DOI: 10.5965/223811711732018377

Revista de Ciências Agroveterinárias 17(3): 2018
Universidade do Estado de Santa Catarina

Pregnancy modulates serum proteins, hematological and blood biochemical variables of the Pêga donkey

Prenhez modula as proteínas séricas, variáveis hematológicas e bioquímicas sanguíneas do jumento Pêga

Annita Morais Girardi*, Carmen Zilda Pereira de Toledo, Renata Lemos Nagib Jorge, José Jurandir Fagliari, Amanda Festa Sabes & Luiz Carlos Marques

[1] Universidade Estadual Paulista, Jaboticabal, SP, Brasil. *Autor para correspondência: annitamgirardi@gmail.com.

Submission: 30/04/2017 | *Acceptance: 13/06/2018*

ABSTRACT

Considering the scarcity of information regarding blood parameters of Pêga donkeys (*Equus asinus*), and the influence of factors such as breed and physiological status on these parameters, this study aimed to evaluate the influence of pregnancy on hematological, blood biochemical and serum protein profiles of the Pêga donkey, using samples from 59 female adult donkeys, 22 pregnant and 37 non-pregnant, kept under field conditions. The parameters measured were counts of red blood cells (RBC), white blood cells (WBC), basophils, eosinophils, bands, segmented neutrophils, lymphocytes, monocytes and platelets; and the levels of packed cell volume, hemoglobin concentration, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatine kinase, gamma-glutamyl transferase, urea, glucose, cholesterol, triglycerides, total calcium, phosphorus, magnesium, chlorides, sodium, potassium, ionized calcium, creatinine, total protein, albumin, total, direct and indirect serum bilirubin. Serum protein fractionation was performed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), which found 10 proteins: immunoglobulins A and G, ceruloplasmin, transferrin, albumin, haptoglobin, α_1-acid glycoprotein, 138 kDa (MWP_{138}), 33 kDa (MWP_{33}) and 23 kDa (MWP_{23}) molecular weight proteins. RBC and triglycerides were higher in pregnant females. Urea, Immunoglobulin A, MWP_{138} and MWP_{23} concentrations were higher in non-pregnant mares. This study presented differences from previous studies, and some differences in blood variables between pregnant and non-pregnant Pêga donkeys, confirming that pregnancy causes significant metabolic changes, which must be considered in the clinical evaluation of these animals.

KEYWORDS: acute phase protein, biochemistry, electrophoresis, Equidae, gestation, hematology.

RESUMO

Considerando a escassez de informações sobre as variáveis hematológicas dos jumentos Pêga e a influência de fatores como raça e estado fisiológico sobre estes parâmetros, este estudo teve como objetivo avaliar a influência da prenhez sobre os perfis hematológico, bioquímico sanguíneo e de proteínas séricas de jumentos Pêga, utilizando amostras de 59 jumentas adultas, 22 prenhes e 37 não prenhes, mantidas sob condições de campo. Foram mensuradas as contagens de hemácias, leucócitos, basófilos, eosinófilos, neutrófilos bastonetes, neutrófilos segmentados, linfócitos, monócitos e plaquetas; volume globular, concentração de hemoglobina e os níveis de alanina aminotransferase, aspartato aminotransferase, fosfatase alcalina, creatina quinase, gama glutamil transferase, ureia, glicose, colesterol, triglicérides, cálcio total, fósforo, magnésio, cloretos, sódio, potássio, cálcio ionizado, creatinina, proteína total, albumina, bilirrubina total, direta e indireta. O fracionamento de proteínas séricas foi realizado pela eletroforese em gel de acrilamida contendo dodecil sulfato de sódio (SDS-PAGE), o qual revelou 10 proteínas: imunoglobulinas A e G, ceruloplasmina, transferrina, albumina, haptoglobina, α_1-glicoproteína ácida, proteína de peso molecular 138 kDa (PPM_{138}), 33 kDa (PPM_{33}) e 23 kDa (PPM_{23}). A contagem de eritrócitos e os triglicérides séricos foram mais altos para as fêmeas prenhes. As concentrações de ureia, IgA, PPM_{138}e PPM_{23} foram mais altas para jumentas não prenhes. Houve diferenças em relação a estudos anteriores, e diferenças entre as variáveis sanguíneas de jumentas Pêga prenhes e vazias confirmam que a prenhez causa mudanças metabólicas significantes, as quais devem ser consideradas na avaliação clínica destes animais.

PALAVRAS-CHAVE: proteínas de fase aguda, bioquímica, eletroforese, Equidae, gestação, hematologia.

INTRODUCTION

The Pêga donkey (*Equus asinus*) is a breed from Iberian origin and has been bred in Brazil since the 18th century, due to requirements for the production of resistant and comfortable mules, being popular in some other South American countries (CANISSO & MCDONNELL 2010).

Changes in hematological parameters for donkey breeds and populations can be associated with age, sex, exercise, geographical and nutritional factors (MORI et al. 2004). Metabolic functions increase during pregnancy to satisfy demands from the activities of the fetus and placenta (FAZIO et al. 2011), resulting in alterations of physiological variables (GRAVENA et al. 2010). The knowledge about these changes can be very useful for handling and treating equine females (GRAVENA et al. 2010), and values obtained from them can help in the assessment of their health status and peripartum diseases (BONELLI et al. 2016).

Considering the few studies on blood variables of Pêga donkeys, and the influence of factors such as age and sex on these parameters (CAMPOS et al. 1968, GIRARDI et al. 2014, GIRARDI et al. 2015, GIRARDI et al. 2016, GIRARDI et al. 2017), the objective of this study was to evaluate differences in the hematological and blood biochemical parameters and serum protein levels of pregnant and non-pregnant Pêga donkeys..

MATERIAL AND METHODS

Blood samples were collected from 59 adult (over three years old) female donkeys, 22 pregnant and 37 non-pregnant, at several stages of pregnancy (5 ± 3 months, diagnosed by reproductive history, rectal palpation and confirmed by the timing of parturition), maintained under field conditions, and bred for reproductive purposes in a donkey herd located in the municipality of Orlândia, São Paulo, Brazil. Animals were fed on mixed pasture (*Cynodon dactylon* cv. Coast-cross and cv. Tifton, *Panicum maximum*, *Brachiaria humidicola* and *Hyparrhenia rufa*) *ad libitum*, supplying 1% body weight per day of concentrate feed (80% ground corn and 20% soybean meal), mineral mixture (Guabiphós Centauro 80, Guabi Nutrição e Saúde Animal S.A., Sales Oliveira, Brazil) and salt *ad libitum*.

Sampling was performed in the morning, in non-fasting animals, under mechanical restraint, using a closed evacuated system (BD Vacutainer, BD Diagnostics – Preanalytical Systems, São Paulo, Brazil), with 4 mL plastic tubes with anticoagulant (K_2EDTA 7.2 mg for complete blood count; NaF 6 mg + Na_2EDTA 12 mg to obtain plasma for glucose measurement), and 10 mL plain tubes to obtain serum for biochemical analysis and serum protein electrophoresis. Collection was achieved via external jugular venipuncture after proper regional antisepsis. Samples were homogenized after collection and packed in a cooler with reusable ice packs for temporary storage and transportation by car to the laboratory, which lasted about two hours. When the samples for complete blood count could not be immediately processed, they were stored at 4°C for a maximum of 12 hours (JAIN 1993). Serum and plasma fractions were separated by centrifugation at 2500 g for 10 minutes, within three hours after collection. Serum samples were stored at -20ºC, in plastic sterile microtubes, for a few days before analysis. Plasma samples were analyzed on the collection day. Each animal was sampled once, from September 2009 to May 2011. The analyses were held at the Department of Veterinary Clinic and Surgery, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Unesp), Jaboticabal, São Paulo, Brazil.

Counts of red blood cells (RBC – $x10^{12}$/L), white blood cells (WBC – $x10^9$/L), platelets ($x10^9$/L), packed cell volume (PCV – L/L) and hemoglobin concentration (Hb – g/L) were measured using an automated veterinary hematology analyzer (ABXVET, Horiba ABX, Montpellier, Hérault, France). Differential leukocyte count (basophils, eosinophils, bands, segmented neutrophils, lymphocytes and monocytes – $x10^9$/L) was performed by blood smear analysis, stained by a modified Rosenfeld method (1947), using light microscopy (Biological Microscope Eclipse E200, Nikon Corporation, Tokyo, Japan).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), gamma-glutamyl transferase (GGT – U/L); urea, glucose, cholesterol, triglycerides, total calcium, phosphorus, magnesium, chlorides (mmol/L); creatinine, total and direct bilirubin (µmol/L); total protein (TP) and albumin (g/L) values were measured using a semi-automatic spectrophotometer (LabQuest, LabTest, Lagoa Santa, Brazil). Sodium, potassium and ionized calcium (mmol/L) levels were determined by an electrolyte analyzer (Roche 9180 Electrolyte Analyzer, Roche, São Paulo, Brazil). Indirect bilirubin values (µmol/L) were obtained by subtracting direct bilirubin levels from total bilirubin values.

As previously described (GIRARDI et al. 2016, GIRARDI et al. 2017), the serum protein fractionation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed based on the technique of LAEMMLI (1970). The percentage of each protein was determined using a computerized scanning densitometer (Shimadzu CS 9301, Tokyo, Japan), with a marker solution (SigmaMarker wide

range, Sigma-Aldrich, St Louis, USA) considering 6.5 to 200 kDa molecular weights as reference. Serum protein fraction concentrations (g/L) were determined by multiplying the percentage of each fraction by TP concentrations obtained through the semi-automatic spectrophotometer, using the biuret method.

Data analysis was performed using a statistical software (GraphPad InStat version 3.10, 32 bit for Windows, GraphPad Software Inc., San Diego, California, USA). Each parameter was tested for normality applying the Kolmogorov-Smirnov test. Values were given as mean ± standard deviation (SD), considering a 95% confidence interval. Unpaired t-tests were used to determine the effect of pregnancy on variables for normally distributed data; for non-normally distributed data, non-parametric tests (Mann-Whitney U test) were performed. Unpaired t-tests were used to compare the results of this study to results from other studies with donkeys. Dixon-Reed and Tukey's tests were performed to help in the identification of additional outliers, using a set of macroinstructions (Reference Value Advisor V 1.4) (GEFFRE et al. 2011) for spreadsheets (Microsoft Excel, Microsoft, Redmond, Washington, USA). The statistical significance level was set at $P<0.05$.

This study was approved by the Research Ethics Committee in Use of Animals of the Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Unesp), protocol no. 6369/10.

RESULTS AND DISCUSSION

The results of hematological variables, biochemical patterns and serum protein fractions of pregnant and non-pregnant Pêga donkeys are shown in Tables 1 to 3. Only ALT activity, basophil and band counts did not have Gaussian distribution for both groups.

RBC values were higher for pregnant donkeys (Table 1), as observed by GRAVENA et al. (2010), possibly due to body response to greater oxygen demand during pregnancy (SOUZA et al. 2002). GRAVENA et al. (2010) also observed that there was no difference between the PCV of pregnant and non-pregnant females. However, disagreeing with these authors, whom observed higher Hb levels for donkeys over 210 days of gestation when compared to non-pregnant females or in previous stages of pregnancy, this study did not observe differences for this variable, possibly because animals over 210 days of pregnancy were a minority in the pregnant group (six animals).

Table 1. Hematological variables of adult female Pêga donkeys (*Equus asinus*), subtyped by pregnancy status.

Variable	Non pregnant (n=22)	Pregnant (n=37)
Red blood cells - RBC ($x10^{12}$/L)	5.54 ± 0.63*/ 5.26 - 5.82	5.96 ± 0.79*/ 5.69 - 6.23
White blood cells - WBC ($x10^9$/L)	10.41 ± 1.63/ 9.68 - 11.13	10.44 ± 1.98/ 9.78 - 11.10
Hemoglobin (g/L)	106.8 ± 11.7/ 101.6 - 112.0	113.2 ± 14.6/ 108.3 - 118.0
Packed cell volume - PCV (L/L)	0.352 ± 0.037/0.335 -0.368	0.370 ± 0.046/0.354 - 0.385
Basophils ($x10^9$/L)†	0.00 ± 0.04/ 0.00 - 0.03	0.00 ± 0.04/ 0.00 - 0.03
Eosinophils ($x10^9$/L)	0.61 ± 0.44/ 0.41 - 0.80	0.51 ± 0.30/ 0.41 - 0.61
Band neutrophils ($x10^9$/L)†	0.00 ± 0.09/ 0.01 - 0.09	0.08 ± 0.07/ 0.04 - 0.09
Segmented neutrophils ($x10^9$/L)	5.33 ± 1.44/ 4.69 - 5.96	4.90 ± 1.20/ 4.50 - 5.30
Lymphocytes ($x10^9$/L)	4.17 ± 1.39 / 3.55 - 4.78	4.61 ± 1.42/ 4.14 - 5.08
Monocytes ($x10^9$/L)	0.25 ± 0.14/ 0.19 - 0.31	0.27 ± 0.14/ 0.22 - 0.32
Platelets ($x10^9$/L)	329 ± 98/ 285 - 372	329 ± 94/ 298 - 361

Values are given as mean ± SD (standard deviation) / 95% confidence limits.
† Values are given as median ± SD (standard deviation) / 95% confidence limits.
Analyzed sample size between parentheses. Means followed by asterisks in rows differ significantly (*p<0.05).

Serum triglycerides level was higher for pregnant donkeys (Table 2). Several maternal endocrine and metabolic adjustments change the use of nutrients to meet the progressive energy requirements caused by gestation and lactation, resulting in changes on lipid metabolism, possibly facilitated by gestational alterations in oestradiol and progesterone concentrations, and making peripartum females vulnerable to the development of hyperlipidaemia (FORHEAD et al. 1994).

Urea serum levels were lower for pregnant animals (Table 2), as also described by FAZIO et al. (2011), who explains this finding by possible decreases in muscle catabolism during pregnancy, reflecting a difference in protein metabolism associated with the presence of the fetus.

WBC, segmented neutrophil, lymphocyte and monocyte counts were higher ($P<0.01$); while hemoglobin concentration ($P<0.01$) and eosinophil count ($P<0.05$) were shown to be lower, for both groups, than the results of GRAVENA et al. (2010). In addition, pregnant donkeys showed lower basophil and higher band counts ($P<0.01$) than those in GRAVENA et al. (2010). Both groups had higher ($P<0.01$) intervals of

serum creatinine, and pregnant donkeys had lower TP and urea (P<0.01) and greater ALT activity (P<0.01) than those described by FAZIO et al. (2011). Since the authors cited above used the same analysis methods as this study, the observed differences probably reflect breed and environmental effects on hematological and blood biochemical values for donkeys.

Table 2. Blood biochemical profile of female adult Pêga donkeys (*Equus asinus*), subtyped by pregnancy status.

Variable	Non pregnant (n=22)	Pregnant (n=37)
ALT (U/L) †	10.48 ± 4.72 / 11.72 - 15.91	15.71 ± 3.08 / 12.85 - 14.90
AST (U/L)	286.17 ± 69.96 / 255.15 - 317.20	274.12 ± 74.91 / 249.13 - 299.12
ALP(U/L)	204.67 ± 58.14 / 178.88 - 230.45	179.66 ± 42.05 / 165.42 - 193.90
GGT (U/L)	61.20 ± 22.90 / 51.05 - 71.36	71.91 ± 17.09 / 63.91 - 79.91
CK (U/L)	247.00 ± 154.00 / 178.71 - 315.29	256.43 ± 147.03 / 207.38 - 305.49
Creatinine (µmol/L)	129.95 ± 15.91 / 122.88 - 137.02	138.79 ± 17.68 / 133.48 - 144.98
Urea (mmol/L)	5.15± 1.83** / 4.34 - 5.96	3.90 ± 1.15** / 3.47 - 4.33
Glucose (mmol/L)	3.95 ± 0.54 / 3.59 - 4.32	3.73 ± 0.25 / 3.59 - 3.88
Cholesterol (mmol/L)	1.75± 0.28 / 1.62 - 1.87	1.76± 0.21 / 1.69 - 1.83
Triglycerides (mmol/L)	0.41 ± 0.17** / 0.33 - 0.48	0.78 ± 0.33** / 0.66 - 0.89
Total protein (g/L)	76.2 ±8.1 / 72.6 - 79.8	74.7 ±6.9 / 72.4 - 77.0
Albumin (g/L)	21.3 ± 1.8 / 20.5 - 22.1	21.5 ± 3.1/20.5 - 22.6
Direct bilirubin (µmol/L)	2.05 ± 0.82 / 1.71 - 2.39	2.05 ± 0.86 / 1.71 - 2.22
Indirect bilirubin (µmol/L)	4.45 ± 2.05 / 3.42 - 5.30	3.93 ± 2.05 / 3.25 - 4.62
Total bilirubin (µmol/L)	6.50 ± 2.74 / 5.30 - 7.70	7.18 ± 2.57 / 6.16 - 8.04
Total calcium (mmol/L)	3.25 ± 0.19 / 3.16-3.33	3.27 ± 0.23 / 3.19 - 3.35
Phosphorus (mmol/L)	1.47 ± 0.37 / 1.30 - 1.63	1.41 ± 0.27 / 1.31 - 1.50
Magnesium (mmol/L)	0.75 ± 0.13/0.69 - 0.81	0.79 ± 0.12 / 0.74 - 0.82
Sodium (mmol/L)	143.95 ± 13.47 / 137.45 - 150.44	149.90 ± 12.63 / 144.15 - 155.66
Potassium (mmol/L)	4.85 ± 0.63 / 4.56 - 5.14	5.11 ± 0.41 / 4.96 - 5.26
Chlorides (mmol/L)	106.54 ± 10.51 / 101.88 - 111.20	108.15 ± 4.31 / 106.44 - 109.85
Ionized calcium (mmol/L)	1.29 ± 0.16 / 1.22 - 1.37	1.29 ± 0.13 / 1.24 - 1.33

ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyl transferase, CK – creatine kinase.

Values are given as mean ± SD (standard deviation) / 95% confidence limits.

† Values are given as median ± SD (standard deviation) / 95% confidence limits.

Analyzed sample size between parentheses. Means followed by asterisks in rows differ significantly (**p<0.01).

As previously reported for donkeys, PCV, WBC, basophils, bands, segmented neutrophils, lymphocytes, monocytes (GRAVENA et al. 2010), TP, ALT and AST (FAZIO et al. 2011) levels were similar between pregnant and non-pregnant animals.

As reported by GIRARDI et al. (2016) and GIRARDI et al. (2017), ten protein fractions were separated by electrophoresis: immunoglobulin A (IgA – 155 kDa), 138 kDa molecular weight protein (MWP_{138}), ceruloplasmin (113 kDa), transferrin (80 kDa), albumin (62 kDa), immunoglobulin G (IgG – heavy chain with 55 kDa and light chain with 26 kDa), haptoglobin (42 kDa), α_1-acid glycoprotein (39 kDa), 33 kDa molecular weight protein (MWP_{33}) and 23 kDa molecular weight protein (MWP_{23}).

Non-pregnant females showed higher IgA, MWP_{138} and MWP_{23} levels (Tabl e 3) than pregnant animals. This finding reinforces the need for more investigations about the three unknown proteins identified, which are not described in scientific literature and showed higher concentrations than many other proteins. There is a demand for new acute phase proteins and their mediators in veterinary science and intensive research about their applications in the innate immunity (MURATA et al. 2004).

Similar to reports of VERONESI et al. (2014), IgA concentrations in donkey maternal serum were lower than one-tenth when compared to IgG, confirming that IgA is found in very low concentrations in the serum of donkeys. Since immunoglobulins are γ-globulins (ECKERSALL 2008), the IgA behavior in this study reinforces the observation of lower γ-globulin values in pregnant donkeys and the linear decrease of this variable during pregnancy (GACEK et al. 1977, PERDIGÃO DE OLIVEIRA et al. 1983). Lower IgA levels for pregnant donkeys cannot be related to immunoglobulin transfer from mother blood to fetus via placenta, since Pêga donkeys have epitheliochorial placentation (TOLEDO et al. 2015), and this type of placenta impedes the transfer of antibodies to the fetus during gestation (GIGUÈRE & POLKES 2005). Moreover, in mares, there is no correlation of immunoglobulin concentrations between serum and uterine fluid (TUNÓN et al. 1998). In humans, the IgA reduction was related to a suppression of the humoral immune system during

pregnancy (AMINO et al. 1978, YASUHARA et al. 1992), a fact that may explain the observations of this study, considering that increases in circulating sex steroid hormones levels in all mammalian species during pregnancy can modify immune responsiveness and may prevent the maternal-fetal rejection response (GROSSMAN 1985). Although reduction of IgG levels during pregnancy was expected, as observed for humans (AMINO et al. 1978, YASUHARA et al. 1992), this study found no differences between the groups for this variable. To our knowledge, serum IgG levels of pregnant donkeys had not been evaluated, previously, separately from other proteins of the γ-globulin fraction, which justifies future research in this regard.

Table 3. Serum protein fractions of adult Pêga donkeys (*Equus asinus*), subtyped by pregnancy status, ordered by decreasing molecular weight.

Serum protein fraction (g/L)	Non pregnant (n=22)	Pregnant (n=37)
Immunoglobulin A	1.49 ±0.77* / 1.15 - 1.82	1.10 ±0.31* /0.99 - 1.22
MWP_{138}	0.66 ± 0.36** /0.48 -0.83	0.37 ± 0.15** /0.32 - 0.43
Ceruloplasmin	0.11 ± 0.08 /0.07 -0.14	0.10 ± 0.06 /0.07 -0.12
Transferrin	3.89 ± 0.97 / 3.46 - 4.32	3.87 ± 0.94 / 3.56 - 4.19
Albumin	43.41 ± 5.98 / 40.76 - 46.06	42.62 ± 5.61 / 40.75 - 44.50
Immunoglobulin G	20.77 ± 4.89 / 18.60 - 22.93	21.27 ± 4.05 / 19.92 - 22.62
Haptoglobin	0.59 ± 0.25 /0.47 -0.70	0.63 ± 0.30 /0.52 -0.73
α1-acid glycoprotein	0.16 ± 0.05 /0.13 -0.18	0.16 ± 0.07 /0.14 -0.19
MWP_{33}	0.19 ± 0.15 /0.13 -0.26	0.15 ± 0.12 /0.11 -0.19
MWP_{23}	3.96 ± 0.99** / 3.52 - 4.41	3.30 ± 0.74** / 3.05 - 3.55

MWP_{138}, MWP_{33}, MWP_{23} – 138 kDa, 33 kDa and 23 kDa molecular weight protein, respectively.
Values are given as mean ± SD (standard deviation) / 95% confidence limits.
Analyzed sample size between parentheses. Means followed by asterisks in rows differ significantly (*p<0.05; **p<0.01).

The absence of changes in most protein fractions may occur due to the increased serum globulin synthesis to compensate for the catabolism imposed by the placenta (PERDIGÃO DE OLIVEIRA et al. 1983). The analysis of the influence of pregnancy on serum protein fractions of donkeys, separated by SDS-PAGE, is unprecedented and was proved to be useful for simultaneous evaluation of several serum proteins in a single analysis.

The sample size is a study limitation, being considered small for the establishment of reference intervals for this donkey breed. The impossibility of monitoring the variables at different stages of pregnancy is another limitation. However, considering the scarcity of hematological and biochemical studies about pregnant donkeys, this research is pioneer in describing differences several variables between pregnant and non-pregnant female Pêga donkeys, particularly regarding the electrophoretic separation of serum proteins by the SDS-PAGE method. Therefore, these results serve as basis for further studies in this and other donkey breeds.

CONCLUSION

Pregnant and non-pregnant female Pêga donkeys had different RBC, triglycerides, urea, IgA, MWP_{138} and MWP_{23} levels, confirming that pregnancy causes significant metabolic changes, which should be considered in the clinical evaluation of these animals.

ACKNOWLEDGEMENTS

This research was funded by São Paulo Research Foundation (FAPESP), grant #2010/02916-4.

REFERENCES

AMINO N et al. 1978. Changes of serum immunoglobulins IgG, IgA, IgM, and IgE during pregnancy. Obstetrics & Gynecology 52: 415-420.

BONELLI F et al. 2016. Hematological and biochemical findings in pregnant, postfoaling, and lactating jennies. Theriogenology 85: 1233-1238.

CAMPOS JM et al. 1968. Hemograma do jumento Pêga. Arquivos da Escola Superior de Veterinária do Estado de Minas Gerais 20 :55-58.

CANISSO IF & MCDONNELL SM. 2010. Donkey breeding behavior with an emphasis on the Pêga breed. In: MATTHEWS NS &TAYLOR TS. Veterinary Care of Donkeys. Ithaca: International Veterinary Information Service. Nº. A2926.0310. Available at: http://www.ivis.org/advances/Matthews/canisso/chapter.asp?LA=1. Access on: Apr. 30, 2017.

ECKERSALL PD. 2008. Proteins, proteomics, and the dysproteinemias. In: KANEKO JJ et al. Clinical Biochemistry of

Domestic Animals. 6.ed. Boston: Elsevier Academic Press. p.117-155
FAZIO E et al. 2011. Changes in the cortisol and some biochemical patterns of pregnant and barren jennies (*Equus asinus*). Veterinarski Arhiv 81: 563-574.
FORHEAD AJ et al. 1994. Endocrine and metabolic responses to transportation in periparturient donkeys. Animal Reproduction Science 36: 315-327.
GACEK F et al. 1977. Evolution of blood serum globulins as determined by pregnancy in the domestic donkey (*Equus asinus* L.). British Veterinary Journal 133: 548-553.
GEFFRE A et al. 2011. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel.Veterinary Clinical Pathology 40: 107-112.
GIGUÈRE S & POLKES AC. 2005. Immunologic disorders in neonatal foals. Veterinary Clinics of North America: Equine Practice 21: 241-272.
GIRARDI AM et al. 2014. Biochemical profile of the Pêga donkey (*Equus asinus*) breed: influence of age and sex. Comparative Clinical Pathology 23: 941-947.
GIRARDI AM et al. 2015. Hematological variables of the Pêga donkey (*Equus asinus*) breed: influence of age and sex. Comparative Clinical Pathology 24: 335-342.
GIRARDI AM et al. 2016. Serum protein fractions, hematological and biochemical variables of the Pêga donkey (*Equus asinus*) breed in the first year of age. Veterinary Archives 86: 335-349.
GIRARDI AM et al. 2017. The effect of age and sex on serum proteins in the Pega donkey (*Equus asinus*). Veterinarni Medicina 62:10-15.
GRAVENA K et al. 2010. Parâmetros hematológicos de jumentas gestantes em diferentes períodos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 62: 1514-1516.
GROSSMAN CJ. 1985. Interactions between the gonadal steroids and the immune system. Science 227: 257-261.
JAIN NC. 1993. Essentials of veterinary hematology. Philadelphia: Lea & Febiger. 417p.
LAEMMLI U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
MORI E et al. 2004. Reference values on hematologic parametes of the Brazilian donkey (*Equus asinus*) breed. Journal of Equine Veterinary Science 24: 271-276.
MURATA H et al. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. The Veterinary Journal 168: 28-40.
PERDIGÃO DE OLIVEIRA FRA et al. 1983. Perfil das globulinas do soro sangüíneo, durante a gestação, na jumenta. Boletim de Indústria Animal 40: 219-227
ROSENFELD G. 1947. Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grunwald e do Giemsa num só corante de emprego rápido. Memórias do Instituto Butantan 20: 329-335
SOUZA AI et al. 2002. Alterações hematológicas e gravidez. Revista Brasileira de Hematologia e Hemoterapia 24: 29-36.
TOLEDO CZP et al. 2015. Morfologia da placenta a termo e da interface materno-fetal da jumenta Pêga. Brazilian Journal of Veterinary Research and Animal Science 52: 195-204.
TUNÓN AM et al. 1998. Concentrations of total protein, albumin and immunoglobulins in undiluted uterine fluid of gynecologically healthy mares. Theriogenology 50: 821-831.
VERONESI MC et al. 2014. IgG, IgA, and lysozyme in Martina Franca donkey jennies and their foals. Theriogenology 81: 825-831.
YASUHARA M et al. 1992. Reciprocal changes in serum levels of immunoglobulins (IgG, IgA, IgM) and complements (C3, C4) in normal pregnancy and after delivery. Journal of Clinical & Laboratory Immunology 38: 137-141.