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.
Key words
Jennies, haematology, biochemistry, pregnancy, foaling, lactation.
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 Ethic 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).
3. Results

None of the samples collected contained clots or were grossly haemolysed. The results for haematological and biochemical parameters expressed as mean ± standard deviation are reported in Tables 1 and 2, respectively. Not all the jennies were sampled at each time point, thus the actual numbers of samples for each time point are indicated in Tables 1 and 2, respectively. In particular, 1 out of 2 jenny died 1 week after delivery due to colic surgery, and 1 out of 2 was excluded after delivery because her foal died due to sepsis. Statistical analysis showed differences related to sampling times for RBC and HCT, WBC, PLT, total protein, blood urea, triglycerides and total cholesterol 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.
Acknowledgements

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References


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<table>
<thead>
<tr>
<th></th>
<th>T-8w</th>
<th>T-6w</th>
<th>T-4w</th>
<th>T-2w</th>
<th>T0</th>
<th>T2w</th>
<th>T4w</th>
<th>T6w</th>
<th>T8w</th>
<th>P</th>
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<tr>
<td>RBC M/µL</td>
<td>5.7±0.3a</td>
<td>6.1±0.4a</td>
<td>6.4±0.6a</td>
<td>6.3±1.1a</td>
<td>5.5±0.6a</td>
<td>6.1±1.2a</td>
<td>6.6±1.5a</td>
<td>5.6±0.6ab</td>
<td>5.7±0.6ab</td>
<td>P=0.02</td>
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<td>WBC K/µL</td>
<td>10.9±0.8a</td>
<td>9.3±3.7a</td>
<td>11.9±1.5ab</td>
<td>11.9±1.7ab</td>
<td>12.8±1.9ab</td>
<td>12.1±2.7a</td>
<td>10.9±1.7ab</td>
<td>10.8±1.9ab</td>
<td>10.0±1.9ab</td>
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<td>Hgb gr/L</td>
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<td>12.1±5.6</td>
<td>12.7±2.0</td>
<td>11.3±0.9</td>
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<td>11.7±1.7</td>
<td>11.8±1.3</td>
<td>NS</td>
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<tr>
<td>Hct %</td>
<td>33.2±1.1a</td>
<td>30.5±5.4a</td>
<td>40.1±12.2a</td>
<td>35.7±5.9ab</td>
<td>31.5±3.2ab</td>
<td>33.7±6.5ab</td>
<td>37.2±7.7ab</td>
<td>32.7±5.5ab</td>
<td>30.4±3.7ab</td>
<td>P=0.02</td>
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<td>MCV fl</td>
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<td>MCH pg</td>
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<td>19.2±1.8</td>
<td>20.1±1.0</td>
<td>20.4±0.7</td>
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<td>MCHC gr/dl</td>
<td>35.7±1.0</td>
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<td>36.0±1.3</td>
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<td>36.0±0.9</td>
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</tr>
<tr>
<td>PLT K/µL</td>
<td>252.0±29.3</td>
<td>245.4±35.2</td>
<td>286.3±77.4</td>
<td>203.7±55.0</td>
<td>238.3±48.7</td>
<td>223.0±44.9</td>
<td>212.6±47.6</td>
<td>244.3±90.1</td>
<td>211.3±50.3</td>
<td>P=0.04</td>
</tr>
</tbody>
</table>

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≠ab≠b: P<0.05).
**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.**

<table>
<thead>
<tr>
<th>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.</th>
<th>T-8w</th>
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<th>T-2w</th>
<th>T0</th>
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<th>T8w</th>
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<tr>
<td>Glucose mg/dl</td>
<td>91.0±9.0</td>
<td>92.0±20.2</td>
<td>78.0±28.2</td>
<td>77.8±18.4</td>
<td>98.1±22.9</td>
<td>79.05±9.7</td>
<td>87.5±32.5</td>
<td>82.0±9.1</td>
<td>78.5±8.9</td>
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<td>Creatinine mg/dl</td>
<td>1.2±0.1</td>
<td>1.3±0.2</td>
<td>1.2±0.1</td>
<td>1.2±0.3</td>
<td>1.4±0.2</td>
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<td>Urea mg/dl</td>
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<td>27.7±8.1</td>
<td>23.3±5.7</td>
<td>35.2±9.6</td>
<td>31.3±14.1</td>
<td>37.6±8.6</td>
<td>33.7±6.8</td>
<td>30.1±7.4</td>
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<td>Triglyceride mg/dl</td>
<td>90.6±16.0</td>
<td>122.8±52.8</td>
<td>103.7±49.3</td>
<td>129.9±41.9</td>
<td>91.0±46.2</td>
<td>40.2±23.0</td>
<td>52.3±12.1</td>
<td>50.7±24.5</td>
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<td>Total cholesterol mg/dl</td>
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<td>71.4±5.7</td>
<td>79.2±13.0</td>
<td>77.6±15.5</td>
<td>94.2±43.0</td>
<td>74.0±15.0</td>
<td>65.0±18.7</td>
<td>57.2±7.0</td>
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<td>Total bilirubin mg/dl</td>
<td>0.2±0.1</td>
<td>0.2±0.003</td>
<td>0.2±0.1</td>
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<td>75.3±21.8</td>
<td>91.1±38.0</td>
<td>120.2±31.7</td>
<td>114.0±31.2</td>
<td>128.0±16.8</td>
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<td>128.5±22.8</td>
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<td>41.4±17.9</td>
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<td>ALP U/L</td>
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<td>205.0±73.0</td>
<td>233.9±47.0</td>
<td>179.2±54.0</td>
<td>215.7±35.3</td>
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<td>P mg/dl</td>
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<td>3.1±0.3</td>
<td>3.4±0.6</td>
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<td>Na mmol/L</td>
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<td>135.9±6.2</td>
<td>136.9±5.8</td>
<td>138.3±2.5</td>
<td>136.3±4.9</td>
<td>134.2±4.4</td>
<td>136.1±4.2</td>
<td>132.6±4.1</td>
</tr>
<tr>
<td>K mmol/L</td>
<td>4.3±0.5</td>
<td>4.2±0.6</td>
<td>4.2±0.4</td>
<td>4.5±0.6</td>
<td>4.3±0.3</td>
<td>4.0±0.6</td>
<td>4.6±0.5</td>
<td>4.3±0.4</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td>PT g/L</td>
<td>8.2±0.4</td>
<td>8.3±0.6</td>
<td>7.4±0.6</td>
<td>7.1±2.0</td>
<td>7.7±0.5</td>
<td>7.4±0.5</td>
<td>7.1±0.4</td>
<td>6.9±0.9</td>
<td>7.3±0.7</td>
</tr>
<tr>
<td>Albumin g/L</td>
<td>2.8±0.2</td>
<td>2.5±0.2</td>
<td>2.4±0.6</td>
<td>3.0±1.2</td>
<td>2.8±0.5</td>
<td>2.8±0.5</td>
<td>2.5±0.7</td>
<td>3.0±0.5</td>
<td>3.3±0.7</td>
</tr>
</tbody>
</table>

**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).