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Original article

CIRCULATING ENDOCRINE, METABOLIC, AND HAEMATOLOGIC INDICES DURING THE PERIPARTUM PERIOD OF DAREHSHORI MARES

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Summary

Chalmeh, A., M. Pourjafar, Kh. Badiei, M. Mazrouei Sebdani, N. Hatami Nasar & S. M. M. Heidari, 2022. Circulating endocrine, metabolic, and haematologic indices during the peripartum period of Darehshori mares. *Bulg. J. Vet. Med.,* **25**, No 1, 113−122.

The characteristics associated with equine peripartum period are different from those of other periods. Information concerning the values and dynamic changes of circulating peripartum endocrine, metabolic, and haematologic indices may improve the understanding of the whole body haemostatic patterns which lead to parturition, lactogenesis, and estrus in the horse. The present study aimed to evaluate these changes around parturition in Darehshori breed. For this purpose, blood sampling was performed on six clinically healthy Darehshori mares (10 years old) about one week prior to parturition, three days following parturition, at the beginning of estrus, and one week after estrus. Circulating values of glucose, estrogen, progesterone, cortisol, T3, T4, TG, cholesterol, HDL, LDL, VLDL, RBC, HGB, HCT, MCV, MCH, MCHC, WBC, neutrophil, lymphocyte, and PLT were assessed in all samples. Progesterone concentrations were minimum before parturition and increased significantly one week after foaling heat (P=0.035). Cortisol level was significantly higher during foaling heat compared with other periods (P=0.029). T4 levels significantly increased from pre- to post-partum periods (P=0.001), but T3 showed increased and decreased values during the study period. All serum lipid profile parameters were significantly reduced from pre to postpartum period (P<0.05). Haematological parameters in mares significantly changed during the peripartum period (P<0.05). Based on the results, the metabolic, haematological, endocrinological, and stress indices in Darehshori mares were found to change around parturition and the first estrus. These changes were largely dependent on hormonal mechanisms related to pregnancy and parturition occurring in normal mares.

Key words: Darehshori mare, endocrinology, haematology, metabolism, parturition, pregnancy

INTRODUCTION

The evaluation of blood parameters such as electrolytes and haematological, hormonal, and coagulation indices is common in clinical practices. For a meaningful interpretation of haematological, biochemical, and hormonal variables as well as milk components, it is necessary to draw a comparison with an established reference interval. However, during particular life phases such as peripartum and, particularly lactation, female mammals face physiological adaptations and demands, which complicate the comparison with established reference intervals (Bazzano *et al.,* 2014b; 2015; Arfuso *et al.,* 2016a,b; Piccione *et al.,* 2017).

Characteristics related to equine peripartum period are different from those of other periods. Researchers believe that hormonal, haematological, and biochemical factors may fluctuate during this period and that these changes are commonly caused by endocrinologic patterns (Blackmore *et al.,* 1978; Bayly *et al.,* 1996). Furthermore, the most apparent fluctuations may be attributed to foetal growth demands and the subsequent lactogenesis (Orozco *et al.,* 2007). Pregnant mares also undergo stressful conditions following parturition and lactation, which may change their immune system function (Moffett & Loke, 2004). The stress caused by parturition along with the risk of post-partum infections impact haematological, biochemical, and immunological parameters of key importance for mares' immunity during this period (Padgett & Glaser, 2003). All mentioned dynamic changes during pregnancy, parturition, and lactogenesis may affect the internal homeostasis of mares. Aoki & Ishii (2012) reported that at the end of pregnancy and in postpartum periods, certain changes occurred in leukocytes distribution in mares. However, some studies have shown that a moderate anaemia takes place during pregnancy (Satue & Domingo, 2008; Satue *et al.,* 2012). In comparison with other farm animals, the postpartum period of mares has unique characteristics because more than half of all mares exhibit a "foal heat" commencing 6 to10 days following parturition (Holtan *et al.,* 1975).

There are many studies on haematologic and biochemical dynamic changes from pre- to post-parturition periods of different breeds of horses (Kramer, 2000; Orozco *et al.,* 2007; Aoki & Ishii, 2012; Satué *et al.,* 2012; Mariella *et al.,* 2014; Krakowski *et al.,* 2017); nonetheless, there is a paucity of information concerning circulating endocrine, metabolic, and haematologic indices during the peripartum period of Darehshori mares. Knowing these values and dynamic changes during that period may help understanding the whole body haemostatic patterns which lead to parturition, lactogenesis, and estrus in this breed. In view of these considerations, the objective of the present study was to evaluate the trend of the circulating levels of the foregoing parameters in Darehshori mares from one week before parturition to one week after the first heat.

MATERIALS AND METHODS

This study was carried out from March to June 2017 on six adult Darehshori pregnant mares (10 years old) in the vicinity of Shiraz, southwest Iran. All six horses, which were in resting period, were kept in individual stalls and fed balanced rations including alfalfa hay, corn silage, and barley grain. The subjects had no history of debilitating diseases and were clinically healthy and free from internal and external parasites based on laboratory parasitic examinations and routine anti-parasitic programmes.

The pregnancy age was calculated based on the date of mating, and the parturition time was further estimated. Via jugular venipuncture, blood samples were obtained from all animals and maintained in plain and EDTA coated tubes (Orom Tajhiz Gostar Co, Iran) one week before parturition, three days after parturition, during the first heat, and one week after the heat signs vanished. Immediately after blood collections, sera were separated by centrifugation for 10 minutes at 3,000 g and stored at – 22 ^{o}C until assayed.

Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method (ZistChem®, Tehran, Iran). The sera were analysed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis & Ashwood, 1994), and triglyceride (TG) by the enzymatic procedure of Mcgowan *et al.* (1983). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. HDL-cholesterol was measured based on the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins sedimented by centrifugation (10,000×g for 5 min). Afterwards, residual cholesterol was measured by the enzymatic method (Burtis & Ashwood, 1994). LDL-cholesterol was calculated as the difference between the total cholesterol measured in the precipitate and in the HDL fraction minus 0.2×triglyceride (LDL=total cholesterol–HDL cholesterol–0.2×TG). VLDL-cholesterol was estimated as one-fifth of triglyceride concentration (Friedewald *et al.,* 1972). Serum triiodothyronine (T3) concentrations were measured through the use of a competitive enzyme immunoassay kit (Padtan Elm Co., Tehran, Iran) and serum thyroxine (T4) via a competitive enzyme immunoassay kit (Monobind Inc., CA, USA). Serum cortisol concentrations were determined by Enzyme Immunoassay Colorimetric method (AccuBind® ELISA kit; Monobind Inc., CA, USA). Estrogen and progesterone were assayed by commercial ELISA kits (Monobind inc. Lake Forest, CA92630, USA).

Through automated cell counter (Exigo®, model Vet50939, Sweden), a complete blood count was conducted on whole blood samples in order to count and evaluate red blood cell (RBC), haemoglobin (HBG), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), neutrophil, lymphocyte and platelet (PLT) counts.

All data were expressed as mean±SD. The Kolmogorov-Smirnov test revealed that the data were normally distributed, and parametric tests were done to assess the data. One-way analysis of variance (ANOVA) analysed the differences among the studied periods regarding the average concentrations of various indices; furthermore, the least significant difference (LSD) was employed as *post-hoc* test to find the differences. Repeated measures ANOVA was used to evaluate the changing pattern of each parameter during the study period using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). The significance level was set at $P<0.05$.

RESULTS

Table 1 presents the dynamic changes of circulating endocrine, metabolic, and haematologic indices (mean±SD) during the periparturient period of adult Darehshori mares. As observed, with the exception of MCH and MCHC ($P>0.05$), the dynamic changes of all studied parameters during this period were significant ($P<0.05$).

Table 1. Circulating endocrine, metabolic and hematologic indices (mean±SD) at peri-parturient period of adult Darehshori mares (n=6).

Parameters	1 week before parturition	3 days after parturition	During the first heat	1 week after the first heat
Estrogen (pg/mL)	22.65±2.36[a]	16.97±1.88[b]	38.76±2.20[c]	19.29±10.78[a]
Progesterone (ng/mL)	2.90±0.39[a]	4.34±0.55[b]	6.81±1.58[c]	12.68±2.28[d]
Cortisol (ng/mL)	3.88±0.40[a]	3.40±0.31[a]	5.52±0.77[b]	4.37±0.68[c]
T3 (ng/dL)	83.00±16.94[a]	61.16±8.42[b]	84.50±16.69[a]	78.50±10.83[c]
T4 (ng/dL)	2.95±0.15[a]	3.28±0.09[b]	3.41±0.14[b]	3.21±0.14[b]
Glucose (mmol/L)	5.56±0.95[a]	4.47±0.41[b]	4.74±0.68[b]	5.68±0.2[a]
TG (mmol/L)	0.48±0.15[a]	0.28±0.08[b]	0.24±0.11[b]	0.15±0.01[c]
Cholesterol (mmol/L)	2.49±0.03[a]	2.06±0.27[b]	2.00±0.39[c]	1.80±0.31[d]
HDL (mmol/L)	3.67±1.25[a]	3.00±0.96[b]	2.45±0.44[c]	2.28±0.35[d]
LDL (mmol/L)	0.96±0.05[a]	1.06±25.31[a]	0.65±0.1[b]	0.58±0.03[b]
VLDL (mmol/L)	0.22±0.07[a]	0.13±0.03[b]	0.11±0.05[c]	0.07±0.01[d]
RBC (10^9/L)	8.62±1.52[a]	9.17±1.21[b]	9.91±2.67[c]	9.34±1.52[b,c]
HCT (%)	40.25±4.38[a]	44.81±3.58[b]	46.33±11.54[b]	45.40±6.85[b]
HGB (mmol/L)	1.99±0.3[a]	2.08±0.29[b]	2.21±0.48[b]	2.17±0.35[b]
MCV (fL)	48.23±3.45[a]	49.86±2.41[a]	43.51±9.04[b]	41.75±6.24[b]
MCH (pg)	15.20±1.22[a]	15.10±1.69[a]	15.31±0.96[a]	15.05±1.91[a]
MCHC (g/dL)	32.38±3.17[a]	29.78±1.45[a]	30.50±0.79[a]	30.01±1.90[a]
WBC (10^9/L)	9.90±1.52[a]	8.14±0.74[b]	10.00±1.41[a]	9.80±0.73[a]
Neutrophil (%)	55.36±4.27[a]	26.26±12.15[b]	48.01±8.11[c]	39.28±4.72[d]
Lymphocyte (%)	36.76±6.45[a]	66.26±12.86[b]	42.96±7.97[c]	53.50±4.69[d]
PLT (10^9/L)	142.01±48.35[a]	116.01±37.22[b]	95.01±17.34[c]	88.25±13.89[d]

[a,b,c,d]: Different letters for each parameter indicate significant differences among all peri-parturient periods (P<0.05).

The highest levels of TG, cholesterol, HDL, VLDL, MCHC, neutrophil, and PLT were detected one week prior to parturition. LDL, MCV, and lymphocyte were at their maximum three days after parturition. Circulating estrogen, cortisol, T3, T4, HCT, HGB, MCH, and WBC were at the highest levels during the first heat. The highest values of progesterone, glucose, and RBC were observed a week after the first heat (Table 1; P<0.05).

Minimum levels of progesterone, T4, RBC, HCT, HGB, and lymphocyte were detected one week prior to parturition. Estrogen, cortisol, T3, glucose, MCHC, WBC and neutrophils were at their lowest three days following parturition. Circulating LDL value was minimum during the first heat. The lowest values of TG, cholesterol, HDL, VLDL, MCV, MCHC, and PLT were observed one week after the first heat (Table 1; P<0.05).

DISCUSSION

Parturition is a unique phenomenon that changes the haemostatic patterns of the whole body. The characteristics of peripartum differ from those of other periods, so it is essential to obtain information regarding these differences for better evaluation of mares in this period. Several literature reports demonstrated haematological and biochemical dynamic changes during parturition and foaling in different horse breeds (Kramer, 2000; Orozco *et al.,* 2007; Aoki & Ishii, 2012; Satué *et al.,* 2012; Mariella *et al.,* 2014; Krakowski *et al.,* 2017); nevertheless, no data are available concerning changes from pre- to post-partum periods of Darehshori mares.

The endocrine control of parturition in mares is similar to that of other species and involves the combined functions of progesterone, estrogen, cortisol, prostaglandins, and oxytocin. Endocrine dynamic changes take place to induce parturition, and they typically begin approximately 300 days of gestation with the onset of an increase in maternal progesterone concentrations. However, the rise in maternal progesterone also appears to have an important role in the final stages of mammary gland development. Moreover, the start of myometrial contractile activities in mares seems to coincide with the precipitous fall in maternal progesterone levels. Contrary to most other species (Haluska & Currie, 1988), delivery in mares takes place under high concentrations of progesterone. Although the onset of foaling is associated with a decline in total progesterone concentration, the initiation of parturition does not require a progesterone reduction to near-zero values. The early postpartum endometrial cells undergo apoptosis; however, the proliferation of cells is predominant during the second week with the increased expression of estrogen receptors, allowing the endometrium to respond to estrogen during foal heat; in the subsequent diestrus, the endometrium is able to respond to progesterone (Jischa *et al.,* 2008). The results of the current study showed that progesterone concentrations were at their lowest prior to parturition while increasing significantly a week after foaling heat (Table 1).

Although physiological foaling is not a major stressor, maternal cortisol concentrations are augmented by the pathways that initiate foaling (Nagel *et al.,* 2014). Increased cortisol release during the final four to five days of pregnancy was reported in the equine foetus (Fowden & Silver, 1995); such increase has not been exhibited in the antepartum mare (Nagel *et al.,* 2010). In mares, cortisol release into plasma is elevated both during the immediate postpartum period and, to a lesser degree, throughout pregnancy with even a decrease towards the end of gestation. Cortisol concentration changes are most likely part of a physiological cascade triggering maturation and preparation for parturition (Satué *et al.,* 2011). In the current research, the cortisol levels of the studied mares were significantly higher during foaling heat in comparison with other periods. It may be suggested that foaling heat is more stressful than other peripartum periods, even parturition.

Late-term pregnancy seems to have various effects on thyroid hormone levels in mares, with studies showing either no change or a decline (Flisińska-Bojanowska *et al.,* 1991) in thyroid hormones in late pregnancy. In humans, on the other hand, thyroid hormone levels increase in late pregnancy as a result of estrogen-induced increase in thyroid-binding globulin and stimulation of the thyroid gland by thyroid-stimulating hormone-like activity of human chorionic gonadotropin (Emerson, 1991). Two studies involving Thoroughbreds showed that resting T4 levels were well below the normal range of values used by the respective laboratories of the investigators; both researches reported the unreliability of T4 measurement as an indication of thyroid function in the Thoroughbred (Blackmore *et al.,* 1978; Bayly *et al.,* 1996). T4 levels in the present study significantly increased from pre- to post-partum periods, but T3 had increased and decreased values throughout the study period.

Watson *et al.* (1993) showed that plasma lipid metabolism was changed in pony mares in late gestation. Most significantly, the TG concentrations increased because VLDL concentrations were higher than the upper limit of the reference range. Stammers *et al.* (1989) reported TG levels in the uterine vein in excess of 1.0 mmol/L in healthy pony mares sampled between 250 and 300 days of gestation. These data indicated that moderate hypertriglyceridaemia is prevalent in the late gestation of pony mares. In pregnant ponies, mean plasma cholesterol concentrations increase mainly due to the rise in HDL-cholesterol. In contrast, the moderate hypercholesterolaemia found in ponies with hyperlipaemia is ascribed to high VLDL-cholesterol concentrations because of the normal concentrations of HDL (Watson *et al.,* 1992).

These dynamic changes have important roles as they reflect the metabolic origins of hyperlipaemia which might occur in late gestation. In the absence of cholesteryl ester transfer protein activity in horses (Watson *et al.,* 1993), these changes are consistent with the large VLDL particles produced by the liver that accommodate a greater mass of TG (Watson *et al,* 1992). In this way, the liver appears to maximise the secretion of TG in response to increased hepatic TG synthesis. The appearance of free cholesterols in VLDL at the expense of cholesteryl esters reflects a decrease in the esterification of cholesterol. Based on the results of the present study, all components of serum lipid profiles were significantly reduced from pre- to post-partum period of Darehshori mares.

The current study also showed that haematological parameters in Darehshori mares significantly changed during the peripartum period. Bazzano *et al.* (2014a) reported significant alterations in HCT, HGB, PLT, WBC, neutrophil, and lymphocyte levels within the pregnant, non-lactating mares during their study. Throughout the experimental period, they further observed a progressive decrease in HCT which started from the 8^{th} week before parturition. Bernstein *et al.* (2001) showed that plasma volume increased by 30–50% in pregnancy in women. The reduction in HCT is always lower in pregnant women than in non-pregnant ones. The increase in plasma volume depends on the increased activity of the renin-angiotensin-aldosterone system, which is a physiological condition of pregnancy in both women (Irani & Xia, 2008) and mares (Satué *et al.,* 2011). This results in increased sodium reabsorption from the kidneys and water retention. The increase in blood volume together with the drop in blood viscosity indicate important adjustments to keep up with the rise in blood flow to organs such as uterus and kidneys (Harm *et al.,* 2012).

The hypervolemia of pregnancy is also crucial to protect the mother and the foetus from the harmful effects of reduced venous return and preventing the mother from suffering the adverse effects of blood loss during delivery (Mcmullin *et al.,* 2003). In the present study, HGB concentrations progressively decreased in pregnant mares, reaching the lowest values in the post-partum period. A similar HGB profile was observed in Brasileirode Hipismo and Breton mares (Orozco *et al.,* 2007) and in Standardbred mares (Mariella *et al.,* 2014).

The water needs of mares start increasing in the last trimester of pregnancy due to placental fluids and the foetus. A further increase in water consumption occurs during lactation when mares require up to 50–80% more water than that required for maintenance (Lewis, 1995). Another physiological cause of the decline in HGB is the reduced lifespan of circulating erythrocytes in pregnant females compared to non-pregnant ones (Lurie, 1993). Weiss & Wardrop (2010) reported that the PLT count in the horses remained within the physiological range. However, the PLT peak recorded at foaling might reflect a hypercoagulable state which physiologically occurs in mares around parturition (Bazzano *et al.,* 2014a). As previously observed in Standardbred (Mariella *et al.,* 2014) and heavy draft mares (Aoki *et al.,* 2013), Bazzano *et al.* (2014a) found a significant increase in WBC count around parturition. In addition, Harvey *et al.* (1994) revealed that the highest leukocyte count was seen after foaling. An adrenocorticoid-mediated leukocytosis was further detected in pregnant women, resulting in white cell count that is particularly noticeable during labour and the puerperium (Tan & Tan, 2013). The differential leukocyte counts underwent significant changes in peripheral blood neutrophil and lymphocyte percentage, which is in accordance with the studies by Aoki *et al.* (2013) on heavy draft mares. A progressive increase was found in neutrophil percentage, starting from the 4th week before foaling although the highest values were recorded at the time of delivery. Conversely, the lymphocyte percentage gradually decreased around parturition (Harvey *et al.,* 1994). In the advanced stages of gestation, an endogenous adrenaline release occurred, increasing the mobilisation of neutrophils in the circulation and the total leukocyte count (Kramer, 2000). In the beginning of parturition, a significant rise was observed in the blood cortisol concentrations in pregnant mares (Nagel *et al.,* 2012). The results of the current study showed that RBC, HCT, and HGB increased significantly during the study, but PLT was reduced from pre- to post-partum period.

Cortisol considerably influences the blood cells through reducing the number of lymphocytes and increasing the number of neutrophils and the total number of leukocytes (Kaneko *et al.,* 1997). Additionally, during peripartum, the hormonal profile undergoes significant alterations as the mare quickly restores a normal cyclicity and fertility following parturition (Cupps, 1991). Although ovarian hormones such as estrogen and progesterone have been associated with a number of physiological changes in the female, studies regarding cycling mares (Roberto Da Costa *et al.,* 2003) reported no noticeable changes in haematological parameters at either phase of the estrus cycle.

CONCLUSION

Based on the results of the present study, circulating endocrine, metabolic and haematological indices in Darehshori mares changed from pre- to post-partum periods. Furthermore, different periods had different dynamic changes which might have naturally occurred due to the physiological alterations from pregnancy (non-lactating) to non-pregnancy (lactating). On the other hand, the dynamic changes in circulating endocrine, metabolic, and haematological indices might differ among different horse breeds; the values presented in this study may assist veterinarians in accurately evaluating the endocrinologic, metabolic, and haematological conditions of Darehshori mares during the peripartum period.

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