus luteum secretes high levels of progesterone comparable to those levels measured in pregnant females [37,41]. However, in canid species such as maned wolves (*Chrysocyon brachyurus*) [42], red foxes (*Vulpes vulpes*)[43], and grey wolves (*Canis lupus*)[44], progestin concentrations were lower in pseudopregnant females than in pregnant females, suggesting that the diagnosis of pregnancy versus pseudopregnancy by endocrine analyses is possible in some species, as long as there are reference hormonal values established for each species.

As carnivore habitats are being threatened with extinction, breeding in captivity is becoming more important [45–50], thus the noninvasive measurement of biomarkers of gestation are useful to refine breeding strategies and to increase their success rates. In a study in ocelots (*Leopardus pardalis*), Blank et al. [26] measured FPM, FEM, and fecal glucocorticoid metabolites (FGCM) from eight females classified as naturally fertilized or with an embryonic transfer. Unlike naturally fertilized females who had consistently high levels of FPM, females with an embryonic transfer had elevated FPM levels in the first trimester, which then decreased until parturition, even though both conditions resulted in successful pregnancies [26]. The embryonic transfer females also secreted decreasing levels of FEM and FGCM throughout the pregnancy, which is the opposite of the natural fertilization pattern [26]. Therefore, while noninvasive sampling was applicable to this study, the use of these hormones for monitoring gestation is limited to natural fertilization in this species.

2.2. Ungulates

The ungulates are a diverse taxonomic group with differing endocrine systems in odd- versus even-toed ungulates. Studies investigating these potential differences are important for gestational monitoring in these species. For example, studies in odd-toed ungulates such as domestic horses (*Equus caballus*) have benefitted from dipstick tests to detect equine chorionic gonadotropin (eCG) from urine in pregnant females [51,52], though blood samples appear to be more reliable than urine samples for this test [53]. However, a false negative diagnosis can occur if the sample is collected before day 40 or after day 120 of pregnancy [52,54]. In addition, a false positive diagnosis could occur if a mare lost her pregnancy after day 40, because the female will not return to estrus until after day 120, when the endometrial cup responsible for eGC secretion has fully regressed [52]. In feral horses, the dipstick method was accurate in detecting pregnancies within 40–140 days of gestation, and a significant elevation in urinary estrone conjugates was

detected after day 35, with a sensitivity and specificity of > 90% [55]. However, in other wild odd-toed ungulates, such as Przewalski's horse (*E. caballus przewalskii*), Grevy's zebra (*E. grevyi*), and Hartman's Mountain zebra (*E. zebra hartmannae*), the eCG dipstick did not reliably correlate with radioimmunoassay (RIA) methods [56]. Moreover, although RIA detected high eCG levels in urine samples from pregnant Przewalski's horses and Hartman's mountain zebras early in gestation, only low eCG levels were detected in tapir (*Tapirus* sp.) and rhinoceros (*Diceros bicornis*, *Ceratotherium simum*) species, indicating that eCG methods are restricted to certain equids [56]. Alternatively, enzyme immunoassay (EIA) methods to measure estrone and progesterone metabolites from urine have been employed in these species, though none of these methods has been reliable in detecting pregnancy from a single sample [56].

In other ungulates, however, urine sample collection is precluded by seasonal ground coverage changes, leaving as alternative the use of fecal samples for the concomitant measurement of FEM and FPM [57]. Similar to other mammals, both estrogens and progestins increase in ungulates following conception until parturition, with progestins increasing at a more dramatic rate [27,58,59]. Thus, FPM had more reliable results in studies on even-toed ungulates such as pronghorn (*Antilocapra americana*) [27], guanacos (*Lama guanicoe*) [60], bighorn sheep (*Ovis canadensis*) [58], tule elk (*Cervus elaphus nannodes*) [61], and forest musk deer (*Moschus berezovskii*) [62], which is probably why it has been used exclusively in bison (*Bison bison*) [57], red brocket deer (*Mazama americana*) [63], peccaries (*Pecari tajacu*) [64], Himalayan musk deer (*M. chrysogaster*) [65], Arabian oryx (*Oryx leucoryx*) [66], and white-tailed deer (*Odocoileus virginianus*) [67]. However, like in felid species, early detection of pregnancy is difficult due to similarities of FPM levels between early gestation and the luteal phase of the estrus cycle, which has been reported in both odd-toed ungulates (Indian rhinos (*Rhinoceros unicornis*) [68], Southern white rhinos (*Ceratotherium simum*) [69]) and even-toed ungulates (Arabian oryxes [66], and white-tail deer (*Odocoileus virginianus*) [67]). In contrast, studies in musk deer (*Moschus berezovskii*) [62] and in pronghorn (*Antilocapra americana*) [27] found a significant difference in FPM levels early in gestation compared to their luteal phases, suggesting this pattern is not conserved across all ungulates. The difference in the rate of progesterone rise during early pregnancy underscores the importance of longitudinal sample collection in hormonal analyses as a pregnancy diagnosis method [27].

2.3. Cetaceans

While considered within the ungulate taxonomic group, monitoring reproduction in cetaceans is unique due to its challenges in non-invasive sampling. In these animals, most endocrine studies have been conducted on serum samples, which have been useful for studies on male and female development [70,71], as well as on pregnancy monitoring by natural breeding or artificial insemination in bottlenose dolphins (*Tursiops truncatus*) [14,16,72,73], killer whales (*Orcinus orca*) [74,75], beluga whales (*Delphinapterus leucas*) [76], and bowhead whales (*Balaena mysticetus*) [77]. For instance, a longitudinal study compared progesterone and estrogen levels between normal and abnormal pregnancies in bottlenose dolphins, and reported lower progesterone concentrations in false pregnancies, early loss and abortion cases than normal pregnancies, but no differences were detected in perinatal loss and failure to thrive conditions [15]. Another study in the same species reported that androstenedione concentrations were higher in pregnancies resulting in calves that failed to thrive compared to normal pregnancies in the early and late stages of gestation [78]. Moreover, cortisol levels were overall higher in failure to thrive and in perinatal loss conditions when compared to normal pregnancies [78]. Another study in killer whales suggested that relaxin may be used to confirm later term pregnancy because its concentrations increase by 800% at late gestation. However, relaxin is not suitable to detect abnormal pregnancies because it is a product of the corpus luteum [74].

Although training techniques have enabled blood collection without the need to capture or cause stress in these animals, those methods are limited to small species and captive populations where human-animal contact is relatively frequent. For this reason, non-invasive or minimally invasive samples have gained attention and enabled the determination of steroid profiles in free-ranging cetaceans (reviewed by Melo et al. [28]). One study in humpback whales (*Megaptera novaeangliae*) reported a method to detect pregnancy by measuring progesterone levels from blubber [79]. However, another study in the same species found that females near parturition did not have high blubber progesterone levels characteristic of gestation, and suggested that androstenedione and testosterone were better biomarkers of late gestation [80]. Additionally, studies have used blow samples (respiratory vapor) to characterize male and female reproduction development in beluga [81] and North Atlantic right whales (*Eubalaena glacialis*) [82], and for measuring cortisol levels in harbor porpoises (*Phocoena phocoena*) [83], beluga whales [84,85], and in humpback whales (*Megaptera novaeangliae*) [86].

Other less commonly used matrixes to measure hormones in cetaceans include urine [78,87,88], saliva [89,90], feces [91–93], earplug [94,95], and ocular secretions [96]. Unfortunately, individual identification is limited to opportunistically collected fecal samples at feeding areas [97] while urine, saliva, earplug, and ocular secretions are only possible or practical in trained captive animals or in dead carcasses. For this reason, blow and blubber have been the methods of choice to investigate reproductive hormones in living wild cetaceans [28].

2.4. Primates

Similar to the aforementioned species, in the early stages of primate pregnancy, progesterone levels are equitable to the levels observed during the luteal phase of the ovarian cycle [89]. However, the timing of elevated progesterone as a result of the luteal phase is different between the infraorders Platyrrhini and Catarrhini. In Platyrrhines, such as cotton-tip tamarins (*Saguinus oedipus*), common marmosets (*Callithrix jacchus*), white-faced saki (*Pithecia pithecia*) and muriqui (*Brachyteles arachnoides*), progesterone metabolites increase after ovulation with delayed excretion of estrogen metabolites [9,98–101], while in Catarrhines, such as pigtailed macaques (*Macaca nemestrina*), yellow baboons (*Papio cynecephalus*), and golden snub-nosed monkeys (*Rhinopithecus roxellana*), the excretion of estrogen metabolites occurs before ovulation and the surge in progesterone metabolites [102,103].

In addition to cycle monitoring, estrogen and progesterone metabolites have been useful in gestational monitoring, with a consistent pattern of elevated steroids during the first trimester and a gradual increase until parturition in several species, including the golden snub-nosed monkey (*Rhinopithecus roxellana;* [104]), rhesus monkeys (*Macaca mulatta*; [105,106], olive baboons (*Papio anubis*; [107,108], yellow baboons (*Papio cynocephalus*; [109]), and Japanese macaques (*M. fuscata*) [5110]. However, gestational endocrine profiles of Wied's black tufted-ear marmosets (*Callithrix kuhli*) showed a drop in urinary estrogen and progesterone levels starting 6 weeks prior to parturition [111].

The measurement of steroid metabolites can be informative of pregnancy viability. In woolly monkeys (*Lagothrix lagotricha poeppigii*), females with shorter gestations and a deceased infant had lower levels of FEM and FPM than females with normal pregnancies [8,9]. The typical increase in FPM and FEM was also found in Japanese macaques that had a stillbirth but not those who had an early miscarriage [5]. Conversely, when measuring both urinary estrogen and progesterone metabolite in black tufted-ear marmosets, both were elevated but only urinary estrogen had a significant difference related to infant survival [111]. Additionally, a difference in FEM levels were found between successful and aborted pregnancies in baboons with no difference in FPM [109]. This suggests that FEM and FPM may not be reliable indicators of fetal health

throughout gestation for every primate species.

While the measurement of progesterone and estrogen metabolites is frequently measured during primate pregnancy, the measurement of adrenal metabolites has also been considered as a potential early biomarker of pregnancy in primates. For example, a study measuring fecal epiandrosterone in wild assamese macaques (*Macaca assamensis*) found that fecal androgens may be able to detect pregnancy earlier than the measurement of fecal progestins because they are substantially higher during the first trimester than in the luteal phase with a subsequent decrease to pre-gestation levels until parturition [112]. However, the opposite pattern was observed in a study on fecal androgen metabolites (FAM) in mandrills (*Mandrillus sphinx*) where FAM levels increased beginning mid-gestation and were at their highest during late gestation [113], which indicates androgen differences in secretion pattern during pregnancy. Therefore, biological or physiological validations must be carried out when using immunoassays in fecal or urine samples to test for androgen specificity and avoid cross-reactivities with different metabolites. Alternatively, more refined techniques such as liquid chromatography-mass spectrometry or gas chromatography can be employed to avoid such problems.

The utilization of noninvasive biomarkers also has the potential to indicate further information regarding gestation, such as sex and number of infants. Previous studies have reported higher FAM levels in mothers carrying male fetuses compared to mothers carrying female fetuses at the end of pregnancy in macaques [112,114,115], baboons [116], and lemurs [117], but no effect was observed in mandrills [118]. Furthermore, a study on golden snub-nosed monkeys reported that a female pregnant with twins had FPM levels approximately 3-fold the levels of single birth females [104]. Additionally, a single birth in marmosets compared to twins or triplets had lower serum levels of LH/CG, progesterone, and estradiol [119], indicating the potential of these hormones in detecting multiple births.

3. Endocrinology of gestation

During gestation, the placenta is a temporary organ that acts as an endocrine exchange between the mother and the fetus. In certain mammals, such as most primates [120], rabbits, and rodents [121], the placenta is hemochorial, which is characterized by contact between the maternal bloodstream and fetal tissue, called the chorion. This hemochorial placenta has the lowest degree of separation between the maternal and fetal tissues in comparison to epitheliochorial (found in equine and porcine), synepitheliochorial (found in ruminants), and endotheliochorial (found in carnivores) placentas [121]. The level of contact between these tissues create an opportunity for the hormones secreted from the placenta to regulate both the mother and the fetal physiology [122–124].

In humans, increasing amounts of human chorionic gonadotropin (hCG) are produced by the placenta and function to maintain the corpus luteum for progesterone synthesis [4125]. While hCG has a dramatic increase in the first trimester, progesterone and estrogen levels gradually increase throughout gestation, with their highest levels around parturition [126–128]. Prior to pregnancy, estrogens are primarily produced by the ovaries, while progestins are produced by the corpus luteum following the spike in luteinizing hormone at ovulation. If fertilization and implantation occur, the corpus luteum will be maintained for a few weeks by hCG produced by syncytiotrophoblastic cells that surround the embryo [129]. Towards mid-late gestation, the main source of progesterone derives from placental utilization of fetal precursors and is integral in the maintenance of fetal life [119]. Similarly, estrogens derived from the placenta during gestation are synthesized through precursors produced in the maternal and fetal adrenal gland (e.g. DHEA(S)) because the placenta is unable to convert C21 steroids to C19 steroids (estrogen precursors) [130].

Placental-derived estrogens result in an upregulation of 11β-hydroxysteroid dehydrogenase (*11β-HSD*), which controls the ability of the placenta to convert cortisol to cortisone [131]. This conversion is important because the maturation of the *zona fasciculata* (ZF) within the fetal adrenal is controlled by fetal exposure to this cortisol [5]. Cortisol then acts on the fetal adrenal gland, activating the negative feedback loop of the Hypothalamic-Pituitary-Adrenal (HPA) axis during early to mid-gestation, and resulting in the low synthesis of glucocorticoids and low expression of 3β-hydroxysteroid dehydrogenase (*HSD3B2*) [4]. Although *HSD3B2* is also involved in the conversion of pregnenolone to progesterone, the fetus relies on the placenta to obtain progesterone [119]. During late gestation, the rise in estrogen production by the placenta stimulates *11β-HSD* to convert cortisol to cortisone, which reduces fetal exposure to cortisol [4]. The resulting decline in cortisol stimulates the HPA axis, which is key for the fetal adrenal gland to mature to the capacity needed to synthesize its own cortisol [132,133].

In addition to steroid hormones, protein hormones are involved with assistance and maintenance of mammalian pregnancy and parturition. In humans, relaxin is stimulated by the release of hCG and is secreted by the ovaries during the luteal phase [78,134–136]. If the luteal phase ends in corpus luteum degradation, relaxin levels will decline until the next luteal phase [134,137]. If the luteal phase ends in pregnancy, relaxin levels begin to increase at the end of the first trimester with the formation of the placenta [134,137,138], and continue rising until parturition [138,139]. In combination with estrogen, relaxin causes depolymerization of hyaluronic acid, which inhibits pelvic stability [140,141] and loosens the pelvic ligaments, specifically, the pubic symphysis during parturition [140,142]. Additional roles of relaxin include the growth of the mammary gland [143], inhibition of myometrium contractions [144], and softening the cervix uteri [145]. Furthermore, circulating levels of maternal oxytocin rise during the final stages of labor causing contractions of the uterine smooth muscle [146–148], in concert with relaxin to facilitate parturition.

In addition to relaxin and oxytocin, the peptide hormone prolactin, which is secreted by the anterior pituitary, increases in concentration beginning in the first trimester until parturition [149]. This increase is promoted by chorionic somatomammotropin (hCS), secreted by the placenta, which supports the secretion of prolactin against the inhibitory effects of estrogen and progesterone [149]. Furthermore, placental lactogen and placental growth hormone are two peptide hormones synthesized in the syncytiotrophoblastic cells early in gestation and continue rising in concentration throughout pregnancy to enhance maternal lipolysis [150,151]. While prolactin and hCS prime the mammary gland for the eventual role of lactation after parturition, placental lactogen and placental growth hormone provide the energy sources for both maternal metabolism and fetal development.

4. The role of DHEA(S) in gestation

Dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAS) are the most abundant circulating steroids in primates and are primarily synthesized within the adrenal cortex, in the *zona reticularis* (ZR), but can be also produced by the gonads and the brain [152]. The synthesis of DHEA(S) is dependent on the co-expression of cytochrome b5 and P450c17 and results from the conversion of pregnenolone through the catalyst complex of P450c17 and NADPH-CPR (Fig. 1) [18]. At late gestation, the fetal adrenal develops a transient layer called the fetal zone (FZ), which is homologous to the ZR and produces high amounts of DHEA(S) due to the expression of cytochrome 17α-hydroxylase/17,20 lyase (CYP17), an enzyme that converts pregnenolone to DHEA [5,10,18,153]. Following the regression of the FZ within the first year of life, the adrenal gland develops the ZR at around 6 years of age in humans, which then begins to secrete DHEA(S) levels [154–156]. The high concentration of DHEA(S) postnatally is possible by the reduced expression of the enzyme *HSD3B2* that normally competes with CYP17 for cortisol production, and by the high expression of P450c17 in the ZR [156,157]. Although the presence of these enzymes was previously thought to be a trait exclusive to some primates, more recent studies have detected

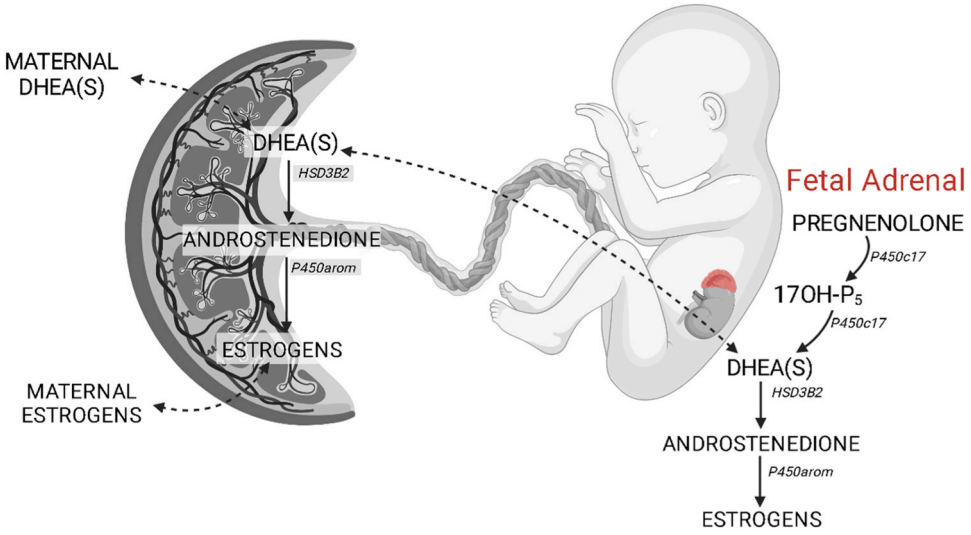

Fig. 1. Overview of the biosynthesis of DHEA(S) and the hormonal exchange between the human placental, maternal, and fetal tissues during late gestation.

DHEA(S)-related enzymes in the spiny mouse (*Acomys cahirinus*) adrenal gland at 76% of their gestational term [158] throughout postpartum, as well as an observed increase in DHEA levels postnatally from 8 to 20 days of age in this species following gonadectomy [158]. This indicates that, like primates, DHEA production in the spiny mouse is independent from gonadal synthesis. In contrast, in common marmosets (*Callithrix jacchus*) DHEAS levels decrease soon after birth [159]. In this species, males lack a functional ZR, whereas the female adrenal gland is able to produce small amounts of DHEA(S) in adult life if stimulated by low or absent gonadal activity [159,160]. However, neonates have a functional FZ, which is highly steroidogenic [160].

The changes in fetal adrenal development in addition to gonadal development during late gestation in some species appear to play a significant role in steroid production, which is crucial for both gestation maintenance and parturition. High DHEA(S) production during the fetal period are the main precursors for estrogen production in species predominantly using the Δ5 steroid synthesis pathway such as higher primates, equine [161,162], and bovine [163] due to the inability of the placenta to synthesize these steroids from C21 precursors [18–20]. In contrast, other species using either Δ4 or Δ5 pathway or exclusively the Δ4 steroid synthesis pathway such as rats [164], polar bears [165], goats [166], rely on the placenta or the gonads for estrogen synthesis during pregnancy. For example, equine placenta lacks significant expression of CYP17A1, which converts C21 to C19 steroids, and rely on ovaries for provision of C19 precursors such as DHEA [167]. In rats, placental-derived androstenedione is converted into active steroids in maternal ovary [168], and sheep and bovine placenta are capable of synthesizing estrogen from C21 steroids [169,170].

Although the evolutionary reasons for the species difference in steroid and DHEA(S) synthesis remain unclear, some studies have suggested that DHEA(S) may be involved in pre- and post-natal brain development due to its function in neurogenesis, neuroprotection, and possibly in memory and cognition [171–173]. Nevertheless, the fact that those precursors originate from fetal adrenal gland makes them ideal candidates as biomarkers of fetal health.

5. DHEA(S) as a biomarker of pregnancy outcome

There is empirical evidence that the fetal adrenal gland is responsible for most DHEA(S) produced at late gestation. One study in pregnant baboons showed that estrogen levels were 5% lower in fetectomized baboons than pregnant controls, and that exogenous estrogen treatment increased estradiol concentrations but decreased DHEA(S) concentrations in maternal serum, indicating that a negative feedback mechanism triggered by high estrogen levels suppresses DHEA(S) synthesis from the maternal adrenal gland during gestation [174]. Similarly, a study in rhesus monkeys showed that DHEAS and cortisol levels were lower in fetectomized females compared to controls, though the circadian rhythm of cortisol was maintained [175]. Furthermore, one study reported high serum DHEA levels in cows at late gestation, with significantly lower levels in cows continuously milked compared to controls [176]. In bovines, however, the main source of DHEA during gestation is the placenta [177], which may be unable to detect fetal death.

One study in humans reported that serum DHEAS concentrations decline with advancing gestation, whereas DHEA levels increase especially in the first and second trimesters, and the DHEA/DHEAS ratio is higher throughout gestation when compared to non-pregnant samples [178]. This result is likely reflective of the differences in the mechanism of the biological action of DHEA and DHEAS, with the former being generally considered the active version, given that it is readily converted into androgens, whereas the latter is more stable ([179]; reviewed by [180]). For this reason, this study suggests that for pregnancy monitoring, DHEA might be a better biomarker of fetal health than DHEAS. However, the distinction between DHEA and DHEAS in this study was possible due to the separation of those steroids via high-performance liquid chromatography (HPLC). When using immunoassays, particularly with fecal or urine samples, both DHEA and DHEAS will be measured due to cross-reactivity between these compounds.

To date, only a few studies have measured fecal DHEA(S) (fDHEAS) non-invasively. A literature review using the keywords “primates”, “DHEAS”, “gestation”, and “fecal” on the search engine Google Scholar revealed only four studies on four species. One study in Japanese macaques (*Macaca fuscata*) reported no changes in fDHEAS levels during the first half of pregnancy in comparison to the baseline, but a significant increase in fDHEAS levels in the second half of successful gestations in comparison to the baseline and to the first half of gestation [5]. DHEAS levels declined in these females after parturition (1.08 ± 0.68 μg/g), and neonates had extremely elevated concentrations of fDHEAS (9.13 ± 7.22 μg/g) as a remaining product from the regressing fetal zone [5]. In contrast, females that had stillbirths did not show any changes in fDHEAS levels, though FEM and FPM remained above non-pregnant levels throughout the gestational period [5]. The same study reported that a female that had a miscarriage during early pregnancy had high FEM and FPM in the first month of pregnancy, followed by a drastic decline to non-pregnant levels after fetal loss was detected by ultrasound [5]. This indicates that longitudinal measurement of FEM and FPM can detect early fetal loss but may mask late fetal loss and stillbirth. Conversely, fDHEAS are not suitable for early loss detection, but are

useful in predicting fetal health at late gestation.

Similarly, previous studies measuring fDHEAS using EIA in orangutans [10], siamangs [181], and in howler monkeys [182] have shown that the mean fDHEAS levels were higher in females at late gestation than non-pregnant/non-lactating females (Table 1). However, we still do not know how accurate DHEAS is in predicting fetal health from one single sample. We propose a method to detect pregnancy outcome in non-human primates at late gestation by establishing basal DHEAS levels per species. Using previously published data (Table 1), we calculated the mean plus 2 standard deviations (SD) of fDHEAS in non-pregnant/non-lactating females, in each species, controlling for environment (captivity, social vs single-housing) and age (adults only), given that these factors influence DHEAS levels [10,183–185]. Following a method similar to the iterative process previously described [31,181,186–190], we predicted that any sample from a pregnant female in the last third of gestation that fell above this threshold would indicate healthy pregnancy, and samples that fell below the threshold would indicate stillbirth. The specificity of the method was determined by the percentage of samples that were correctly identified as non-pregnant and the sensitivity of this method was determined by the percentage of samples that were correctly identified as successful/unsuccessful gestation. In addition, we conducted ROC curve analyses in R software (version 4.2.2) using the function "roc" (package pROC) [191] to test the accuracy of fecal DHEAS in predicting pregnancy and fetal health.

The method had 100% specificity for all species, and 100% sensitivity for orangutan, siamangs, and Japanese macaques housed individually at late gestation, given that all samples from successful pregnancies were above the threshold, and all samples from stillbirth were below the threshold (Fig. 2 – A, B, E). However, the sensitivity was 80% in howler monkeys (Fig. 2 – C) and 50% in Japanese macaques housed socially (Fig. 2 – D). The lower sensitivity in these species may have been caused by the fact that both species were housed in social groups. DHEAS is affected by stress levels and has been implicated in the regulation of dominance rank in female Japanese macaques [192]. In addition, a previous study in Japanese macaques comparing the age-related changes in fecal DHEAS levels between single- and socially-housed females reported that the relationship between age and DHEAS levels was stronger in females housed individually than socially, suggesting that social factors influence DHEAS levels [184]. Thus, future studies that compare successful versus unsuccessful pregnancies in macaques living under the same housing conditions would be valuable to test the accuracy of fecal DHEAS in predicting fetal health. Nevertheless, the ROC curve analyses using this preliminary data suggests that fDHEAS is a good predictor of both pregnancy (AUC = 0.8039) and fetal health (AUC= 0.8137) (Fig. 3).

Another possible reason for the lower sensitivity in howler monkeys compared to other species is that the two samples that fell below the threshold were collected prior to the complete development of the fetal zone (37 and 85 days prior to parturition), but further studies on fetal development in the species are needed to confirm this hypothesis. However, we acknowledge that the high sensitivity for the other species may have been achieved due to the small sample size of our preliminary data. For this reason, while this method might be useful to predict gestation outcome, we suggest caution when applying it to species in which the DHEAS pattern has not been well investigated, a bigger sample size to establish baseline levels per population, and we do not recommend its use on a single sample.

6. Other potential applications of DHEA(S) in wildlife reproduction

6.1. Maternal behavior

One of the most important factors that determine infant survival is parental care, which has been negatively associated with stress levels [193–195]. DHEA(S) are involved in stress regulation [196] in addition to their role as an estrogen precursor. During the stress response, the activation of the HPA axis results in secretion of glucocorticoids (GC) and DHEA(S). While short-term elevations in GC will be beneficial by enhancing the ability of the body to cope with the stressor (e.g, glucose mobilization and analgesia during "fight or flight"), prolonged stress will be detrimental to the individual (e.g immunosuppression, neurotoxicity, cardiac diseases) [197–199]. In contrast, DHEA(S) act as GC-antagonists by competing with GC for glucocorticoids and mineralocorticoid receptors, which helps the body restore homeostasis with their beneficial effects, such as neuroprotection, immune-enhancement and mood improvement [196,200]. However, in prolonged stress, GC are secreted at higher levels than DHEA(S), which can lead to immunodepression, cognitive impairment, and mood disorders (e.g anxiety and depression), characteristics of chronic stress [201]. For this reason, the GC:DHEA(S) ratio has been considered an important index to evaluate stress levels in wildlife [10,192,202].

One recent study in humans measured cortisol and DHEAS levels during the last trimester of pregnancy in patients exhibiting severe anxiety (ANX). The authors observed that the ANX group had significantly higher cortisol and lower DHEAS levels when compared to healthy pregnant women, resulting in a higher cortisol:DHEAS ratio in the ANX group [203]. Although this study did not investigate maternal behavior, previous studies have reported an association between anxiety or stress levels and postpartum depression in humans [204–206], as well as high stress levels and maternal rejection in lowland gorillas (*Gorilla gorilla gorilla*)[195]), rhesus monkey [193] and in Japanese macaques [207]. Considering the beneficial effects of DHEA(S) in mood and anti-depression [208] as opposed to the negative effects of dysregulated GC secretion in increasing anxiety [209], the co-measurement of these two adrenal hormones can be useful in evaluating the severity of mood disorders, which may affect maternal behavior.

Stress levels during the postpartum are also associated with reproductive experience. Multiparous females generally have lower risk of peripartum stress and provide higher infant investment than nulliparous females [210,211]. In monogamous species, this relationship may be extended to fathers. Bardi et al.[212] investigated the response of urinary cortisol, DHEA, and the DHEA:cortisol ratio to a cognitive-foraging

Table 1
Mean ± standard deviation (SD) of fecal DHEAS levels in captive primates all measured using EIA. Data was controlled for environment, age, and species.

Species		Mean ± SD fecal DHEAS levels (ng/g)				Reference
	Age (Years)	Baseline	Late Gestation[a]	Fold change	Outcome	
Pongo pygmaeus	28 – 58	172.66 ± 102.48 N = 2 (*11*)	511.45 ± 137.35 N = 1 (*3*)	2.96	Successful	[10]
Symphalangus syndactilus	13 – 28	2534.17 ± 1035.88 N = 4 (13)	15625.85 ± 7867.06 N = 1 (*6*)	6.16	Successful	[181]
Macaca fuscata (socially living)	5 – 12	886.67 ± 490.51 N = 4 (12)	2545 ± 1687.71 N = 2 (*4*)	2.87	Successful	[5]
Macaca fuscata (singly living)	5 – 13	2411.25 ± 1410.28 N = 3 (*9*)	1747.14 ± 1606.01 N = 2 (*7*)	0.72	Stillbirth	[5]
Alouatta caraya	5 – 12	13281.15 ± 4092.38 N = 1 (*3*)	57843.86 ± 37160.31 N = 4 (*10*)	4.35	Successful	[182]

N = number of females (total number of fecal samples)

[a] Sample collection start for each species: *P. pygmaeus,* last trimester; *S. syndactilus*, 46 days prepartum; *M. fuscata* (both), 83 days prepartum; *A. caraya*, 90 days prepartum

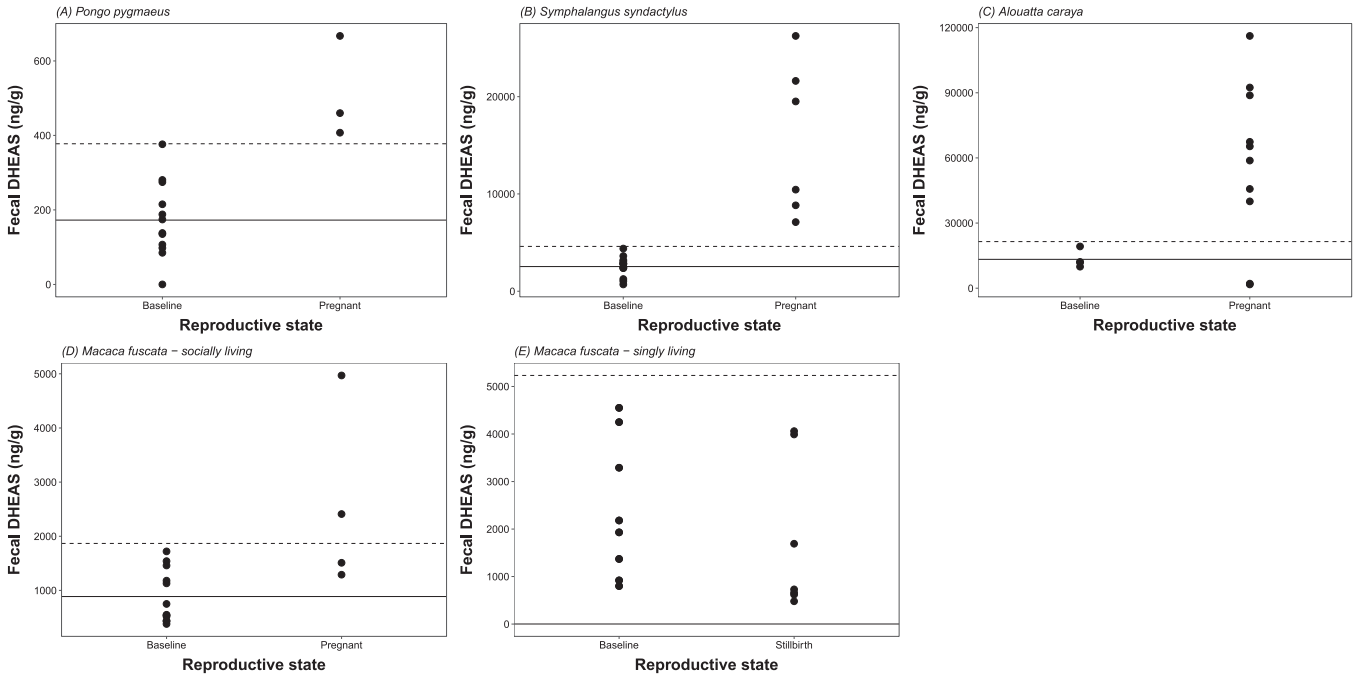

Fig. 2. Fecal DHEAS concentrations in four primate species by reproductive state. Each dot represents one fecal sample. The solid line is the mean baseline (non-pregnant/non-lactating) levels. The dashed line refers to 2 standard deviations above the mean.

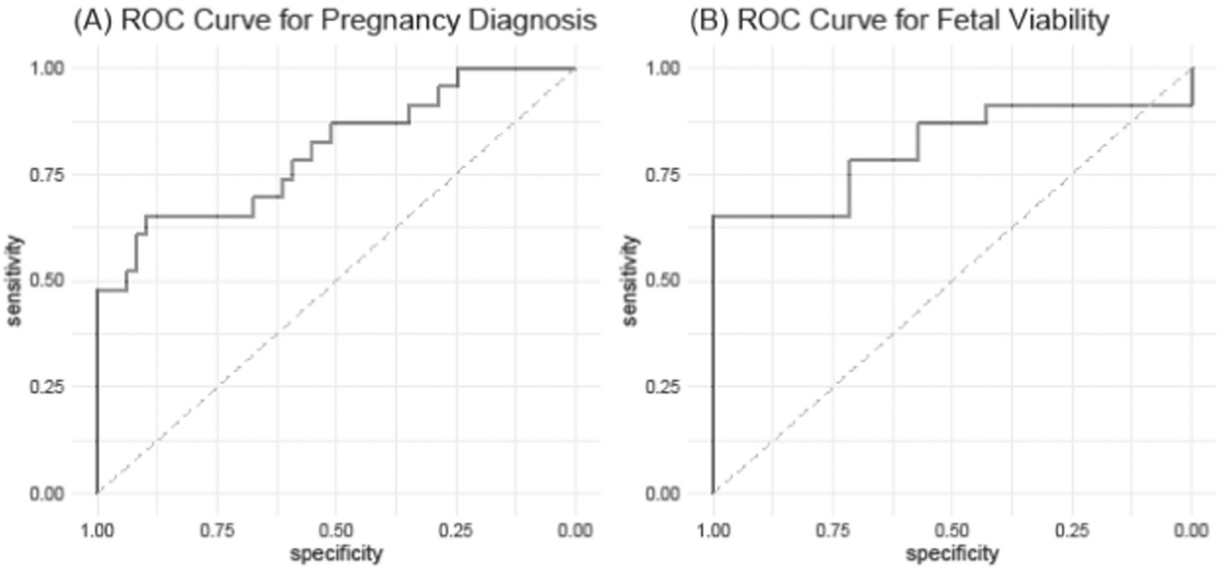

Fig. 3. *(A) The receiver operating characteristic curve (ROC)* of samples from all females in the study (N = 72; Baseline = 49; Pregnant = 23) excluding the unsuccessful births showing the diagnostic ability of using fecal DHEAS to determine pregnancy at late gestation. AUC = 0.8039 (B) The ROC of samples from all pregnant females in the study (N = 30; Successful = 23; Unsuccessful = 7) showing the diagnostic ability of using fecal DHEAS to determine fetal viability at late gestation. AUC = 0.8137.

task in pairs of a monogamous species (owl monkey (*Aotus* spp.)), with and without reproductive experience (RE). The authors observed that RE did not affect cortisol concentrations, but experienced parents had a higher DHEA:cortisol ratio after exposure to habituation training and in the first day of testing than non-experienced parents. Pairs with RE also had 4-fold more efficient foraging strategies than did non-RE pairs. These results showed that in this species, RE reduces the stress response and enhances cognitive skills in monogamous pairs, consequently improving the likelihood of infant survival.

6.2. Infant development

Several studies have focused on fetal and neonatal HPA axis activity to investigate how early exposure to stressful events can impact the offspring's development through epigenetic mechanisms [213,214]. For those studies, the use of non-invasive matrixes characterized by a long-time lag (e.g hair and nails from neonates) can provide important past information about fetal physiology because hormonal metabolites from these samples represent changes that can occur months prior to collection, as opposed to feces and urine, which have a relatively shorter time lag (6–24 h) [215,216].

One study in 80 humans collected nails from infants to measure whether fetal DHEA and DHEAS were affected in gestations characterized by stressful life events compared to normal gestations [213]. The authors observed that infants of mothers with stressful life events during pregnancy had higher DHEA levels than controls, but no apparent changes in DHEAS levels. Furthermore, DHEA levels were not related to maternal stress before pregnancy.

Another study in capuchin monkeys (*Cebus apella*) investigated whether photoperiod stress (maternal constant light exposure during the last third of gestation) affect infant cortisol and DHEAS levels at birth, at one month and at ten months of age [214]. The resulting effect was a marked reduction in DHEAS concentrations in these infants and almost twice the concentration of cortisol than controls. The authors highlighted the influence of maternal chronodisruption during late gestation on primate adrenal gland maturation and suggest further investigations regarding these changes during postnatal and particularly adult life [214].

Studies in domestic species have also shown the potential of DHEAS and GC:DHEAS as biomarkers of chronic stress, of prenatal HPA function, and in gestation monitoring, especially during the third trimester. For example, Lanci et al. [217] measured hair cortisol and DHEAS levels in foals and mares to investigate the feto-maternal relationship, as well as differences in hormonal concentrations between healthy and sick foals. The authors found a correlation between foal and mare hair cortisol concentrations, as well as a positive correlation between cortisol and DHEAS concentrations in both. Hair cortisol concentrations did not differ between groups and was not influenced by clinical parameters. However, sick foals had higher DHEAS concentrations (43.1 $\pm$ 69.0 pg/mg) than healthy ones (19.2 $\pm$ 44.0 pg/mg) and a lower cortisol:DHEAS ratio (4.1 $\pm$ 3.8 pg/mg versus 7.8 $\pm$ 6.7 pg/mg, respectively). The authors associated this increase in DHEAS with the neuroprotective effect of this hormone because most of the sick foals had Hypoxic-Ischemic Encephalopathy/Neonatal Syndrome. Moreover, they found increased levels of both cortisol and the cortisol:DHEAS ratio in females pregnant with sick foals (0.8 $\pm$ 0.5 pg/mg) compared to those pregnant with healthy foals (0.6 $\pm$ 0.4 pg/mg). The authors suggest that high maternal cortisol levels could have led to impaired HPA development in the fetus, which resulted in sick foals [217]. Another study by Fusi et al. [218] in 126 newborn puppies investigated the relationship between DHEA concentrations and fetal birth condition (premature, stillbirth, and puppies that died between days 1–30 postbirth) using hair and claws. The authors found that premature puppies had higher DHEA claw concentrations (33.8 $\pm$ 13.15 pg/mg) when compared with stillbirth (26.6 $\pm$ 13.20 pg/mg) and failure to thrive (24.7 $\pm$ 15.80 pg/mg), but no effects in hair DHEA concentrations. These studies suggest that the relationship between GC, DHEA(S), and fetal health may vary with species and sample matrix, which reinforces the need for comparative studies. Moreover, early exposure to life-stress events may affect fetal programming of the HPA axis, therefore a good understanding of these mechanisms is vital to adopt these hormones as biomarkers of fetal health.

Another factor that must be considered is the relation between GC and its impact in the reproductive system, which indicates the intrinsic connection between the HPA and the Hypothalamic–Pituitary–Gonadal (HPG) axis. While the stress during gestation can affect fetal and postnatal periods, resulting in reduced birth weight, anxiety, and impaired maternal and fetal HPA axis activity [219,220], in adult life, stress suppresses important reproductive hormones, such as testosterone, LH, and estrogen secretion, as well as their action on gonads due to the downregulation of their receptors [219,221]. Thus, monitoring stress levels has been pivotal in wildlife management for improving breeding strategies [222,223]. In this context, DHEA(S) is a promising biomarker for wildlife monitoring due to their regulatory roles in both stress and reproduction.

7. Summary

The reported studies have demonstrated that DHEA(S) are potential biomarkers of reproduction and fetal health due to the intrinsic role of these steroids as both an estrogen precursor (in the Δ5 metabolic pathway) and a stress regulator. On the one hand, the ability to measure DHEA(S) at late gestation as biomarkers of fetal health might be restricted to some taxa, and due to the high interspecies variation in adrenal steroid metabolic pathways, it requires further comparative studies to establish the endocrine pattern of gestation for each species. On the other hand, the use of DHEA(S) as biomarkers of stress has been expanded to numerous wild and domestic species, which can be helpful in developing breeding management protocols and in evaluating conservation strategies. Furthermore, the advance of noninvasive matrixes has enabled studies to investigate the effect of environment and early stress exposure on fetal development, maternal behavior, and infant survival. Although the use of DHEA(S) in predicting fetal outcome is limited to late gestation, it complements the information provided by progesterone and estrogens. Combined, these hormones can detect both early and late miscarriage events and may be helpful in identifying possible causes of decreased fertility in some populations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The study was funded by the National Science Foundation (Award nº 2123574) and by the Wenner-Gren Foundation (Grant nº 10088). Additionally, we thank two anonymous reviewers for their suggestions to improve the manuscript.

References

[1] L.J. Salomon, Z. Alfirevic, F. Da Silva Costa, R.L. Deter, F. Figueras, T. Ghi, P. Glanc, A. Khalil, W. Lee, R. Napolitano, A. Papageorghiou, A. Sotiriadis, J. Stirnemann, A. Toi, G. Yeo, ISUOG Practice Guidelines: ultrasound assessment of fetal biometry and growth, Ultrasound Obstet. Gynecol. 53 (2019) 715–723, https://doi.org/10.1002/uog.20272.

[2] A. Vernunft, A. Eggert, K.-P. Brüssow, Ultrasonographic monitoring of fetal growth and fetal weight calculation in sows during gestation, Agriculture 13 (2022) 16, https://doi.org/10.3390/agriculture13010016.

[3] F.O.B. Monteiro, M.B. de Koivisto, W.R.R. Vicente, R. de Amorim Carvalho, C. W. Whiteman, P.H.G. Castro, C.E. Maia, Uterine evaluation and gestation diagnosis in owl monkey (Aotus azarai infulatus) using the B mode ultrasound, J. Med. Primatol. 35 (2006) 123–130, https://doi.org/10.1111/j.1600-0684.2006.00155.x.

[4] W. Saltzman, D. Maestripieri, The neuroendocrinology of primate maternal behavior, Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (2011) 1192–1204, https://doi.org/10.1016/j.pnpbp.2010.09.017.

[5] R.S.C. Takeshita, M.A. Huffman, K. Mouri, K. Shimizu, F.B. Bercovitch, Dead or alive? Predicting fetal loss in Japanese macaques (Macaca fuscata) by fecal metabolites, Anim. Reprod. Sci. 175 (2016) 33–38, https://doi.org/10.1016/j.anireprosci.2016.10.006.

[6] A. Kumar, S. Mehrotra, S. Dangi, G. Singh, S. chand, L. Singh, A. Mahla, S. Kumar, K. Nehra, Faecal steroid metabolites assay as a non-invasive monitoring of reproductive status in animals, Vet. World 6 (2013) 59, https://doi.org/10.5455/vetworld.2013.59-63.

[7] J.R. Herrick, G. Agoramoorthy, R. Rudran, J.D. Harder, Urinary progesterone in free-ranging red howler monkeys (Alouatta seniculus): preliminary observations of the estrous cycle and gestation, Am. J. Primatol. 51 (2000) 257–263, https://doi.org/10.1002/1098-2345(200008)51:4<257::AID-AJP5>3.0.CO;2-6.

[8] A.M. Dettmer, K.L. Rosenberg, S.J. Suomi, J.S. Meyer, M.A. Novak, Associations between Parity, Hair Hormone Profiles during Pregnancy and Lactation, and Infant Development in Rhesus Monkeys (Macaca mulatta), PLoS One 10 (2015), e0131692, https://doi.org/10.1371/journal.pone.0131692.

[9] L.A. Abondano, T.E. Ziegler, A. Di Fiore, Reproductive endocrinology of wild female woolly monkeys (*Lagothrix lagotricha poeppigii*) during puberty, ovarian cyclicity, and pregnancy, Am. J. Prima 84 (2022), https://doi.org/10.1002/ajp.23303.

[10] R.S.C. Takeshita, R.S. Mendonça, F.B. Bercovitch, M.A. Huffman, Developmental changes in the endocrine stress response in orangutans (Pongo pygmaeus),

J. Comp. Physiol. B 189 (2019) 659–672, https://doi.org/10.1007/s00360-019-01235-7.
[11] I.S. Fraser, K.M. Nicholson, G. Graham, H. Boyle, Hormone changes in relation to the time of fetal death after prostaglandin-induced abortion, Prostaglandins 13 (1977) 1161–1177, https://doi.org/10.1016/0090-6980(77)90142-3.
[12] K.C. Tan, V.H.H. Goh, S.M.M. Karim, S.S. Ratnam, S.R. Kottegoda, Maternal plasma estradiol and progesterone levels during therapeutic abortion induced by 16, 16 dimethyl PGE2 p-benzaldehyde semicarbazone ester, Prostaglandins Leukot. Med. 14 (1984) 215–224, https://doi.org/10.1016/0262-1746(84)90205-1.
[13] J.-O. Lindell, H. Kindahl, L.-E. Edqvist, Prostaglandin induced early abortions in the bovine. Clinical outcome and endogenous release of prostaglandin F2α and progesterone, Anim. Reprod. Sci. 3 (1981) 288–289, https://doi.org/10.1016/0378-4320(81)90004-X.
[14] J.K. O'Brien, T.R. Robeck, The relationship of maternal characteristics and circulating progesterone concentrations with reproductive outcome in the bottlenose dolphin (Tursiops truncatus) after artificial insemination, with and without ovulation induction, and natural breeding, Theriogenology 78 (2012) 469–482, https://doi.org/10.1016/j.theriogenology.2012.02.011.
[15] T.R. Robeck, K.J. Steinman, C.B. Parry, F.M. Gomez, E.D. Jensen, Comparisons of serum progesterone and progestagen concentrations in normal and abnormal bottlenose Dolphin (Tursiops truncatus) pregnancies, Front. Mar. Sci. 8 (2021), 630563, https://doi.org/10.3389/fmars.2021.630563.
[16] D.R. Bergfelt, J.L. Blum, B.G. Steinetz, K.J. Steinman, J.K. O'Brien, T.R. Robeck, Relaxin as a hormonal aid to evaluate pregnancy and pregnancy loss in bottlenose dolphins (Tursiops truncatus), Gen. Comp. Endocrinol. 242 (2017) 24–29, https://doi.org/10.1016/j.ygcen.2015.12.024.
[17] W.E. Rainey, K.S. Rehman, B.R. Carr, The human fetal adrenal: making adrenal androgens for placental estrogens, Semin. Reprod. Med. 22 (2004) 327–336, https://doi.org/10.1055/s-2004-861549.
[18] A.J. Conley, J.C. Pattison, I.M. Bird, Variations in adrenal androgen production among (nonhuman) primates, Semin. Reprod. Med. 22 (2004) 311–326, https://doi.org/10.1055/s-2004-861548.
[19] S.W. Walsh, F.Z. Stanczyk, M.J. Novy, Daily hormonal changes in the maternal, fetal, and amniotic fluid compartments before parturition in a primate species, J. Clin. Endocrinol. Metab. 58 (1984) 629–639, https://doi.org/10.1210/jcem-58-4-629.
[20] J. Rege, S. Garber, A.J. Conley, R.M. Elsey, A.F. Turcu, R.J. Auchus, W.E. Rainey, Circulating 11-oxygenated androgens across species, J. Steroid Biochem. Mol. Biol. 190 (2019) 242–249, https://doi.org/10.1016/j.jsbmb.2019.04.005.
[21] W.B. Ballard, R.W. Tobey, Decreased calf production of moose immobilized with anectine administered from helicopter, Wildl. Soc. Bull. 9 (1981) 207–209.
[22] D.A. Jessup, R.K. Clark, R.A. Weaver, M.D. Kock, The safety and cost-effectiveness of net-gun capture of desert bighorn sheep (Ovis canadensis nelsoni), J. Zoo. Anim. Med 19 (1988) 208, https://doi.org/10.2307/20094889.
[23] D.G. Larsen, D.A. Gauthier, Effects of capturing pregnant moose and calves on calf survivorship, J. Wildl. Manag. 53 (1989) 564, https://doi.org/10.2307/3809177.
[24] N. Peterson, R.R. Lopez, P.A. Frank, M.J. Peterson, Evaluating capture methods for urban white-tailed deer, Wildl. Soc. Bull. 31 (2003) 1176–1187.
[25] P. Valkenburg, R.D. Boertje, J.L. Davis, Effects of darting and netting on caribou in Alaska, J. Wildl. Manag. 47 (1983) 1233, https://doi.org/10.2307/3808201.
[26] M.H. Blank, C.H. Adania, W.F. Swanson, D. de Souza Ramos Angrimani, M. Nichi, M. Alcindo de Barros Vaz Guimarães, R.C. Barnabe, Comparative fecal steroid profile during pregnancy, parturition, and lactation between natural fertilization and embryo transfer in ocelots (Leopardus pardalis), Theriogenology 182 (2022) 26–34, https://doi.org/10.1016/j.theriogenology.2022.01.026.
[27] C.A. Bleke, E.M. Gese, S.S. French, Variations, validations, degradations, and noninvasive determination of pregnancy using fecal steroid metabolites in free-ranging pronghorn, Gen. Comp. Endocrinol. 312 (2021), 113841, https://doi.org/10.1016/j.ygcen.2021.113841.
[28] D.M.D. de Mello, C.A. De Oliveira, Biological matrices for sampling free-ranging cetaceans and the implications of their use for reproductive endocrine monitoring, Mammal. Rev. 46 (2016) 77–91.
[29] B.L. Lasley, J. Kirkpatrick, Monitoring ovarian function in captive and free-ranging wildlife by means of urinary and fecal steroids, J. Zoo. Wildl. Med 22 (1991) 23–31.
[30] C. Touma, R. Palme, Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation, Ann. N. Y. Acad. Sci. 1046 (2005) 54–74, https://doi.org/10.1196/annals.1343.006.
[31] J.L. Brown, S.K. Wasser, D.E. Wildt, L.H. Graham, Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces1, Biol. Reprod. 51 (1994) 776–786, https://doi.org/10.1095/biolreprod51.4.776.
[32] C.J. Andrews, D.G. Thomas, J. Yapura, M.A. Potter, Reproductive biology of the 38 extant felid species: a review, Mammal. Rev. 49 (2019) 16–30, https://doi.org/10.1111/mam.12145.
[33] S. Bristol-Gould, T.K. Woodruff, Folliculogenesis in the domestic cat (Felis catus), Theriogenology 66 (2006) 5–13, https://doi.org/10.1016/j.theriogenology.2006.03.019.
[34] E. Malandain, D. Rault, E. Froment, S. Baudon, L. Desquilbet, D. Begon, S. Chastant-Maillard, Follicular growth monitoring in the female cat during estrus, Theriogenology 76 (2011) 1337–1346, https://doi.org/10.1016/j.theriogenology.2011.06.002.
[35] J.G. Howard, M.A. Barone, A.M. Donoghue, D.E. Wildt, The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats, Reproduction 96 (1992) 175–186, https://doi.org/10.1530/jrf.0.0960175.
[36] S.L. Monfort, K.D. Dahl, N.M. Czekala, L. Stevens, M. Bush, D.E. Wildt, Monitoring ovarian function and pregnancy in the giant panda (Ailuropoda melanoleuca) by evaluating urinary bioactive FSH and steroid metabolites, Reproduction 85 (1989) 203–212, https://doi.org/10.1530/jrf.0.0850203.
[37] E. Curry, L.J. Browning, P. Reinhart, T.L. Roth, Integrating trans-abdominal ultrasonography with fecal steroid metabolite monitoring to accurately diagnose pregnancy and predict the timing of parturition in the red panda (*Ailurus fulgens styani*), Zoo. Biol. 36 (2017) 193–200, https://doi.org/10.1002/zoo.21358.
[38] R.A. Mead, M. Bowles, G. Starypan, M. Jones, Evidence for pseudopregnancy and induced ovulation in captive wolverines (Gulo gulo), Zoo. Biol. 12 (1993) 353–358, https://doi.org/10.1002/zoo.1430120405.
[39] C.J. Andrews, D.G. Thomas, M.V. Welch, J. Yapura, M.A. Potter, Monitoring ovarian function and detecting pregnancy in felids: a review, Theriogenology 157 (2020) 245–253, https://doi.org/10.1016/j.theriogenology.2020.06.036.
[40] G.-R. Chang, C.-C. Yang, S.-H. Hsu, C. Lin, C.-L. Chiu, F.-T. Chan, F.C. Mao, Fecal reproductive steroid profiles for monitoring reproductive patterns in female formosan black bears (*Ursus thibetanus Formosanus*), Ann. Zool. Fenn. 48 (2011) 275–286, https://doi.org/10.5735/086.048.0502.
[41] H.G. Verhage, N.B. Beamer, R.M. Brenner, Plasma levels of estradiol and progesterone in the cat during polyestrus, pregnancy and pseudopregnancy, Biol. Reprod. 14 (1976) 179–1585, https://doi.org/10.1095/biolreprod14.5.579.
[42] A.L. Velloso, S.K. Wasser, S.L. Monfort, J.M. Dietz, Longitudinal fecal steroid excretion in maned wolves (Chrysocyon brachyurus), Gen. Comp. Endocrinol. 112 (1998) 96–107, https://doi.org/10.1006/gcen.1998.7147.
[43] M. Bonnin, M. Mondain-Monval, B. Dutourne, Oestrogen and progesterone concentrations in peripheral blood in pregnant red foxes (Vulpes vulpes), Reproduction 54 (1978) 37–41, https://doi.org/10.1530/jrf.0.0540037.
[44] U.S. Seal, E.D. Plotka, J.M. Packard, L.D. Mech, Endocrine correlates of reproduction in the wolf. I. Serum Progesterone, Estradiol and LH during the estrous cycle, Biol. Reprod. 21 (1979) 1057–1066, https://doi.org/10.1095/biolreprod21.5.1057.
[45] J.L. Brown, Female reproductive cycles of wild female felids, Anim. Reprod. Sci. 124 (2011) 155–162, https://doi.org/10.1016/j.anireprosci.2010.08.024.
[46] J.D. Mellen, Factors influencing reproductive success in small captive exotic felids (Felis spp.): a multiple regression analysis, Zoo. Biol. 10 (1991) 95–110.
[47] W.F. Swanson, Research in nondomestic species: experiences in reproductive physiology research for conservation of endangered felids, ILAR J. 44 (2003) 307–316, https://doi.org/10.1093/ilar.44.4.307.
[48] W.F. Swanson, Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats, Theriogenology 66 (2006) 49–58, https://doi.org/10.1016/j.theriogenology.2006.03.024.
[49] F. Dalerum, S. Creel, S.B. Hall, Behavioral and endocrine correlates of reproductive failure in social aggregations of captive wolverines (*Gulo gulo*), J. Zool. 269 (2006) 527–536, https://doi.org/10.1111/j.1469-7998.2006.00116.x.
[50] B.S. Durrant, N. Ravida, T. Spady, A. Cheng, New technologies for the study of carnivore reproduction, Theriogenology 66 (2006) 1729–1736, https://doi.org/10.1016/j.theriogenology.2006.02.046.
[51] L. Couture, J.P. Lemonnier, F. Troalen, J.F. Roser, G.R. Bousfield, D. Bellet, J. M. Bidart, Immunochemical studies of equine chorionic gonadotropin (eCG), eCG alpha, and eCG beta, Endocrinology 132 (1993) 205–211, https://doi.org/10.1210/endo.132.1.7678214.
[52] P.M. McCue, Endocrine evaluation of pregnancy, in: J.J. Dascanio, P.M. McCue (Eds.), Equine Reprod. Proced, John Wiley & Sons, Inc, Hoboken, NJ, USA, 2014, pp. 235–239, https://doi.org/10.1002/9781118904398.ch71.
[53] J.F. Roser, R.M. Lofstedt, Urinary eCG patterns in the mare during pregnancy, Theriogenology 32 (1989) 607–622, https://doi.org/10.1016/0093-691X(89)90282-3.
[54] T.M. Nett, D.W. Holtan, V.L. Estergreen, L.H. Oestrogens, PMSG, and prolactin in serum of pregnant mares. J. Reprod. Fertil. (1975) 457–462.
[55] J.F. Kirkpatrick, B.L. Lasley, S.E. Shideler, J.F. Roser, J.W. Turner, Non-instrumented immunoassay field tests for pregnancy detection in free-roaming feral horses, J. Wildl. Manag. 57 (1993) 168, https://doi.org/10.2307/3809014.
[56] E.C. Ramsay, F. Moran, J.F. Roser, B.L. Lasley, Urinary steroid evaluations to monitor ovarian function in exotic ungulates: X. Pregnancy diagnosis in Perissodactyla, Zoo. Biol. 13 (1994) 129–147, https://doi.org/10.1002/zoo.1430130205.
[57] S.L. Cain, M.D. Higgs, T.J. Roffe, S.L. Monfort, J. Berger, Using fecal progestagens and logistic regression to enhance pregnancy detection in wild ungulates: a bison case study, Wildl. Soc. Bull. 36 (2012) 631–640, https://doi.org/10.1002/wsb.178.
[58] K.A. Schoenecker, R.O. Lyda, J. Kirkpatrick, Comparison of three fecal steroid metabolites for pregnancy detection used with single sampling in bighorn sheep (Ovis canadensis), J. Wildl. Dis. 40 (2004) 273–281, https://doi.org/10.7589/0090-3558-40.2.273.
[59] M.A. Stoops, K.M. MacKinnon, T.L. Roth, Longitudinal fecal hormone analysis for monitoring reproductive activity in the female polar bear (Ursus maritimus), Theriogenology 78 (2012) 1977–1986, https://doi.org/10.1016/j.theriogenology.2012.07.005.
[60] A. Marozzi, V.I. Cantarelli, F.M. Gomez, A. Panebianco, L.R. Leggieri, P. Gregorio, M.F. Ponzio, P.D. Carmanchahi, A predictive model to diagnose pregnancy in guanacos (*Lama guanicoe*) using non-invasive methods, Can. J. Zool. 98 (2020) 13–20, https://doi.org/10.1139/cjz-2019-0070.
[61] M.A. Stoops, G.B. Anderson, B.L. Lasley, S.E. Shideler, Use of fecal steroid metabolites to estimate the pregnancy rate of a free-ranging herd of tule elk, J. Wildl. Manag. 63 (1999) 561–569, https://doi.org/10.2307/3802643.

[62] Y.-H. Wang, S.-Q. Liu, S. Yang, T.-X. Zhang, Y.-T. Wei, J.-T. Zhou, D.-F. Hu, L.-H. Li, Determination of ovarian cyclicity and pregnancy using fecal progesterone in forest musk deer (Moschus berezovskii), Anim. Reprod. Sci. 170 (2016) 1–9, https://doi.org/10.1016/j.anireprosci.2016.03.002.

[63] V.G. Krepschi, B.F. Polegato, E.S. Zanetti, J.M.B. Duarte, Fecal progestins during pregnancy and postpartum periods of captive red brocket deer (Mazama americana), Anim. Reprod. Sci. 137 (2013) 62–68, https://doi.org/10.1016/j.anireprosci.2012.11.016.

[64] P. Mayor, D.A. Guimaraes, J. da Silva, F. Jori, M. Lopez-Bejar, Reproductive monitoring of collared peccary females (Pecari tajacu) by analysis of fecal progesterone metabolites, Theriogenology 134 (2019) 11–17, https://doi.org/10.1016/j.theriogenology.2019.05.008.

[65] C. Mithileshwari, T. Srivastava, V. Kumar, A. Kumar, G. Umapathy, Non-invasive assessment of fecal progestagens and pregnancy detection in Himalayan musk deer (Moschus chrysogaster), Theriogenology 85 (2016) 216–223, https://doi.org/10.1016/j.theriogenology.2015.09.009.

[66] S. Ostrowski, C. Blanvillain, P. Mésochina, K. Ismail, F. Schwarzenberger, Monitoring reproductive steroids in feces of Arabian oryx: toward a non-invasive method to predict reproductive status in the wild, Wildl. Soc. Bull. 33 (2005) 965–973, https://doi.org/10.2193/0091-7648(2005)33[965:MRSIFO]2.0.CO;2.

[67] W.D. Walter, P.J. Pekins, A.T. Rutberg, H.J. Kilpatrick, Evaluation of immunocontraceptive adjuvants, titers, and fecal pregnancy indicators in free-ranging white-tailed deer, Wildl. Soc. Bull. 30 (2002) 908–914.

[68] C. Borque, S.S. Pérez-Garnelo, M. Delclaux, E. Martínez, J. De la Fuente, Fecal steroid evaluation to monitor reproductive status in wild ungulate females using enzyme immunoassay commercial kits, J. Zoo. Wildl. Med. 42 (2011) 537–551, https://doi.org/10.1638/2009-0187.1.

[69] M.L. Patton, R.R. Swaisgood, N.M. Czekala, A.M. White, G.A. Fetter, J. P. Montagne, R.G. Rieches, V.A. Lance, Reproductive cycle length and pregnancy in the southern white rhinoceros (Ceratotherium simum simum) as determined by fecal pregnane analysis and observations of mating behavior, Zoo. Biol. 18 (1999) 111–127, https://doi.org/10.1002/(SICI)1098-2361(1999)18:2<111::AID-ZOO3>3.0.CO;2-0.

[70] J.K. O'Brien, K.J. Steinman, G.A. Fetter, T.R. Robeck, Androgen and glucocorticoid production in the male killer whale (*Orcinus orca*): influence of age, maturity, and environmental factors, Andrology 5 (2017) 180–190, https://doi.org/10.1111/andr.12254.

[71] G.A. Montano, T.R. Robeck, K.J. Steinman, J.K. O'Brien, Circulating anti-Müllerian hormone concentrations in relation to age and season in male and female beluga (Delphinapterus leucas, Reprod. Fertil. Dev. 29 (2017) 1642, https://doi.org/10.1071/RD15537.

[72] J.E. Sawyer-Steffan, V.L. Kirby, W.G. Gilmartin, Progesterone and estrogens in the pregnant and nonpregnant dolphin, tursiops truncatus, and the effects of induced ovulation1, Biol. Reprod. 28 (1983) 897–901, https://doi.org/10.1095/biolreprod28.4.897.

[73] K.L. West, J. Ramer, J.L. Brown, J. Sweeney, E.M. Hanahoe, T. Reidarson, J. Proudfoot, D.R. Bergfelt, Thyroid hormone concentrations in relation to age, sex, pregnancy, and perinatal loss in bottlenose dolphins (Tursiops truncatus), Gen. Comp. Endocrinol. 197 (2014) 73–81, https://doi.org/10.1016/j.ygcen.2013.11.021.

[74] T.R. Robeck, J.L. Blum, K.J. Steinman, J.R. Ratner, D.R. Bergfelt, J.K. O'Brien, Longitudinal profiles of relaxin and progestagens during pregnancy, pregnancy loss and false pregnancy in the killer whale (Orcinus orca), Gen. Comp. Endocrinol. 267 (2018) 98–108, https://doi.org/10.1016/j.ygcen.2018.06.008.

[75] E.L. Legacki, T.R. Robeck, K.J. Steinman, A.J. Conley, Comparative analysis of steroids in cyclic and pregnant killer whales, beluga whales and bottlenose dolphins by liquid chromatography tandem mass spectrometry, Gen. Comp. Endocrinol. 285 (2020), 113273, https://doi.org/10.1016/j.ygcen.2019.113273.

[76] C.E.C. Goertz, K. Burek-Huntington, K. Royer, L. Quakenbush, T. Clauss, R. Hobbs, N.M. Kellar, Comparing progesterone in blubber and serum to assess pregnancy in wild beluga whales (Delphinapterus leucas), Conserv. Physiol. 7 (2019) coz071, https://doi.org/10.1093/conphys/coz071.

[77] C. Rosa, T.M. O'Hara, P.F. Hoekstra, K.R. Refsal, J.E. Blake, Serum thyroid hormone concentrations and thyroid histomorphology as biomarkers in bowhead whales (Balaena mysticetus), Can. J. Zool. 85 (2007) 609–618, https://doi.org/10.1139/Z07-035.

[78] K.J. Steinman, G.A. Montano, T.R. Robeck, Characterization of circulating androgens, cortisol and estrogens during normal, abnormal and false pregnancy in bottlenose dolphins (Tursiops truncatus) under managed care, Front. Mar. Sci. 8 (2021), 737926, https://doi.org/10.3389/fmars.2021.737926.

[79] C.T. Clark, A.H. Fleming, J. Calambokidis, N.M. Kellar, C.D. Allen, K.N. Catelani, M. Robbins, N.E. Beaulieu, D. Steel, J.T. Harvey, Heavy with child? Pregnancy status and stable isotope ratios as determined from biopsies of humpback whales, Conserv. Physiol. 4 (2016) 1–13, https://doi.org/10.1093/conphys/cow050.

[80] G. Dalle Luche, A.S.P. Boggs, J.R. Kucklick, J. Groß, D.W. Hawker, S. Bengtson Nash, Androstenedione and testosterone but not progesterone are potential biomarkers of pregnancy in Humpback Whales (Megaptera novaeangliae) approaching parturition, Sci. Rep. 10 (2020) 2954, https://doi.org/10.1038/s41598-020-58933-4.

[81] J.T. Richard, T.R. Robeck, S.D. Osborn, L. Naples, A. McDermott, R. LaForge, T. A. Romano, B.L. Sartini, Testosterone and progesterone concentrations in blow samples are biologically relevant in belugas (Delphinapterus leucas), Gen. Comp. Endocrinol. 246 (2017) 183–193, https://doi.org/10.1016/j.ygcen.2016.12.006.

[82] E.A. Burgess, K.E. Hunt, S.D. Kraus, R.M. Rolland, Quantifying hormones in exhaled breath for physiological assessment of large whales at sea, Sci. Rep. 8 (2018) 10031, https://doi.org/10.1038/s41598-018-28200-8.

[83] A. Reckendorf, M. Schmicke, P. Bunskoek, K. Anderson Hansen, M. Thybo, C. Strube, U. Siebert, Is harbor porpoise (phocoena phocoena) exhaled breath sampling suitable for hormonal assessments? Animals 11 (2021) 907, https://doi.org/10.3390/ani11030907.

[84] L.A. Thompson, T.R. Spoon, C.E.C. Goertz, R.C. Hobbs, T.A. Romano, Blow collection as a non-invasive method for measuring cortisol in the beluga (Delphinapterus leucas), PLoS One 9 (2014), e114062, https://doi.org/10.1371/journal.pone.0114062.

[85] J.M. Hudson, W.G. Anderson, M. Marcoux, Measurement of cortisol in blow samples collected from free-swimming beluga whales (Delphinapterus leucas), Mar. Mammal. Sci. 37 (2021) 888–900.

[86] C.J. Hogg, T.L. Rogers, A. Shorter, K. Barton, P.J.O. Miller, D. Nowacek, Determination of steroid hormones in whale blow: It is possible, Mar. Mammal. Sci. 25 (2009) 605–618, https://doi.org/10.1111/j.1748-7692.2008.00277.x.

[87] H. Muraco, A. Cheng, N. Ravida, D. Arn, J. Hudson, B. Durrant, A. New, Approach to detection of luteinizing hormone in a bottlenose dolphin (<I>Tursiops truncatus</I>), Aquat. Mamm. 35 (2009) 386–393, https://doi.org/10.1578/AM.35.3.2009.386.

[88] N. Birukawa, H. Ando, M. Goto, N. Kanda, L.A. Pastene, H. Nakatsuji, H. Hata, A. Urano, Plasma and urine levels of electrolytes, urea and steroid hormones involved in osmoregulation of cetaceans, Zool. Sci. 22 (2005) 1245–1257, https://doi.org/10.2108/zsj.22.1245.

[89] T. Monreal-Pawlowsky, A. Carbajal, O. Tallo-Parra, M. Sabés-Alsina, L. Monclús, J. Almunia, H. Fernández-Bellon, M. Lopez-Bejar, Daily salivary cortisol levels in response to stress factors in captive common bottlenose dolphins (Tursiops truncatus): a potential welfare indicator, Vet. Rec. 180 (2017) 593.

[90] D. Rickert, R. Simon, L. von Fersen, K. Baumgartner, T. Bertsch, C. Kirschbaum, M. Erhard, Saliva and blood cortisol measurement in bottlenose dolphins (Tursiops truncatus): methodology, application, and limitations, Animals 12 (2021) 22, https://doi.org/10.3390/ani12010022.

[91] R.M. Rolland, K.E. Hunt, S.D. Kraus, S.K. Wasser, Assessing reproductive status of right whales (Eubalaena glacialis) using fecal hormone metabolites, Gen. Comp. Endocrinol. 142 (2005) 308–317, https://doi.org/10.1016/j.ygcen.2005.02.002.

[92] L.J. Miller, L.K. Lauderdale, M.T. Walsh, J.L. Bryant, K.A. Mitchell, D.A. Granger, J.D. Mellen, Reference intervals and values for fecal cortisol, aldosterone, and the ratio of cortisol to dehydroepiandrosterone metabolites in four species of cetaceans, PLOS ONE 16 (2021), e0250331, https://doi.org/10.1371/journal.pone.0250331.

[93] K.E. Hunt, J. Robbins, C.L. Buck, M. Bérubé, R.M. Rolland, Evaluation of fecal hormones for noninvasive research on reproduction and stress in humpback whales (Megaptera novaeangliae), Gen. Comp. Endocrinol. 280 (2019) 24–34, https://doi.org/10.1016/j.ygcen.2019.04.004.

[94] S.J. Trumble, E.M. Robinson, M. Berman-Kowalewski, C.W. Potter, S. Usenko, Blue whale earplug reveals lifetime contaminant exposure and hormone profiles, Proc. Natl. Acad. Sci. 110 (2013) 16922–16926, https://doi.org/10.1073/pnas.1311418110.

[95] D.D. Crain, A. Thomas, F. Mansouri, C.W. Potter, S. Usenko, S.J. Trumble, Hormone comparison between right and left baleen whale earplugs, Conserv. Physiol. 8 (2020) coaa055, https://doi.org/10.1093/conphys/coaa055.

[96] S. Atkinson, C. Combelles, D. Vincent, P. Nachtigall, J. Pawloski, M. Breese, Monitoring of progesterone in captive female false killer whales, Pseudorca crassidens, Gen. Comp. Endocrinol. 115 (1999) 323–332, https://doi.org/10.1006/gcen.1999.7319.

[97] K.M. Parsons, J.W. Durban, D.E. Claridge, Comparing two alternative methods for sampling small cetaceans for molecular analysis, Mar. Mammal. Sci. 19 (2003) 224–231, https://doi.org/10.1111/j.1748-7692.2003.tb01104.x.

[98] T.E. Zieglar, G. Scheffler, C.T. Snowden, The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, saguinus oedipus, Horm. Behav. 29 (1995) 407–424.

[99] T.E. Ziegler, Metabolism of reproductive steroids during the ovarian cycle in two species of callitrichids, Saguinus oedipus and Callithrix jacchus, and estimation of the ovulatory period from fecal steroids, Biol. Reprod. 54 (1996) 91–99, https://doi.org/10.1095/biolreprod54.1.91.

[100] T.E. Ziegler, D.J. Wittwer, Circulating and excreted hormones during the ovarian cycle in the cotton-top tamarin, Saguinus oedipus, Am. J. Primatol. 31 (1993) 55–65.

[101] T.E. Ziegler, C.V. Santos, A. Pissinatti, K.B. Strier, Steroid excretion during the ovarian cycle in captive and wild muriquis, *Brachyteles arachnoides*, Am. J. Prima 42 (1997) 311–321, https://doi.org/10.1002/(SICI)1098-2345(1997)42:4<311::AID-AJP6>3.0.CO;2-#.

[102] C.L. Thompson, B.L. Powell, S.H. Williams, G. Hanya, K.E. Glander, C.J. Vinyard, Thyroid hormone fluctuations indicate a thermoregulatory function in both a tropical (*Alouatta palliata*) and seasonally cold-habitat (*Macaca fuscata*) primate, Am. J. Primatol. 79 (2017), https://doi.org/10.1002/ajp.22714.

[103] S.K. Wasser, L. Risler, R.A. Steiner, Excreted steroids in primate feces over the menstrual cycle and pregnancy1, Biol. Reprod. 39 (1988) 862–872, https://doi.org/10.1095/biolreprod39.4.862.

[104] S.Kusuda Muren, O. Doi, H. Naito, H. Hashikawa, Puberty, ovarian cycle, pregnancy, and postpartum ovulation in captive Sichuan golden monkeys (Rhinopithecus roxellana) based on changes in urinary and fecal gonadal steroid metabolites, Theriogenology 87 (2017) 179–186, https://doi.org/10.1016/j.theriogenology.2016.08.020.

[105] C. Bielert, J.A. Czaja, S. Eisele, G. Scheffler, J.A. Robinson, R.W. Goy, Mating in the rhesus monkey (Macaca mulatta) after conception and its relationship to oestradiol and progesterone levels throughout pregnancy, Reproduction 46 (1976) 179–187, https://doi.org/10.1530/jrf.0.0460179.

[106] G.D. Hodgen, M.L. Dufau, K.J. Catt, W.W. Tullner, Estrogens, progesterone and chorionic gonadotropin in pregnant rhesus monkeys, Endocrinology 91 (1972) 896–900, https://doi.org/10.1210/endo-91-4-896.
[107] E.D. Albrecht, G.W. Aberdeen, G.J. Pepe, The role of estrogen in the maintenance of primate pregnancy, Am. J. Obstet. Gynecol. 182 (2000) 432–438, https://doi.org/10.1016/S0002-9378(00)70235-3.
[108] J.A. French, T. Koban, M. Rukstalis, S.M. Ramirez, M. Bardi, L. Brent, Excretion of urinary steroids in pre- and postpartum female baboons, Gen. Comp. Endocrinol. 137 (2004) 69–77, https://doi.org/10.1016/j.ygcen.2004.02.008.
[109] J.C. Beehner, N. Nguyen, E.O. Wango, S.C. Alberts, J. Altmann, The endocrinology of pregnancy and fetal loss in wild baboons, Horm. Behav. 49 (2006) 688–699, https://doi.org/10.1016/j.yhbeh.2005.12.016.
[110] M. Bardi, A.J. Petto, D.E. Lee-Parritz, Parental failure in captive cotton-top tamarins (Saguinus oedipus), Am. J. Primatol. 54 (2001) 159–169, https://doi.org/10.1002/ajp.1020.
[111] J.E. Fite, J.A. French, Pre-and postpartum sex steroids in female marmosets (Callithrix kuhlii): is there a link with infant survivorship and maternal behavior? Horm. Behav. 38 (2000) 1–12, https://doi.org/10.1006/hbeh.2000.1607.
[112] I. Fürtbauer, M. Heistermann, O. Schülke, J. Ostner, Brief communication: fecal androgen excretion and fetal sex effects during gestation in wild assamese macaques (Macaca assamensis), Am. J. Phys. Anthropol. 147 (2012) 334–339, https://doi.org/10.1002/ajpa.21646.
[113] J.M. Setchell, T.E. Smith, L.A. Knapp, Androgens in a female primate: relationships with reproductive status, age, dominance rank, fetal sex and secondary sexual color, Physiol. Behav. 147 (2015) 245–254, https://doi.org/10.1016/j.physbeh.2015.04.051.
[114] V.J. Grant, M. Konečná, R.-S. Sonnweber, R.J. Irwin, B. Wallner, Macaque mothers' preconception testosterone levels relate to dominance and to sex of offspring, Anim. Behav. 82 (2011) 893–899, https://doi.org/10.1016/j.anbehav.2011.07.029.
[115] K. Wallen, Hormonal influences on sexually differentiated behavior in nonhuman primates, Front. Neuroendocrinol. 26 (2005) 7–26, https://doi.org/10.1016/j.yfrne.2005.02.001.
[116] J. Altmann, J.W. Lynch, N. Nguyen, S.C. Alberts, L.R. Gesquiere, Life-history correlates of steroid concentrations in wild peripartum baboons, Am. J. Primatol. 64 (2004) 95–106, https://doi.org/10.1002/ajp.20064.
[117] C.M. Drea, Endocrine correlates of pregnancy in the ring-tailed lemur (Lemur catta): Implications for the masculinization of daughters, Horm. Behav. 59 (2011) 417–427, https://doi.org/10.1016/j.yhbeh.2010.09.011.
[118] J.M. Setchell, E.J. Wickings, Social and seasonal influences on the reproductive cycle in female mandrills (Mandrillus sphinx), Am. J. Phys. Anthropol. 125 (2004) 73–84, https://doi.org/10.1002/ajpa.10375.
[119] R. Torii, H. Nigi, H. Koizumi, Yoshikunitanioka, serum chorionic gonadotropin, progesterone, and Estradiol-17~ levels during pregnancy in the common marmoset, callithrixjacchus, Primates 30 (1989) 207–215.
[120] C.L. Coe, G.R. Lubach, Vital and vulnerable functions of the primate placenta critical for infant health and brain development, Front. Neuroendocrinol. 35 (2014) 439–446, https://doi.org/10.1016/j.yfrne.2014.03.004.
[121] A.M. Parsons Aubone, R. Evans, G.J. Bouma, Androgen signaling in the placenta, in: C. Marsh (Ed.), Reprod. Horm., IntechOpen, 2021.
[122] D. Haig, Placental hormones, genomic imprinting, and maternal-fetal communication, J. Evol. Biol (1996) 357–380, https://doi.org/10.1046/j.1420-9101.1996.9030357.x.
[123] J.N. Rutherford, Fetal signaling through placental structure and endocrine function: Illustrations and implications from a nonhuman primate model, Am. J. Hum. Biol. 21 (2009) 745–753, https://doi.org/10.1002/ajhb.20923.
[124] D.E. Wildman, C. Chen, O. Erez, L.I. Grossman, M. Goodman, R. Romero, Evolution of the mammalian placenta revealed by phylogenetic analysis, Proc. Natl. Acad. Sci. 103 (2006) 3203–3208, https://doi.org/10.1073/pnas.0511344103.
[125] A.J. Zeleznik, D.F. Benyo, Control of follicular development, corpus luteum function and the recognition of pregnancy in higher primates, in: E. Knobil, J. D. Neill (Eds.), Physiol. Reprod., 2nd ed..,, Raven Press, New York, NY, 1994, pp. 751–782.
[126] P.L. Chambers, J.P. Hearn, Peripheral plasma levels of progesterone, oestradiol-17 beta, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey, Callithrix jacchus, J. Reprod. Fertil. 56 (1979) 23–32.
[127] F.I. Reyes, J.S.D. Winter, C. Faiman, W.C. Hobson, Serial serum levels of gonadotropins, prolactin and sex steroids in the nonpregnant and pregnant chimpanzee, Endocrinology 96 (1975) 1447–1455, https://doi.org/10.1210/endo-96-6-1447.
[128] D. Tulchinsky, C.J. Hobel, E. Yeager, J.R. Marshall, Plasma estrone, estradiol, estriol, progesterone and 17-hydroxyprogesterone in human pregnancy, Am. J. Obstet. Gynecol. 112 (1972) 1095–1100, https://doi.org/10.1016/0002-9378(72)90185-8.
[129] T. Fournier, Human chorionic gonadotropin: Different glycoforms and biological activity depending on its source of production, Ann. Endocrinol. 77 (2016) 75–81, https://doi.org/10.1016/j.ando.2016.04.012.
[130] W.P. McNulty, M.J. Novy, S.W. Walsh, Fetal and postnatal development of the adrenal glands in Macaca mulatta, Biol. Reprod. 25 (1981) 1079–1089.
[131] E.D. Albrecht, G.J. Pepe, Central integrative role of oestrogen in modulating the communication between the placenta and fetus that results in primate fetal–placental development, Placenta 20 (1999) 129–139, https://doi.org/10.1053/plac.1998.0359.
[132] S. Mesiano, R.B. Jaffe, Developmental and functional biology of the primate fetal adrenal, Cortex 18 (1997) 26.
[133] G.J. Pepe, E.D. Albrecht, Regulation of the primate fetal adrenal cortex, Endocr. Rev. 11 (1990) 151–176, https://doi.org/10.1210/edrv-11-1-151.
[134] E.S. Hayes, [No title found], Reprod. Biol. Endocrinol. 2 (2004) 36. https://doi.org/10.1186/1477–7827-2–36.
[135] A. Einspanier, M.R. Zarreh-Hoshyari-Khah, M. Balvers, L. Kerr, K. Fuhrmann, R. Ivell, Local relaxin biosynthesis in the ovary and uterus through the oestrous cycle and early pregnancy in the female marmoset monkey (Callithrix jacchus), Hum. Reprod. 12 (1997) 1325–1337, https://doi.org/10.1093/humrep/12.6.1325.
[136] J. Garibay-Tupas, K. Csiszar, M. Fox, S. Povey, G. Bryant-Greenwood, Analysis of the 5'-upstream regions of the human relaxin H1 and H2 genes and their chromosomal localization on chromosome 9p24.1 by radiation hybrid and breakpoint mapping, J. Mol. Endocrinol. 23 (1999) 355–365, https://doi.org/10.1677/jme.0.0230355.
[137] D.R. Stewart, A.C. Celniker, C.A.J. Taylor, J.R. Cragun, J.W. Overstreet, B. Lasley, Relaxin in the peri-implantation period, J. Clin. Endocrinol. Metab. 70 (1990) 1771–1773.
[138] D. Aldabe, D.C. Ribeiro, S. Milosavljevic, M. Dawn Bussey, Pregnancy-related pelvic girdle pain and its relationship with relaxin levels during pregnancy: a systematic review, Eur. Spine J. 21 (2012) 1769–1776, https://doi.org/10.1007/s00586-012-2162-x.
[139] R.J. Bell, L.W. Eddie, A.R. Lester, E.C. Wood, P.D. Johnston, H.D. Nial, Relaxin in human pregnancy serum measured with an homologous radioimmunoassay, Obstet. Gynecol. 69 (1987) 585–589.
[140] R. Hagen, Pelvic girdle relaxation from an orthopaedic point of view, Acta Orthop. Scand. 45 (1974) 550–563, https://doi.org/10.3109/17453677408989178.
[141] X. Hall, An evaluation of the roles of oestrogen, progesterone, and relaxin in producing relaxation of the symphysis pubis of the ovariectomized mouse, using the technique of metachromatic staining with toluidine blue, J. Endocrinol. 13 (1956) 384–393.
[142] J.P. Manning, B.G. Steinetz, M.C. Butler, S. Priester, Th effect of steroid and relaxin on acid phosphatase in the pubic symphysis of the ovariectomized mouse, 501-5, J. Endocrinol. 33 (1965), https://doi.org/10.1677/joe.0.0330501.
[143] M. Hamolsky, R.C. Sparrow, Influence of relaxin on mammary development in sexually immature female rats, Proc. Soc. Exp. Biol. Med. 60 (1945) 8–9, https://doi.org/10.3181/00379727-60–15074 P.
[144] J.C.J. Krantz, H.H. Bryant, C.J. Carr, The action of aqueous corpus luteum extract upon uterine activity, Surg. Gynecol. Obstet. 90 (1950) 372–375.
[145] E.F. Graham, A.E. Dracy, The effect of relaxin and mechanical dilation on the bovine cervix, J. Dairy Sci. 36 (1953) 772–777, https://doi.org/10.3168/jds.S0022-0302(53)91559-8.
[146] P. Kumaresan, G.S. Han, P.B. Anandarangam, A. Vasicka, Oxytocin in maternal and fetal blood, Obstet. Gynecol. 46 (1975) 272–274.
[147] T. Chard, C.N. Hudson, C.R.W. Edwards, N.R.H. Boyd, Release of oxytocin and vasopressin by the human foetus during labour, Nature 234 (1971) 352–354.
[148] M.S. Soloff, M. Alexandrova, M.J. Fernstrom, Oxytocin receptors: triggers for parturition and lactation, Science 204 (1979) 1313–1315, https://doi.org/10.1126/science.221972.
[149] J.E. Hall, M.E. Hall, Guyton and hall textbook of medical physiology, 14th ed., 2021.
[150] D. Newbern, M. Freemark, Placental hormones and the control of maternal metabolism and fetal growth, Curr. Opin. Endocrinol. Diabetes Obes. 18 (2011) 409–416, https://doi.org/10.1097/MED.0b013e32834c800d.
[151] B. Huppertz, E. Schleußner, The Placenta: Basics and Clinical Significance (eds.), Springer Berlin Heidelberg, Berlin, Heidelberg, 2023, https://doi.org/10.1007/978-3-662-66256-4.
[152] E.E. Baulieu, Dehydroepiandrosterone (DHEA): a fountain of youth, J. Clin. Endocrinol. Metab. 81 (1996) 3147–3151.
[153] Y. Xing, A.M. Lerario, W. Rainey, G.D. Hammer, Development of adrenal cortex zonation, Endocrinol. Metab. Clin. North Am. 44 (2015) 243–274, https://doi.org/10.1016/j.ecl.2015.02.001.
[154] R.J. Auchus, W.E. Rainey, Adrenarche - physiology, biochemistry and human disease, Clin. Endocrinol. (Oxf.). 60 (2004) 288–296, https://doi.org/10.1046/j.1365-2265.2003.01858.x.
[155] B. Campbell, Adrenarche and the evolution of human life history, Am. J. Hum. Biol. 18 (2006) 569–589, https://doi.org/10.1002/ajhb.20528.
[156] W.E. Rainey, B.R. Carr, H. Sasano, T. Suzuki, J.I. Mason, Dissecting human adrenal androgen production, Trends Endocrinol. Metab. 13 (2002) 234–239, https://doi.org/10.1016/S1043-2760(02)00609-4.
[157] J.C. Havelock, R.J. Auchus, W.E. Rainey, The rise in adrenal androgen biosynthesis: adrenarche, Semin. Reprod. Med. 22 (2004) 337–347, https://doi.org/10.1055/s-2004-861550.
[158] T.A. Quinn, U. Ratnayake, H. Dickinson, T.-H. Nguyen, M. McIntosh, M. Castillo-Melendez, A.J. Conley, D.W. Walker, Ontogeny of the adrenal gland in the spiny mouse, with particular reference to production of the steroids cortisol and dehydroepiandrosterone, Endocrinology 154 (2013) 1190–1201, https://doi.org/10.1210/en.2012-1953.
[159] J.C. Pattison, W. Saltzman, D.H. Abbott, B.K. Hogan, A.D. Nguyen, B. Husen, A. Einspanier, A.J. Conley, I.M. Bird, Gender and gonadal status differences in zona reticularis expression in marmoset monkey adrenals: cytochrome b5 localization with respect to cytochrome P450 17,20-lyase activity, Mol. Cell. Endocrinol. 265– 266 (2007) 93–101, https://doi.org/10.1016/j.mce.2006.12.023.

[160] J. Levine, L.G. Wolfe, R.J. Schiebinger, D.L. Loriaux, G.B. Cutler, Rapid regression of fetal adrenal zone and absence of adrenal reticular zone in the marmoset, Endocrinology 111 (1982) 1797–1802, https://doi.org/10.1210/endo-111-6-1797.

[161] A.J. Conley, Review of the reproductive endocrinology of the pregnant and parturient mare, Theriogenology 86 (2016) 355–365, https://doi.org/10.1016/j.theriogenology.2016.04.049.

[162] R.L. Pashen, E.L. Sheldrick, W.R. Allen, A.P. Flint, Dehydroepiandrosterone synthesis by the fetal foal and its importance as an oestrogen precursor, J. Reprod. Fertil. Suppl. 32 (1982) 389–397.

[163] R.W. Estabrook, J.I. Mason, C. Martin-Wixtrom, M. Zuber, M.R. Waterman, Some enzymatic vagaries of a bovine adrenal microsomal cytochrome P-450 introduced and expressed in transformed monkey kidney cells, Prog. Clin. Biol. Res. 274 (1988) 525–540.

[164] H.R. Fevold, M.C. Lorence, J.L. McCarthy, J.M. Trant, M. Kagimoto, M. R. Waterman, J.I. Mason, Rat P450 $_{17\alpha}$ from testis: characterization of a full-length cDNA encoding a unique steroid hydroxylase capable of catalyzing Both Δ^4 - and Δ^5 -steroid-17,20-lyase reactions, Mol. Endocrinol. 3 (1989) 968–975, https://doi.org/10.1210/mend-3-6-968.

[165] L. Gustavson, B.M. Jenssen, J. Bytingsvik, B. Styrishave, M. Hansen, J. Aars, G. S. Eggen, T.M. Ciesielski, Steroid hormone profile in female polar bears (Ursus maritimus), Polar Biol. 38 (2015) 1183–1194, https://doi.org/10.1007/s00300-015-1682-3.

[166] R.J. Bobes, M. Pérez-Martínez, Y. Gómez, M.C. Romano, Metabolism of progesterone to estrogens and androgens by individual follicles of the goat ovary, Small Rumin. Res. 47 (2003) 233–242, https://doi.org/10.1016/S0921-4488(02)00278-X.

[167] J.I. Raeside, A. Brief, Account of the discovery of the fetal/placental unit for estrogen production in equine and human pregnancies: relation to human medicine, Yale J. Biol. Med. 90 (2017) 449–461.

[168] J.A. Jackson, E.D. Albrecht, Estrogen regulates placental androstenedione production during rat pregnancy, Endocrinology 119 (1986) 1052–1057.

[169] B.M. John, C.G. Pierrepoint, Demonstration of an active C17-C20 lyase in the sheep placenta, Reproduction 43 (1975) 559–562.

[170] G. Evans, W.C. Wagner, In vitro oestrogen synthesis by bovine placenta during pregnancy and induced parturition, Eur. J. Endocrinol. 98 (1981) 119–125.

[171] L. Stárka, M. Dušková, M. Hill, Dehydroepiandrosterone: a neuroactive steroid, J. Steroid Biochem. Mol. Biol. 145 (2015) 254–260, https://doi.org/10.1016/j.jsbmb.2014.03.008.

[172] R.F. Greaves, S.A. Wudy, E. Badoer, M. Zacharin, J.J. Hirst, T. Quinn, D. W. Walker, A tale of two steroids: the importance of the androgens DHEA and DHEAS for early neurodevelopment, J. Steroid Biochem. Mol. Biol. 188 (2019) 77–85, https://doi.org/10.1016/j.jsbmb.2018.12.007.

[173] T.A. Quinn, S.R. Robinson, D. Walker, Dehydroepiandrosterone (DHEA) and DHEA sulfAte: roles in brain function and disease, Sex. Horm. Neurodegener. Process. Dis. (2018) 41–68.

[174] E.D. Albrecht, G.J. Pepe, Suppression of maternal adrenal dehydroepiandrosterone and dehydroepiandrosterone sulfate production by estrogen during baboon pregnancy, J. Clin. Endocrinol. Metab. 80 (1995) 3201–3208.

[175] H. Umezaki, D.L. Hess, G.J. Valenzuela, C.A. Ducsay, Fetectomy alters maternal pituitary-adrenal function in pregnant rhesus macaques1, Biol. Reprod. 65 (2001) 1616–1621, https://doi.org/10.1095/biolreprod65.5.1616.

[176] L. Marinelli, E. Trevisi, L. Da Dalt, M. Merlo, G. Bertoni, G. Gabai, Dehydroepiandrosterone secretion in dairy cattle is episodic and unaffected by ACTH stimulation, J. Endocrinol. 194 (2007) 627–635, https://doi.org/10.1677/JOE-07-0226.

[177] E. Möstl, T. Janowski, R. Palme, A. Rás, S. Zduńczyk, E. Bamberg, Dehydroepiandrosterone and epitestosterone in the blood of cows at term, J. Vet. Med. Ser. A 36 (1989) 104–109.

[178] N. Tagawa, Y. Hidaka, T. Takano, Y. Shimaoka, Y. Kobayashi, N. Amino, Serum concentrations of dehydroepiandrosterone and dehydroepiandrosterone sulfate and their relation to cytokine production during and after normal pregnancy, Clin. Chim. Acta 340 (2004) 187–193, https://doi.org/10.1016/j.cccn.2003.10.018.

[179] C. Longcope, Dehydroepiandrosterone metabolism, J. Endocrinol. 150 (1996).

[180] P.D. Kroboth, F.S. Salek, A.L. Pittenger, T.J. Fabian, R.F. Frye, DHEA and DHEA-S: a review, J. Clin. Pharm. 39 (1999) 327–348.

[181] R.S.C. Takeshita, Validation of an enzyme immunoassay for measurement of fecal dehydroepiandrosterone sulfate in gibbons and siamangs, Zoo. Biol. 41 (2022) 544–553, https://doi.org/10.1002/zoo.21687.

[182] G.P. da Silva, J.T. de Melo, F.O.B. Monteiro, A.K.P. Ferreira, L.A. Carneiro, R.S. C. Takeshita, Validation of a dehydroepiandrosterone-sulfate assay in three platyrrhine primates (Alouatta caraya, Aotus azarae infulatus, and Sapajus apella), Int. J. Primatol. 42 (2021) 722–736, https://doi.org/10.1007/s10764-021-00239-x.

[183] R.S.C. Takeshita, M.A. Huffman, F.B. Bercovitch, K. Mouri, K. Shimizu, The influence of age and season on fecal dehydroepiandrosterone-sulfate (DHEAS) concentrations in Japanese macaques (Macaca fuscata), Gen. Comp. Endocrinol. 191 (2013) 39–43, https://doi.org/10.1016/j.ygcen.2013.05.019.

[184] R.S.C. Takeshita, F.B. Bercovitch, M.A. Huffman, K. Mouri, C. Garcia, L. Rigaill, K. Shimizu, Environmental, biological, and social factors influencing fecal adrenal steroid concentrations in female Japanese macaques (*Macaca fuscata*): adrenal Steroids in Japanese Macaques, Am. J. Prima 76 (2014) 1084–1093, https://doi.org/10.1002/ajp.22295.

[185] S.P. Prall, L. Ambu, S. Nathan, S. Alsisto, D. Ramirez, M.P. Muehlenbein, Androgens and innate immunity in rehabilitated semi-captive orangutans (*Pongo pygmaeus morio*) from Malaysian Borneo: androgens and Immune Function in Orangutans, Am. J. Primatol. 77 (2015) 642–650, https://doi.org/10.1002/ajp.22387.

[186] A. Leeds, P.M. Dennis, K.E. Lukas, T.S. Stoinski, M.A. Willis, M.W. Schook, Validating the use of a commercial enzyme immunoassay to measure oxytocin in unextracted urine and saliva of the western lowland gorilla (Gorilla gorilla gorilla), Primates 59 (2018) 499–515, https://doi.org/10.1007/s10329-018-0678-3.

[187] J.L. Brown, D.L. Schmitt, A. Bellem, L.H. Graham, J. Lehnhardt, Hormone secretion in the asian elephant (Elephas Maximus): characterizationof ovulatory and anovulatory luteinizing hormone surges, Biol. Reprod. 61 (1999) 1294–1299, https://doi.org/10.1095/biolreprod61.5.1294.

[188] M.R. Heintz, R.M. Santymire, L.A. Parr, E.V. Lonsdorf, Validation of a cortisol enzyme immunoassay and characterization of salivary cortisol circadian rhythm in chimpanzees (Pan troglodytes), Am. J. Primatol. 73 (2011) 903–908, https://doi.org/10.1002/ajp.20960.

[189] N. Moreira, E.L.A. Monteiro-Filho, W. Moraes, W.F. Swanson, L.H. Graham, O. L. Pasquali, M.L.F. Gomes, R.N. Morais, D.E. Wildt, J.L. Brown, Reproductive steroid hormones and ovarian activity in felids of theLeopardus genus, Zoo. Biol. 20 (2001) 103–116, https://doi.org/10.1002/zoo.1010.

[190] J.D. Wark, L. Amendolagine, K.E. Lukas, C.W. Kuhar, P.M. Dennis, C.T. Snowdon, T. Schoffner, M.W. Schook, Fecal glucocorticoid metabolite responses to management stressors and social change in four species of callitrichine monkeys, Primates 57 (2016) 267–277, https://doi.org/10.1007/s10329-016-0514-6.

[191] X. Robin, N. Turck, A. Hainard, N. Tiberti, F. Lisacek, J.-C. Sanchez, M. Müller, pROC: an open-source package for r and s+ to analyze and compare roc curves, BMC Bioinforma. 12 (2011) 77, https://doi.org/10.1186/1471-2105-12-77.

[192] E. Hart, R.S.C. Takeshita, Effect of social dominance and reproductive state on adrenal steroids in female Japanese macaques (Macaca fuscata) (under review), Int. J. Primatol. (2023).

[193] D. Maestripieri, C.L. Hoffman, G.M. Anderson, C.S. Carter, J.D. Higley, Mother–infant interactions in free-ranging rhesus macaques: relationships between physiological and behavioral variables, Physiol. Behav. 96 (2009) 613–619, https://doi.org/10.1016/j.physbeh.2008.12.016.

[194] W. Saltzman, D.H. Abbott, Effects of elevated circulating cortisol concentrations on maternal behavior in common marmoset monkeys (Callithrix jacchus), Psychoneuroendocrinology 34 (2009) 1222–1234, https://doi.org/10.1016/j.psyneuen.2009.03.012.

[195] N.I. Bahr, C.R. Pryce, M.D. Beli, R.D. Martin, Evidence from urinary cortisol that maternal behavior is related to stress in gorillas, Physiol. Behav. 64 (1998) 429–437.

[196] S.P. Prall, E.E. Larson, M.P. Muehlenbein, The role of dehydroepiandrosterone on functional innate immune responses to acute stress, Stress Health 33 (2017) 656–664, https://doi.org/10.1002/smi.2752.

[197] H.S. Kamin, D.A. Kertes, Cortisol and DHEA in development and psychopathology, Horm. Behav. 89 (2017) 69–85, https://doi.org/10.1016/j.yhbeh.2016.11.018.

[198] N. Wielebnowski, Stress and distress: evaluating their impact for the well-being of zoo animals, J. Am. Vet. Med. Assoc. 223 (2003) 973–977.

[199] E. Iob, A. Steptoe, Cardiovascular disease and hair cortisol: a novel biomarker of chronic stress, Curr. Cardiol. Rep. 21 (2019) 116, https://doi.org/10.1007/s11886-019-1208-7.

[200] N. Pluchino, P. Drakopoulos, F. Bianchi-Demicheli, J.M. Wenger, P. Petignat, A. R. Genazzani, Neurobiology of DHEA and effects on sexuality, mood and cognition, J. Steroid Biochem. Mol. Biol. 145 (2015) 273–280, https://doi.org/10.1016/j.jsbmb.2014.04.012.

[201] T.G. Guilliams, L. Edwards, Chronic stress and the HPA axis, Standard 9 (2010) 1–12.

[202] J.C. Whitham, J.L. Bryant, L.J. Miller, Beyond glucocorticoids: integrating dehydroepiandrosterone (DHEA) into animal welfare research, Animals 10 (2020) 1381, https://doi.org/10.3390/ani10081381.

[203] P. Leff-Gelman, M. Flores-Ramos, A.E.Á. Carrasco, M.L. Martínez, M.F. S. Takashima, F.M.C. Coronel, B.F. Labonne, J.A.Z. Dosal, P.B. Chávez-Peón, S. G. Morales, I. Camacho-Arroyo, Cortisol and DHEA-S levels in pregnant women with severe anxiety, BMC Psychiatry 20 (2020) 393, https://doi.org/10.1186/s12888-020-02788-6.

[204] C. Razurel, B. Kaiser, J.-P. Antonietti, M. Epiney, C. Sellenet, Relationship between perceived perinatal stress and depressive symptoms, anxiety, and parental self-efficacy in primiparous mothers and the role of social support, Women Health 57 (2017) 154–172, https://doi.org/10.1080/03630242.2016.1157125.

[205] M. Qobadi, C. Collier, L. Zhang, The effect of stressful life events on postpartum depression: findings from the 2009–2011 mississippi pregnancy risk assessment monitoring system, Matern. Child Health J. 20 (2016) 164–172, https://doi.org/10.1007/s10995-016-2028-7.

[206] C. Chojenta, D. Loxton, J. Lucke, How do previous mental health, social support, and stressful life events contribute to postnatal depression in a representative sample of australian women? J. Midwifery Women's. Health 57 (2012) 145–150, https://doi.org/10.1111/j.1542-2011.2011.00140.x.

[207] M. Bardi, K. Shimizu, G.M. Barrett, S.M. Borgognini-Tarli, M.A. Huffman, Peripartum cortisol levels and mother-infant interactions in Japanese macaques (https://doi.org/DOI), Am. J. Phys. Anthropol. 120 (2003) 298–304, https://doi.org/10.1002/ajpa.10150.

[208] R.K. Sripada, C.E. Marx, A.P. King, N. Rajaram, S.N. Garfinkel, J.L. Abelson, I. Liberzon, DHEA enhances emotion regulation neurocircuits and modulates memory for Emotional Stimuli, Neuropsychopharmacology 38 (2013) 1798–1807, https://doi.org/10.1038/npp.2013.79.
[209] G.B. Raglan, L.A. Schmidt, J. Schulkin, The role of glucocorticoids and corticotropin-releasing hormone regulation on anxiety symptoms and response to treatment, Endocr. Connect 6 (2017) R1–R7, https://doi.org/10.1530/EC-16-0100.
[210] M. Arlet, L.-L. Veromann-Jürgenson, L. Isbell, R. Mänd, A. Lemasson, Maternal care in free-ranging arboreal grey-cheeked mangabeys (Lophocebus albigena johnstoni) in kibale national park, Uganda, Folia Primatol. 90 (2019) 441–455, https://doi.org/10.1159/000499656.
[211] G. Green, R. Tesler, A. Marques, Primiparous and multiparous women's mode of birth and negative emotions, Int. J. Environ. Res. Public. Health 19 (2022) 5189, https://doi.org/10.3390/ijerph19095189.
[212] M. Bardi, M. Eckles, E. Kirk, T. Landis, S. Evans, K.G. Lambert, Parity modifies endocrine hormones in urine and problem-solving strategies of captive owl monkeys (Aotus spp.), Comp. Med. 64 (2014) 10.
[213] M. Tegethoff, J.-S. Raul, C. Jamey, M.B. Khelil, B. Ludes, G. Meinlschmidt, Dehydroepiandrosterone in nails of infants: a potential biomarker of intrauterine responses to maternal stress, Biol. Psychol. 87 (2011) 414–420, https://doi.org/10.1016/j.biopsycho.2011.05.007.
[214] H.G. Richter, N. Mendez, L. Abarzua-Catalan, G.J. Valenzuela, M. Seron-Ferre, C. Torres-Farfan, Developmental programming of capuchin monkey adrenal dysfunction by gestational chronodisruption, BioMed. Res. Int. 2018 (2018) 1–11, https://doi.org/10.1155/2018/9183053.
[215] R. Palme, P. Fischer, Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock, Anim. Reprod. Sci. 43 (1996) 43–63.
[216] N.I. Bahr, R. Palme, U. Möhle, J.K. Hodges, M. Heistermann, Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates, Gen. Comp. Endocrinol. 117 (2000) 427–438, https://doi.org/10.1006/gcen.1999.7431.
[217] A. Lanci, J. Mariella, N. Ellero, A. Faoro, T. Peric, A. Prandi, F. Freccero, C. Castagnetti, Hair cortisol and DHEA-S in foals and mares as a retrospective picture of feto-maternal relationship under physiological and pathological conditions, Animals 12 (2022) 1266, https://doi.org/10.3390/ani12101266.
[218] J. Fusi, M.C. Veronesi, A. Prandi, T. Meloni, M. Faustini, T. Peric, Hair and claw dehydroepiandrosterone concentrations in newborn puppies spontaneously dead within 30 days of age, Animals 12 (2022) 3162, https://doi.org/10.3390/ani12223162.
[219] D. Joseph, S. Whirledge, Stress and the HPA axis: balancing homeostasis and fertility, Int. J. Mol. Sci. 18 (2017) 2224, https://doi.org/10.3390/ijms18102224.
[220] G. Valsamakis, G. Chrousos, G. Mastorakos, Stress, female reproduction and pregnancy, Psychoneuroendocrinology 100 (2019) 48–57, https://doi.org/10.1016/j.psyneuen.2018.09.031.
[221] R.J. Witorsch, Effects of elevated glucocorticoids on reproduction and development: relevance to endocrine disruptor screening, Crit. Rev. Toxicol. 46 (2016) 420–436, https://doi.org/10.3109/10408444.2016.1140718.
[222] J. Cameron, Stress and behaviorally induced reproductive dysfunction in primates, Semin. Reprod. Med. 15 (1997) 37–45, https://doi.org/10.1055/s-2008-1067966.
[223] K.L. Edwards, A.N. Edes, J.L. Brown, Stress, well-being and reproductive success, in: P. Comizzoli, J.L. Brown, W.V. Holt (Eds.), Reprod. Sci. Anim. Conserv., Springer International Publishing, Cham, 2019, https://doi.org/10.1007/978-3-030-23633-5.