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HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN PREGNANT, POSTFOALING
AND LACTATING JENNIES

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19 Abstract

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

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

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

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

51 Key words

52 Jennies, haematology, biochemistry, pregnancy, foaling, lactation.

53

54 1. Introduction

55 Donkeys (*Equus asinus*) have been close companions to humans for millennia and have
56 been used as working animals all over the world. Donkey milk could possibly be used in
57 children with intolerance to cow's milk [1,2] or in animal-assisted therapy [3]. The renewed
58 interest in these animals is demonstrated by the number of studies on establishing the
59 base-line data of both haematological and biochemical variables in the blood of adult [4-
60 26], as well as in newborn donkeys [27-28].

61 Pregnancy and lactation are physiological periods that result in increased metabolic
62 demands. Although homeostatic mechanisms keep substances in the blood at relatively
63 constant levels, some changes in the concentrations of routine clinical chemistry analytes
64 are likely to occur [29]. During pregnancy, an expansion in plasma volume and erythrocyte
65 mass occurs, as well as an increase in plasma protein synthesis [30]. In women, it is well
66 known that during pregnancy reference intervals are different from the non-pregnant state
67 [31-33].

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

75 The aims of this study were to: 1) verify if significant changes occur in hematological and
76 biochemical parameters in jennies during the last two months of pregnancy and the first
77 two months of lactation; and 2) determine any differences with respect to equine species
78

79 2. Material and Methods

80 2.1. Animals

81 The study was conducted on 9 jennies belonging to the Amiata donkey breed for a total of
82 18 pregnancies and 16 foals born during a two-year study. Jennies were from 5 to 13
83 years old, weighed 300 to 350 kg, and were kept in collective paddocks at the Veterinary
84 Teaching Hospital, Department of Veterinary Sciences, Pisa University. Approval for this
85 study was obtained from the Ethical Committee on Animal Experimentation of the
86 University of Pisa, and the protocol was sent to the Ministry of Health.

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

93 Jennies were included in this study according to the following criteria: 1) pregnancy length
94 353.4±13.0 days [43]; 2) unassisted delivery; 3) treatment against gastrointestinal
95 parasites and vaccinated against equine influenza, tetanus, and equine herpes virus-1, in
96 accordance with the guidelines of the American Association of Equine Practitioners
97 Infectious Disease Committee [44].

98

99 2.2. Sample collection and handling

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

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108 2.3. Complete blood count

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

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118 2.4. Biochemical analysis

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

124 The parameters analysed were: 1) glucose concentration (Glucose SL, enzymatic
125 colorimetric method, cod. ASR01202, Assel Srl, Rome, Italy); 2) creatinine (Creatinine,
126 kinetic modified Jaffè method, cod. ASR01150, Assel Srl, Rome, Italy); 3) blood urea
127 (Urea UV SL, kinetic enzymatic method, cod. ASR01143, Assel Srl, Rome, Italy); 4)
128 triglycerides (Triglycerides-SL, enzymatic colorimetric method, cod. ASR01134, Assel Srl,
129 Rome, Italy); 5) total cholesterol (Cholesterol liquid, trinder method-endpoint, cod. 7050,
130 FAR, Verona, Italy); 6) total bilirubin (Total Bilirubin, colorimetric method without DMSO,
131 cod. ASR01034/1, Assel Srl, Rome, Italy); 7) aspartate aminotransferase (AST) (AST SL,
132 kinetic method UV -IFCC- cod. ASR01220, Assel Srl, Rome, Italy); 8) gamma glutamyl
133 transferase (γ GT) (Gamma GT SL, kinetic method-Szasz-Tris, cod. ASR01194, Assel Srl,
134 Rome, Italy); 9) creatine-phosphokinase (CK) (CK NAC SL, kinetic method UV, cod.
135 ASR01074, Assel Srl, Rome, Italy); 10) alkaline phosphatase (ALP) (Alkaline Phosphatase
136 SL-DGKC- kinetic method, cod. ASR01162, Assel Srl, Rome, Italy); 11) total calcium (Tot-
137 Ca) (Calcium OCPC, colorimetric method, cod. ASR01050, Assel Srl, Rome, Italy); 12)
138 potassium (K) (pHox Plus L, Stat Profile, Nova Biochemical, Milan, Italy); 13) sodium (Na)
139 (pHox Plus L, Stat Profile, Nova Biochemical, Milan, Italy); 14) phosphorus (P)
140 (Phosphorus UV, direct method with molibdate, cod. 90009800, Seac-Radim, Florence,
141 Italy); 15) total protein (TP); 16) albumin (Albumin BCG, bromocresol green method, cod.
142 90009781, Seac-Radim, Florence, Italy). The same operator always performed the
143 biochemistry profile, according to standard methods.

144

145 2.5. Statistical analysis

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

153

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 \pm 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.

164 4. Discussion and Conclusions

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

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

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

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

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

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

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

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

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

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

212 The AST activity trend showed an increase near to parturition and early lactation. This
213 might be due to a predominance of anabolic over catabolic processes during pregnancy
214 [55]. Compared to activity values obtained in mares, our results were always higher
215 [38,42].

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

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

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

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

231 lactating jennies. To the authors' knowledge, no data have been reported for P either in
232 jennies or mares, but our results are in line with findings reported for women [56-57].
233 Na decreased during lactation and concentrations were always comparable to donkey
234 reference intervals [21]. The higher Na concentrations during pregnancy might be due to
235 an increased renal Na retention mediated by aldosterone release during pregnancy, as
236 already reported in mares [58]. K showed the same trend as Na and concentrations are in
237 line with this species [21]. The higher K values during pregnancy in jennies may be related
238 to the reabsorption by the gut due to the enlarged pregnant uterus, as suggested for
239 mares [42].

240 The TP trend showed a decreased concentration during the post-partum period and
241 lactation than the gestational period, in contrast with mares in which higher TP
242 concentrations were found in late pregnancy and early lactation compared to parturition
243 [42].

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

251

252 Acknowledgements

253 The authors wish to thank the Regional Stud Centre of Tuscany, Italy. This work was
254 supported by MIUR funds.

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415

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

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

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

425

	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	24.0±12.0 ^a	19.5±5.5 ^a	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±0.5 ^{bc}	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	

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428

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

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

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