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Evaluation of Cortisol and Stress in Captive Animals

Vahdettin Bayazit, PhD

Muş Alparslan University, Faculty of Sciences & Arts,
Department of Biology, Station Street,49100, Muş, Turkey,

Abstract: The purpose of this study was to investigate the relationship between stres and cortisol levels in captive animals. Stress is defined as a physiologic response to events perceived as potentially or actually threatening the integrity of the body. Serum, saliva or urine cortisol has been extensively used as a stress indicator in many mammalian species. Stereotypic behavior is exhibited by a wide range of captive animals. Its association with hormones, especially elevated cortisol level and lack of naturalistic stimuli in the environment, has been little studied. Therefore, Cortisol levels were reviewed after short-term stressors and compared them to cortisol levels in captive animals in this article. It was found some significant important the relationships among the stess and cortisol level in captive animals. Recently, fecal cortisol metabolites have been identified as an index of stress in animals. Since many captive and wild populations of endangered species are intensively managed, measuring fecal cortisol metabolites will be useful to identify factors affecting animal well-being in captivity.

Key words: Cortisol, stres, captive, animal

INTRODUCTION

Since that time its popularity has increased due to the attractiveness of non-invasive, repeated and simple stress-free sampling. It has proved a popular sampling fluid for psychobiology, sports medicine, pharmacology and paediatric studies as well as in the area of complementary medicine. In the diagnostic laboratory, salivary progesterone and oestradiol have been used for assessing ovarian function and 17α-OH progesterone for the diagnosis of congenital adrenal hyperplasia (CAH). Salivary cortisol is used for investigating adrenal function and recently there has been considerable interest in the use of bedtime salivary cortisol levels as a screening test for Cushing's disease. However, there are several caveats on the use of saliva including collection techniques, the variable matrix of saliva, sensitivity, steroid stability, the presence of binding proteins and reference range anomalies. This brief review will attempt to address these issues and provide a balanced approach to steroid analysis in Saliha (Fell *et.al.,* 1986; Negrão *et.al.,*2004; Safwate, *et.al.,* 1982). Cortisol is produced in the cortex of the adrenal glands. The lipophilic steroid hormone is released into circulation and bound to proteins: 90% to corticoid binding globulin (CBG) and 8% to albumin. Only about 4% of the total cortisol in the blood is free. Only this free hormone fraction in blood is actively available for the target cells. These facts have to be taken into account when assessing correlation studies of cortisol in blood and in saliva. At 145 – 180 ng/mL (400 – 500 nmol/L) of total cortisol plasma levels the CBG is saturated. Above this concentration the percentage of free cortisol increases. Therefore the plasma level of total cortisol depends from the CBG concentration. Any increased CBG level leads to an elevated cortisol plasma level, whereas the free cortisol concentration in plasma and saliva still is normal. The CBG concentration is affected by various conditions such as pregnancy, liver disease, inflammation, polycystic ovary syndrome and application of different drugs such as contraceptives (Chernecky and Berger, 2008; Elizabeth ana Breslau, 2004)

Salivary and Fecal Cortisol Concentrations:

As a biological medium saliva is a variable and complex fluid and is mostly produced by three pairs of salivary glands (parotid, submandibular and sublingual) with a small contribution from the buccal glands which line the mouth. In addition, saliva contains variable amounts of gingival crevicular fluid, which leaks from the tooth-gum margin, as well as plasma exudates, or blood, from oral abrasions or lesions. Hormones can enter saliva by a variety of mechanisms but for the neutral steroids the most common route is rapid diffusion

Corresponding Author: Vahdettin Bayazit,PhD, Muş Alparslan University, Faculty of Sciences & Arts, Department of Biology, Station Street,49100, Muş,Turkey,
Phone : +4362127456,
E-mail: bvahdettin@yahoo.com

through the acinar cells and as such their concentration is independent of the rate of saliva flow. For charged steroids, like DHEAS, the mode of entry is by diffusion between the tight junctions of the acinar cells and its concentration is inversely related to saliva flow rate. Saliva pH also alters with saliva flow rate and hence affects the partitioning of charged steroids. Steroids can also enter saliva from blood or plasma via oral abrasions or directly from foodstuffs by contamination with exogenous steroids (Chernecky and Berger, 2008; Elizabeth ana Breslau, 2004 Gaab *et.al.,* 2005). The prime advantage of saliva is that it offers non-invasive, stress-free and real-time repeated sampling where blood collection is either undesirable or difficult. It is well suited for paediatric, time-shift and psychobiological studies. In addition, no special training or equipment is needed and subjects can conveniently collect samples themselves, if required. Salivary steroid levels can reflect the circulating level of free steroid rather than total circulating levels, which are confounded by the presence of circulating high affinity binding proteins. There are, however, disadvantages in the use of saliva. It can be a difficult matrix to deal with experimentally and may require physical or chemical disruption. Freeze/thaw cycles and centrifugation are often used to break up mucins and dithiothreitol treatment can facilitate filtration. The analysis of steroids in saliva can present analytical problems since they are present at far lower levels than in circulation. Existing assays may need to be adapted to improve sensitivity, although kits are available for measuring some steroids in saliva. Some use manual methods or automated platforms, designed for plasma, and care is required to address standardisation issues as well as the differing matrices of plasma and saliva. The possibility of blood contamination and its likely interference can be quantified but the presence of both sex hormonebinding globulin and corticosteroid-binding globulin in uncontaminated saliva casts doubt on the reliability of salivary steroids to accurately reflect circulating free steroid levels. The presence of 11β-hydroxysteroid dehydrogenase type 2 and 17-hydroxysteroid dehydrogenase in salivary glands also complicates the relationship between salivary and plasma free steroids. During saliva sampling there is also the possibility of oral contamination by exogenously administered steroids. Another point, often overlooked, is that during the course of clinical investigation the physician is likely to investigate other analytes, besides salivary steroids, and more often than not a blood sample is likely to be taken (Chernecky and Berger, 2008; Cirilele *et.al.,* 2000 ; Fischbach *et.al.,* 2009; Klein *et.al.,* 2004; Keesd *et.al.*, 2005 ; Koren *et.al.,* 2002 ; Raul *et.al.,* 2004; Taddio *et.al.,* 2007).

Posttraumatic stress disorder (PTSD) is characterized by traumatic memories that can manifest as daytime recollections,traumatic nightmares, or flashbacks in which components of the event are relived. These symptoms reflect excessive retrieval of the traumatic memory, which usually retains its vividness and power to evoke distress for decades or even a lifetime. During stressful periods, glucocorticoid (cortisol and corticosterone) production and release, by the adrenal glands, is increased. These hormones and their metabolites are found in blood, urine and feces. Previous studies have found measurements of fecal glucocorticoid metabolites (FGM) accurately detect long-term stressors. The goal of the present study was to determine if FGM measurements are a reliable means of detecting short-term (<30 min) stressors in two animal models. Animals respond to stressors through a variety of mechanisms. A stressor is anything that causes an animal to stray from homeostasis. Autonomic responses mediate the “fight or flight” response and are beneficial for short activities and include changes in heart rate, blood pressure, and gastrointestinal motility. During periods of stress, the HPA axis increases its production of stress hormones, cortisol and corticosterone.These hormones and their metabolites can be measured in blood, tissues, urine and feces of animals. To avoid the stress inherent in blood sample collection, measurement of fecal glucocorticoid metabolites (FGM) has gained popularity. Such techniques make it is possible to collect fresh fecal samples in the field without disturbing the animal, allowing for a noninvasive assessment of stres (Burke *et.al.,* 2005; Chernecky and Berger, 2008; Cirilele *et.al.,* 2000; Gaab *et.al.,* 2005; Tu *et.al.,* 2006). Glucocorticoids are metabolized in the liver before passage through the biliary system and gastrointestinal tract before accumulation and excretion in the feces (Figure 1).

Stress and Glucocorticoids:

Thus, FGM measurements may show net effects accumulated over time, which may be missed by a single plasma sample. Long-term and short-term stressors are of different significance to the well-being of the animal. The response to an acute stressor can be beneficial (e.g. glucocorticoid release can mobilize glucose and provide energy to escape a predator). Chronic stress, on the other hand, can prove maladaptive by decreasing reproductive and immune function over time. Both acute and chronic stressors can cause increases in plasma glucocorticoid levels. On the other hand, an inability to detect short-term stressors in fecal samples might lead to a false conclusion that the animal is under no stress whatsoever. Therefore, Thus, the effects of short-term (<30 min) stressors on blood corticosterone and FGM levels can investigate. An acute stressor may cause a rapid increase in plasma corticosterone levels and a smaller, delayed increase in the fecal corticosterone

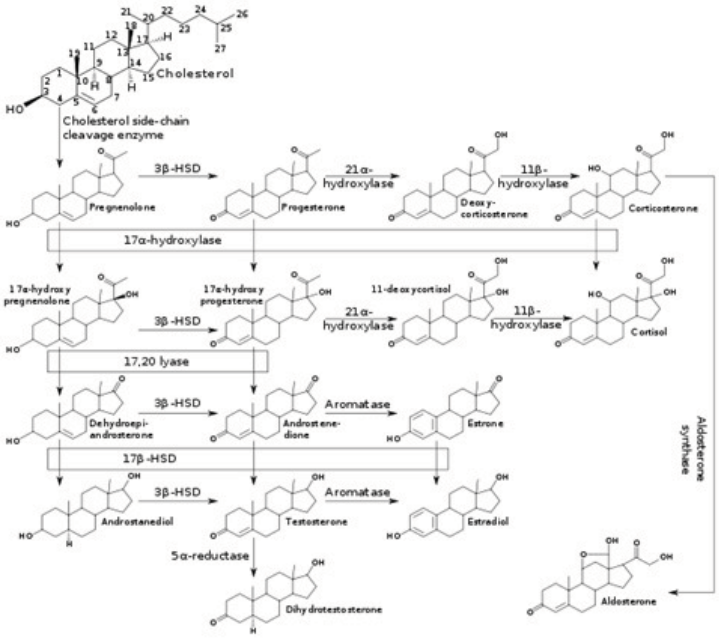

Fig. 1: Steroidogenesis.

metabolites (Burke *et.al.,* 2005; Davenport *et.al.,* 2006; Gaab *et.al.,* 2005; Fischbach *et.al.,* 2009). Laboratory cages are physically confining and socially restrictive living spaces for mamalian species, and these conditions impose unreasonable stresses upon them. Recent studies have confirmed the causes and effects of housing and social stressors on mamalian, including primates who are subjected to solitary lives in cages or those who are housed in cramped, crowded conditions. Some investigations have shown the harmful consequences of separating primates from their cage mates and placing them together arbitrarily into new groups, altering power dynamics and systems of social support. Imposing unnatural physical and social configurations on animals resulted in profound disruptions of species-specific behavior and physiological issues. Laboratory condidations differ enormously from natural habitats, a a few study has demonstrated that several of a laboratory's environmental conditions contribute to unacceptable levels of stress in primates, including ambient temperature, lighting conditions, loud noises, cage locations, and even the mere presence of humans in primate rooms. On the other hand, some investigating laboratories have been able to make some small modifications in the environmental conditions of their laboratories, it is not possible for some animals to live in laboratories and participate in experiments without suffering from environmental stres (Davenport *et.al.,* 2006; Gordon *et.al,* 1992 ; Koren *et.al.,* 2002; Morato *et.al.,* 2004; Reinhardt 1997; Taddio *et.al.,* 2007; Wildt, *et.al.,* 1984). Enormous of animals in laboratories are subjected to a variety of routine animal husbandry procedures, all of which are experienced as stressful even when a laboratory follows best practices. The most sensitively conducted non-invasive and non-experimental procedures can create stressful conditions in captive animals. Capture is especially stressful for animals, specially rabbits and rats, and they frequently reveal their distress in obvious ways such as crouching, assuming defensive postures, diarrhea, fear grinning, attempting to flee or making aggressive displays. Rabbits are frequently restrained and captured in laboratories, and they always experience restraint as stressful regardless of the method used. Common methods of restraint and studies that have demonstrated their stressful effects include anesthetics, manual restraint, squeeze cages, table restraints, tethering, and transfer boxes. In addition to capture and restraint, recent studies have demonstrated that animals are also significantly stressed by other routine husbandry procedures such as feeding, medical procedures, palpation, pregnancy examinations, and weighing (Graham and Brown, 1996; Mellen, 1991; Saco *et.al.,* 2008). All research protocols are stressful to all animals, for example behavioral testing, blood sampling, novel situations and environmental manipulation, stool sampling, reproduction techniques such as penile vibratory stimulation or electroejaculation, venipuncture, and saliva or urine sampling. The known physiological effects of stress in mammalian in laboratories include arteriosclerosis, osteoporosis, diabetes, changes in blood pressure, body temperature, circadian rhythms, ECG patterns, enzymatic shifts, heart rate, leukocytosis, metabolism, respiratory rates, sleep patterns, and weight gain or loss. Usually only the most extreme forms of

fear, pain, or suffering will cause primates to show the visible effects of their distress. Stress is a complicated phenomenon, affecting multiple, interconnected systems, so that it is difficult to isolate as a single variable or effect. Animals, for example, the primate react to stress in highly individualized and complex ways, especially at the biochemical level where the sympathetic nervous system, the hormonal systems, and the immune systems all interact with each other in response to stressful conditions. Stress is a highly variable phenomenon affecting individual animals in unique ways and making statistically reliable data problematic. Further complicating stress measurements are the intra-animal differences in how the four general defense systems respond in attempting to cope with the stressor (Graham and Brown, 1996; Mellen, 1991; Morato, *et.al.,* 2001; Pérez *et.al.,* 2004; Wildt, *et.al.,* 1984). There is an important discrepancy between animal models of anxiety and human anxiety patients: While experimental animals are usually unstressed, patients usually have a long history of stres. However, an equivalent mistake is the assumption that stress research on primate models can be meaningfully extrapolated to humans. Just as pharmacological efficacy has great variation between nonhuman and human primates, the experimental data obtained from nonhuman primates have little generalizability beyond the simple, tautological recognition that induced stressors cause symptoms of stress. For many years saliva has been used as a biological fluid for the detection of different biomarkers such as electrolytes, hormones, drugs and antibodies in human and veterinary medicine. Sample collection is non-invasive, painless and very convenient especially for the patients. Collecting saliva samples is possible any time, day or night because of the overall convenience of collection. Saliva collection can be accomplished under circumstances where blood collection is difficult or inadvisable. Saliva is the specimen of choice in a variety of the subsets of traditional medical care as well as emerging areas of health measurement and monitoring. With the ability to evaluate a pooled saliva sample from multiple collections it is possible to get a reliable assessment of hormone concentration even if distinct diurnal fluctuations ocur (Fell *et.al.*, 1986; Negrão *et.al.,*2004; Safwate, *et.al.,* 1982; Saltzman *et.al.,* 2004). Chronic stress has been demonstrated by elevated blood cortisol levels in mammal species. Recently, It has been demonstrated that adrenocortical activity can be monitored noninvasively in the cat by measuring cortisol metabolites excreted in the feces, a useful procedure for evaluating responses to physiological and psychological stressors associated with environmental conditions and husbandry practices. Since many captive and wild populations of endangered species such as the jaguar are intensively managed, measuring fecal cortisol metabolites will be useful to identify factors affecting animal well-being in captivity. In addition, adrenal activity could have been measured in wild jaguars by closely monitoring radio-collared animals to collect a fresh sample or by obtaining fecal samples during capture for radio-collaring. Increases in fecal corticoid excretion in domestic cats have been typically observed in the first fecal sample collected as early as 24 h after an ACTH challenge (Graham and Brown, 1996; Mellen, 1991; Morato *et.al.,* 2004; Saco *et.al.,* 2008). In a study, mean fecal cortisol metabolites have been increased 2- to 4-fold over the pre-chemical restraint and electroejaculation levels, while they have been increased 6- to 12-fold over the pre-ACTH challenge and 2- to 9-fold after immobilization in the cheetah. In general, cortisol metabolism appears to be conserved among felid species. Since cat species produce cortisol and corticosterone, it is possible that the difference among studies is related to the use of different RIA in each. Fecal cortisol metabolite concentrations has been ranged from 4.0 to 1276.6 ng/g dry feces. Mean overall fecal cortisol metabolite concentration has been 418.0 ± 35.0 ng/g dry feces, mean baseline concentration has 307.8 ± 17.5 ng/g dry feces, and mean peak concentration has been found 820.9 ± 86.8 ng/g dry feces. On the other hand, this study shows that determination of fecal cortisol and androgen metabolites can be very useful for noninvasively assessing an animal's well-being and for complementing behavioral, physiologic, and pathologic studies. At the conservation strategy level, this technique is clearly useful to assess whether housing conditions, handling or other manipulatory procedures can affect the reproductive and/or general physiological status of captive animals (Gaab *et.al.,* 2005 ; Pérez *et.al.,* 2004; Saco *et.al.,* 2008; Soltis *et.al.,* 2003;Wildt, *et.al.,* 1984). In an other study, Social subordination in a number of animal species is associated with elevated circulating levels of glucocorticoid hormones, presumably reflecting high levels of stress in subordinate individuals . In recent years, however, subordinate animals in an increasing number of species have been found to undergo significant reductions in circulating or excreted glucocorticoid levels, as compared to dominant individuals. Investigation of these contrasting socioendocrine profiles may be particularly informative both in understanding the psychosocial, physical, and physiological sequelae of social status that influence endocrine function, and in identifying the neuroendocrine mechanisms leading to chronic dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis. However, the mechanisms underlying socially induced suppression of HPA activity have not been elucidated clearly in any species.

In a study investigating the mechanisms of social suppression of HPA activity in the common marmoset (*Callithrix jacchus*), low baseline cortisol levels in anovulatory subordinate female marmosets have been found associated with reduced adrenocortical responsiveness to adrenocorticotropic hormone (ACTH). Following suppression of endogenous cortisol and ACTH with dexamethasone, a potent synthetic glucocorticoid, subordinate females have been found showed impaired cortisol responses to exogenous ACTH, as compared to dominant females in the follicular phase of the ovarian cycle. In this study , It has been report initial attempts to identify such a neural mechanism by characterizing: (1) baseline circulating ACTH levels; and (2) ACTH and cortisol responses to negative feedback by dexamethasone in anovulatory subordinate females as compared to dominant females undergoing ovulatory cycles. It has been used a total of 28 captive-born, adult female common marmosets (*Callithrix jacchus jacchus*), including 14 socially dominant females undergoing ovulatory cycles and 14 anovulatory subordinates. Each animal was tested no more than once with each dose of dexamethasone (DEX) or saline.Results concerned with this study were given below Figure 2 and 3 (Chase *et.al.,* 2000; Pines *et.al.,* 2004).

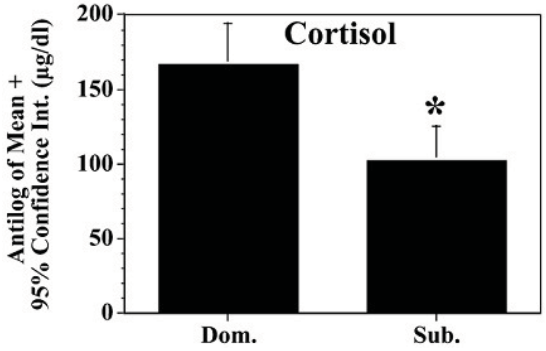

Fig. 2: Morning baseline concentrations of plasma cortisol, prior to DEX or saline treatment,for all doses combined, in dominant female during the follicular phase of the ovarian cycle (N=14) and anovulatonary subordinate females (N= 14).

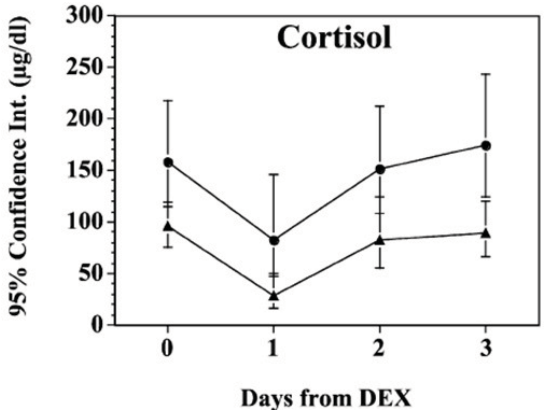

Fig. 3: Plasma cortisol concentartions before and after with 0.5 mg / kg DEX in dominant (N=8) and subordinate (N=8) female marmosets.

Anovulatory, socially subordinate female marmosets exhibit pronounced and persistent reductions in circulating basal cortisol levels. Previously, It has been showed that one mechanism of this cortisol suppression may be reduced adrenocotical responsiveness to ACTH, which in turn might be mediated by suppression of reproductive hormones (Pérez *et.al.,* 2004; Pines *et.al.,* 2004). There is little information on 11-deoxycortisol in saliva, presumably due to its very low level, although it can be detected following metyrapone. On the other hand, salivary cortiso determinations are more promising. Since the earliest reports, there is evidence that saliva levels reflect unbound concentrations in plasma. Direct measurement in saliva may be comparable to extraction but extraction has the advantage of allowing the analysis of low volume saliva samples. This can avoid the use of stimulants and the loss of data due to insufficient sample volume. Salivary cortisol

determinations have proved popular in psychobiology, stress and sports medicine studies. Their use is based on the assumption that salivary cortisol is a reasonable reflection of hypothalamicpituitary- adrenal (HPA) axis function. Indeed, in the diagnostic setting, salivary cortisol levels parallel those in plasma following ACTH and CRH stimulation, and following exercise induced-stress. However, the correlation of salivary cortisol levels with total plasma cortisol is confounded by the presence of corticosteroid-binding globulin in plasma which is largely saturated up to 500–600 nmol/L of cortisol. Salivary cortisol correlates better with measured plasma free cortisol than total plasma cortisol. However, it appears to be subject-specific as considerably variability is found between individuals for daily paired samples (Figure 4). Salivary cortisol determinations were used as markers of metabolic disturbances in obese and diabetic patients and used to investigate changes in glucocorticoid control of the HPA axis following oral prednisolone. Conversely, salivary cortisol measurement is not a useful tool in determining dose adequacy in subjects on oral glucocorticoid replacement therapy. Blood spots or serum is preferable, due to contamination of saliva by oral hydrocortisone. The diurnal variation of plasma cortisol is reflected by similar changes in salivary cortisol and hence timed salivary cortisols have been used in a diagnostic setting. Early morning salivary cortisols are useful as a screening tool for adrenal suppression and salivary cortisol is helpful to investigate the control of diurnal cortisol secretion following exposure to darkness and light. Recently, there has been considerable interest in the use of night time salivary cortisols for the initial screening for Cushing's syndrome. However, despite the optimism, an element of caution is required. Reported cut-off values differ considerably (Brown, *et.al.,* 2004; Chernecky and Berger, 2008; Cirilele *et.al.,* 2000; Elizabeth ana Breslau, 2004; Fischbach *et.al.,* 2009; Klein *et.al.,* 2004; Keesd et.al., 2005; Koren *et.al.,* 2002; Oliveira, *et.al.,* 2008; Olsen *et.al.,* 1994; Pérez *et.al.,* 2004; Raul *et.al.,* 2004; Taddio *et.al.,* 2007).

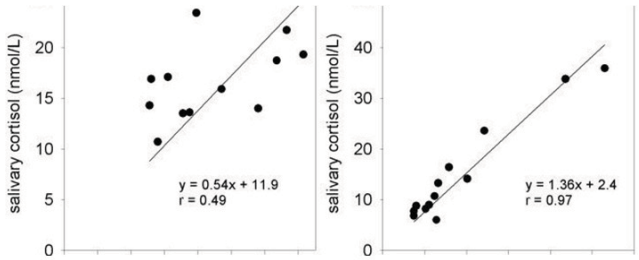

Fig. 4: Correlation of salivary cortisol and plasma free cortisol in paired samples from two normal individuals (a and b). Salivary cortisol was measured by ELISA and plasma free cortisol by ligand binding/ultrafiltration.

For both plasma and saliva, the cortisol response was significantly lower after milking than after ACTH administration. This confirms the concept that a maximal response can be obtained with the ACTH dose used, whereas the response caused by milking is small. As previously observed by other authors, usually only severe stressors are found to have effects on cortisol concentrations similar to those measured after ACTH administration. In blood, the assay measures total cortisol concentration, but this may not necessarily reflect the biological action of the hormone. In saliva, the cortisol concentration that is measured a direct reflection of the free fraction in the blood. Furthermore, under stress conditions, the binding capacity of cortisol-binding protein becomes saturated, resulting in a disproportionate increase in free cortisol, the fraction that is biologically active. These facts partly explain the differences between concentrations of plasma and salivary cortisol. Concentrations of cortisol in saliva were substantially lower than those found in plasma, and this sometimes produced sampling problems. Although, It is concentrated the saliva samples 10-fold, some cortisol concentrations in the samples remained lower than the minimal concentrations measured by the kit that is used. In general, it was easy to collect saliva from experimental calves because they readily sucked the cotton swab that was placed in their mouths. However, it was also necessary to manually restrain the calf's head. In contrast, it was substantially more difficult to take saliva samples from adult cows. Although the cows remained tied in tie stalls throughout the experiment, it was necessary to restrain the cows' heads with a halter and to hold their mouths open. Furthermore, it took substantially more time to collect saliva samples than blood samples (3 min vs. 30 s). As a study concerning with the calves, below, It was given the cortisol concentrations in the calves in Figure 5 (Fell et.al., 1986; Gaab *et.al.,* 2005; Negrão *et.al.,*2004; Pérez *et.al.,* 2004; Safwate, *et.al.,* 1982).

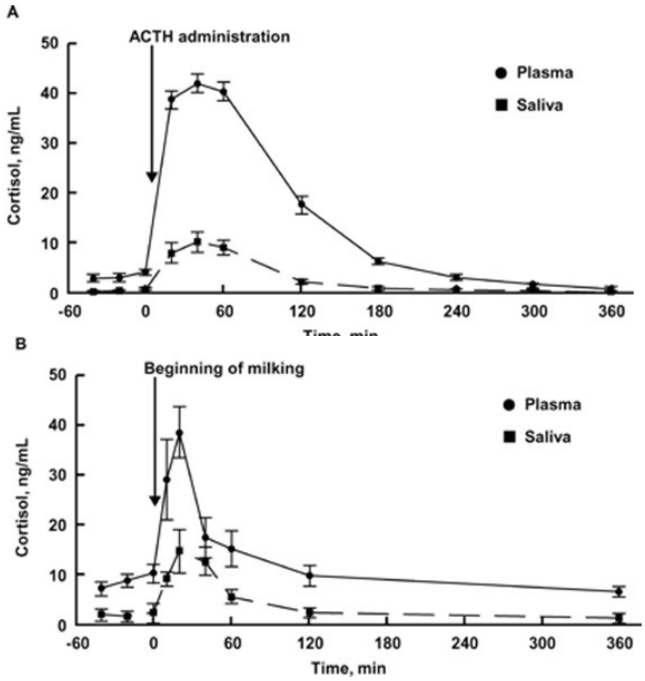

Fig. 5: Cortisol levels (ng/mL) in plasma (•) and saliva (■) before and after ACTH administration in calves (A) or machine milking in cows (B). Values are means and standard errors of the mean.

Discussion:

Cortisol is a lipophilic steroid with low molecular weight (MW ~362 Dalton). Following ACTH binding to membrane receptors on cells of the adrenal cortex, cortisol is synthesized and released into the blood stream. Up to 95% of the secreted cortisol will be bound to large proteins (CBG, albumin) and carried throughout the body in the blood. Since the vast majority of cortisol actions rely on binding to its cytosolic mineralocorticoid and glucocorticoid receptors, only the small fraction of unbound, i.e., free cortisol is thought to be biologically active. Due to its low molecular weight and lipophilic nature, unbound cortisol enters cells by passive diffusion which makes it feasible to measure the free cortisol fraction in all bodily fluids. While the assessment of cortisol in sweat or tears is only of theoretical importance and urinary cortisol of decreasing interest, salivary cortisol has become an invaluable tool for both basic scientists and clinicans. A number of significant advantages over the assessment of cortisol in blood has resulted in an steadily increasing interest in salivary cortisol. Late night salivary cortisol measurement is nevertheless very promising for the diagnosis of Cushing's although elevation above threshold values can occur in the elderly, diabetics and women in late pregnancy. The variations in reported late night salivary cortisol cut-off values could result from the relatively small numbers in each of the study control groups, which may have varying degrees of obesity, non-adrenal disorders and pseudo-Cushing states, which themselves may be influenced by periodic hypercortisolism. Methodological and standardisation issues are also likely contributors to differences in reported cut-off values but a major factor is probably differing specificities of cortisol antibodies towards cortisone. The salivary gland has abundant 11 β-hydroxysteroid dehydrogenase type 2 activity and as a consequence, saliva, unlike plasma, has up to three times the level of cortisone compared to cortisol. Depending on the relative cross-reactivity of cortisol antibodies towards cortisone, there could be quite different values of salivary cortisol measured by different immunoassays. Conversely, differences in plasma would be expected to be minimal as cortisone levels are normally only 10% of circulating cortisol levels. It is therefore desirable that laboratories establish their own method-specific reference ranges before using salivary cortisol for diagnostic purposes (Chernecky and Berger, 2008; Cirilele *et.al.,* 2000 ; Fischbach *et.al.,* 2009; Klein *et.al.,* 2004; Keesd et.al., 2005 ; Koren *et.al.,* 2002 ; Raul *et.al.,* 2004; Taddio *et.al.,* 2007).

Salivary steroid testing has a recognised place in research and diagnostic medicine although its limitations must be acknowledged. It is clearly not desirable for androgen assays as well as assays to assess ovarian function and the monitoring of absorption of steroids from transdermal creams. Caution must be exercised for these applications. The use of salivary cortisol for measuring endogenous cortisol is the most encouraging. It can be successfully applied to research studies, adrenal stimulation tests, investigating diurnal variation as well as night time samples as a screening test for Cushing's syndrome. However, there is a need to establish methodspecific reference ranges and sample stimulation, collection and storage should remain consistent across study groups with a preference for the collection of whole unstimulated saliva, if possible (Gaab *et.al.,* 2005). Ecological or "ecosocial" approaches that include biomarkers in large-sample psychosocial studies are advocated to advance the understanding of complex causal disorders and to attend to disparities across gender, race, ethnicity, and economic strata. When large community samples are involved, logistic and cost considerations often dictate departures from laboratory study norms. Instead of intensive protocols that apply inclusion and exclusion criteria to the sample, that standardize procedures and timing, and that have staff oversee specimen collection, biomarker specimens often are provided by participants from home according to written instructions and mailed to a laboratory. There currently are few methodological reports of the "ecological validity" of biomarker data collected and modeled using this approach (Burke *et.al.,* 2005; Chernecky and Berger, 2008; Elizabeth ana Breslau, 2004; Soltis *et.al.,* 2003).

Data are from an ongoing perinatal mental health study that uses salivary cortisol as a biomarker. Cortisol is a stress-response hormone that reflects hypothalamic-pituitary-adrenal (HPA) axis functioning, making it a potentially useful biomarker for health outcome studies where stress-related disorders are of interest. Cortisol can be validly measured in saliva, even during pregnancy. The reliability of assays conducted when specimens are collected at home and mailed has been established. Cortisol is a marker of the HPA axis function of interest for mental health and health (including perinatal) outcomes research because it has long been associated with stress regulation. Cortisol is found in blood, urine, and saliva. Saliva specimens are easily obtained, feasible to mail, and reliably assayed if frozen within 14 days. Stress and HPA axis dysregulation evidenced in plasma cortisol have been considered in relation to adverse pregnancy outcomes, including labor processes and prematurity and the validity of salivary cortisol measures has been affirmedin pregnant women. Cortisol is currently of interest for mental health research because alterations in the diurnal cortisol profile, including both lower and higher levels have been associated with stressand trauma-related psychopathology, including major depressive disorder (MDD) and, our team's main area of interest, posttraumatic stress disorder (PTSD). Recent studies have most often found differences between stress and psychopathology case and control groups in evening cortisol levels, thus the following analyses focus on that sample time point (Burke *et.al.,* 2005; Chernecky and Berger, 2008; Cirilele *et.al.,* 2000 ; Fischbach *et.al.,* 2009; Gaab *et.al.,* 2005; Klein *et.al.,* 2004; Keesd et.al., 2005 ; Koren *et.al.,* 2002 ; Raul *et.al.,* 2004; Taddio *et.al.,* 2007). For both plasma and saliva, the cortisol response has been found significantly lower after milking than after ACTH administration. This confirms the concept that a maximal response can be obtained with the ACTH dose used, whereas the response caused by milking is small. As previously observed by other authors, usually only severe stressors are found to have effects on cortisol concentrations similar to those measured after ACTH administration. In blood, the assay measures total cortisol concentration, but this may not necessarily reflect the biological action of the hormone. In saliva, the cortisol concentration that is measured is a direct reflection of the free fraction in the blood. Furthermore, under stress conditions, the binding capacity of cortisol-binding protein becomes saturated, resulting in a disproportionate increase in free cortisol, the fraction that is biologically active. These facts partly explain the differences between concentrations of plasma and salivary cortisol. The significant positive correlations between salivary and plasmatic concentrations and the parallel responses to ACTH and milking show that it is possible to estimate cortisol concentrations in the plasma from salivary concentrations in both calves and adult cattle. The increase in cortisol concentration in response to ACTH administration or machine milking are representative of adrenal activity or milking stimulus, respectively. The present study shows that determination of fecal or saliva cortisol and androgen metabolites can be very useful for noninvasively assessing an animal's well-being and for complementing behavioral, physiologic, and pathologic studies. However, the relationship between adrenal activity and reproductive performance still remains to be investigated in the jaguar. At the conservation strategy level, this technique is clearly useful to assess whether housing conditions, handling or other manipulatory procedures can affect the reproductive and/or general physiological status of captive animals. In addition, it is possible to use these techniques to evaluate changes in the reproductive and/or physiological status of free-living populations in the presence of the pressures due to

human intervention (Chernecky and Berger, 2008; Cirilele *et.al.,* 2000 ; Fischbach *et.al.,* 2009; Gaab *et.al.,* 2005; Soltis *et.al.,* 2003).

REFERENCES

Brown, J.L., SL. Walker and T. Moeller, 2004. Comparative endocrinology of cycling and non-cycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants, General and Comparative Endocrinology, 36: 360–370.

Burke, H.M., M.C. Davis, C. Otte and D.C. Mohr, 2005.Depression and cortisol responses to psychological stress: a meta-analysis, Psychoneuroendocrinology, 30: 846–856.

Chase, W.K., L.M. Marinus, M.A. Novak, 2000. A behavioral comparison of male rhesus macaques (*Macaca mulatta*) in four different housing conditions. American Journal of Primatology, 51: 51.

Chernecky, C.C. and B.J. Berger, 2008. Laboratory Tests and Diagnostic Procedures, 5th ed. St. Louis: Saunders.

Cirilele, V., P. Kinyz, V. Dumestre, V. Goulle, J.P. ana B. Ludes, 2000. Identification of tem corticosteroids in human hair by liquid chromatography-ionspray mass spectrometry. For. Sci. Int., 107: 381-388.

Davenport, M.D., S. Tıefenbacher, C.K. Lutz, C., M.A. Novak ana J.S. Meyer, 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen. Comp. Endocrinol, 147: 255-261.

Elizabeth, A.Y., Breslau, N., 2004. Cortisol and Catecholamines in Posttraumatic Stress Disorder. An Epidemiologic Community Study. Arch Gen Psychiatry, 61: 394-401.

Fell, L.R. and D.A. Shutt, 1986. Adrenocortical response of calves to transport stress as measured by salivary cortisol. Can. J. Anim. Sci., 66: 637–641.

Fischbach, F.T., M.B. Dunning, III, eds. 2009. Manual of Laboratory and Diagnostic Tests, 8th ed. Philadelphia: Lippincott Williams and Wilkins.

Gaab, J., N. Rohleder, U.M. Nater and U. Ehlert, 2005. Psychological determinants of the cortisol stress response: The role of anticipatory cognitive appraisal. Psychoneuroendocrin., 30: 599-610.

Graham, L.H., J.L. Brown, 1996. Cortisol metabolism in the domestic cat and implications for non-invasive monitoring of adrenocortical function in endangered felids. Zoo Biology, 15: 71-82.

Gordon, T.P., D.A. Gust, M.E. Wilson, A. Ahmed-Ansari, A.R. Brodie, H.M. McClure, 1992. Social separation and reunion affects immune system in juvenile rhesus monkeys. Physiology and Behavior, 51: 467-472.

Keesd, F., R.H. Straub ana R. Paus, 2005. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. FASEB Jour., 19: 1332-1334.

Klein, J., T. Karaskov, B. Stevens, J. Yamada ana G. Koren, 2004. Hair cortisol- a potential biological marker for chronic stress. Clin. Pharm. Therap., 75: 44.

Koren, L., O. Mokady, T. Karaskov, J. Klein, G. Koren and E. Geffen, 2002. A novel method using hair for determining hormonal levels in wildlife. Animal Behav., 63: 403-406.

Mellen, J.D., 1991. Factors influencing reproductive success in small captive exotic felids (*Felis* ssp.): a multiple regression analysis. Zoo Biology, 10: 95-110.

Morato, R.G., M.G. Bueno, P. Malmheister, I.T.N. Verreschi and R.C. Barnabe, 2004. Changes in the fecal concentrations of cortisol and androgen metabolites in captive male jaguars (*Panthera onca*) in response to stres. Brazilian Journal of Medical and Biological Research, 37(12): 1903-1907.

Morato, R.G., V.A. Conforti, F.C. Azevedo, A.T.A. Jacomo, L.Silveira, D.Sana, A.L.V. Nunes, M.A.B.V. Guimarães ana R.C. Barnabé, 2001. Comparative analyses of semen and endocrine characteristics of free-living versus captive jaguars (*Panthera onca*). Reproduction, 122: 745-751.

Negrão, J.A., M.A. Porcionato, A.M. de Passillé and J. Rushen, 2004. Cortisol in Saliva and Plasma of Cattle After ACTH Administration and Milking. American Dairy Science Association, J. Dairy Sci., 87: 1713-1718.

Oliveira, C.A., E.C.G. Felippe and M.O.M. Chelini, 2008. Serum cortisol and progestin concentrations in pregnant and non-pregnant Asian elephants (*Elephas maximus*). Research in Veterinary Science, 84(3): 361-363.

Olsen, J.H., C.L. Chen, M.M. Boules, L.S. Morris and B.R. Coville, 1994. Determination of reproductive cyclicity and pregnancy in Asian elephants (*Elephas maximus*) by rapid radioimmunoassay of serum progesterone, Journal of Zoo and Wildlife Medicine, 25: 349–354.

Pérez, G.C., S. García-Belenguer Laita, J.C. Illera del Portal and J. Palacio Liesa, 2004. Validation of an EIA technique for the determination of salivarycortisol in cattle. Spanish Journal of Agricultural Research, 2 (1): 45-51.

Pines, M.K., G. Kaplan, L.J. Rogers, 2004. Stressors of common marmosets (*Callithrix jacchus*) in the captive environment: effects on behavior and cortisol levels. Folia Primatologica, 75 (Supplement 1): 317-318.

Raul, J.S., V. Cirimele, B. Ludes and P. Kintz, 2004. Detection of physiological concentrations of cortisol and cortisone in human hair. Clin. Biochem., 37: 1105-1111.

Reinhardt, V., 1997. Refining the traditional housing and handling of laboratory rhesus macaques improves scientific methodology. Primate Report, 49: 93-112.

Saco, Y., Fina, M. Giménez, R. Pato, J. Piedrafita and A. Bassols, 2008. Evaluation of serum cortisol, metabolic parameters, acute phase proteins and faecal corticosterone as indicators of stress in cows. The Veterinary Journal 177(3): 439-441.

Safwate, A., E. Richet, J.P. Barlet. and P. Delost, 1982. ACTH and plasma corticosteroid levels in young calves. J. Physiol., 78: 195–197.

Saltzman, W., S.L. Prudom, N.J. Schultz-Darken, D.J. Wittwer, D.H. Abbott, 2004. Social suppression of cortisol in female marmoset monkeys: role of circulating ACTH levels and glucocorticoid negative feedback. Psychoneuroendocrinology, 29: 141–161.

Soltis, J., F.H. Wegner, J.D. Newman, 2003. Adult cortisol response to immature offspring play in captive squirrel monkeys. Physiology and Behavior, 80: 217-23.

Taddio, A., C. Newman and G. Koren, 2007. Hair cortisol as a potential biologic marker of chronic stress in hospitalized neonates. Neonatol., 92: 42-49.

Tu, M.T., S.J. Lupien and C.D. Walker, 2006. Diurnal salivary cortisol levels in postpartum mothers as a function of infant feeding choice and parity.Psychoneuroendocrinology, 31(7): 812-824.

Wildt, D.E, D. Meltzer, P.K. Chacraborty ana M. Bush, 1984. Adrenal-testicular-pituitary relationships in the cheetah subjected to anesthesia/electroejaculation. Biology of Reproduction, 30: 665-672.