Chapter 8

Action Mechanisms and Pathophysiological Characteristics of Cortisol in Horses

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Abstract

Cortisol (CORT), also known as stress hormone, plays a vital role in physiological processes such as electrolyte and fluid balance, cardiovascular homeostasis, carbohydrate, protein and lipid metabolism, immune and inflammatory responses, and sexual development and reproduction. Cortisol levels are influenced by various physiological factors such as race, age, circadian rhythm, seasonality, exercise and pregnancy. Also, some stressful conditions including isolation or transport, among others, modify levels of this hormone in the body. Excesses or deficiencies of cortisol cause important clinical problems such as Cushing's and Addison's syndromes, which contribute substantially to morbidity in equine medicine. Thus, in this review, we will develop the mechanisms of synthesis and regulation, as well as the physiological factors involved and the most important diseases related to the alteration of cortisol secretion in horses and foals.

Keywords: cortisol, horse, pathophysiology, regulatory mechanisms

1. Introduction

1.1. Synthesis of cortisol, regulatory mechanisms and participation in physiological functions in the horse

The glucocorticoid activity of adrenocortical cortex secretion comes from cortisol (CORT) almost entirely. The adrenal synthesis of CORT is regulated by the hypothalamic-pituitary-adrenal axis (HPA) and plays an important role in the integral endocrine response to stress. The HPA axis is activated when various physiological, pathophysiological or environmental stress factors drive the signals of peripheral components and the central nervous system,

which are interpreted and integrated into the hypothalamus. The activation of the hypothalamic paraventricular nuclei promotes the release of the corticotropin-releasing hormone (CRH) in the hypothalamus-hypophysis support system. CRH acts on the anterior pituitary gland to activate type 1 CRH receptors on the surface of corticotrophic cells and thereby induces the release of adrenocorticotropic hormone (ACTH) into systemic circulation [1]. These hormones are important for the health of the body and help control both physical and mental stress [1, 2]. Thus, chronic responses to stress are mediated by glucocorticoids.

The hormone ACTH binds to the melanocortin 2 (MC2R) receptors located in adrenocortical cells and stimulates the adrenal glands to synthesize and secrete mainly CORT and to a lesser extent also aldosterone. MC2R is a transmembrane receptor coupled to the G protein that acts through adenylate cyclase to increase the levels of cyclic AMP. Cyclic AMP activates a variety of critical enzymes for the synthesis of CORT [1, 3]. Currently, the expression of this subtype of melanocortin receptor in the equine adrenal cortex has not been characterized, but it is presumed to be similar to that described in humans.

The critical enzymes necessary for the synthesis of CORT are expressed in cells of the fasciculated area of the adrenal cortex. These enzymes include 3-β-hydroxysteroid dehydrogenase (3-β-HSD), 17-α-hydroxylase, 21-α-hydroxylase and 11-β-hydroxylase [4]. The last enzyme catalyzes the final step in the synthesis of CORT from the precursor molecule of 11-deoxycortisol, and is present only in glucocorticoid-producing cells.

CORT is not stored in adrenocortical cells, but is secreted into the systemic circulation immediately after synthesis is induced by ACTH [5]. CORT is lipophilic and, therefore, is transported in plasma predominantly bound to plasma proteins, including cortisol-binding globulin (CBG) and albumin [6].

In most adult mammals, including horses, approximately 90% of circulating CORT is bound to CBG [6]. Nicolaides et al. [7] reported that, since CORT receptors are located in the cytoplasm of steroid-sensitive cells, only the free and free portion of circulating CORT is available to enter the cells by diffusion through the plasma membrane and bind to these intracellular glucocorticoid receptors (GR).

The binding of CORT to the cytoplasmic GR causes conformational changes that allow the dissociation of heat shock regulatory proteins (HSP), allowing the GR-CORT complex to dimerize, localize in the nucleus, bind to the DNA in glucocorticoid-response elements (GRE) and regulate transcription of genes that respond to glucocorticoids [8]. However, the equine GR isoforms and their respective activities are not well characterized [9, 10]. In addition, CORT itself acts through negative feedback mechanisms at the HPA axis to regulate this activity [11].

In healthy horses, approximately 90% of plasma CORT is bound to CBG and albumin [10, 12]. It is the remaining 10% of unbound, free CORT that is considered biologically active, and is available to bind cytoplasmic steroid receptors to mediate the majority of CORT's systemic effects [5].

In the organism, there are many cell types sensitive to glucocorticoids. In this way, the CORT has different effects, necessary for the responses to stress to both health and illness. CORT also regulates vital functions such as blood glucose, maintenance of normal vascular tone and blood pressure [13]. Likewise, CORT increases the absorption of electrolytes by direct action on the

renal tubules and indirectly, through the secretion of atrial natriuretic peptide (ANP) at the cardiac level. CORT is a lipolytic agent that induces hyperglycemia and leads to fat mobilization and protein catabolism (amino acids mobilization) to support higher energy requirements and a high demand for protein biosynthesis in compromised situations [14]. Proteins with few critical functions are degraded into amino acids for mobilization into circulation before proteins with essential functions such as brain neurotransmitters and muscle contractile proteins.

CORT stimulates the production of erythrocytes and platelets. Another effect of CORT is the reversal and low regulation of inflammatory responses resulting from a stressful event [15]. The production of CORT increases in response to stress and is a physiological adaptation that promotes survival [16]. A stress response mediated by CORT is to ensure that adequate nutrients are delivered to the brain and other areas of the body that could be compromised by a stressful event or injury. Glucocorticoids are powerful inhibitors of the immune system, which limits the secretion of cytokines by macrophages and the production of antibodies. In fact, it has been demonstrated that different stressful situations such as resistance exercise, fatigue, lack of food or water and extreme temperatures induce the release of glucocorticoids and immunosuppression [17].

1.2. Reference values for cortisol levels

The CORT levels in the circulation reflect the activity of the HPA axis. Therefore, excretion in saliva and feces allows non-invasive sampling of CORT metabolites [18, 19]. Plasma CORT binds mainly to transporter proteins, while salivary CORT is not bound, that is, it is found as free CORT [20]. CORT levels in saliva and plasma reflect acute changes in release [21]. Fecal CORT as a circulating CORT index has a delay of 24 hours until excretion. Therefore, the collection protocols should uniformly sample the total fecal mass due to the unequal distribution of the hormone [22]. Compared to plasma levels, the salivary CORT is clearly lower. In saliva, only free CORT is produced, that is, unbound, whereas in the plasma both free CORT and CBG are measured [23].

Fureix et al. [24] and Pawluski et al. [25] described that there is a positive correlation between nocturnal plasma CORT levels and concentrations of fecal CORT metabolites in horses. Salivary CORT can be used to measure acute stress responses and identify stress triggers. Fecal cortisol can be used to compare levels of general stress with long-term conditions [25]. While the determination of CORT metabolites in saliva allows the detection of small and transient changes in the release of CORT, the levels of fecal CORT metabolites increase only in response to marked or prolonged release of this hormone [18]. However, contradictory results have been reported when comparing salivary and blood samples. This discrepancy is related to the limited sensitivity and specificity of saliva samples and the role of corticosteroid-binding globulins in CORT plasma levels. However, Pawluski et al. [25] reported correlations between plasma and fecal CORT levels.

CORT is susceptible to be modified by the manipulation of stressful and painful stimuli, circadian rhythm, exercise, transport, hypoglycemia and stress [26–29]. Therefore, establishing a reference interval for the basal CORT is difficult. Plasma levels ranging from 12.32 ± 2.0 to 68.1 ± 22.8 ng/ml have been reported in healthy adult horses at rest [11, 28, 30–32].

2. Physiological factor that modifies cortisol levels in horses

2.1. Breed

Although it is unknown whether breed is a modifier of CORT, Söder et al. [33] reported significantly lower CORT levels in Icelandic horses compared to Standardbred horses. However, these variations were not only attributed to the genetic configuration. They were also related to the level of training and management conditions between both equine breeds.

2.2. Age

Plasma CORT levels usually change with the age of the horse. In comparison to foals born at term, premature foals have lower serum CORT concentrations before 2 hours after birth. These low basal concentrations of CORT and also of ACTH imply that foals may have either altered adrenocortical sensitivity to ACTH, the ability to synthesize limited CORT, or both [34].

It has been established that the adrenocortical function may not be fully mature at birth, even in term foals. At 12–24 hours of age, mean baseline concentrations of CORT are lower in healthy foals compared to levels reported in healthy adult horses despite the fact that in foals there are higher concurrent concentrations of ACTH [35–37].

In neonatal foals, the CORT concentration increases during the first week of life, up to about half that in adult horses in response to a comparable dose of ACTH [38, 39]. During the first year of life, great changes occur in CORT in response to the stress of weaning and growth [25].

The advance of age is associated with a loss of adrenal sensitivity to dexamethasone and greater sensitivity to CRH and ACTH. Older horses are also more prone to diseases such as Cushing's syndrome which alters the episodic and circadian rhythm of CORT [35, 40]. Cushing's disease in adult equines originates more frequently in an adenoma of the pars intermedia of the pituitary gland [41]. This adenoma stimulates the production of ACTH and thus more CORT is secreted by the adrenal glands. Hart et al. [42], indicated an increase in free CORT and nearly twice as much in the stool with this endocrine disease. However, the total CORT observed was not affected in sick animals compared to horses and healthy ponies of the same age. Likewise, it was demonstrated that the increase in CORT in feces could be related to the decrease in the capacity of CORT binding in plasma and that this fact could be a component of these endocrine disorders in horses. However, other investigations in this species disagree [32, 43, 44].

2.3. Circadian and ultradian rhythms

Horses that live in undisturbed natural habitats and trained horses, which have adapted to their environment, show a normal oscillation in CORT blood concentrations. These concentrations are generally higher in the morning and decrease throughout the day [45, 46]. These same authors have reported maximum levels between 6:00 and 10:00 am and minimum between 6:00 and 9:00 pm. Rendle et al. [47, 48], identified a circadian rhythm in horses and ponies with the highest ACTH plasma values at 8:00 am that subsequently decrease throughout the day. The circadian rhythm can be affected by various factors such as exercise, mating, training,

stress, sleep patterns and individual activities [49]. The response of CORT to these factors is immediate, proportional and quickly exceeds the normal plasma concentration [12].

Ultradian rhythms with average periods ranging from 105 to 128 and from 24 to 31 minutes overlap the circadian rhythm [50]. In contrast, the loss of the circadian rhythm of CORT occurs in animals suffering from chronic stress, disease and old age [20]. For example, in horses with Cushing's disease, the circadian rhythm is lost and the CORT is constantly high [41]. For this reason, no ultradian [47] or circadian [26, 51] rhythms have been found in horses and ponies affected by intermediate pituitary dysfunction. Also, alterations of the circadian rhythm in CORT can be observed during situations of chronic stress in many species such as pigs and humans with different types of psychological disorders such as certain types of depression, chronic fatigue syndrome and post-traumatic stress disorder [52, 53].

2.4. Seasonality

CORT levels show a marked seasonality, detecting maximum values between the months of May and September [35, 54, 55]. This seasonal pattern could reflect the physiological adaptations to the lower availability of nutrients during the winter and increase the food reserves for the period of greatest reproductive activity [35, 55]. However, the seasonal patterns of CORT and ACTH are not correlated, since the peak of ACTH occurs during the fall [35, 37, 55]. This asynchrony in the HPA axis could be the result of alterations in adrenal sensitivity, changes in the metabolism of CORT or seasonal variations in the bioactivity of ACTH. In fact, Donaldson et al. [35] have described a loss of sensitivity of the HPA axis to dexamethasone during the autumnal period. On the contrary, Haritou et al. [26] showed in horses that the plasma CORT levels did not change during the year and were different only in the summer when they obtained higher values along 24 hours.

2.5. Transport

It has been shown that transport [54, 56–60], loading of horses in a trailer [61] and social stress [12] increase the synthesis of CORT. The transport of horses at short and medium distances leads to a greater release of CORT [18]. In fact, CORT levels correlate positively with transport duration [56]. In addition, the secretion of CORT depends on the transport conditions [21] and the new environment.

At the same time, this raises the possibility that both psychological and physical stress may have a negative effect on embryo recovery rates of competition mares. Tischner et al. [62], measured stress responses to transport in mares at different stages of the estrous cycle and gestation. These authors reported that "the most intense stress reaction (to transport), measured by the maximum increase in noradrenaline, adrenaline and CORT, was shown in the mares in the right and during the winter anestrus". This suggests that competition mares subjected to embryo transfer procedures could be particularly susceptible to stress if transported in the interval between insemination and uterine lavage. There are also negative effect of heat on the recovery rates of equine embryos [63]. It is possible that the combined effect of stress and heat in mares that are "bad travelers" are other factors that could limit the rates of embryo recovery in sport mares. However, the concentrations of salivary CORT and fecal glucocorticoids were not modified during transport of horses in New York [60].

2.6. Environmental factors

The variations of the CORT levels during short periods of time depend on the adaptation of the horse to its environment [45]. In addition, there is evidence in some countries of seasonal dynamics and variations between annual periods in the same geographical region [64, 65]. Therefore, environmental factors, weather or the presence of insects, cause transient changes in the diurnal pattern of cortisol release [66].

2.7. Feeding

Today's horse management practices often include restricted access to forage and feeding large quantities of concentrates in a limited number of meals throughout the day [67]. Higher concentrations of CORT were observed in serum 30 minutes before the morning food was administered compared to 30 minutes after the feed intake. A significant postprandial increase in endogenous ACTH has also been documented. This suggests that the animal's feeding status can also be a co-founder for both endogenous and dynamic ACTH tests [51].

In a study conducted on adult horses with overweight, Glunk et al. [68] determined if limit feeding combined with a slow-feed hay net could affect morphometric measurements and patterns of postprandial hormones and metabolites. The results of the study conducted during 28 days, showed that the glucose and insulin values increased, while the levels of CORT and leptin decreased. In conclusion, it could be said that when overweight adult horses are fed, the use of a slow feeding hay network together with a diet with limit of feeding seems to be an effective method to reduce body weight and maintain more homeostatic levels of postprandial metabolites and hormones.

However, it has been shown that CORT levels increase before feeding. This elevation could be due to the anticipation of receiving the morning meal after a period of several hours without grain or hay or without acclimating to the daily feeding routine. This finding may have important implications in the way a horse is handled. At times when it is important to take into account the negative impact of stress, such as times of illness or reproduction. At times like during the reproductive season, stress can affect both the immune system and reproduction. Therefore, care must be taken to avoid other circumstances that intensify the stress already experienced half an hour before feeding [69].

2.8. Exercise

CORT is frequently used to assess stress levels induced by exercise [70, 71]. Different studies have been carried out in relation to stress in horses such as the load stress in tow [61], participation in equestrian dressage competition [72–74], competition of resistance [75] jumping [76], tourist driving and education [77]. It has been shown that moderate exercise in horse increases CORT by up to 29% compared to baseline levels through the stress response. Also, the plasma concentration of CORT was more than double the normal value 60 minutes after exercise [78]. In stress-induced exercise, a marked increase in CORT levels was attributed to exercise duration and not to intensity [79]. In addition, the secretion of CORT depends on the animal's experience in competitions [80], different head and neck positions [81] and the horse character [82].

The hormonal response during exercise is also influenced by hemodilution or hemoconcentration actions related to the displacement of plasma fluids inside and outside the vascular beds. A greater secretion of CORT can be expected during and after exercise on horses during resistance competitions. This greater secretion occurs mainly in the case of mares or horses that cover longer distances or that take place at high temperatures. Janczarek et al. [83] suggested that a high level of CORT can adversely affect the heart rate of horses, but at the same time stimulates the body to combat dehydration. The permissive action of this substance enables the animal to react favorably to situations of stress and exhaustion, since the main metabolic effects of CORT are the increase in hepatic gluconeogenesis, the mobility of free fatty acids and lipolysis [79]. During exercise, CORT is also useful in suppressing insulin release and maximizing blood glucose utilization [14, 80, 84]. Thus, the availability of energy resources necessary during physical exercise is favored. On the other hand, it has been shown that high concentrations of CORT after exercise episodes can alter the anabolic responses of testosterone and growth hormone (GH) [79]. On the contrary, Zuluaga and Martínez [44] showed no significant differences in horse performance.

2.9. Sexual excitation and reproductive state

Although sexual arousal [85, 86] and mating [87] increase CORT levels in stallions, in sexually experienced and well-trained animals, ejaculation and semen collection is perceived as no more than a modest temporary stressor [88].

In the mare, the physiological status significantly alters CORT concentrations. Based on previous studies conducted in intact and ovariectomized mares, in which it was determined that the administration of synthetic analogue of ACTH (tetracosactide) stimulates the synthesis of CORT [89, 90], Satué et al. [91] described that in the natural estral cycle, the increase in ACTH secretion stimulates the synthesis of CORT at the time of ovulation in Spanish Purebred mares. However, Ginther et al. [92] found increased levels of CORT during the luteal phase, followed by a decrease during the periovulatory period at the time of follicular deviation. This decrease in CORT may be necessary for correct follicular development and LH release. This dynamic during the corpus luteum period could partially confirm the results of the investigations carried out by Satué et al. [93, 94] during the same period of the cycle. In fact, although the relationship between CORT and progesterone is not very close, the correlations obtained between both parameters ($r = 0.47$) may suggest a certain stimulation of CORT in the synthesis of luteal progesterone in Spanish Purebred mares.

Generically, non-pregnant mares show CORT concentrations 20% higher than pregnant ones [93, 94]. These differences in the adrenocortical response between non-pregnant and pregnant mares could be interpreted in terms of variations in the metabolism of this glucocorticoid. In fact, with respect to non-pregnant mares, fetal CORT levels induce a negative feedback mechanism of maternal levels during pregnancy [82].

In mares of different breeds, as in Spanish Purebred mares, Quarter Horse, Standardbred, Thoroughbred and arabians [32, 54, 95, 96] CORT levels increase during the first half of gestation. The gestational period is associated with a state of insulin resistance, due to the anti-insulin effects of CORT, GH, lactogen and placental GH [97, 98]. The purpose is to increase blood glucose to improve placental transfer and meet fetal demands [99]. In fact, mares under

restriction regimes and food presage a higher incidence of abortions. These facts do not correlate with alterations in CORT levels but rather with the metabolic changes associated with the lower glucose bioavailability and the increase of free fatty acids that could stimulate the synthesis of prostaglandins and arachidonic acid [100].

On the contrary, the elevation of the CBG [101], the decrease in production and the increase in the volume of distribution, the increase in fetal metabolism and the antiglucocorticoid effects of progesterone, could reduce CORT during the last gestation period, describing an inverse correlation between both steroid hormones [32].

At the end of pregnancy, the maternal CORT rises substantially before delivery due to the increased activity of fetal adrenal and the maturational changes necessary for the correct adaptation of the fetus to extrauterine life [102]. Furthermore, CORT release during and after foaling is most likely part of the endocrine pathways regulating parturition and not a labor-associated stress response [103].

In addition, different patterns are established in the CORT cyclicity between pregnant and empty mares, establishing differences that can be between 400 and 700% between them. Compared to the usual circadian pattern characterized by the morning increase of CORT in physiologically normal mares [37, 45], CORT levels decrease in the morning [45] and increase at night [36] in ovariectomized mares. Pregnancy is considered an additional factor that modifies the diurnal and annual pattern of CORT [89, 90], and can even mask cyclicality during the second half of pregnancy in the mare [32]. These changes in the acrofase are related to the action of the gestation and lactation hormones, exerting a different influence on the secretion and use of CORT in the mare. Thus, in a pregnant and lactating mare, the increase in CORT is related to the need for glucocorticoids during the period of fetal development and intensive lactation.

2.10. Fertility

In women it has been described that CORT inhibits the release of pituitary gonadotropins and makes the gonads become resistant to sex steroids through inactivity of the receptor [104]. Along with these suppressive effects on the gonads, CORT has shown in the mare that they have inhibitory effects on steroid hormone receptors [105]. In addition, overexposure of the fetus to excess glucocorticoids could be implicated in the restriction of fetal growth [106].

In pregnant women, abnormally high levels of CORT contributed to miscarriage by altering normal reproductive function at both the tissue and hormonal levels [107]. Likewise, in a study carried out on sheep, it was determined that high levels of CORT lead to the premature activation of growth regulation mechanisms in the fetus that have deleterious prenatal and postnatal consequences [108]. It has also been determined in sheep that high levels of CORT suppress insulin-like growth factors found in the liver, skeletal muscles and adrenal glands in fetuses [109]. It has been shown that CORT in sheep is also able to reduce the activity of the gonadotropin-releasing hormone (GnRH) receptor through the improvement of negative feedback mechanisms in estradiol [110]. In adult sows it was determined that the chronic administration of cortisol delays ovulation through the deterioration of the LH peak during the estrous cycle [111]. However, research in horses has not yet established a threshold in the systemic circulation of CORT before it presents harmful effects in pregnancy.

It has been shown that hormones are important factors that contribute to the differentiation of the conceptus in the uterus. Hormones indirectly affect fetal growth, either through genetic programming or fetoplacental growth and maturation. During pregnancy, hormones are produced at maternal and fetal levels with direct effects on their outcome. Glucocorticoids have programming action in the uterus and affect the development of the tissues and organs of the fetus [108]. Kapoor et al. [112], determined that excessive exposure of the human fetus to glucocorticoids can reprogram the fetal HPA and thus permanently change the HPA activity of the offspring. Fetal exposure to glucocorticoids can occur simply by initiating the mother's response to stress. It has also shown that high concentrations of glucocorticoids impair fetal growth and are a major determinant of intrauterine growth restriction [108]. Challis et al. [113] reported that fetal HPA is responsible for the maturation of the organ systems essential for postnatal survival.

Endocrine changes initiated by elevated CORT levels may be transient, although some alterations persist after glucocorticoid concentrations return to baseline [108]. Changes initiated by chronic exposure to glucocorticoids include underdevelopment of fetal HPA and placental hormone deficiency. The critical window of fetal HPA maturation is specific to the species [112]. Fowden et al. [114] reported that the activation of fetal HPA is an essential process for delivery in the mare. Pregnancy in equines is unique since fetal CORT levels increase rapidly very close to the term. This, in turn, increases the synthesis of uteroplacental prostaglandins and initiates myometrial contractions.

In addition, transrectal ultrasound examination in non-lactating mares induces a significant increase in salivary CORT. This reflects an activation of the HPA axis and a shift toward a sympathetic domain. On the contrary, transvaginal follicular punctures guided by ultrasound did not modify the salivary levels of CORT [115]. Also, the diagnosis of transabdominal gestation does not induce an activation of the HPA axis. This finding affirms what was previously described by Schönbom et al. [116], who indicated that controls of advanced pregnancies can be easily performed by transabdominal ultrasound.

2.11. Other factors

Other factors such as painful stimulation, water or food deprivation, contraction restriction or immobilizers [117], stabling and isolation [28, 73], weaning [118] or social stress [12] have also been linked to elevation in CORT levels. Leal et al. [119] showed that horses stabled in the urban environment were in a state of stress. Likewise, report stated that the confinement type (partial full-time), type bed (big place with chips, as small without bedding) as well as the type of work (patrol or sports) did not change the ability of the horses to cope with these housing conditions.

3. Cortisol related with equine clinic

Adrenocortical dysfunction may manifest as either abnormal increases or decreases in activity. Increased adrenocortical activity (hyperadrenocorticism) may occur in horses with PPDI, but primary hyperadrenocorticism is rare in horses. Other pathological inflammatory conditions also are related with alterations with CORT levels [1].

3.1. Hyperadrenocorticism

Cushing's disease or Pituitary Pars Intermedia Dysfunction (PPID) is the most common in horses and you put over 15 years of age with a prevalence of 15–20%. As reported by McGowan et al. [74], all breeds and types of horses may be affected by the PPID, although Morgan horses and ponies seem to be at greater risk. The corticoadrenal hyperplasia that accompanies equine Cushing's disease is relatively rare and occurs in approximately 20% of affected horses [1, 120]. In fact, there is only one well-described case of functional adrenocortical adenoma in horses. This animal showed different clinical signs such as voracious appetite, loss of muscle mass, bulging supraorbital fat, delayed coat shedding, hyperhidrosis and lethargy [41, 121].

In horses with PPID, the pars intermedia of the pituitary gland enlarge over time due exclusively to hyperplasia or adenoma formation on melanotrope cell population. This pathology produces an excessive and autonomous secretion of peptides derived from proopiomelanocortin (POMC), which include ACTH, α-melanocyte-stimulating hormone (α-MSH), β-endorphin and the intermediate peptide similar to corticotropin [120].

The increase of the hormone ACTH leads to secondary hyperadrenocorticism and the increase of CORT due to hypothalamic innervation lapses. In turn, hypothalamic dopamine exerts an inhibitory control on the production and secretion of POMC peptides by melanotropes located in the pars intermedia. In horses, abnormal pars intermedia tissue contains significantly reduced amounts of dopamine. Thus, about 10% of the tissue of the pars intermedia is normal, which means a specific loss of hypothalamic dopaminergic innervation. This loss of dopaminergic innervation is due to an oxidant-induced injury in the hypothalamic tissue. Therefore, a risk factor for affected horses could be the reduction of antioxidant defense mechanisms in neural tissue. In addition, insoluble aggregates of the neural protein α-synuclein have been found in dopaminergic nerve endings in horses affected by PPID [120].

Horses with PPID present lethargy, marked hypertrichosis together with recurrent laminitis, muscle wasting, pendulous abdomen. Also it was described additional problems such as polydipsia, polyuria, recurrent infections and abnormal sweating patterns that probably represented end-stage disease. In recent years the early recognition of the disease has been an important achievement. The clinical picture is often more subtle and the symptoms include decreased performance, loss of the superior line, slight changes in attitude, lamellar changes in the hoof in the absence of pain and mild delayed coat shedding in spring time and/or regional hypertrichosis [74, 122].

3.2. Hypoadrenocorticism

Addison's disease or hypoadrenocorticism consists of permanent adrenocortical insufficiency and, in general, is rare in the horse. This syndrome is also called relative adrenal insufficiency (RAI) or critical illness related to corticosteroid failure (CIRCI). This disease can contribute substantially to the morbidity and mortality associated with the primary disease [1].

The CORT insufficiency can be transient or permanent could be a consequence of the deterioration of the HPA axis in one or several levels [5]. The permanent dysfunction of the HPA axis results in the destruction of one or more glandular components of the shaft. Despite being rare in human and veterinary medicine, adrenocortical destruction mediated by immunity (Addison's disease) is the most common manifestation of permanent HPA axis hypofunction. Patients with

Addison's disease cannot develop an appropriate CORT response to stress. Therefore, these patients are frequently present with hemodynamic instability and collapse. Aldosterone deficiency, which is added to CORT deficiency, is a typical characteristic of Addison's disease. In affected individuals, it produces fluid and electrolyte disorders that contribute to hypovolemia, hypotension and cardiovascular collapse [1, 5].

Bacterial components such as endotoxin (a lipopolysaccharide component of Gram negative bacterial cell walls) and host pro-inflammatory cytokines participate in initiating and maintaining the HPA axis response to sepsis. These factors can directly stimulate HPA axis activity at the multiple levels, ultimately resulting in stimulation of CORT synthesis and secretion [123].

In the presence of overwhelming bacterial infection or excessive host inflammatory response, HPA axis function can also be suppressed at one or more levels. For example, in patients who died from septic shock, nitric oxide-mediated induction in the death of hypothalamic neurons of cardioregulatory centers, which may be involved in HPA axis dysfunction, has been described. The bacterial endotoxin directly decreases gene expression of the pituitary CRH receptor in both rats and cattle [124]. In addition, tumor necrosis factor-α (TNF-α) can directly affect the release of pituitary ACTH and adrenal CORT synthesis [123]. Several reduced levels of high density lipoprotein (HDL) in plasma have been demonstrated in critically ill individuals. Therefore, the availability of cholesterol for the synthesis of corticosteroids may be limited during sepsis, since decreased levels of HDL are related to attenuate CORT responses to ACTH stimulation [123].

While irreversible HPA axis hypofunction due to component destruction is uncommon, recent evidence suggests that transient HPA axis dysfunction (RAI/CIRCI) can occur in a substantial number of critically ill patients with a variety of conditions. It has also been suggested that RAI/CIRCI can occur in septic neonatal foals. Couëtil and Hoffman [125] described a clinical case in a neonate foal with septicemia, a transient dysfunction of the HPA axis. This dysfunction is evidenced by a low basal CORT concentration and an altered CORT response to a high dose ACTH stimulation test. In addition, two independent studies that measured basal concentrations of ACTH and CORT in healthy and septic neonatal foals found a significant increase in the proportion of ACTH:CORT in foals with septicemia that did not survive [126, 127]. These high concentrations of ACTH and low CORT concentrations suggest that HPA axis dysfunction can occur in septic foals at term.

In two studies conducted by Hart et al. [39] and Wong et al. [128], the HPA axis function has been characterized in hospitalized foals that use stimulation tests with ACTH. None of the studies identified a significant difference in CORT peak responses between groups of healthy and diseased foals of similar age. These results were in response to a low-dose ACTH stimulation test (0.1 μg/kg) [128] or in response to a paired low-dose ACTH stimulation test (10 μg)/high dose (100 μg) [39]. However, when the criteria for human diagnosis for RAI/CIRCI [123] were adapted and applied to a group of hospitalized foals, approximately 50% fulfilled these criteria [39]. In addition, the greater severity of the disease and the worse prognosis were correlated with the decrease in CORT responses to stimulation with ACTH. Specifically, foals that did not survive had lower CORT responses to low-dose ACTH stimulation compared to survivors [128]. Likewise, foals that met the RAI/CIRCI criteria had a significantly higher incidence of shock, multiple organ dysfunction syndrome and non-survival compared to foals with an adequate CORT response to ACTH [39]. These studies provide evidence that RAI/CIRCI occurs in critically ill and septic neonatal foals with frequency and impact comparable to humans with septicemia.

In adult horses, insufficiency of the adrenal cortex is not well described. Transient adrenal insufficiency is characterized by low basal levels of ACTH and CORT and altered responses of this hormone to the stimulation test with ACTH. This situation has been described in a horse after the abrupt cessation of long-term anabolic steroid supplementation [129]. A syndrome of adrenal exhaustion that produces lethargy, anorexia and poor performance is also described anecdotally in racehorses. This syndrome has been attributed to adrenal insufficiency associated with prolonged steroid administration or chronic stress [120].

Before the ACTH stimulation test, horses with adrenal insufficiency have reduced CORT concentrations and do not respond or respond minimally. However, measurement of ACTH levels may be important in determining other causes of hypoadrenocorticism. It is suggested that the exogenous administration of glucocorticoids decreases the concentrations of ACTH (secondary hypoadrenocorticism). Likewise, adrenal insufficiency (primary adrenocorticism) results in a higher concentration of ACTH due to the decrease in endogenous glucocorticoid concentrations due to the lack of negative feedback [1].

In mares with abnormal behavior related to estrus, a diminished response of CORT to ACTH has been described [36]. However, the clinical importance of this behavior is unknown. In horses treated with chronic glucocorticoids or anabolic steroid supplements, the potential for iatrogenic adrenal insufficiency associated with the suppression of the HPA axis by exogenous steroids should be considered. In the same way, care must be taken to avoid abrupt cessation of this type of treatment.

In horses as in many other animal species, the adrenal gland is extremely vulnerable to the ischemic injury associated with endotoxic or hypovolemic shock. It is a common finding in the necropsy of adult horses with acute gastrointestinal disease and other diseases associated with endotoxic shock, adrenocortical hemorrhage and necrosis similar to Waterhouse-Friedrichsen syndrome in humans [123]. In theory, although this has not been documented to date, in surviving horses, this damage to the adrenals could contribute to long-term adrenocortical insufficiency. Furthermore, to the knowledge of the authors, classic hypoadrenocorticism or Addison's disease has not been described in horses. This disease is responsible for the adrenocortical destruction mediated by immune mechanisms and manifested by deficiency of glucocorticoids and mineralocorticoids.

In general, horses with adrenal insufficiency have a history of depression, anorexia, exercise intolerance, weight loss, bad hair or lameness. Therefore, it is necessary to obtain a complete history, including, among other things, the performance, the previous illnesses, the administration of medications and those conditions that may cause stress. Endogenous and exogenous glucocorticoids suppress the HPA axis. This produces atrophy of the fasciculate area of the adrenal gland due to the decrease in ACTH concentrations. Although there may be electrolyte disturbances in some cases of adrenal insufficiency, the glomerulosa zone is minimally affected. The clinical signs of these alterations include depression, anorexia, scanty hair, abdominal deformity and lameness. The biochemical analysis may be normal or there may be hyponatremia, hypochloremia, hyperkalemia and hypoglycemia. Severe damage from sepsis, hemorrhage, venous thrombosis and cortical necrosis may lead to atrophy and dysfunction of the adrenal gland. Therefore, hypoadrenocorticism can occur in critically ill horses with septicemia, colic, enterocolitis, endotoxemia, disseminates intravascular coagulation [10].

3.3. Other conditions

In a study conducted by Martos et al. [130], the existing CORT concentrations were compared in four groups of animals that had the following pathologies: (1) postoperative hernia, anorexia, diarrhea, castration, chronic inflammation, babesiosis, laminitis, proximal enteritis, Horner syndrome, umbilical hernia and control group (17.55–37.56 ng/ml); (2) displacement of the major colon, idiopathic ileus and obstruction of the small intestine (49.40–53.02 ng/ml); (3) impaction of the large intestine [68, 91] and (4) acute inflammation (151.08 ng/ml). The group of animals with postoperative hernia, anorexia, diarrhea, castration, chronic inflammations, babesiosis and chronic anemia had lower CORT levels compared with control group. However, animals that present significant colonic displacement, idiopathic ileus, strangulated small bowel obstruction, impaction of the large intestine, acute inflammation and obstruction of the large intestine represented with visceral pain, functional gastrointestinal disorders, hypovolemic shock, dehydration, acidic-base anomalies and the electrolyte showed acute response to stress. Recently, Ayala et al. [28] reported elevated CORT levels in horses with laminitis, acute abdominal syndrome, castration, surgery and acute and chronic diseases than control group. The major changes in the activity of the HPA axis occurred mainly in acute diseases, laminitis and abdominal syndrome.

Elevated concentrations of CORT in serum have been associated with the presentation of colic and the severity of the disease. Therefore, CORT levels can provide additional information about decision making and prognosis and thus predict the survival of horses with colic [53, 131]. Leal et al. [119] described a significant association between abnormal circadian rhythm and the incidence of colic in horses. The results show that horses with <30% circadian rhythm are more prone to colic episodes. In addition, pain and plasma CORT in clinical and surgical colic provide a physiological validation of pain scores as a marker of underlying stress in horses [132, 133].

Finally, Keating et al. [134] showed that stress management and CORT levels have an ability to influence and manage fecal egg count levels without having to use a deworming agent. Further studies may be done regarding the factors that influence CORT and determine which potential factors, if any, can be controlled. Combined with management practices that are already known to lower the levels of eggs in the feces, it has the potential to be another method that could alleviate and curb cyathostome infestation without ever having to resort to a deworming agent.

4. Conclusion

The activation of the HPA axis in stressful situations triggers behavioral and physiological changes that improve the body's adaptability and increase its chances of survival. Unlike chronic stress, acute stress subjected to various stressful conditions including isolation, transport or exercise increase significantly plasma, saliva or feces concentrations of this hormone. Diverse physiological factors such as age, circadian and ultradian rhythms, season, feeding or reproductive state influencing cortisol levels, so it will have to take it into account when interpreting this parameter. Clinical elevation of cortisol is related with Cushing's syndrome in older horses. Deficiencies of cortisol are related to serious pathologies such as sepsis or endotoxemia in foals or adult horses.

Abbreviations

3-β-HSD	3-β-Hydroxysteroid dehydrogenase
ACTH	Adrenocorticotropic hormone
CBG	Cortisol-binding globulin
CIRCI	Critical illness related to corticosteroid failure
CORT	Cortisol
CRH	Corticotropin-releasing hormone
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
HDL	High density lipoprotein
HPA axis	Hypothalamic-pituitary-adrenal axis
HSP	Heat shock regulatory proteins
POMC	Proopiomelanocortin
PPID	Pituitary pars intermedia dysfunction
RAI	Relative adrenal insufficiency
α-MSH	α-Melanocyte-stimulating hormone

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