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HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN PREGNANT, POSTFOALING AND LACTATING JENNIES

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Abstract

The aims of this study were to: 1) verify if significant changes occur in hematological and biochemical parameters in jennies during the last two months of pregnancy and the first two months of lactation, and 2) determine any differences with equine species.

Materials and methods. Hematological and biochemical parameters were evaluated in jennies every 15 days during late pregnancy, parturition, and early lactation. The Kolmogorov-Smirnov test, ANOVA for repeated measurements and Tukey's multiple comparison test as *post hoc* were applied. The significance level was set at $p<0.05$.

Results. Statistical analysis showed differences related to time for RBC and HCT, WBC, PLT, total proteins (TP), blood urea, triglycerides and total cholesterol concentrations, AST, GGT, CK activities, sodium (Na) and potassium (K).

Discussion and conclusions. RBC and HCT were higher in late pregnancy than at foaling and during lactation. The relative anaemia might be due to increased water ingestion due to fluid losses. The WBC count was higher at foaling than during late pregnancy and lactation. This could be related to the release of cortisol and catecholamine during delivery. The PLT trend showed lower values from delivery to the first two months of lactation compared to late gestation. Blood urea increased near parturition, and then remained constant during delivery and lactation, which might be due to the high-energy demand at the beginning of lactation. Triglycerides and total cholesterol showed a decrease from delivery through the lactation period. Thus jennies seem to have a similar metabolism of fats to ponies and draft horse mares, characterized by a greater fat content and mobilization than light breed horses. AST activity decreased at parturition and early lactation, probably due to a predominance of anabolic over catabolic processes during pregnancy. GGT activity was lower at delivery and during lactation than at late gestation. This could be due to a physiological load on the liver in the perinatal period. GGT activity was always higher than in mares, but within the normal range for adult donkeys. CK decreased near delivery, then was constant from parturition through the first two months of lactation. Na decreased during lactation, probably due to an increased renal retention mediated by aldosterone release during pregnancy. K showed the same trend as Na, and concentrations are in line with the species. The higher K during pregnancy may be due to reabsorption by the gut. TP decreased more during the post-partum period and lactation than in the gestational period.

51 Key words
52 Jennies, haematology, biochemistry, pregnancy, foaling, lactation.
53

1. Introduction

Donkeys (*Equus asinus*) have been close companions to humans for millennia and have been used as working animals all over the world. Donkey milk could possibly be used in children with intolerance to cow's milk [1,2] or in animal-assisted therapy [3]. The renewed interest in these animals is demonstrated by the number of studies on establishing the base-line data of both haematological and biochemical variables in the blood of adult [4-26], as well as in newborn donkeys [27-28].

Pregnancy and lactation are physiological periods that result in increased metabolic demands. Although homeostatic mechanisms keep substances in the blood at relatively constant levels, some changes in the concentrations of routine clinical chemistry analytes are likely to occur [29]. During pregnancy, an expansion in plasma volume and erythrocyte mass occurs, as well as an increase in plasma protein synthesis [30]. In women, it is well known that during pregnancy reference intervals are different from the non-pregnant state [31-33].

The effects of pregnancy, parturition and lactation on haematological parameters have only been studied in horses to a limited extent [34-44] in different breeds. Moreover, only one study [42] has reported a thorough investigation of the last months of pregnancy and first month of lactation and no data are present in literature concerning haematological and biochemical values in jennies during the same period. Therefore, more detailed parameters in hematology and biochemistry during late pregnancy, parturition and lactation in jennies could be useful for accurate diagnosis of diseases.

The aims of this study were to: 1) verify if significant changes occur in hematological and biochemical parameters in jennies during the last two months of pregnancy and the first two months of lactation; and 2) determine any differences with respect to equine species

2. Material and Methods

2.1. Animals

The study was conducted on 9 jennies belonging to the Amiata donkey breed for a total of 18 pregnancies and 16 foals born during a two-year study. Jennies were from 5 to 13 years old, weighed 300 to 350 kg, and were kept in collective paddocks at the Veterinary Teaching Hospital, Department of Veterinary Sciences, Pisa University. Approval for this study was obtained from the Ethical Committee on Animal Experimentation of the University of Pisa, and the protocol was sent to the Ministry of Health.

In order to provide NRC recommendations for energy, jennies were fed with meadow hay *ad libitum* along with commercial equine feed This feeding procedure began at two months pre-partum, and continued through post-partum and the first two months of lactation. Jennies were housed together during pregnancy in a paddock 10x20 m. Close to parturition jennies were housed in 4x4 m boxes for the first 15 days of lactation, and then returned to the paddock.

Jennies were included in this study according to the following criteria: 1) pregnancy length 353.4±13.0 days [43]; 2) unassisted delivery; 3) treatment against gastrointestinal parasites and vaccinated against equine influenza, tetanus, and equine herpes virus-1, in accordance with the guidelines of the American Association of Equine Practitioners Infectious Disease Committee [44].

2.2. Sample collection and handling

Blood samples were obtained from the jugular vein. Each jenny was sampled every 15 days during late pregnancy, approximately two months before parturition, at parturition, and every 15 days during the first two months of lactation. Blood was collected in test tubes containing K2-EDTA (cod. 22056, FL Medical, Padua, Italy) and lithium-heparin test tubes (cod. 22304, FL Medical, Padua, Italy). To avoid alterations related to diurnal variations, blood samples were collected at the same time each day (8:00-9:00 am), except for the sample collected at parturition.

2.3. Complete blood count

K2-EDTA samples were analysed with a cell counter (Hecovet C 01030360/ITA, and CAL-SEAC 71010810 multiparametric haematology calibrator, SEAC-RADIM Co, Florence, Italy) at least 5 minutes after the collection. The aim was to determine: 1) erythrocyte count (RBC), 2) leukocyte count (WBC), 3) haemoglobin concentration (Hgb), 4) mean corpuscular volume (MCV), 5) mean corpuscular haemoglobin (MCH), 6) mean corpuscular haemoglobin concentration (MCHC), 7) platelet count (PLT). Specimens containing clots or grossly haemolysed were excluded. A quality control of the cell counter was performed each day.

2.4. Biochemical analysis

Heparinised samples were centrifuged at 3000 g for 10 minutes, as recommended by the manufacturer. Plasma was then frozen at -18 °C and analysed within 15 days after collection. Clinical chemistry was performed with an autoanalyzer (Liasys, Analyzer Medical System-AMS, Rome, Italy; quality control normal level: ASR02010 and pathologic level: ASR02020, Assel Srl, Rome, Italy).

The parameters analysed were: 1) glucose concentration (Glucose SL, enzymatic colorimetric method, cod. ASR01202, Assel Srl, Rome, Italy); 2) creatinine (Creatinine, kinetic modified Jaffè method, cod. ASR01150, Assel Srl, Rome, Italy); 3) blood urea (Urea UV SL, kinetic enzymatic method, cod. ASR01143, Assel Srl, Rome, Italy); 4) triglycerides (Triglycerides-SL, enzymatic colorimetric method, cod. ASR01134, Assel Srl, Rome, Italy); 5) total cholesterol (Cholesterol liquid, trinder method-endpoint, cod. 7050, FAR, Verona, Italy); 6) total bilirubin (Total Bilirubin, colorimetric method without DMSO, cod. ASR01034/1, Assel Srl, Rome, Italy); 7) aspartate aminotransferase (AST) (AST SL, kinetic method UV -IFCC- cod. ASR01220, Assel Srl, Rome, Italy); 8) gamma glutamyl transferase (γGT) (Gamma GT SL, kinetic method-Szasz-Tris, cod. ASR01194, Assel Srl, Rome, Italy); 9) creatine-phosphokinase (CK) (CK NAC SL, kinetic method UV, cod. ASR01074, Assel Srl, Rome, Italy); 10) alkaline phosphatase (ALP) (Alkaline Phosphatase SL-DGKC- kinetic method, cod. ASR01162, Assel Srl, Rome, Italy); 11) total calcium (Tot-Ca) (Calcium OCPC, colorimetric method, cod. ASR01050, Assel Srl, Rome, Italy); 12) potassium (K) (pHox Plus L, Stat Profile, Nova Biochemical, Milan, Italy); 13) sodium (Na) (pHox Plus L, Stat Profile, Nova Biochemical, Milan, Italy); 14) phosphorus (P) (Phosphorus UV, direct method with molibdate, cod. 90009800, Seac-Radim, Florence, Italy); 15) total protein (TP); 16) albumin (Albumin BCG, bromocresol green method, cod. 90009781, Seac-Radim, Florence, Italy). The same operator always performed the biochemistry profile, according to standard methods.

2.5. Statistical analysis

Average (X) and standard deviation (SD) values were calculated for each haematological and biochemical parameter collected at all sampling times, along with minimum and maximum values and the 95% of confidence interval (CI). The Kolmogorov-Smirnov test was carried out to verify data distribution. Results showed an approximately Gaussian distribution, thus analysis of variance for repeated measurements and Tukey's multiple comparison test as *post hoc* were applied. The significance level was set at $p<0.05$. All analyses were performed using commercial software (GraphPad Prism, USA).

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154 3. Results

155 None of the samples collected contained clots or were grossly haemolysed. The results for
156 haematological and biochemical parameters expressed as mean ± standard deviation are
157 reported in Tables 1 and 2, respectively. Not all the jennies were sampled at each time
158 point, thus the actual numbers of samples for each time point are indicated in Tables 1 and
159 2, respectively. In particular, 1 out of 2 jenny died 1 week after delivery due to colic
160 surgery, and 1 out of 2 was excluded after delivery because her foal died due to
161 septicaemia. Statistical analysis showed differences related to sampling times for RBC and
162 HCT, WBC, PLT, total protein, blood urea, triglycerides and total cholesterol
163 concentrations, AST, GGT, CK activities, P, Na, and K.

4. Discussion and Conclusions

All animal species need specific reference intervals of haematological and biochemical parameters for a good interpretation of blood sample analyses. The aim of the present paper was to study haematological and biochemical profiles in jennies during late pregnancy, post-partum period and lactation.

Jennies enrolled in this study had a wide age range (5-13 years) because age seems not to influence haematological and biochemical parameters [42,44]. The RBC and HCT trend showed higher values in late pregnancy than foaling time and lactating period. Thus, jennies did not seem to be affected by anaemia in late gestation, as in mares [42], but not as women [46] and other animal species [47-49]. The relative anaemia (lower HCT) during lactation might be related to a large increase in water ingestion secondary to fluid losses with the beginning of lactation and subsequent over-hydration and erythrocyte dilution, as already reported for mares [42]. Compared to values in mares, the results in jennies were lower throughout the study period [37-39,42].

The trend in WBC count showed higher values at foaling than late pregnancy and lactation, in agreement with previous studies on mares [37,42]. This could be related to the release of cortisol and catecholamine during delivery or to neutrophil margination into the uterus in the post-partum period [42,50]. In terms of WBC values, jennies had similar values compared to mares throughout the study period [37,42].

The PLT trend showed lower values from delivery to the first two months of lactation compared to late gestation, thus the post-partum period seems to exert significant effects on circulating platelet numbers. These results are not in agreement with previous studies on mares where PLT were found to be constant over time [32,38-39].

The results on MCV, MCH and MCHC showed a constant tendency over time, as reported for mares [39], but with higher values, while MCHC was similar for the two species during the all study period [37-38,42].

Glucose concentration was constant throughout the study period in jennies and values were in line to those reported in pregnant and lactating mares [42], but higher compared to adult donkeys [6,15]. This could be due to the development of insulin resistance, as reported for horses [39,51] and dogs [52], as well as for women [53].

Blood urea increased near parturition (-2 weeks), and then remained constant during delivery and lactation. This trend might be due to the high-energy demand at the beginning of lactation, as observed in mares [42].

Creatinine values were constant throughout the study period and within normal ranges for adult species [15,17-18,20-21,26], thus jennies do not seem to be influenced by the increase in energy demand in late gestation and by the quota produced by the foetus, as reported in mares [42].

Triglycerides and total cholesterol concentrations showed an important decrease from delivery through the lactation period, in particular two weeks after parturition. Our data are in line with previous studies on pony and draft mares in which triglycerides showed lower values during lactation than before parturition [41,54]. However they are in contrast with studies on light breed mares [29], in which triglycerides were constant and concentrations were always similar to reference values for adult horses. Our results showed a more similar metabolism of fats to ponies and draft horse mares, which have a greater fat content and mobilization than light breed horses.

Total bilirubin was constant throughout the study period. In mares, total bilirubin increases in late pregnancy due to secondary cholestasis because of the enlarged uterus [42], while in jennies this does not seem to occur.

The AST activity trend showed an increase near to parturition and early lactation. This might be due to a predominance of anabolic over catabolic processes during pregnancy [55]. Compared to activity values obtained in mares, our results were always higher [38,42].

The GGT activity trend showed lower values at delivery and during lactation than late gestation. Our results are in contrast to findings in mares in which the GGT activity increased around delivery and decreased gradually after foaling [55], which could be related to a physiological load on the liver in the perinatal period. GGT activity in jennies was always higher than in mares [42].

CK decreased near delivery, than remains constant after parturition for the first two months of lactation. These results are not in agreement with Marella et al. [42], who found an increase in CK activity at delivery and during lactation compared to pregnancy. It is difficult to explain the decreased activity of this enzyme because to the authors' knowledge there are no studies on CK activity during pregnancy, lactation and parturition in donkeys.

ALP activity values were constant and in line with pregnant and lactating light breeds mares [39], but not with draft mares in which ALP activity was found to increase around delivery and decrease gradually after foaling [41].

Tot Ca values were constant throughout the study period, while a decrease has been observed in mares at parturition [42]. P was higher in late gestation and parturition than in

lactating jennies. To the authors' knowledge, no data have been reported for P either in jennies or mares, but our results are in line with findings reported for women [56-57].

Na decreased during lactation and concentrations were always comparable to donkey reference intervals [21]. The higher Na concentrations during pregnancy might be due to an increased renal Na retention mediated by aldosterone release during pregnancy, as already reported in mares [58]. K showed the same trend as Na and concentrations are in line with this species [21]. The higher K values during pregnancy in jennies may be related to the reabsorption by the gut due to the enlarged pregnant uterus, as suggested for mares [42].

The TP trend showed a decreased concentration during the post-partum period and lactation than the gestational period, in contrast with mares in which higher TP concentrations were found in late pregnancy and early lactation compared to parturition [42].

In conclusion, our results showed significant changes in hematological and biochemical parameters in jennies during the last two months of pregnancy and the first two months of lactation. These changes are only partially comparable to mares. Thus, values obtained in jennies could be useful in clinical practice to assess the health status in jennies and to check peri-partum diseases. The results contribute to a better understanding of the biochemical processes in pregnant and lactating jennies, in order to estimate their physiological status and to be used for diagnostic purposes.

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References

[1] Carroccio A, Cavataio F, Montalto G. Intolerance to hydrolysed cow's milk proteins in infants: clinical characteristic and dietary treatment. Clin Exp Allergy 2000; 30: 1597-1603.

[2] Muraro MA, Giampietro PG, Galli E. Soy formulas and non bovine milk. Ann Allergy Asthma Immunol 2002; 89(S): 97-101.

[3] Borioni M, Marinaro P, Celestini S, Del Sole F, Magro R, Zoppi D, et al. Effect of equestrian therapy and onotherapy in physical and psycho-social performances of adults with intellectual disability: a preliminary study of evaluation tools based on the ICF classification. Disabil Rehabil 2012, 34(4): 279-97.

[4] Nayieri GD. Blood characteristics of the adult donkey. Zbl Vet Med A 1978; 25: 541-7.

[5] Zinkl JC, Mae D, Guzman P, Farver TB, Humble JA. Reference ranges and the influence of age and sex on haematological and serum biochemical values in donkey (*Equus asinus*). Am J vet Res 1990; 51: 408-13.

[6] French JM, Patrick VH. Reference values for physiological, haematological and biochemical parameters in domestic donkeys (*Equus asinus*). Equine vet Educ 1995; 7: 33-5.

[7] Orlandi M, Curadi MC, Leotta R, Berni P. Metabolic profile in the Amiata donkey. Annali Facoltà Medicina Veterinaria di Pisa 1997; L: 47-53. ISSN: 0365-4729

[8] Folch P, Jordana J, Cuenca R. Reference ranges and the influence of age and sex on haematological values of the endangered Catalonian donkey. Vet J 1997; 154: 163-8.

[9] Jordana J, Folch P, Cuenca R. Clinical biochemical parameters of the endangered Catalonian donkey breed: normal values and the influence of sex, age, and management practices effect. Res Vet Sci 1998; 64: 7-10.

[10] Mushi EZ, Binta MG, Ndebele RT. Haematological studies on apparently healthy donkeys in Oodi, Kgatleng district Botswana. Proceedings of an ATNESA workshop, South Africa, 1999; 163-5.

[11] Mori E, Fernandes WR, Mirandola RMS, Kubo G, Ferreira RR, Oliveira JV, Gacek F. Reference values on serum biochemical parameters of Brazilian donkey (*Equus asinus*) breed. J Equine Vet Sci 2003; 23(8): 358-64.

[12] Carcangiu V, Vacca GM, Cubeddu GM, Bini PP. Rilievi ematici in asini bianchi dell'Asinara sottoposti a trattamento per il controllo delle ectoparassitosi. J Mt Ecol 2003; 7(S): 207-10.

[13] Mori E, Mirandola RMS, Ferreira RR, Oliveira JV, Gacek F, Fernandes WR. Reference values on hematologic parameters of the Brazilian donkey (*Equus asinus*) breed. J Equine Vet Sci 2004; 24(7): 271-6.

[14] Al-Busadah KA, Homeida AM. Some physical variables, biochemical and haematological parameters in Hassawi ass. Scientific Journal King Faisal University (Basic and Applied Sciences) 2005; 6(1): 145-52.

[15] Caldin M, Furlanello T, Solano-Gallego L, De Lorenzi D, Carli E, Tasca S, et al. Reference ranges for hematology, biochemical profile and electrophoresis in a single herd of Ragusana donkeys from Sicily (Italy). Comp Clin Path 2005; 14: 5-12.

[16] Lopez I, Estepa JC, Mendoza FJ, Rodriguez M, Aguilera-Tejero E. Serum concentrations of calcium, phosphorus, magnesium and calciotropic hormones in donkeys. J Am Vet Res 2006; 67(8): 1333-6.

[17] Pitel PH, Moulin M. Approche des valeurs hematologiques et biochimiques chez deux races asinines. Pratique Veterinaire Equine 2006; 38(149): 19-25.

[18] De Aluja AS, Mondragon RL, Castillo DA, Ochoa P. Hematological and biochemical reference values in the donkey (*Equus asinus*) in Mexico. In: Matthews NS, Taylor TS, editors. Veterinary Care of Donkeys, International Veterinary Information Service, Ithaca, New York, (www.ivis.org), Last update: 21/06/2006.

[19] Ahemed AE, Abdel-Hamid HY, Abdel-Rahim AA, Ismail MN. The influence of age and sex on some blood parameters in healthy donkey in south Valley Egypt. Bs Vet Med J, 2007; 151-8.

[20] Couroucè-Malblanc A, Fortier G, Moulin M, Valette JP, Petit L, Dumontier S, et al. Reference values on hematologic and biochemical parameters in french donkeys. Proceedings 10th WEVA 2008; 458-60.

[21] Lemma A, Moges M. Clinical, hematological and serum biochemical reference values of working donkeys (*Equus asinus*) owned by transport operators in Addis Ababa, Ethiopia. Livest Res Rural Dev 2009; 21(8): 1-8.

[22] Etana KM, Jenbere TS, Bojis E, Negussie H. Determination of Reference Hematological and Serum-Biochemical Values for Working Donkeys of Ethiopia. Vet Res 2011; 4(3): 90-4.

[23] Alberghina D, Fazio F, Arfuso F, Scianò S, Zumbo A, Piccione G. Reference Intervals of Serum Protein Concentrations from Clinically Healthy Female Ragusana Donkeys (*Equus asinus*) Determined by Cellulose Acetate Electrophoresis. J Equine Vet Sci 2013; 33(6): 433-6.

[24] Zakari FO, Ayo JO, Rekwot PI, Karu MU. Effects of age and season on haematological parameters of donkeys during the rainy and cold–dry seasons. Int J Biometereol 2015; 10.1007/s00484-015-0989-7.

[25] Morais Girardi A, Marques LC, Pereira de Toledo CZ, Barbosa JC, Maldonado Jr W, Lemos Nagib Jorge R, Aparecida da Silva Nogueira C. Biochemical profile of the Pêga donkey (*Equus asinus*) breed: influence of age and sex. Comp Clin Pathol 2014; 23(4): 941-7.

[26] Laus F, Spaterna A, Faillace V, Paggi E, Serri E, Vullo C, Cerquetella M, Tesei B. Reference values for hematological and biochemical parameters of mixed breed donkeys *(Equus asinus).*Wulfenia J 2015; 22(1): 294-304.

[27] Sgorbini M, Bonelli F, Rota A, Baragli P, Marchetti V, Corazza M. Hematology and Clinical Chemistry in Amiata Donkey Foals from Birth to 2 Months of Age. J Equine Vet Sci 2013; 33(1): 35-9.

[28] Veronesi MC, Gloria A, Panzani S, Sfirro MP, Carluccio A. Blood analysis in newborn donkeys: hematology, biochemistry and blood gases analysis. Theriogenol 2014; 82(2): 294-303.

[29] Harvey JW, Pate M, Kivipelto J, Asquisth R. Clinical biochemistry of pregnant and nursing mares. Vet Clin Pathol 2005; 3(3): 248-54.

[30] Larsson A, Palm M, Hansson LO, Axelsson O. Reference values for clinical chemistry test during normal pregnancy. Int J Obstet Gynaecol 2008; 115: 874-881.

[31] Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr 71:1256–1.

[32] Hafez SA, Freeman LE, Caceci T, Smith BJ. Study of the vasculature of the caprine reproductive organs using the tissue clearing technique, with special reference to the angioarchitecture of the utero ovarian vessels and the adaptation of the ovarian and/or vaginal arteries to multiple pregnancies. Anat Rec 2007; 290: 389-405.

[33] Satué K, Montesinos P. Plasma biochemistry profile in pregnant Spanish purebred broodmares. Comp Clin Pathol 2013; 22: 113-7.

[34] Hansen MF, Todd, AC, McGee WR. Blood picture of lactating and non lactating Throughbred mares. Vet Med 1950; 45: 228-30.

[35] Meuten DJ, Kociba G, Therelfall WR, Nagode LA. Serum alkaline phosphatase in pregnant mares. Vet Clin Path, 1980; 9: 27-30.

[36] Gill J, Kompanowska-Jezierska E. Seasonal changes in the red blood cell indices in Arabian brood mares and their foals. Comp biochem physiol 1986; 83(4): 643-51.

[37] Harvey JW, Asquith RL, Pate MG, Kivipelto J, Chen CL, Ott EA. Haematological findings in pregnant, postparturient and nursing mare. Comp Haematol Int 1994; 4: 25-9.

[38] Taylor-Macallister C, Macallister CG, Walker D, Aalseth D. Haematology and serum biochemistry evaluation in normal postpartum mares. Equine vet J 1997; 29 (3): 234-5.

[39] Hoffman RM, Kronfeld DS, Cooper WL, Harris P. Glucose clearance in grazing pregnant mares is affected by diet, pregnancy and lactation. J Anim Sci 2003; 81:1764-71.

[40] Berlin D, Aroch I. Concentrations of ionized and total magnesium and calcium in healthy horses: Effects of age, pregnancy, lactation, pH and sample type. Vet J 2009; 181: 305-11.

[41] Aoki T, Ishii M. Hematological and biochemical profiles in peripartum mares and neonatal foals (Heavy draft horses). J Equine Vet Sci 2012; 32: 170-6.

[42] Mariella J, Pirrone A, Gentilini F, Castagnetti C. Hematological and biochemical profiles in Standardbred mares during peripartum. Theriogenol 2014; 81(4): 526-34.

[43] Crisci A, Rota A, Panzani D, Sgorbini M, Ousey JC, Camillo F. Clinical, ultrasonographic, and endocrinological studies on donkey pregnancy. Theriogenol 2014; 81: 275-83.

[44] Available at: http://www.aaep.org/vaccination_guidelines.htm. Accessed April 10, 2010.

[45] Gurgoze S, Icen H. The influence of age on clinical biochemical parameters in pure-bred Arabian mares. J Equine Vet Sci 2010; 30(10): 569-574.

[46] Bailit JL, Daty E, Jodie W. Repeated hematocrit measurements in low-risk pregnant women. J Reprod Med 2007; 52(7): 619-22.

[47] Allard RL, Carlos AD, Faltin EC. Canine hematological changes during gestation and lactation. Compan Anim PRact 1989; 19: 3-6.

[48] Berman E. Hemograms of the cat during pregnancy and lactation and after lactation. Am J Vet Res 1974; 35: 457-60.

[49] Wells MY, Decobecq CP, Decouvelaere DM, Justice C, Guittin P. Changes in clinical pathology parameters during gestation in the New Zealand white rabbit. Toxicol Pathol 1999; 27: 370-9.

[50] Blanchard TL, Orsini JA, Garcia MC, Elmore RG, Youngquist RS, Bierschwal CJ. Influence of dystocia on white blood cell and blood neutrophil counts in mares. Theriogenol 1986; 25: 347-52.

[51] Evans JW. Effect of fasting, gestation, lactation and exercise on glucose turnover in horses. J ANim Sci 1071; 33: 1001-4.

[52] Johnson CA. Glucose homeostasis during canine pregnancy: insulin resistance, ketosis, and hypoglycemia. Theriogenol 2008; 70(9): 1418-23.

[53] Dahlgren J. Pregnancy and insulin resistance. Metab Syndr Relat Disord 2006; 4(2): 149-52.

[54] Watson TD, Burns L, Packard CJ, Sheperd J. Effect of pregnancy and lactation on plasma lipid and lipoprotein concentrations, lipoprotein composition and post-heparin lipase activities in Shetland pony mares. J Reprod Fertil 1993; 97: 563-8.

[55] Davies Morel MC. Equine reproductive physiology, breeding and stud management. Ipswich, England: Farming Press Books; 1993.

[56] Sultana MS, Begum R, Akhter KS, Lovely NS, Akhter S, Islam MT. Serum calcium and phosphate level in normal pregnant women. Bangladesh Journal of Medical Science; 2012; 11(3): 217-20.

[57] Mansmann, HC Jr. Phosphorus, Calcium and Magnesium Interactions. http://barttersite.org/phosphorus-calcium-and-magnesium-interactions/

	T-8w	T-6w	T-4w	T-2w	T0	T2w	T4w	T6w	T8w	
RBC M/µL	5.7±0.3^{ab}	6.1±0.4^{a}	6.4±0.6^{a}	6.3±1.1^{a}	5.5±0.6^{b}	6.1±1.2^{a}	6.6±1.5^{a}	5.6±0.6^{ab}	5.7±0.6^{ab}	P=0.02
WBC K/µL	10.9±0.8^{a}	9.3±3.7^{a}	11.9±1.5^{ab}	11.9±1.7^{ab}	12.8±1.9^{b}	12.1±2.7^{b}	10.9±1.7^{a}	10.8±1.9^{a}	10.0±1.9^{a}	P=0.03
Hgb gr/L	10.7±2.8	11.1±2.3	12.1±5.6	12.7±2.0	11.3±0.9	12.1±2.1	13.2±2.9	11.7±1.7	11.8±1.3	NS
Hct %	33.2±1.1a	30.5±5.4^{a}	40.1±12.2^{a}	35.7±5.9^{ab}	31.5±3.2^{ab}	33.7±6.5^{ab}	37.2±7.7^{ab}	32.7±5.5^{ab}	30.4±3.7^{b}	P=0.02
MCV fl	55.6±2.3	53.6±4.8	56.0±2.7	56.1±2.7	56.9±3.2	55.1±3.6	54.1±4.9	55.0±2.1	54.1±1.6	NS
MCH pg	20.1±0.9	20.0±0.9	19.2±1.8	20.1±1.0	20.4±0.7	19.9±1.2	20.2±0.8	20.0±2.3	20.6±0.8	NS
MCH C gr/dl	35.7±1.0	34.5±3.2	33.7±3.0	36.0±1.3	36.1±1.5	36.0±0.9	36.6±2.1	38.3±2.2	38.5±1.6	NS
PLT K/µL	252.0±29.3^{a}	245.4±35.2^{a}	286.3±77.4^{a}	203.7±55.0^{b}	238.3±48.7^{b}	223.0±44.9^{b}	212.6±47.6^{b}	244.3±90.1^{ab}	211.3±50.3^{b}	P=0.04
n=	18	18	18	18	18	16	16	16	16	

Table 1. Haematological parameters expressed as mean± standard deviation in 9 jennies from 2 months before delivery up to 2 months after delivery, for a total of 18 pregnancies and 16 foals born.

Legend - T-8w: 8 weeks before presumptive delivery; T-6w: 6 weeks before presumptive delivery; T-4w: 2 weeks before presumptive delivery; T-2w: 2 weeks before presumptive delivery; T0: delivery; T2w: 2 weeks after delivery; T4w: 4 weeks; T4w: 4 weeks after delivery; T6w: 6 weeks after delivery; T8w: 8 weeks after delivery. Within row, different superscripts denote a significant difference ($a \neq ab \neq b$: $P<0.05$).

	T-8w	T-6w	T-4w	T-2w	T0	T2w	T4w	T6w	T8w	
Glucose mg/dl	91.0±9.0	92.0±20.2	78.0±28.2	77.8±18.4	98.1±22.9	79.05±9.7	87.5±32.5	82.0±9.1	78.5±8.9	NS
Creatinine mg/dl	1.2±0.1	1.3±0.2	1.2±0.1	1.2±0.3	1.4±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.2±0.2	NS
Urea mg/dl	25.3±2.7[a]	27.7±8.1[ab]	23.3±5.7[a]	35.2±9.6[b]	31.3±14.1[ab]	37.6±8.6[b]	33.7±6.8[b]	30.1±7.4[ab]	38.3±9.5[b]	P=0.0006
Triglyceride mg/dl	90.6±16.0[a]	122.8±52.8[a]	103.7±49.3[a]	129.9±41.9[a]	91.0±46.2[ab]	40.2±23.0[ab]	52.3±12.1[b]	50.7±24.5[b]	41.2±25.7[b]	P=0.0001
Total cholesterol mg/dl	69.0±11.6[a]	71.4±5.7[a]	79.2±13.0[a]	77.6±15.5[a]	94.2±43.0[ab]	74.0±15.0[abc]	65.0±18.7[abc]	57.2±7.0[c]	64.3±5.7[a]	P=0.003
Total bilirubin mg/dl	0.2±0.1	0.2±0.003	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.04	0.2±0.02	0.2±0.05	0.2±0.04	NS
AST U/L	82.9±26.8[a]	75.3±21.8[a]	91.1±38.0[ab]	120.2±31.7[ab]	114.0±31.2[ab]	128.0±16.8[b]	101.6±34.5[ab]	128.5±22.8[b]	118.2±36.0[ab]	P=0.002
GGT U/L	38.6±11.4[a]	41.4±17.9[a]	31.5±15.4[a]	20.2±12.3[a] [b]	24.0±12.0[a] [b]	19.5±5.5a[b]	11.4±5.0[b]	18.0±12.4[b]	19.1±10.8[b]	P=0.001
CK U/L	60.6±13.1[a]	40.3±9.0[a]	44.7±12.8[ab]	39.0±22.7[b]	40.2±14.0[b]	43.9±17.6[b]	41.8±15.6[b]	30.5±12.9[b]	38.0±14.7[b]	P=0.003
ALP U/L	168.6±27.0	205.0±73.0	233.9±47.0	179.2±54.4	215.7±35.3	226.8±44.4	211.3±90.5	234.4±78.4	184.7±32.4	NS
Total Ca mg/dl	11.4±1.1	11.1±1.2	11.2±0.8	11.2±2.0	12.0±1.2	10.5±1.6	10.8±2.5	11.5±1.4	11.0±1.4	NS
P mg/dl	3.7±0.5[a]	3.1±0.3[a]	3.4±0.6[a]	3.3±0.6[a]	3.5±0.9[ab]	3.6 ±1.4[ab]	3.4±0.4[ab]	3.8±1.3[ab]	3.1±0.2[b]	P=0.009
Na^+ mmol/L	134.9±7.1[a]	135.1±6.9[a]	135.9±6.2[a]	136.9±5.8[a]	138.3±2.5[a]	136.3±4.9[ab]	134.2±4.4[ab]	136.1±4.2[ab]	132.6±4.1[b]	P=0.02
K^+ mmol/L	4.3±0.5[a]	4.2±0.6[a]	4.2±0.4[a]	4.5±0.6[ab]	4.3±0.3[ab]	4.0±0.6[ab]	4.6±0.5[abc]	4.3±0.4[abc]	3.7±0.5bc	P=0.03
PT gr/L	8.2±0.4[a]	8.3±0.6[ab]	7.4±0.6[b]	7.1±2.0[ab]	7.7±0.5[ab]	7.4±0.5[abc]	7.1±0.4[c]	6.9±0.9[c]	7.3±0.7[bc]	P=0.0002
Albumin gr/L	2.8±0.2	2.5±0.2	2.4±0.6	3.0±1.2	2.8±0.5	2.8±0.5	2.5±0.7	3.0±0.5	3.3±0.7	NS
n=	18	18	18	18	18	16	16	16	16	

Table 2. Clinical chemistry parameters expressed as mean±standard deviation in 9 jennies from 2 months before delivery up to 2 months after delivery, for a total of 18 pregnancies and 16 foals born.

Legend - T-8w: 8 weeks before presumptive delivery; T-6w: 6 weeks before presumptive delivery; T-4w: 2 weeks before presumptive delivery; T-2w: 2 weeks before presumptive delivery; T0: delivery; T2w: 2 weeks after delivery; T4w: 4 weeks; T4w: 4 weeks after delivery; T6w: 6 weeks after delivery; T8w: 8 weeks after delivery. Within row, different superscripts denote a significant difference (a≠ab≠abc≠b≠c: $P<0.05$).