Adrenocorticotrophic hormone and cortisol levels during late pregnancy and post-foaling period in mares

Giuseppe **Piccione***, Francesca **Arfuso**, Francesco **Abbate**,
Claudia **Giannetto**, Michele **Panzera**, Maria **Rizzo**, Francesco **Fazio**

Department of Veterinary Sciences, University of Messina
Polo universitario dell'Annunziata, 98168, Messina, Italy

The aim of this study was to evaluate the trend of adrenocorticotrophic hormone (ACTH) and cortisol levels in mares during peripartum period. Twelve pregnant mares (Group A) were weekly monitored from the last 6 weeks before foaling (6BF-1BF) until the first 3 weeks after foaling (1AF-3AF). Twelve non-pregnant non-lactating mares constituted the control (Group B). Jugular blood samples were analyzed for plasma ACTH and serum cortisol concentration. ACTH showed higher values (P<0.05) at 1BF compared to the postpartum data points (1AF, 2AF and 3AF) in Group A. Cortisol levels were higher (P<0.05) at 1BF and 2BF compared to the 3AF in Group A. A significant positive correlation between ACTH and cortisol values was found in mares from Group A throughout the peripartum period (Pearson's r=0.40; P=0.0028). The Dunnet's test showed lower ACTH values in Group A at postpartum data points than control, and higher cortisol levels in Group A throughout prepartum times and at 1AF than control (P<0.0001). The decrease of ACTH and cortisol levels found during the early postpartum period could indicate a reduced HPA response to physical and/or psychological stress during this physiological phase. This could help the mare to protect against stress-associated inhibition of lactation, relieve psychological stress, and enhance her immune function. Further studies involving the evaluation of prolactin and sex steroid hormones values are needed to fully understand the dynamic hormonal changes occurring in pregnant and lactating mares in order to permit clinicians to make appropriate interpretation of the results.

KEY WORDS: ACTH / cortisol / postpartum / prepartum /mares

---

*Corresponding author: giuseppe.piccione@unime.it

The ability of an animal to produce an appropriate response to a stimulus that provokes a threat to homestosasis is essential to survival. At this regards, the activation of Hypothalamic-Pituitary-Adrenal axis (HPA) is one of the important neuroendocrine responses that occur following a stress condition and it helps an organism re-establish homeostasis [Herman *et al.* 2003]. When an individual is exposed to stress, neural signals are sent to the hypothalamus in the brain and it secretes corticotrophin releasing hormone (CRH) [Engelmann *et al.* 2004]. This hormone activates the anterior pituitary gland to produce adrenocorticotrophic hormone (ACTH) [Engelmann *et al.* 2004]. ACTH stimulates the adrenal cortex to secrete glucocorticoid hormones that can provide a front line of defence against threats to homeostasis [Mills *et al.* 1997].

Pregnancy and lactation are physiological periods that result in increased metabolic demands and the maternal body system needs to adapt in order to ensure foetus growth and development [Bonelli *et al.* 2016]. Despite the action of homeostatic mechanisms to maintain blood parameters within physiologic levels, physiological biochemical changes are likely to occur during the peripartum period [Harvey *et al.* 2005; Bazzano *et al.* 2014a,b,c; Bazzano *et al.* 2015, Arfuso *et al.* 2016]. It has been showed that hormonal adaptations during late pregnancy have effects on endocrine mechanisms associated with physiological functioning of the mother postpartum, including lactation and maternal behavior [Brunton and Russell 2008; Meinlschmidt *et al.* 2010]. In particular, during pregnancy, maternal-endocrine regulation undergoes profound adaptive changes in the framework of a functional organization structured in three interrelated compartments: the mother, placenta and fetus [Ousey 2004, Berg *et al.* 2007]. Many of these changes are initially induced by estradiol and progesterone produced by the corpus luteum, combined with chorionic gonadotropin. As the pregnancy progresses, steroid and peptide hormones produced by the fetoplacental unit, took over. The neuroendocrine changes that take place during peripartum period affect the stress-induced activation of the HPA [Torner *et al.* 2002] in order to optimize fetal growth and development, protect the fetus from adverse exposures, and prepare the mother for timely parturition [Meinlschmidt *et al.* 2010]. The maternal HPA axis and associated "fetal, prenatal, or perinatal programing" of the stress response in the offspring have received a great deal of attention during recent years [Meaney *et al.* 2007, Phillips 2007]. Studies carried out on human, ovine and murine species showed an increased HPA activity followed by increased plasma glucocorticoids levels during pregnancy with lasting effects persisting throughout the postpartum period [Neumann *et al.* 2005; Keller-Wood and Wood 2008, Meinlschmidt *et al.* 2010]. Conflicting results are available about the influence of pregnancy on HPA activity and glucocorticoids levels in mares. Some authors have reported that most phases of pregnancy were without effect on blood cortisol and total corticoid concentrations in unstressed mares [Silver and Fowden 1994, Hoffmann *et al.* 1996, Satuč *et al.* 2011], while other authors have observed significantly higher cortisol concentration during the first half of pregnancy than those of the second half in mares [Satuč *et al.* 2007]. Moreover, an

increase in cortisol levels 48 h before foaling and synchronization between ultradian patterns of foetal and maternal plasma ACTH were reported [Cudd *et al.* 1995].

In view of such considerations, the aim of the present study was to evaluate the trend of plasma ACTH and serum cortisol levels in mares during the last 6 weeks of gestation and the first 3 weeks of postpartum period.

Materials and methods

Animals

Twenty-four healthy mares (12 pregnant mares, 12 non-pregnant mares) of different breed (Thoroughbred and Standardbred) and aged between 8 and 11 years were enrolled in the study. Before starting the sampling period, all the mares were subjected to clinical examination (evaluation of body temperature, heart rate, and respiratory rate), routine biochemistry, and transrectal ultrasound examination of the reproductive system, in order to ensure their health status. Animals from the same breeding centre were housed in individual straw-bedded boxes (latitude 37.46 N; longitude 14.93 E) under natural spring photoperiod (sunrise 5:15 AM and sunset 7:00 PM).

During the study animals were fed twice a day (07.30 AM; 05.00 PM) and water was available *ad libitum*. Pregnant mares received 6±1 kg/day hay and 5±0.5 kg/day concentrates (crude protein 16%, crude fat 6%, crude fibre 7.35%, ash 10.09%, sodium 0.46%, lysine 0.85%, methionine 0.35%, omega-3 0.65%), non-pregnant mares received 5±0.5 kg/day hay and 2±0.5 kg/day concentrates. Twelve pregnant mares of mean body condition score (BCS) 6.0-7.5 were monitored from 6th week of pregnancy until 3rd week after foaling (Group A). Twelve non-pregnant, non-lactating mares, mean BCS 5.5-7.0, were used as control group (Group B).

Mares of Group B were chosen from the same breeding centre with known medical history.

Table 1. Breed, age (years), gestation length (days) and parity (+, multiparous; -, primiparous) of pregnant mares (Group A) enrolled in the study

Breed	Age (years)	Parity	Gestation length (days)
Standardbred	9	+	338
Standardbred	10	+	340
Standardbred	8	-	342
Standardbred	9	+	343
Standardbred	10	+	341
Standardbred	9	-	343
Thoroughbred	11	+	340
Thoroughbred	9	-	333
Thoroughbred	10	+	345
Thoroughbred	8	-	345
Thoroughbred	11	+	338
Thoroughbred	10	+	337
Mean±Standard Deviation	10±1		340±4

All deliveries occurred within March and mid-May and the mean gestation length of pregnant mares was 340 ± 5 days (Tab. 1).

All treatments, housing and animal care were carried out in accordance with the standards recommended by EU Directive 2010/63/EU for animal experiments.

Blood sampling and hormone determination

From each animal of Group A blood samples were weekly collected by jugular venepuncture on the same day in the morning (8.00 AM) until the time of parturition. All mares delivered within a week (5 ± 2 days) from the last prepartum sampling. Therefore, we expressed each time point before foaling (BF) as weeks. Additional samples were taken from each mare 7, 14 and 21 days after foaling (AF). From mares of Group B blood samples were collected at the beginning of the study. Samples were drawn into 8ml vacutainer tubes (Terumo Corporation, Tokyo, Japan) containing ethylenediaminetetraacetic acid (EDTA) to assay plasma ACTH, and into 10ml tubes containing clot activators (Terumo Corporation, Tokyo, Japan) to assay serum cortisol.

Blood samples collected in EDTA tubes were centrifuged at 300 rpm for 10 minutes in order to obtain plasma samples, whereas the blood samples collected in tubes containing clot activators were centrifuged at 3000 rpm for 10 minutes in order to obtain serum samples. Both plasma and serum samples were stored at -20°C until analysis. ACTH plasma concentrations as well as the cortisol serum concentrations were measured using a solid phase 2-site chemiluminescent immunometric assay on an Immulite 2000 analyser (Siemens Healthcare Diagnostic, Deerfield, IL, USA). All samples were analysed in duplicate. Samples exhibited parallel displacement to the standard curve. The overall intra-essay coefficient of variation has been calculated to be <5%. The used assay has been validated for plasma ACTH and serum cortisol in equine species [Giannetto *et al.* 2013, 2015].

Statistical analysis

All data are expressed as mean values ± standard error of the mean (±SEM).

Data were tested for normality using the Shapiro-Wilk normality test. The Dunnett's test was performed to evaluate whether significant differences between pregnant and control mares occurred. On the data obtained from the animals of Group A, one-way repeated-measures analysis of variance (ANOVA) was applied to evaluate the significant effects of time along the peripartum period on ACTH and cortisol levels. When significant differences were found, Bonferroni-corrected post hoc comparisons were conducted. Pearson's correlation coefficients were computed to evaluate the relationship between ACTH and cortisol values in mares of Group A throughout the peripartum period. P values <0.05 were considered statistically significant. The statistical analysis was performed using the software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

Results and discussion

None of the animals included in the study showed clinical signs of disease during the monitoring period. Pregnant mares delivered healthy, viable full-term foals, without assistance. They passed a normal and intact placenta spontaneously within two hours and achieved the complete involution of the uterus within two weeks after foaling. The Shapiro-Wilk analysis showed a normal distribution of the data (P>0.05).

Statistical analysis showed a significant effect of peripartum time (P<0.0001) on ACTH and cortisol values measured in mares of Group A (Fig. 1). In particular, ACTH showed higher values at 1BF compared to 1AF, 2AF and 3AF; moreover, lower ACTH values were found at 2AF and 3AF respect to 3BF, and at 2AF compared to 2BF. Cortisol levels were statistically significant lower at 2AF compared to 1BF, and at 3AF respect to 6BF, 4BF, 3BF, 2BF and 1BF. A significant positive correlation
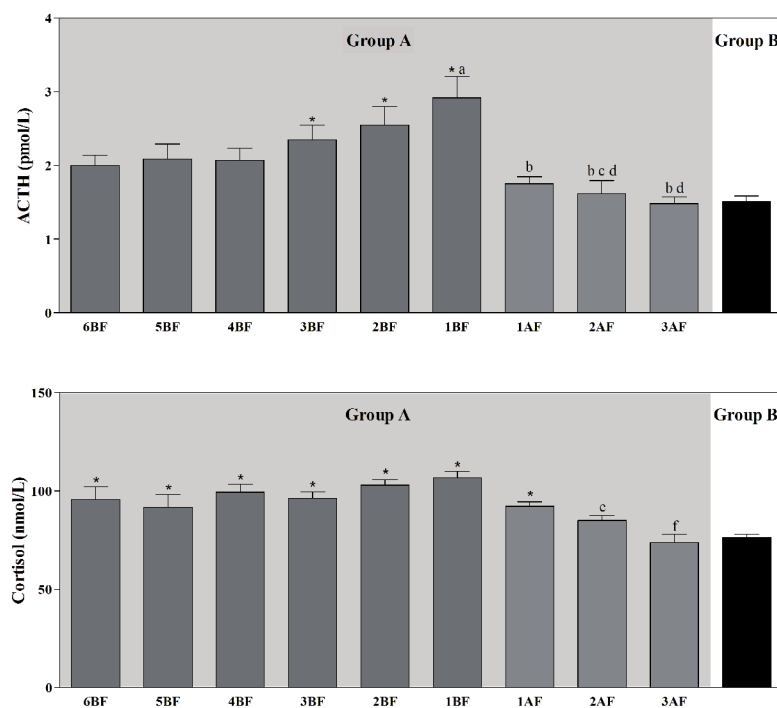
Significancant effect of peripartum time (P<0.0001): [a]vs 6BF; [b]vs 1BF; [c]vs 2BF; [d]vs 3BF; [e]vs 1BF; [f]vs 6BF, 4BF, 3BF, 2BF and 1BF
Significancant effect of group (P<0.0001): * vs Group B

Fig. 1. Mean values±standard error of the mean (SEM) with statistical significances of plasma adrenocorticotrophic hormone (ACTH) and serum cortisol recorded during the study from pregnant (Group A) and non-pregnant mares (Group B). Time is expressed as weeks before foaling (BF) and after foaling (AF).

between ACTH and cortisol values was found in mares throughout the peripartum period (coefficients of correlation: r=0.40; P<0.0001). The Dunnet's test revealed significant differences (P<0.0001) between Group A and Group B (Fig. 1).

The hormonal changes that occur in pregnant mares are quite different from those in other species and the maintenance of the pregnancy, as well as the health of the fetus, is dependent on equine physiology. The deep knowledge of hormonal dynamics in periparturient mares is crucial to promptly and correctly diagnose mare's diseases that could jeopardize both the mare's and the fetus' life, and to monitor mare's response to treatments. In the present study, plasma ACTH and serum cortisol levels are thoroughly investigated beginning from the 6th week of pregnancy until 3rd week after foaling. The same trend was followed by the studied hormones during the monitoring period as highlighted by the significant positive correlation found between ACTH and cortisol levels throughout the peripartum period. A gradual increase in the values of ACTH and cortisol was observed from the beginning of the monitoring period until the last week of the prepartum period in Group A. ACTH showed statistically significant higher values the last week of pregnancy compared to the beginning of the monitoring period (6BF) whereas, in agreement with previous studies [Silver and Fowden 1994; Hoffmann *et al.* 1996, Satuč *et al.* 2011], no statistically significant changes in cortisol values were found throughout the considered prepartum period. The increase of ACTH and cortisol is likely to occur in pregnant animals in comparison to non-pregnant. During pregnancy, plasma CRH increases due to its production by placenta and fetal membranes rather than of hypothalamic origin [Hoffman *et al.* 2003] and it stimulates maternal ACTH secretion. The ACTH binds to high-affinity membrane receptors on the adrenal cell and activates the adenylate cyclase system, resulting in a net increase of cholesterol transport into the cell and to biosynthesis of cortisol. The production of cortisol follows, within minutes, the ACTH secretion. Plasma ACTH levels rises and follow in parallel the rise of cortisol levels. Elevated estrogen levels in pregnancy lead to a doubling of corticosteroid binding globulin levels [Taylor and Badell 2001] resulting in low catabolism of cortisol by the liver and a doubling of cortisol's half-life in plasma. Moreover, as suggested for human species [Mastorakos and Ilias 2003], during pregnancy maternal adrenal glands gradually become hypertrophic as cortisol production in the adrenal zona fasciculata is increased, because of the relatively increased maternal ACTH secretion. This could justify the higher ACTH and cortisol levels found in Group A at prepartum period compared to control group.

Group A showed a statistically significant decrease of ACTH and cortisol levels at the postpartum period. The decrease in the concentration of these hormones found after foaling could indicate an attenuated stress response of the postpartum mares that it is likely due to the neuroendocrine changes that take place in postpartum female mammals [Torner *et al.* 2002, Ousey 2004]. One major change that occurs in postpartum, lactating females is that they have sustained, elevated prolactin levels and increased prolactin receptors expression in the choroid plexus due to sucklinginduced hyperprolactinemia [Pi and Voogt 2000]. This postpartum hyperprolactinemia seems

to cause attenuation of the HPA axis to a stressor [Torner *et al.* 2002]. Studies carried out on human species have been proposed that the HPA axis attenuation in postpartum females is essential in order to reduce anxiety so that maternal care is optimized [Ousey 2004, Bosch *et al.* 2007].

In conclusion, the decrease of plasma ACTH and serum cortisol levels found after foaling could indicate a reduced HPA response to physical and/or psychological stress during the postpartum period. This phenomenon could help the mare to conserve energy required for lactation, protect against stress-associated inhibition of lactation, relieve psychological stress, and enhance her immune function. Further studies involving the evaluation of prolactin and sex steroid hormones values are needed to fully understand the dynamic hormonal changes occurring in pregnant and lactating mares in order to permit clinicians to make appropriate interpretation of the results.

REFERENCES

1. ARFUSO F., GIANNETTO C., RIZZO M., FAZIO F., GIUDICE E., PICCIONE G., 2016 – Serum levels of mitochondrial uncoupling protein 1, leptin, and lipids during late pregnancy and the early postpartum period in mares. *Theriogenology* 86, 1156-1164.
2. BAZZANO M., GIANNETTO C., FAZIO F., ARFUSO F., GIUDICE E., PICCIONE G., 2014a – Metabolic profile of broodmares during late pregnancy and early post-partum. *Reproduction in Domestic Animals* 49, 947-953.
3. BAZZANO M., GIANNETTO C., FAZIO F., RIZZO M., GIUDICE E., PICCIONE G., 2014b – Physiological adjustments of haematological profile during the last trimester of pregnancy and the early postpartum period in mares. *Animal Reproduction Science* 149, 199-203.
4. BAZZANO M., GIANNETTO C., FAZIO F., MARAFIOTI S., GIUDICE E., PICCIONE G., 2014c – Hemostatic profile during late pregnancy and early postpartum period in mares. *Theriogenology* 81, 639-643.
5. BAZZANO M., ARFUSO F., GIUDICE E., DI PIETRO S., PICCIONE G., 2015 – Platelet aggregation percentage increased in healthy broodmares during the peripartum. *Journal of Equine Veterinary Science* 35, 573-576.
6. BERG E.L., MCNAMARA D.L., KEISLER D.H., 2007 – Endocrine profiles of periparturient mares and their foals. *Journal of Animal Science* 85, 1660-1668.
7. BONELLI F., ROTA A., CORAZZA M., SERIO D., SGORBINI M., 2016 – Hematological and biochemical findings in pregnant, postfoaling, and lactating jennies. *Theriogenology* 85, 1233-1238.
8. BOSCH O.J., MUSCH W., BREDEWOLD R., SLATTERY D.A., NEUMANN I.G., 2007 – Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: Implication for postpartum mood disorder. *Psychoneuroendocrinology* 32, 267-278.
9. BRUNTON P. J., RUSSELL J.A., 2008 – The expectant brain: Adapting for motherhood. *Nature Reviews Neuroscience* 9, 11-25.
10. CUDD T.A., LEBLANC M., SILVER M., NORMAN W., MADISON J., KELLER-WOOD M., WOOD C.E., 1995 – Ontogeny and ultradian rhythms of adrenocorticotropin and cortisol in the late gestation fetal horse. *Journal of Endocrinology* 144, 271-283.
11. ENGELMANN M., LANDGRAF R., WOTJAK C.T., 2004 – The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: An old concept revisited. *Frontiers in Neuroendocrinology* 25, 132-149.
12. GIANNETTO C., FAZIO F., ALBERGHINA D., ASSENZA A., PANZERA M., PICCIONE G., 2015 – Different daily patterns of serum cortisol and locomotor activity rhythm in horses under natural photoperiod. *Journal of Veterinary Behavior* 10, 118-121.

13. GIANNETTO C., FAZIO F., ALBERGHINA D., PANZERA M., PICCIONE G., 2013 – Constant darkness disrupt daily rhythm of adrenocorticotrophin in horses. *Journal of Applied Biomedicne* 11, 41-45.

14. HARVEY J.W., PATE M.G., KIVIPELTO J., ASQUITH R.L., 2005 – Clinical biochemistry of pregnant and nursing mares. *Veterinary Clinical Pathology* 34, 248-254.

15. HERMAN J.P., FIGUEIREDO H., MUELLER N.K., ULRIGH-LAI Y., OSTRANDER M.M., CHOI D.C., CULLINAN W.E., 2003 – Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology* 24, 151-180.

16. HOFFMAN R.M., KRONFELD D.S., COOPER W.L., HARRIS P.A., 2003 – Glucose clearance in pregnant mares is affected by diet, pregnancy and lactation. *Journal of Animal Science* 81, 1764-771.

17. HOFFMANN B., GENTZ F., FAILING K., 1996 – Investigations into the course of progesterone-, oestrogen-and eCG- concentrations during normal and impaired pregnancy in the mare. *Reproduction in Domestic Animals* 31, 717-723.

18. KELLER-WOOD M., WOOD C.E., 2008 – Regulation of maternal ACTH in ovine pregnancy: does progesterone play a role? *The American Journal of Physiology - Endocrinology and Metabolism* 295, E913-E920.

19. MASTORAKOS G., ILIAS I., 2003 – Maternal and fetal Hypothalamic–Pituitary–Adrenal Axes during pregnancy and postpartum. *Annals of the New York Academy of Sciences* 997, 136-149.

20. MEANEY M.J., SZYF M., SECKL J.R., 2007 – Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends in Molecular Medicine* 13, 269-277.

21. MEINLSCHMIDT G., MARTIN C., NEUMANN I.D., HEINRICHS M., 2010 – Maternal cortisol in late pregnancy and hypothalamic–pituitary–adrenal reactivity to psychosocial stress postpartum in women. *Stress* 13(2), 163-171.

22. MILLS P.C., KRAMER H., AUER D.E., 1997 – Stress response to chronic inflammation in the horse. *Equine Veterinary Journal* 6, 483-486.

23. NEUMANN I.D., KROMER S.A., BOSCH O.J., 2005 – Effects of psycho-social stress during pregnancy on neuroendocrine and behavioural parameters in lactation depends on the genetically determined stress vulnerability. *Psychoneuroendocrinology* 30, 791-806.

24. OUSEY J.C., 2004 – Peripartal endocrinology in the mare and foetus. *Reproduction in Domestic Animals* 39, 222-231.

25. PHILLIPS D.I., 2007 – Programming of the stress response: A fundamental mechanism underlying the long-term effects of the fetal environment? *Journal of Internal Medicine* 261, 453-460.

26. PI X.J., VOOGT J.L., 2000 – Effect of suckling on prolactin receptor immunoreactivity in the hypothalamus of the rat. *Neuroendocrinology* 71, 308-317.

27. SATUÉ K., DOMINGO R., MUŃOZ A., 2007 – Serum cortisol concentrations in Spanish mares during pregnancy. *Ippologia* 18(3), 21-25.

28. SATUÉ K., DOMINGO R., REDONDO J.I., 2011 – Relationship between progesterone, oestrone sulphate and cortisol and the components of renin angiotensin aldosterone system in Spanish purebred broodmares during pregnancy. *Theriogenology* 76, 1404-1415.

29. SILVER M., FOWDEN A.L., 1994 – Prepartum adrenocortical maturation in the fetal foal: responses to ACTH. *Journal of Endocrinology* 142, 417-425.

30. TAYLOR R.N., BADELL M.L., 2001 – The endocrinology of pregnancy. In: Gardner D.G., Shoback D. eds.Greenspan's basic and clinical endocrinology. Prentice Hall, Hartford, CT, pp. 575-602.

31. TORNER L., TOSCHI N., NAVA G., CLAPP C., NEUMANN I.D., 2002 – Increased hypothalamic expression of prolactin in lactation: involvement in behavioral an neuroendocrine stress responses. *European Journal of Neuroscience* 15, 1381-138.