CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

INSTITUTE OF TROPICS AND SUBTROPICS

Metabolites of hormones: A non invasive determination of seasonal hormonal dynamics from ungulate's faeces

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Prague 2012

Declaration

I hereby confirm that I wrote bachelor thesis entitled **„Metabolites of hormones: A non invasive determination of seasonal hormonal dynamics from ungulate's faeces"** myself and used only references cited in the text and reported in bibliography.

In Dobříkov of the day 10th of May 2012 ………………….

Zuzana Klapalová

Acknowledgments

I would like to thank to bachelor thesis supervisor Radim Kotrba, Ph.D for his valuable advices, comments and methodical contribution. Further I would like to thanks to my friend for correction of translation into the English.

Content

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Summary

Non invasive methods were evaluated as useful instruments for observation of endocrinology of free living or captive animals with the least disturbance. The most common method used for determination of hormons metabolites from feces is enzyme immunoassay. The most frequently studied factors affecting concentration of hormonal metbolites in studied animals was reproductive state, environment, availability and quality of nutrition, age and in polyestric species seasonality. The goal of this review was to evaluate methods used for determination of hormones metabolites from feces on the base of extraction, reproductive cycle, behaviour, nutrition, environmental effects, sample size, frequency of collection and seasonality. In females the most important factor on metabolites of steroid hormones has ovulation. On the contrary environment was not influencing greatly the hormonal metabolites. In males the most frequent were studies on deer rut and antler development. Surprisingly the increase of glucocorticoids was greater during the winter not the rut at the autumn. This review encourage my plans to follow theoretical part by experimental evaluation of deer and eland endocrinology via fecal sampling.

Keywords: non-invasive, hormones, metabolites, faceal sampling

Abstrakt

Neinvazivní metody byly v minulosti vyhodnoceny jako vhodné pro stanovení metabolitu hormonů z důvodů nejméně ovlivňující jejich hladiny a široké použitelnosti jak u volně žijících tak v zajetí chovaných kopytníků. Nejčastěji používanou metodou pro stanovování hormonůz trusu je enzymatická imunoesej (ELISA). Na koncentrace studovaných metabolitů hormonů měl nejčastěji vliv reprodukční cyklus zvířat, prostředí, výživa, věk a u polyestrických druhů sezónnost. Cílem této práce bylo na základě literárního přehledu představit metody použité pro stanovení hormonů a jejich metabolitů na základě techniky extrakce, reprodukčního cyklu, chování, výživy, prostředí, počtu zvířat ve studii a sezónnosti. U samic měla na hodnotu hladiny metabolitů největší vliv fáze jejich ovulačního cyklu, ale naopak prostředí nepůsobilo takové výkyvy v koncentraci daného hormonu či metabolitu. U samců se studie nejvíce zaměřovaly na jelenovité, kde byly hladiny nejvyšší v období říje a určitých fázích parožního cyklu. Nejvíce překvapivá byla zjištěná vysoká hladina glukokortikoidů v zimním období oproti letním měsícům a období říje. Tento přehled je povzbudivý pro plánovaný experiment zaměřený na hodnocení sociálního prostředí a dvojího parožního cyklu u jelenovitých a sociálního prostředí a termoregulace u antilopy losí.

Klíčová slova: neinvazivní, hormony, metabolity, faceal sampling

1. Introduction

Scientists are always interested, what causes and shape behaviour of animals. In many cases behaviour is effected by changes of hormone levels, which circulate in blood of animal. Hormones bind on it's receptors and influence whole series of physiological functions, for example pubescence individuality, development of sexual behaviour, initiation of lactation near etc.

This work focuses on review of Publisher results literature of published results studying nonivasively endocrinology of ruminants via fecal sampling with special attention to eland (*Taurotragus oryx*) and cervids (*Cervidae*)..

Eland is with several cervids large ungulate with sexual dimorphism especially in size (Papas, 2002). It is not seasonal breeder. On the contrary to eland, most of cervids from temperate climate zone have seasonal reproduction.

Hormones and the nervous system are the key coordinators of physiological processes and synchronizing internal functions with the external environment. Endocrine glands release hormones into the blood, that is transported to receptive cells, tissues or organs (Janský & Novotný, 1981). In final phase original excrected hormones are detected as metabolites, which considerably varies. These metabolites, as well as their quantity, are species specific (Palme et al., 2005) and in contradiction to hormones, from of which derived, are increasingly washable.

To detect hormones in its liquid state usually the species specific enzyme immunoassay has to be prepared (Muir et al., 2001; Palme, 2005). Advantage of these method is the ease of procedure, promptness of analysis and low cost. Fundamental principle of this immunoassay is in competition of measured hormone with labeled known hormone for binding place on polyclonal antiboby. Starting medium for analysis can be blood, saliva, urine, feces etc. According to that method sampling can be invasive or noninvasive. Invasive methods were used earlier, nevertheless in the process from them began step back. Non invasive method apply mainly on the ground of that the at analyse surface of hormones, presents reality that the at sample collection for metering happens to no physical interference with bodies animals that the so arent disturbed or even koled. The range of application of these methods is very wide for studying animals in the wild (Strier et al., 1999; Fichtel et al., 2007) and captive ones (Rabiee et al, 2002a; Denhard et al., 2002).

Sex hormones govern action pair with once from basic characteristic of organism, with their reproduction. After chemical page are it steroids and near of each of sex find how male, so female hormones, but in by other rates. Sex hormones make adolescence individuality and are involved into development of sexual organs, production of sexual sentinel node and development of typical sexual behaviour for leadership to mount (Pereira et al., 2005). Seasonal establishment changes in the levels of sex hormones deal with already several studies. Animal seasonally polyhedral entails that the sexual cycle is impressed with photoperiod. Mark polyestric animal employs when near individual will slip rut several times per annum. Mating season in our conditions lasts from January to October (Stella, 2004). Of reproduction behaviour interlocks even spreading individuals of a kind in territories

2. Objective and methods

Aim my work was to review literature on evaluation of metabolites of hormones from feces in ruminants as a noninvasive method to study endocrinology with special attention to common eland and cervids.

When writing a thesis I used the book sources from Agricultural and Food Library in Prague, Library of Study and Information Centre of the Czech University of Life Sciences in Prague.Throughout the thesis I search information from Internet databases and Google Scholar databases accessed by Study and Information Centre of the Czech University of Life Sciences in Prague - 360 Search, Web of Knowledge, ScienceDirect, BioOne, EBSCO, Ovid, ProQuest, PMC Scopus - where I was using particular keywords in search work and drew the necessary information. When searching for scientific papers (articles), I used following key words: endocrinology, hormones, metabolites, non-invasive methods, reproduction, and seasonality. From the collected scientific evidence I prepared tables summarizing specific information of each study. The citations followed the requirements of norm ISO 690, adjusted to the requirements of the Czech University of Life Sciences in Prague. Styles and requirements for thesis compilation I followed recommendations Boldiš, Petr. Citace a citování. According to the instructions of the Study and Information Centre of the Czech University of Life Sciences in Prague, 2004 (Boldiš, 2004).

3. Literature review

3.1 Eland (*Taurotragus oryx*, Pallas 1766)

Eland is as well as other large ungulates with sexual dimorphism suited to life according to the size (Papas, 2002). Similarly is that in cervids of temperate climatic zone with exception of seasonality in reproduction.

Eland has shorter neck, massive in males, and chunkier legs than its relatives, what gives bigger resemblance to bovine animals (Kingdon, 1982). African elands form transient group among antelopes and true bovinae (Treus, 1983).

3.2 Endocrionology of ruminants

Hormones and the nervous system are the key coordinators of physiological processes and synchronize internal life functions with the external environment. Endocrine tissues and glands secrete more than 40 different hormones in mammals that regulate processes such as reproduction, osmoregulation, intermediary and mineralmetabolism, growth, development as well as control or activate other endocrine glands (Schwarzenberger, 1999).

Endocrine glands release hormones into blood, which transports them to particular cells, tissues or organs (Janský & Novotný, 1981). After release of hormones into blood, where they may circulate a couple of hours, and impingement upon objective tissues, their conversions by conjugation, deconjugation, oxidation, metabolism or bacterial degradation happens (Janský & Novotný, 1981). In the final stage, hormones they do not affect as were secreted, but mostly by their metabolites, which varies considerably. These metabolites, as well as their quantity, are species specific (Palme et al., 2005) and in contradiction to hormones, from of which were to be derived, are increasingly soluble in water.

Most considerable exercise at hormonal regulation vertebrates have a complex of hypothalamus- pituitary. Together forms one functional unit that is the last straw to nervous - tissue regulation. Hypophysis (pituary gland) during embryonal development is formed from two folds.

- Anterior pituitary gland is glandular part, to which evolutionary belongs to also middle lobe; being present in adult fish, amphibians and reptiles. Somatotropin as product of this gland effects metabolism of all basic nutrient, but especially on production of albumins. Two others hormones governs activity of other endocrine glands (thyroid gland and adrenal cortex). Next three hormones glandular parts have important function at regulation reproduction. Effects on gonads. Next effects of one of these hormones - prolactin display only near females of mammals; during pregnancy averting maturing of other eggs, prepares milk gland on production milks and later regulates other glands.
- Middle lobe pituary gland produces hormone that the channels ability of lower vertebrates to discolour bodies
- Back lobe pituary gland is not there as genuine endocrine glands, but store two hormones, that forms with hypothalamus and into back lobe pituary gland get nervous grains. One hormone regulates capacity body fluids and second (oxytocin) effects on systoles sarcotome gravid uteri and activates milk producing glands (Stárka, 1997).
- Parathyroid glands form usually four objects located caudally on back lobes. Major cells produce polypeptide hormone parathormone which regulates concentration of calcium and phosphorus ionts in blood.

3.3 Determination of hormones via metabolites

For determination of hormones in their liquid state there is several methods, that we can divide into two basic groups. These are invasive and noninvasive methods. Every group employs specific enzyme immunoassay, from which is possible given metabolite measure. Their separation is based on effect of each method have on physical state of studied animal (Kobelt et al., 2003).

3.3.1 Invasive methods

Since early time the interest of monitoring endocrinous state of animals was based on invasive methods evolving to measure liquid level of steroid hormones. These methods cover process of sample collection when physical interference of animal bodies is penetrated, such as for example cut, puncture, and so on. The blood collection is the most frequent invasive method with subsequent analysis of hormone‘s concentration from blood plasma. Advantage of those method is the direct measurement of instantaneous hormones in blood, without influence of their conversions, but it brings other troubles and imperfections (Kobelt et al., 2003). In wild animals or in some zoology gardens can be blood taking dangerous or impossible (Palme a Möstl, 2000).

3.3.2 Non-Invasive methods

Among noninvasive methods belong such processes during which physical interference with bodies is not breaked. Hormones are not determined from blood plasma, but from alternative sources as are saliva (Greenwood & Shutt, 1992), urine (Bamberg et al., 2001), feces (Pereira et al., 2005), milk (Rabiee et al., 2002a), feathers (Bortolotti et al., 2008) or coat.

Nevertheless, also these media have some limitation. At analysis of milk or urine is yet necessary to define manipulation of animals for sample collection and application of these methods have one's limits that only on some individual animals. Using urine in free living animals, where experimenter has not suitable conditions to obtained sample, is nearly impossible. Similar limitation is for collection of saliva where the size of animal can be another difficulty. Using milk, it is possible to apply only to lactating female, therefore for analysis of hormone levels like optimal of all media seems to be feces (Möstl & Palme, 2002). Metabolites are extracted from feces preserved by freezing shortly after defaecation or after lyophilisation or cure in alcohol (Palme, 2005). Usage gives exhibits freshly and after cure, freshly are however more suitable because of simpler manipulation (Möstl and Palme 2002; Palme et al., 2005; Palme, 2005). Advantage and preferred application of noninvasive methods of collection before invasive is reality that the designs of collection is more simple, repeatable and wihout necessary equipment or handling facilities (Touma & Palme, 2005; Möstl et al., 2005). It means that after application of those methods will not get to influence level of measured hormones. In addition make possible to long - term observe one animal, without get to his physical detrimental effect (Hirschenhauser et al., 2005).

3.3.3 Enzyme Immunoassay (EAI)

Enzyme immunoassay is one of most often used immunochemistry methods applied at assessment concentration of hormones or their metabolites, measurable from a variety media, such as for example blood, urine, dropping, saliva, and so on. Principle of those methods consists at that that the EIA employs polyclonal antibodies take up surface micro - titrating laminae. O binding place upon this anti - matters together completes known quantity enzyme marked by hormones (so - called conjugate) and unknown quantity measured steroid hormone (Möstl et al., 2005). Because of changes in concentrations after release of hormone into blood and metering their surface from take away media to be necessary enjoyment analysis and biological validation enzyme immunoassay (Palme, 2005), by the help of which surface measured materials investigate with.

3.4 Steroid hormones

Hormones are biologically active substances, exploited for intercellular communication, whose performance interlock endocrines or component cells in different tissues. Endocrine glands release hormones into blood, that is transporting them to objective tissues (Janský and Novotný, 1981). After release of hormones into blood, where they may circulate for couple of hours, and impingement upon objective tissues, their conversions among, by e.g. conjugation, deconjugation, oxidation or metabolism bacterias can happen. Hormones are metabolized in liver, from here wander either into kidneys and are secreted by urine, or gall into intestines and are secreted by feces (Palme, 2005).

Time delay among release of hormones into blood and their growth in measured medium is species specific and nearly bears with it, how long does it take alleyway food from intestines into back passage. In big mammals lasts alleyway food to the extent some days (Goymann et al., 1999; Wasser et al., 2000; Denhard et al., 2001), compared to that in birds only of several hours (Kikuchi et al., 1994 podle Hirschenhauser et al., 2005; Denhart et al., 2003). For example Palme et al. (1995) found out differences at the time delay among concentration of hormones in blood and their excreta in livestock animals, when highest level of hormones metabolites in feces of ewes discovered duodenary after one hour. In Galloway cattle after twenty four hours and near pigs after as much as forty eight hours. Levels of hormones in animals show us valuable information about their state, for example it is possible monitor oestrus (Garnier et al., 2002) and pregnancy in females (Heistermann et al., 1996; Kuckelkorn, 1994) and measure, whether testosterone level in males is dependent upon season (Strier et al., 1999). Further they may bear a hand to evaluate, whether human activity effects animals as stress factor (Barja et al., 2007; Gorgasser et al., 2007) or if turns level stress hormones near animals that the live in social group (Fichtel et al., 2007; Foley et al., 2001).

3.5 Hormones and behavior

Scientist always interest, what causes worth while breed animals. In a many cases is behaviour effected by changes of hormone’s level, that circulate in blood of animal. Hormones scales on it's receptors and influence many physiological functions, for example adolescence individuality, development of sexual behaviour, initiation of lactation in females (Cavigelli 1999; Fichtel et al., 2007).

Living organism is an open system that communicate with outdoor environment, trucks material, energy and information desk. In spite of these dynamics the organism is trying to hold up steady internal environment, homoeostasis. External environment of organism prepares various situations, with unfavourable conditions which organism cannot balance out and respond on them abnormally. It can evocate stress and response referred to as stressed (Möstl and Palme, 2002). Although stress is common state for each individuality, there is no its accurate definition (Hofer and East 1998; Moberg and Mench 2000; Sapolsky et al., 2000; McEwen and Wingfield 2003; Wielebnowski 2003; Romero 2004), perhaps in different disciplines the definitions are stated.

Glucocorticoids or stress hormones are releases from adrenal medulla under the thumb of hypothalamus- pituitary complex. Play an important role when animal meets stress factor. After that glucocorticoids as cortisol and corticosterone are release into blood (Cavigelli 1999; Fichtel et al., 2007). Increased concentration of these hormones in blood leads to mobilization of energetic reserves. Also general readiness of organism to flight or defense increases under stress (Schwarzenberger et al., 1996; Garnier et al., 2002; Schwarzenberger et al., 2004; Patzl et al., 1998). Stress is required for the development of conditioned taste aversions and extend it to ruminants. It was suggested that activity of the hypothalamic-pituitary-adrenal axis is integral to food aversion learning in ruminants (Kronberg, Walker, Fitzgerald, 1993).

3.6 Reproduction and hormones

Animal's reproductive systems can be dividend into the internal reproductive organs and to the external genitalia. The gonads are the achal organs that produce the gametes. In the male, testes produce sperm, and in the female, ovaries make eggs. In biological terms sexual reproduction involves the union of gametes - the sperm and the ovum - produced by two parents. Each gamete is formed by meiosis . This means each contains only half the chromosomes of the body cells (haploid). Fertilization results in the joining of the male and female gametes to form a zygotewhich contains the full number of chromosomes (diploid). The zygote then starts to divide by mitosis to form a new animal with all its body cells containing chromosomes that are identical to those of the original zygote (Lawson, 2008).

Sex hormone governs action pair with once from basic characteristic of organism, with their reproduction. Chemicaly steroids are it and near of each of sex find how male, so female hormones, but in by other rates. Sex hormone determine adolescence individuality and development of sexual organ, production sexual sentinel node and develops typical sexual behaviour leadership to embrace. After insemination work development ova, embryos and fruit and later also channel processes incidental care of brood (Pereira et al., 2005).

Male sex hormones give a name androgens and their central representative is testosterone. Originate in interstitial Leydig cells of male gonads, testicles (test instrument) (Mann and Lutwak-Mann, 1981). Testosterone is the most important "male's" hormone. Is synthesized in testicles in Leydig cells after stimulation by LH. Testosterone is fundamental to development and ageing prime and secondary sexual organs, sexual dimormfism and typical sexual behaviour. At the same time stimulates performance FSH (Gamčík and Kozumplík, 1984). Secretion of androgens especially lutropin, that is of produced by anterior pituitary of hypophysis. That stimulates Leydigovy cell, governs performance androgens at all and especially then testosterone. Performance of lutropin is controlled by gonadotrophin excitant hormone from hypothalamus. That releases every two as far as four hours and turns levels of testosterone into blood. Male gamete - sperm, rise along spermatogenesis (Mann and Lutwak-Mann, 1981).

Female gonad are a pair of ovaries (ovary), which put out gamete (ova) and female sex hormone. Ova develop in cortical layer of ovaries from so - called oocytes and are saved in pouch, so - called Graaph folliculus. Most considerable female sex hormones are estrogens (oestradiol, estrone, estriol) and gerontoxons. Estrogens put out especially cell Graaph folliculus, except it forms also in yellow corpuscle, in placenta, in adrenals estrogens effects on development fabrics incidental reproduction, development secondary sexual signs and molding female sexual behaviour, further somnifacient menstrual cycle and support build - up endometrium. To other important female hormones are gerontoxons, of which is most considerable luteal hormone. That is produced by especially yellow corpuscle. Prepares endometrium (endometrium) to nidation (nidation) ova by that the she translate into secretion phase. From of other female¨s sex hormone we can name relaxin, that is secreted by corpus luteum during pregnancy - yew and placenta and makes relaxin ligament saucepan and symphysis thereby relieves childbirth (Bao and Garverick, 1998).

Fertility is influenced by 20 % hereditary base and minimally from 80 % by external factors. external factor quit of have decisive position alimentary influences, further climatic factor (sheds period, light, warm, moisture level etc.), way of breeding and stabling, level of nursing care, system proceeding, organization planning and care about reproduction. Further then age animal, racial PI propriety, health and condition state (Rensis and Scaranuzzi, 2003).

3.6.1 Effect of feed on better results reproduction

Heterotrophic animals develop of power, organic material (mostly carbonaceous allied substances) and basic nutrient from food. Food further process in biochemical of the process-yclept digestion, to obtain nutrients and energy. In general digested food degrades on simplier particles, that are enough small, to allow to be absorbed in the body of and further to be used for example in cellulate breathing or at biosynthesis (Mann, 1981).

Non- genetic (outer) factors is the most important factor level of nutrition – inadequate nutrition or deficient value of diet can deteriorate females fertility eventually cause impotence, near with calf will spawn higher decline embryos and lowered germinative quality nastal brood. However not even excessive supply of nutrients that the causes fat female isn't for reproduction fit (Majzlík, 2000). Negative energetical balance causes reduction of body weight, decrease activities folliculated and has impact on production of reproductive hormones. Increasing concentration of energy, intake of dry matter and microbial fermentation in rumen can reduce energy losses. Cow should be in a positive nutritional state 4 – 6 weeks postpartum (increasing body condition, cut in non-esterified fatty acids in blood, normal fatness milks) (Winston, 2009). Higher surface protein in feeding can cut pregnancy, due to higher concentration of blood urea and uterine liquids, higher power requirement on converting ammoniac on carbamide or negative influence over immunity function. Monitoring of urea in milk can be used protein from feeding dues (Winston, 2009).

The relationships between hormone levels and boarding could also study Joëlle Taillon at the white-tailed deer (Taillon, 2008), which quality were surprising: Fawns fed the control diet presented higher glucocorticoid and lower testosterone levels then fawns fed the poor diet, suggesting that control fawns faced a higher nutritional stress than those on the poor diet. Similarly to other studies on social mammals, we found no relationship between faecal glucocorticoid levels and social rank, suggesting that social stress was similar for dominant and subordinate fawns during winter. Testosterone levels were not correlated to social rank as found previously in groups of individuals forming stable social hierarchies and maintaining stable dominance relationships. The simultaneous suppression of glucocorticoid and testosterone levels suggests for the first time that young ungulates present a hormonal strategy to prevent fast depletion of limited proteins and fat resources during winter (Taillon, 2008).

3.6.2 *Effect of seasonality and the environment on reproduction*

Animal seasonally polyhedral entails that the sexual cycle is impressed with photoperiod. Mark polyestric animal employs when near individual will slip rut several times per annum (Stella, 2004). Deer mating season proceeds from halves September by the middle October on heat, where for hinds come deers. In rutting season deers can hear shouting. The course heat depensd sex ratio, weather and lull in hunting. Of reproductive behaviour interlocks even spreading individuals of a kind in territories. Near some territories wear well, near by other create only for a definite period of time – time courting (rut harts). Fellow behaviour begins insinuation, that precluding mating. Partners together acquaint by the help of effluvial, sound and optical signal. Societa is group individuals of a kind, among which is definite hierarchy. These insider advantage to defence before predator (McNab, 2002).

Seasonal establishment change in the levels of sex hormones deal with already several studies, some record a little odd. Koubasov et al. (2006), except thyroid hormones, studied also levels of sex hormones and their romance with quantity thyroid hormones. In his results apportioned sex hormone into two groups. Primary group includes gonadotrophin, prolactin and luteal hormone, that to its quantity positively correlate seasonal establishment levels hormone trijodthyroxine. Alternative group hormones, testosterone and oestradiol, had anologous course seasonal establishment changes like thyroxine. In addition concentration testosterone matched changes in length photo - periods.

According to Koubasova et al. (2006) are cold and spring preparatory period, in of which body create reserves. You are used to anabolic activity in summer, when is endocrinous maintainance homoeostasis effected strong anabolic effects testosterone and oestradiol, that so replaces dominant effects trijodthyroxine.

According to Prof. MVDr. Rudolfa Dvořáka, DrSc (2009)dair ycows inseminate during hot months in the year, come to downward tendency of fertility.

Different factors contribute to this situation; the most important are a consequence of increased temperature and humidity that result in a decreased expression of overt estrus and a reduction in appetite and dry matter intake. Heat stress reduces the degree of dominance of the selected follicle and this can be seen as reduced steroidogenic capacity of its theca and granulosa cells and a fall in blood estradiol concentrations (Rensis, 2008). Plasma progesterone levels can be increased or decreased depending on whether the heatstress is acute or chronic, and on the metabolic state of the animal. These endocrine changes reduce follicular activity and alter the ovulatory mechanism, leading to a decrease in oocyte and embryo quality. The uterine environment is also modified, reducing the likelihood of embryo implantation. Appetite and dry matter intake are both reduced by heat stress thus prolonging the postpartum period of negative energy balance and increasing the calving-conception interval, particularly in high producing dairy cows. The utilization of cooling systems may have a beneficial effect on fertility but dairy cows cooled in this way are still unable to match the fertility achieved in winter. Recent studies suggest that the use of gonadotropins to induce follicular development and ovulation can decrease the severity of seasonal postpartum infertility in dairy cost (Rensis, 2008).

Study of Huber et al (2003) showed as though, female glucocorticoid excretion varied seasonally with a peak during December and January. Out of several potential predictor variables investigated, minimum ambient temperature and snow proved to be the only factors exerting a significant effect on fecal glucocorticoid excretion. They suggest that high winter glucocorticoid levels may act via catabolic function during adaptation of deer to cold winter month when resources are limited.

4. Results

The review of studies published on fecal hormonal metabolites of ungulates are summarised in Table 1 and 2 in Appendix. In Table 1 describing methods used for determination of hormonal metabolites was possible to use 25 studies found on Web of Science. To fulfil Table 2 on evaluation of factors influencing endocrinology of studied species was used 30 studies. The most of the studies was done on free ranging animals and most freqently studied was bighorn sheep (*Ovis canadensis*).

The most common method used for determination of hormons metabolites from feces is enzyme immunoassay. The most frequently studied factors affecting concentration of hormonal metbolites in studied animals was reproductive state, environment, availability and quality of nutrition, age and in polyestric species seasonality. The goal of this review was to evaluate methods used for determination of hormones metabolites from feces on the base of extraction, reproductive cycle, behaviour, nutrition, environmental effects, sample size, frequency of collection and seasonality. In females the most important factor on metabolites of steroid hormones has ovulation. On the contrary environment was not influencing greatly the hormonal metabolites. In males the most frequent were studies on deer rut and antler development. Surprisingly the increase of glucocorticoids was greater during the winter not the rut at the autumn.

5. Discusion

Evaluation of validity of reproductive strategies is based on evaluation of steroid hormones. Those variability oscilates because of events like seasonality, reproductive cycle, age, pregnancy, rut etc. Thus resulted in scientific discussion about evaluation results from fecal sampling and determination of hormone‘s metabolites. Fecal sampling for detection of steroids was used as a tool for management of free ranging or captive ungulates. The most frequent method was enzyme immunoassay (Tab. 1), which was evaluated as most valid method. For better extraction and proper concentration of hormones is recommended to store after collection in cold and keep frozen.

The validation of techniques in studies of stress is more difficult and usually involves questions such as is an animal stressed or non-stressed and does this present as acute or chronic stress, positive or negative stress (distress) (Whitten et al., 1998; Möstl and Palme, 2002; von der Ohe and Servheen, 2002; Wielebnowski, 2003; Millspaugh and Washburn, 2004; Touma and Palme, 2005; Keay et al., 2006; Lane, 2006). Studies in free-ranging species are often confronted with difficulties in locating samples, usually involving observation of known individuals and collecting samples upon defecation. However, non-invasive faecal steroid analysis also offers the opportunity to study free-ranging animals for which direct observation of defecation is difficult or impossible.

Like all laboratory-based methods, assay validation is most important for obtaining useful and accurate results. However, the particularity with faecal steroid analysis is that the parent hormones progesterone, testosterone, cortisol or corticosterone are not (or only barely if at all) present in the faeces. Consequently, it is inaccurate to speak of faecal-progesterone or faecal-cortisol analysis, although this designation is common practice in a considerable proportion of the published literature. Proper faecal steroid assay validation is all related to steroid metabolism (Palme et al., 1996, 2005; Schwarzenberger et al., 1996, 1997; Möstl et al., 2005; Palme, 2005). How is showed in study of Shargal et al. (2008) high level of testosterone of Nubian ibex (*Capra nubiana*) correspond with increase of dominant behaviour in herd (Tab.1). On high level of testosterone functions in males seasonal establishment executant behaviour is high pair with circulation of testosterone, that is of most often visible in Pe`re David's deer (*Elaphurus davidianus*). Conversely observed levels of progesterone in Holstein–Friesian cattle (*Bos primigenius* f. *taurus*), was found to be low as concentrations of fecal progestagen in females aged less than 18 months of age indicated that sexual maturity in captivity is not attained before that age (Mohammed et al., 2011).

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6. Conclusion

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

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8. Appendices

Tables 1- methods used for determination of hormonal metabolites

Hormone	Laboratory method	Metabolite	Species	Sex	Conservation/ storing of the sample	Concentration (hormone)	Volume/weight of the sample	Preparation of the sample	Solution with XXXX and volume	Source (Citation)
Progesterone	Enzyme immunoassay	20-oxo-pregnanes	Cattle(*Bos primigenius f. taurus*)	Female	stored -20°C	41,8 ± 3,8ng/g	0,5g	drying, mixing, centrifugation	fecal steroids are more time consuming than the results of the findings from milk	(Schwarzenberger, Son, Pretting, Arbeiter, 1996)
Progesterone	Enzyme immunoassay	20-oxo-pregnanes	American bison (*Bison bison*)	Female	in plastic cups in the cold	258,8 ± 40,3ng/g	100 - 300g	samples were homogenized mixed	confirmation that the bison are seasonally polyestrous	(Viervacke, Schwarzenberger, 2006)
Progesterone	Enzyme immunoassay	20α -OH-pregnanes	Holstein–Friesian cattle(*Bos primigenius f. taurus*)	Female	stored -20°C	47 ± 22,9ng/g	0,5 g	drying, mixing, centrifugation	concentrations of progesterone were not affected by the level of daily milk yields	(Rabiee, Macmillan, Schwarzenberger, 2002)
Progesterone	Enzyme immunoassay	20β -OH-pregnanes	Holstein–Friesian cattle(*Bos primigenius f. taurus*)	Female	stored -20°C	47 ± 22,9ng/g	0,5 g	drying, mixing, centrifugation	concentrations of progesterone were not affected by the level of daily milk yields	(Rabiee, Macmillan, Schwarzenberger, 2002)
Progesterone	Enzyme immunoassay	20-oxo-pregnanes	Holstein–Friesian cattle(*Bos primigenius f. taurus*)	Female	stored -20°C	47 ± 22,9ng/g	0,5 g	drying, mixing, centrifugation	concentrations of progesterone were not affected by the level of daily milk yields	(Rabiee, Macmillan, Schwarzenberger, 2002)
Progesterone	Enzyme immunoassay	pregnanediol, 20-oxo-pregnanes	Arabian oryx (*Oryx leucoryx*)	female	stored -20°C	35,7 ± 19,1ng/g	8 - 10g	wet, mixing, centrifugation	Reanalysis of results after correcting for difference in water content of feces did not give different conclusions.	(Ostrowski, Blanvillain, et al 2005)
Progesterone	Enzyme immunoassay	20-oxo-pregnanes	Mountain gazelles (*Gazella gazella*)	Female	in plastic cups in -20°C	6497 ± 1245,5 ng/g	5 g	drying, mixing, centrifugation	Low concentrations of fecal progestagen in females aged less than 18 months old indicated that sexual maturity in captivity is not attained before that age.	(Mohammed, Green, Holt, 2011)
Progesterone	Enzyme immunoassay	20-oxo-pregnanes	Mountain gazelles (*Gazella gazella*)	Female	in plastic cups in -20°C	6497 ± 1245,5 ng/g	5 g	drying, mixing, centrifugation	Low concentrations of fecal progestagen in females aged less than 18 months old indicated that sexual maturity in captivity is not attained before that age.	(Mohammed, Green, Holt, 2011)
Progesterone	Enzyme immunoassay	5α-pregnane-3,20-dione	Brown brocket deer (*Mazama gouazoubira*)	Female	stored -20°C	290,6 ± 65,5 ng/g	0,5 ± 0,02 g	drying, mixing, centrifugation	For all hinds, behavioral estrus was associated with nadirs in fecal progestagen concentrations.	(Pereira, Polegato, Souza, Negra˜o, Duarte, 2006)
Progesterone	Radioimmunoassay	pregnanediol-3α-glucuronide	Pe`re David's deer (*Elaphurus davidianus*)	Female	stored -20°C	235,26 ± 86,71 ng/g	0,5g	drying, mixing, centrifugation	reprodiction activity hind was largery connected with ovary secretion estrogen	(Li, Jiang, Jiang, Fang, 2001)
Progesterone	Radioimmunoassay	17β-estradiol	Goats- Toggenburg and Alpine (*Capra* sp.)	Female	stored -20°C	153,48±206,84 ng/g	0,3 g	homogenized mixed, centrifugation	the profiles of progesterone fecal metabolites reflect the serum concentrations of the same	(Capezzuto, Chelini, Felippe, Oliveira, 2008)
Progesterone	Radioimmunoassay	5α-pregnane-3β-ol-20-one	Sable antelope (*Hippotragus niger*)	Female	stored -20°C	1,6 – 6,6 ng/g	0,025 g	drying, mixing, centrifugation	data indicate that they exhibit considerable interspecific variability in estrous cycle length and reproductive seasonalit	(Thompson, Mashburn, Monfort, 1998)
Progesterone	Radioimmunoassay	4-pregnen-20α-ol-3-one	Mohor gazelle (*Gazella dama mhorr*)	Female	stored -20°C	4048 ± 407 ng/g	5 g	mixed, skimmed	Therefore, it was assumed that the cyclic variability in progesterone metabolite excretion genuinely reflected changes in the peripheral progesterone concentrations associated with	(Pickard, Abáigar, Green, Holt, Cano, 2001)

Table 1a

Hormone	Laboratory method	Metabolite	Species	Sex	Conservation/ storing of the sample	Concentration (hormone)	Volume/weight of the sample	Preparation of the sample	Solution with XXXX and volume	Source (Citation)
Testosterone	Enzyme immunoassay	17βhydroxy-4androsten 3	White-tailed deer (Odocoileus virginianus)	Male	in plastic cups in -20°C	138,6 ± 5,6ng/g	0,5 ± 0,02g	drying, mixing, centrifugation	higher concentrations of testosterone after chudčim feed, but lower in winter	(Taillon, 2008)
Testosterone	Enzyme immunoassay	5-androstene-3β,5α-dihydrotestosterone,5-α-androstano-3a-ol-17-one	Pampas deer (*Ozotoceros bezoarticus bezoarticus*)	Male	in dry in 8°C	343,9 ± 211,4ng/g	0,5 ± 0,02g	drying, mixing, centrifugation	stags in hard antler had higher concentrations of fecal testosterone when compared to males in antler casting or antlers in velvet	(Pereira, Duarte, Negra˜o, 2005)
Testosterone	Radioimmunoassay	5α-dihydrotestosterone	Nubian ibex (*Capra nubiana*)	Male	in dry in 8°C	211 ± 12ng/g	0,5g	mixed, skimmed	Testosterone affects aggression and dominance in animals and because of its higher concentration is more male descendants	(Shargal, Shore, Roteri, et al 2008)
Testosterone	Radioimmunoassay	17βhydroxy-4androsten 3	Pe`re David's deer (*Elaphurus davidianus*)	Male	stored -20°C	553,06 ± 165.63 ng/g	0,5g	drying, mixing, centrifugation	near males seasonal establishment executant behaviour is high pair with circulation testosterone	(Li, Jiang, Jiang, Fang, 2001)
Testosterone	Radioimmunoassay	5-α-dihydrotestosterone,17βhydroxy-4androsten 3	Bighorn sheep (*Ovis canadensis*)	Male	srored ethanold and H2O in -20°C	5,9 ± 3,9%	2g	drying, mixing, centrifugation	conspicuous seasonal cycle in fecal testosterone pattern associated with the mating season	(Pelletier, Bauman, Festa-Bianchet, 2003)
Glucocorticoids	Radioimmunoassay	17-dioxoandrostanes	Pe`re David's deer (*Elaphurus davidianus*)	Male and Female	in plastic cups in -20°C	268,98 ± 15,21 ng/g	0,5g	drying and wet, mixing, centrifugation	Fecal cortisol concentration of the display group was significanty higher than that of the free-ranging group	(Li, Jiang, Tang, Zeng, 2007)
Glucocorticoids	Radioimmunoassay	l-corticosteron	White-tailed deer (*Odocoileus virginianus*)	Male	in plastic cups in -20°C	67,2 ± 1,9ng/g	0,05 ± 0,02g	drying, mixing, centrifugation	better when the diet is higher glucocorticoid concentration is reduced in winter	(Taillon, 2008)
Glucocorticoids	Radioimmunoassay	l-corticosteron	White-tailed deer (*Odocoileus virginianus*)	Male	stored -20°C	97 ng/g	57,7g	mixed, skimmed	fecal samples exposed to rainfall for one week may artificially inflate fecal glucocorticoid measurement	(Washburn, Millspaugh, 2002)
Glucocorticoids	Enzyme immunoassay	3α,11-ox cortisol	Red deer (*Cervus elaphus*)	Female	stored -20°C	1,06 ± 5,7 ng/g	0,5g	mixed, skimmed	The time past defecation may therefore be overestimated in droppings lying in the sun and underestimated in feces lying in the shade.	(Huber, Palme, Zenker, Möstl, 2003)
Glucocorticoids	Enzyme immunoassay	11-oxoetiocholanolone	Red deer (*Cervus elaphus*)	Male and Female	in plastic cups in -20°C	27,601 ng/g	0,5g	drying, mixing, centrifugation	minimum ambient temperature and snow proved to be the only factors exerting a significant effect on fecal glucocorticoid excretion	(Huber, Palme, Arnold, 2003)
Glucocorticoids	Enzyme immunoassay	11-oxoetiocholanolone	Red deer (*Cervus elaphus*)	Male and Female	in plastic cups in -20°C	27,601 ng/g	0,5g	drying, mixing, centrifugation	minimum ambient temperature and snow proved to be the only factors exerting a significant effect on fecal glucocorticoid excretion	(Huber, Palme, Arnold, 2003)
Prostaglandin F2α	Radioimmunoassay	15-Ketodihydroprostaglandin F2α	Reindeer (*Rangifer tarandus tarandus*)	Female	stored -20°C	1,2 nmol/L	200 ml	centrifugation	high prostaglandin production most likely be related to development of the placenta	(Ropstad, et al 2005)

Table 1.b

Tables 2- evaluation of factors influencing endocrinology of studied species

Metabolite	Species	Area of the study, applied for (reproduction, behaviour etc.)	Captive or wild	Sex	Age of animals	Amount of the metabolite (range or mean- specified)	Units	Season when collected	Reproductive season	No. of animals covered by	Sampling frequency	Data on behaviour yes/no	Source (Citation)
20-oxo-pregnanes	American bison (*Bison bison*)	Farm in Belgium	captive on pasture	Male	9 year	39,5 ± 2,2 in fecal	ng/g	July and early August	the mating season	13	after discharge, 2 weeks	yes, associated with hormonal ovulation	(Viervacke, Schwarzenberger, 2006)
20-oxo-pregnanes	Cattle (*Bos primigenius f. taurus*)	Farm in Vienna	captive	Female	3 - 6 year	39,5 ± 2,2 in fecal	ng/g	two ovulatory periods	ovulatory period	12	morning and evening	yes, quiet behaviour	(Schwarzenberger, Son, 1996)
20-oxo-pregnanes	Cattle (*Bos primigenius f. taurus*)	Farm in Vienna	captive	Female	3 - 6 year	34,1 ± 15,2 in milk	ng/g	two ovulatory periods	ovulatory period	12	morning and evening	yes, quiet behaviour	(Schwarzenberger, Son, 1996)
20-oxo-pregnanes	Arabian oryx (*Oryx leucoryx*)	reserve of Mahazat as-Sayd	captive	Female	Adult	15,5	ng/g	in 1998-1999 and 2003	estrous cycle	25	collected their feces twice a week	no	(Ostrowski, Blanvillain, et al 2005)
20-oxo-pregnanes	Holstein–Friesian (*Bos primigenius f. taurus*)	Milk-Farm	captive	Female	4 - 9 year	38,2	ng/g	period of one estrous cycle	group of cycling cows	16	5 weeks	yes, quiet behaviour	(Rabiee, Macmillan, Schwarzenberger, 2002)
20-oxo-pregnanes, 5α-dihydrotestosterone	Nubian ibex (*Capra nubiana*)	Safari—Zoological Center, Ramat Gan, Israel	captive	Male and Female	Adult	332,5 ± 112,2	ng/g	20-month period	post-partum	26 (M 17, F 9)	2 weeks between 5:00 - 8:00 h	yes, effect of dominance and aggression by testosterone on offspring sex	(Shargal, Shore, Roteri, et al 2008)
17βhydroxy-4androsten 3	White-tailed deer (*Odocoileus virginianus*)	Island St. Lawience and Canada	captive	Male	6 - 7 month	4,8	ng/g	January 2004	dominant and subordinate	13	every 10 day	yes, action quality and quantity of food	(Taillon, 2008)
17βhydroxy-4androsten 3	Pe´re David's deer (*Elaphurus davidianus*)	Dafeng, China	captive	Male	Adult	38	ng/g	From July 1997 to September 1997 and from March 1998to July 1998	during the pre-rut	17	every 5 days, from 06:00 to 08:00	yes,rutting behavior includes anogenital sniffing, urine sniffing, flehmen,bellowing, wallowing, antler adorning,	(Li, Jiang, Jiang, Fang, 2001)
5α-dihydrotestosterone	Red brocket deer (*Mazama americana*)	Southeast Brazil	captive	Male	Adult	302,85 ± 152,29	ng/g	all year	anther cycle	6	every week between 8:00 - 9:00 h	yes, behaviour with exchange antlers	(Versiani, Pereira, Duarte, 2009)
5-α-dihydrotestosterone	Bighorn sheep (*Ovis canadensis*)	Sheep River Provincial Park (Alberta, Canada)	wild	Male	1 - 3 year	5,9 ± 3,9	%	2 year (2001/2002)	during the pre-rut.	11	every day, afternoon	yes, aggressiveness was weakly correlated with fecal testosterone	(Pelletier, Bauman, Festa-Bianchet, 2003)
5α-dihydrotestosterone	Pampas deer (*Ozotoceros bezoarticus bezoarticu* s)	free-ranging stags from ENP	wild	Male	2 - 6 year	6.l	%	From October 2000 to September 2001	anther cycle	15	between days 10 and 20 of each month	yes, behaviour with exchange antlers	(Pereira, Duarte, Negra´o, 2005)
20α -OH-pregnanes	Holstein–Friesian cattle (*Bos primigenius f. taurus*)	Milk-Farm	captive	Female	4 - 9 year	48,2	ng/g	period of one estrous cycle	group of cycling cows	16	5 weeks	yes, quiet behaviour	(Rabiee, Macmillan, Schwarzenberger, 2002)
20β -OH-pregnanes	Holstein–Friesian cattle (*Bos primigenius f. taurus*)	Milk-Farm	captive	Female	4 - 9 year	17	ng/g	period of one estrous cycle	group of cycling cows	16	5 weeks	yes, quiet behaviour	(Rabiee, Macmillan, Schwarzenberger, 2002)
5-androstene-3β	Pampas deer (*Ozotoceros bezoarticus bezoarticus*)	free-ranging stags from ENP	wild	Male	2 - 6 year	2.l	%	From October 2000 to September 2001	anther cycle	15	between days 10 and 20 of each month	yes, behaviour with exchange antlers	(Pereira, Duarte, Negra´o, 2005)
5-androstene-3β	Bighorn sheep (*Ovis canadensis*)	Sheep River Provincial Park (Alberta, Canada)	wild	Male	1 - 3 year	12,2 ± 4	%	2 year (2001/2002)	during the pre-rut.	11	every day, afternoon	yes, aggressiveness was weakly correlated with fecal testosterone	(Pelletier, Bauman, Festa-Bianchet, 2003)

***Table** 2a*

Metabolite	Species	Area of the study, applied for (reproduction, behaviour etc.)	Captive or wild	Sex	Age of animals	Amount of the metabolite (range or mean- specified)	Units	Season when collected	Reproductive season	No. of animals covered by	Sampling frequency	Data on behaviour yes/no	Source (Citation)
11-oxotestosterone	Bighorn sheep (*Ovis canadensis*)	Sheep River Provincial Park (Alberta, Canada)	wild	male	1 - 3 year	25,6 ± 15,1	%	2 year (2001/2002)	during the pre-rut.	11	every day, afternoon	yes, aggressiveness was weakly correlated with fecal testosterone	(Pelletier, Bauman, Festa-Bianchet, 2003)
11-oxoetiocholanolone	Red deer (*Cervus elaphus*)	Research Institute of Wildlife Ecology in Vienna,	semi-natural	Male and Female	Adult and young	471,644	ng/g	in 1999 and 2000	during the pre-rut	17	once a week for one year	yes, seasonal variation, sex differences, and invasive sample collection may confound glucocorticoid measures as indices of stress	(Huber, Palme, Arnold, 2003)
5-α-androstano-3a-ol-17-one	Pampas deer (*Ozotoceros bezoarticus bezoarticus*)	free-ranging stags from ENP	wild	male	2 - 6 year	0,2	%	from October 2000 to September 2001	anther cycle	15	between days 10 and 20 of each month	yes, behaviour with exchange antlers	(Pereira, Duarte, Negra˜o, 2005)
androstenedione	Bighorn sheep (*Ovis canadensis*)	Sheep River Provincial Park (Alberta, Canada)	wild	male	1 - 3 year	18,6 ± 10,7	%	2 year (2001/2002)	during the pre-rut.	11	every day, afternoon	yes, aggressiveness was weakly correlated with fecal testosterone	(Pelletier, Bauman, Festa-Bianchet, 2003)
pregnanediol	Arabian oryx (*Oryx leucoryx*)	reserve of Mahazat as-Sayd	captive	Female	Adult	14,5	ng/g	in 1998-1999 and 2003	estrous cycle	25	collected their feces twice a week	no	(Ostrowski, Blanvillain, et al 2005)
17-dioxoandrostanes	Pe`re David's deer (*Elaphurus davidianus*)	Dafeng Nature Reserve, China	captive	Male and Female	Adult and young	81,4 ± 4,4	ng/g	February 15 to April 16 in 2004	-	19	every 6 days, from 6:00 to 8:00	yes, frequency of conflict behavior in the display group was significantly higher than those in the free-ranging group	(Li, Jiang, Tang, Zeng, 2007)
pregnanediol-3α-glucuronide	Pe`re David's deer (*Elaphurus davidianus*)	Dafeng, China	captive	Female	Adult	37,64	ng/g	From July 1997 to September 1997 and from March 1998to July 1998	estrous cycle	25	every 5 days, from 06:00 to 08:00	yes, estrous behavior includes frequent urinating, receptivity, and permitting mount	(Li, Jiang, Jiang, Fang, 2001)
17β-estradiol	Goats Toggenburg and Alpine (*Capra* sp.)	south-east of Brazil	captive	Female	Adult	18,543	ng/g	between November and May	post-partum	11	weekly from each animal between 09:00 and 11:30	yes, associated with hormonal ovulation	(Capezzuto, Chelini, Felippe, Oliveira, 2008)
15-Ketodihydroprostaglandin F2α	Reindeer (*Rangifer tarandus tarandus*)	in Oulu, Finland	captive	Female	Adult	47,7 ± 83,5	nmol/L	from September 1997 until May 1998	estrous cycle	13	three times a week	yes, associated with hormonal ovulation	(Ropstad, et al 2005)
5α-pregnane-3,20-dione	Brown brocket deer (*Mazama gouazoubira*)	Wild Animal Section of the Sa˜o Paulo State University	captive	Female	5 - 8 year	91,3± 9,6	ng/g	From February through May 2002,	pregnancy and the estrous cycle	5	collected daily (12:00–18:00 h)	yes, the period in which hinds permitted copulation	(Pereira, Polegato, Souza, Negra˜o, Duarte, 2006)
20-oxo-pregnanes	Pregnant mountain gazelles (*Gazella gazella*)	Thumamah, Saudi Arabia	wild	Female	Adult	13.l	ng/g	the summer months (July-August),winter season (December–February)	reproductive cycling	8	every day	yes, associated with hormonal ovulation	(Mohammed, Green, Holt, 2011)
l-corticosteron	White-tailed deer (*Odocoileus virginianus*)	Charles Green Conservation Area, near Ashland	captive	Male	5 - year	47±59,81	ng/g	March 2001	-	15	10–30 h after they received a 50 IU injection of ACTH	yes, quiet behaviour	(Washburn, Millspaugh, 2002)
5α-pregnane-3β-ol-20-one	Sable Antelope (*Hippotragus niger*)	National Zoological Park's Conservation and Research Center	captive	Female	4–14 year	13,8± 6 1,9	ng/g	all year	estrous cycle	18	every week	yes, quiet behaviour	(Thompson, Mashburn, Monfort, 1998)
4-pregnen-20α-ol-3-one	Mohor gazelle (*Gazella dama mhorr*)	Spain	captive	Female	Adult	24,38 ± 1,77	ng/g	period of one estrous cycle	estrous cycle	7	once a week	yes, courtship behaviour	(Pickard, Abáigar, Green, Holt, Cano, 2001)
3α,11-ox cortisol	Red deer (*Cervus elaphus*)	Research Institute of Wildlife Ecology in Vienna, Austria	captive	Female	Adult	3,9 ± 20,62	ng/g	June 2000	ovulatory period	6	3 times a day	yes, experiment as fol- lows	(Huber, Palme, Zenker, Möstl, 2003)

***Table** 2b*