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Biochemical blood analysis along pregnancy in Martina Franca jennies

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Abstract

The availability of biochemical blood data specific for the pregnant condition are essential for the correct management of both normal pregnancies and also for the prompt recognition of every abnormality. Because of the lacking knowledge about biochemical blood analysis in the donkey along the entire pregnancy, the study was designed to provide first preliminary data about the values and possible changes of blood alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), alkaline phosphatase (ALP), creatine-kinase (CK), blood urea nitrogen (BUN), creatinine (CREA), uric acid (UA), amylase (AMY), gamma-glutamyl transferase (γ-GT),

triglycerides (TRI), cholesterol (CHOL), total protein (TP), albumin (ALB), glucose (GLU), phosphorus (P), calcium (Ca), occurring from the beginning of pregnancy until parturition. The study was performed on 10 Martina Franca healthy jennies with normal pregnancy course and giving birth to mature, healthy and viable foals. Blood samples were collected monthly from the 1st to the 6th month of pregnancy, then twice a month from the 6th to the 9th month and afterwards weekly until parturition. The results showed a significant slight increase of glucose and creatinine in the second quarter of pregnancy and a minor decrease of cholesterol near to parturition, while all the other parameters did not significantly change along pregnancy.

1. Introduction

Pregnancy is a very dynamic physiologic condition in which the female mammals undergoes several changes, mainly related to the adjustments of the genital system, the metabolic changes, and the fetal growth. All the physiologic changes must be well known in order to promptly recognize abnormalities that could impair the health of the pregnant female, of the fetus, or both. Among the wide variety of physiologic changes, the knowledge of the normal biochemical blood changes, as markers of organs activity and efficiency, are required. Because every species is characterized by particular physiologic changes related to pregnancy, specie-specific knowledge are necessary for a correct management of each animals species gestation. In the horse mare, several studies reported the physiological blood biochemical changes during pregnancy [1-5] but, to the authors knowledge, only one study reported the blood biochemical characteristics of the pregnant Amiata jennies, but only related to the last 8 weeks before foaling [6]. Thus a detailed information about the biochemical blood values and changes occurring along the whole donkey pregnancy is lacking.

The donkey has for long time been considered very similar to the horse, but, in spite of some similarities, a number of reproductive different figures between the two species were reported [7-11]. Therefore, also for the donkey, species-specific pregnancy-associated blood biochemical profiles are necessary for providing practical tools for the prompt disturbances diagnosis and management.

Moreover, in the horse, some pregnancy-associated blood biochemical differences were reported in the different breeds, probably because of the different metabolic conditions.

Italian donkeys overall population consists of several breeds, mainly characterized by a marked difference in body size, and used for different purposes, such as onotherapy, milk production, etc..

Among them, the Martina Franca donkey breed has been greatly appreciated in the past for the high stature (135–148 cm of height at the withers in females, and 135-153 cm of height at the withers in males), in comparison to other breeds. At present, this breed, consisting of 68 approved for breeding jackasses and 292 jennies (Food and Agriculture Organization Domestic Animal Diversity Information System, 2014) [12], is considered endangered. Within an endangered population, the exact knowledge about the physiological features of reproduction is essential to allow the preservation program application.

In order to add useful knowledge for a better reproductive management in an endangered Italian donkey breed, the present study was aimed to provide the biochemical blood analysis data during the whole normal pregnancy course in Martina Franca jennies.

2. Material and methods

2.1 Animals

The project was approved by the Committee on Animal Research and Ethics of the Universities of Chieti-Pescara and Teramo (http://www.unich.it/unichieti/appmanager/federati/CEISA), Protocol #45/2013/CEISA/COM, approval date July 16, 2013.

The study was performed on 10 Martina Franca jennies, 4-12 (mean ± SD: 8.9 ± 2.18) years old, 320-380 (mean ± SD: 343 ± 20.03) kg body weight, housed in the Veterinary Teaching Farm, of the University of Teramo, Italy, and fed daily with standard hay *ad libitum* and commercial equine fodder (4 kg). The jennies were healthy, dewormed before breeding, and regularly vaccinated and kept in open paddocks. At the time of the ultrasonographic detection of a follicle greater than 30 mm in size, the jennies were artificially inseminated with semen collected from stallions of proven fertility, every 48 hours, until ovulation. Pregnancy diagnosis was done at 14 days after ovulation, considered as the first day of pregnancy, and confirmed at 45 days after ovulation. The jennies general condition, the pregnancy course, the fetal development and well-being, were fully monitored by routine clinical and ultrasonographic examinations until parturition. The body condition score ranged between 3/5 and 4/5 and remained unchanged along the entire pregnancy.

Jennies were kept in open paddocks for most of the pregnancy and, when the udder enlargement was detected, moved to individual delivery boxes and monitored via a close circuit television system [7,13]. After delivery the foals were immediately evaluated for maturity, health and viability. Foalings were defined as normal and spontaneous, and donkey foals defined as mature, healthy and viable, according to the criteria reported by [14].

2.2 Blood sampling and biochemical analysis

Starting from the first month of pregnancy, blood samplings were performed with the following schedule: monthly until the end of the 6^{th} month of pregnancy, twice a month from the 7^{th} to the end of 9^{th} month of pregnancy, and then weekly until foaling. Blood samples were collected always in the morning, between 8.00 and 10.00 AM, from the jugular vein into plain vacutainer and, after centrifugation at 1500 x g for 10 min, serum was withdrawn and frozen at -80° C until analysis, performed by an automated biochemistry analyzer (Olympus AU 400, Olympus-diagnostic, Hamburg, Germany).

The analysed parameters included: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), alkaline phosphatase (ALP), creatine-kinase (CK), blood urea nitrogen (BUN), creatinine (CREA), uric acid (UA), amylase (AMY), gamma-glutamyl transferase (γ-GT), triglycerides (TRI), cholesterol (CHOL), total protein (TP), albumin (ALB), glucose (GLU), phosphorus (P), calcium (Ca).

2.3 Statistical analysis

Data were assessed for normality by Kolmogorov-Smirnov. Data about the biochemical blood parameters changes along pregnancy were analysed by the Analysis of Variance for repeated measures (ANOVA), followed by the Tukey test for multiple comparisons. For each parameter, differences recorded at each sampling time were considered significant with $p<0.05$. Data were analysed using SPSS 15.0 for Windows platform (SPSS Inc. Chicago, IL, USA).

3. Results

3.1 Clinical findings

All the jennies foaled spontaneously and unassisted, at the physiological end of pregnancy (mean 361.6 ± 12.47 days long, range 346-381 days), and gave birth to mature, healthy and viable foals. Therefore, data about the biochemical parameters along pregnancy in all the 10 Martina Franca jennies, were considered suitable to provide preliminary normal data about biochemical blood parameters during pregnancy.

3.2 Biochemical blood findings

Data about biochemical blood changes recorded monthly from the 1st to the 6th month, and then twice-a-month from the 6th to the 9th month of pregnancy are reported in table 1. Data about biochemical blood changes recorded weekly from the 13rd week before parturition until foaling, are reported in table 2. Each parameter is expressed as mean ± SD and (min-max).

4. Discussion

Although belonging to the same family *Equidae*, genus *Equus*, horses and donkeys share some physiological similarities, but however showed some specie-specific differences. Therefore for a better management of the pregnancy condition, data about the donkey specie are required.

To the authors knowledge this is the first study reporting the biochemical blood changes occurring during the whole pregnancy course in donkeys. Indeed, only one study previously reported the hematologic and biochemical changes occurring in the last 8 weeks of pregnancy and during lactation, in Amiata breed jennies. Therefore, the present study results could be considered as the first, preliminary, pregnancy associated reference data for this species, although data were collected from a small number of only one donkey breed. However, although the number of pregnant jennies enrolled in the present study could seem very small, it should be highlighted that, according to the Martina Franca breed consistency, a number of 10 animals on a total of 292 total jennies may be considered adequate. Because all the 10 jennies showed a normal pregnancy course and foaled spontaneously at the physiologic term of pregnancy, giving birth to mature, healthy and viable foals, obtained data can be considered as indicative of the normal pregnant condition in Martina Franca jennies. Although the apparently wide range of pregnancy duration (346-381 days), the mean ± SD pregnancy length (371±12 days) and range, were in agreement with data previously reported for the same donkey breed [13]. Although the paternal effects on fetal growth are well known, the interplay between maternal and paternal effects on pregnancy-associated biochemical blood changes could also be taken in consideration, but at present not investigated. Under this perspective, in the present study it could have been valuable to use the same stallion for all the 10 jennies, but this was not feasible in a preservation program application of endangered population. When the biochemical blood parameters were evaluated, only glucose, creatinine and cholesterol showed statistically significant changes, while all the other parameters remained almost constant along the whole pregnancy course, without statistically significant changes.

4.1 Glucose

Glucose serum levels (mean levels ranging between 62 and 112 mg/dl) were very similar to those reported for the Amiata jennies from 2 months before foaling [6], and also to the data reported for the Ragusano adult donkey [15], but a bit higher than the mean value reported for the Brazilian donkey [16]. In the present study a significant ($p<0.01$) slight increase in blood glucose levels was observed from the 1st month (67±8.91 mg/dl) to the 6th and half month of pregnancy (82±5.52 mg/dl), without any further significant change. This difference was not reported in the study on Amiata jennies [6], but that study started in the last 2 months of pregnancy, when the change could have already been occurred. Bonelli et al [6], found however higher blood glucose levels in pregnant jennies as compared to reference values for adult donkeys, and suggested that that finding could be attributable to the development of insulin resistance, recognized for other species such as the horse, the dog and the human. In the present study, therefore, the significant increase of blood glucose values in Martina Franca jennies from the beginning to about half of pregnancy course, could be supposed to be related to a similar condition of insulin resistance, even if this hypothesis need to be in deep investigated. When compared to data reported in the horse mare, although [1] did not find pregnancy associated blood glucose changes, a study performed on Lipizzaner mares reported higher blood glucose levels in late-term pregnant mares than those in early or mid-gestation [2]. Aoki and Ishii [17] and [5] reported higher glucose levels at parturition, supposed to be related to the physical stress at parturition [17], or as the consequence of the progressive development of insulin resistance that allows the glucose placental transfer to the fetus [5]. It must be highlighted that, different to the study performed in the horse mares, in the present study the last sampling time before foaling was never performed the exact day of parturition, but always at least 2 days before parturition.

4.2 Blood urea nitrogen, creatinine, uric acid

Blood urea nitrogen serum values (mean levels ranging between 14 and 22 mg/dl) were always a bit lower when compared to adult donkey of different breeds [15, 16, 18], and the difference resulted more pronounced when data were compared to those reported for the Catalan donkey breed (the donkey breed closest to the Martina Franca breed) [18], suggesting a possible effect of each breed on the BUN values. When the BUN whole pregnancy course profile was evaluated, no significant changes were observed, likely to what reported for the horse mare [1], but different to the trend of decrease observed in Lipizzaner mares from about the fifth month of gestation until parturition [2]. This finding was different from data reported in pregnant Amiata jennies, in which blood urea significantly increased during the last 2 weeks of pregnancy. Moreover, the BUN values recorded in the last 2 months of pregnancy in Amiata jennies were higher in comparison to the present study results.

Creatinine serum concentrations showed a significant increase ($p<0.01$) between the 1^{st}-4^{th} (mean levels ranging between 0.9 and 1.0 mg/dl) month and the 9^{th} month of pregnancy (mean±SD, 1.2±0.11 mg/dl), and then remained constant until the end of pregnancy. This increase could be explained, as supposed by [5] for the horse mare, by a different energy request by the jenny in that phase of gestation or, also associated to the creatinine production by the fetus, that is however excreted by the mother. Unfortunately, this trend is not comparable with the study reported in jennies [6], because of the different time-frame of that study. However, when compared to the horse mare, a term-pregnancy associated creatinine increase was reported [1,2,5]. When compared to data reported for adult donkeys, in the present study the mean levels (ranging between 0.9 and 1.6 mg/dl) was lower in comparison to those reported for the Amiata donkeys, but very similar to those reported for the Catalan donkey [18] and for the Ragusano breed [15], but higher than data reported for the Balcan donkey [19].

In the present study UA concentrations remained unchanged along the entire pregnancy course (mean values ranging between 0.2 and 0.4 mg/dl). Unfortunately this data could not be compared to other studies in pregnant donkeys or horses, because the authors did not find reference about UA concentrations in pregnant horses and the study in late pregnant Amiata jennies [6] did not consider this parameter.

4.3 Lipid metabolism and enzymes

Triglycerides serum levels remained unchanged throughout pregnancy (mean values ranging between 80 and 139 mg/dl), and resulted very variable among subjects, as highlighted by the wide standard deviations. This finding is however very similar to data reported in the Amiata jennies [6]. In the horse, [1] reported the highest concentration of triglycerides at mid-gestation in comparison to early-gestation, while in Lipizzaner mares triglycerides were found increasing from about the fifth month of pregnancy until parturition [2]. When compared to values reported for the adult donkey, triglycerides mean concentrations resulted higher in comparison to the Ragusano donkey [15], but rather similar to data reported in the Catalan breed [18].

Cholesterol serum levels showed a significant ($p<0.05$) decrease between the sampling performed 10 weeks before foaling (83±9.09 mg/dl) in comparison to the one performed at the last week before foaling (66±7.90 mg/dl). This finding is in contrast con the absence of significant cholesterol concentrations in the last 8 weeks of pregnancy in the Amiata jennies [6], even if the values resulted very similar in the two breeds, as well as when data were compared to mean values reported for the Catalan [18] and Ragusano [15] adult donkeys.

All together, those different species- and breed-related findings seem to suggest that the lipid metabolism is one of the most influenced by several factors, and therefore reference data should also consider the specific physiologic conditions, such as pregnancy.

Total bilirubin serum levels remained unchanged throughout pregnancy with mean values ranging between 0.0 and 0.1 mg/dl. These trend and values are in agreement with data reported in the Amiata jennies [6] and also similar to data reported for the Catalan [18] and Ragusano [15] adult donkeys. However, these data are in contrast to the total bilirubin increases reported in the pregnant horse mare [1], in which the late pregnancy enlarged uterus was supposed to induce a secondary cholestasis. AST plasma concentrations did not significantly change along the entire pregnancy (mean levels ranging between 133 and 198 U/L). This is in contrast to the trend of increase near to foaling reported in the Amiata jennies [6], but also with the decreasing trend of AST activity reported in the final third of pregnancy in the mare [1-4]. The mean values obtained in the present study were however higher than those reported in the Amiata jennies [6] in the last 8 weeks of pregnancy, but on average lower when compared to data reported for the adult Catalan [18], Ragusano [15], and Brasilian [16] donkey breeds. The values appeared very similar to the data reported for the Ethiopian donkey [20]. The AST activity appears to be marked different among breeds within the same species and deserves interest from a clinical stand point, needing further investigation in the specific condition of pregnancy.

Also ALT serum mean levels (ranging between 2 and 6 U/L) remained unchanged along the whole pregnancy course, but a comparison with the Amiata pregnant jennies is not possible because the authors did not analyse that enzyme. Unfortunately few studies reported the data in the adult donkey, so that when the values were compared to data reported for the adult Ragusano [15] and the Ethiopian donkey breed [20], the present study data resulted about two and four times lower, respectively. Also in the mare no significant changes along pregnancy were detected [3,4].

Gamma-glutamyl transferase activity (mean serum values ranging between 13.6 and 25.7 U/L) did not significantly change along pregnancy, differently to the decreases reported close to parturition in the Amiata jennies [6], that reported values a bit higher in comparison to the mean values observed in the present study. However, also in the pregnant mares a significant decrease of γ-GT associated in the third in relation to the first and the second period of pregnancy [1,4] was reported, while in Holstein breed mares [3] significant γ-GT changes along pregnancy were not detected. On the opposite, [5] reported an increased γ-GT activity around delivery. Therefore the effect of breed on γ-GT changes associated to particular physiologic conditions such as pregnancy should be carefully considered. When data were compared to values reported for the adult donkeys, the present study results were very similar to the mean values reported for the adult Ragusano breed [15] and the Balcan breed [19], but about one half lower when compared to data reported for the Catalan breed [18] and the Brazilian breed [16].

4.4 Amylase

Amylase activity remained unchanged along the whole pregnancy course (mean serum values ranging between 3 and 4 U/L) and this finding is not comparable with the Amiata pregnant jennies because this parameter was not studied by those authors. Also in the pregnant horse the authors did not find data for comparison. The comparison of obtained values with data reported for the adult donkey, the present study mean values was very similar to the mean value reported in the Ragusano breed [15].

4.5 Muscular enzymes

The creatine-kinase activity did not show significant changes along the whole pregnancy course (mean serum values ranging between 36 and 77 U/L, with wide standard deviations), in contrast to data reported in the Amiata jennies [6], in which a significant decrease was observed from the last 4 weeks before parturition. However, the mean CK values were similar between the two studies. In comparison to data reported for the mare, [17] found a significant CK increase at delivery, while a CK decrease in the third period of pregnancy was reported in Spanish mares [4]. When the mean values were compared to data reported for the adult donkeys, the present study results were more than two times lower [15,16,18,19].

The alkaline phosphatase activity also remained unchanged (mean serum values ranging between 104 and 137), similarly to what reported in the last 8 weeks of pregnancy in the Amiata jennies [6], even if the present study mean values were a bit lower in comparison to data obtained in that study. The absence of significant changes agree with most of data reported for the mare, except for the ALP activity increase observed around delivery in heavy draft mares [17], and in Lipizzaner mares [2], and, on the opposite the decrease between early gestation and the last third of pregnancy [1].

4.6 Total proteins, albumin

As previously mostly reported in the horse, also in the present study nor total proteins neither albumin serum concentrations did not change significantly along pregnancy in Martina Franca jennies, with mean values ranging between 6.5 and 8.2 g/dl and 2.9 and 3.7 g/dl, respectively. However, a slight TP concentrations decrease in the last 8 weeks of pregnancy, was found in the Amiata jennies [6], while albumin concentrations remained unchanged. Also in mares, TP were found to increase in the second and third period of pregnancy respect the first period [4], or at parturition in comparison to late pregnancy [5], while albumin remained unchanged [4,5], or decreased in late-term pregnancy than in Lipizzaner mares in early and mid-gestation [2], while a study on several horse breeds did not report pregnancy associated changes nor for TP neither for albumin [1]. When the mean values were concerned, the results of the present study were very similar to data reported in the Amiata late pregnant jennies [6], and also to data reported for the adult donkey [15,16,18,19].

4.7 Calcium, phosphorus

Nor calcium neither phosphorus showed a significant change during pregnancy, with mean serum values ranging between 8.3 and 15.2 mg/dl and 2.6 and 4.1 mg/dl, respectively, while a significant phosphorus concentrations increase at delivery respect the previous last 8 weeks of pregnancy in Amiata jennies [6] was reported. In the horse a calcium decrease at parturition was reported [5], while [1] found lower calcium concentrations in the first two months of pregnancy in comparison to mid-gestation. Serum phosphate concentrations was found lowest at mid-gestation [1]. The mean values observed in the present study resulted however similar to those reported in late pregnant Amiata jennies [6], but also in line with data reported for the adult Ragusano breed [15].

5. Conclusion

In conclusion, the present study provided the first data about the biochemical blood analysis in Martina Franca jennies along the entire normal pregnancy course. The study shared some similarities and displayed some differences with data previously reported for the last 8 weeks of pregnancy in the Amiata donkey breed, and with data obtained in pregnant horse mares, evidencing once more, the differences existing between the two equine species, and also within the donkey species, as demonstrated by the finding of some differences in comparison to data obtained in other breeds adult donkeys. Taken together, the results from the present study seem to confirm the need for specie-specific reference data, but also suggest that breed-specific reference are necessary for a better interpretation of laboratory analysis also in donkeys. Moreover, data and changes related to the pregnancy condition are also necessary for the adequate management of normal gestations, but even more for the prompt recognition of every abnormalities, pivotal in every reproductive process and even more during program of endangered population preservation.

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Month of pregnancy	GLU mg/dl	BUN mg/dl	CREA mg/dl	TRI mg/dl	CHOL mg/dl	AMY U/L	AST U/L	ALT U/L	TBIL mg/dl	ALP U/L	γGT U/L
1	67 ± 8.9[a] (58-90)	18 ± 3.83 (15-24)	0.9 ± 0.15[a] (0.6-1.1)	84 ± 28.08 (56-133)	63 ± 6.84 (55-78)	3 ± 1.25 (1-5)	141 ± 19.14 (122-176)	2 ± 0.67 (1-3)	0.0 ± 0.05 (0-0.1)	121 ± 29.04 (86-175)	13.6 ± 2.38 (10.8-18.2)
2	69 ± 8.97 (59-83)	19 ± 4.86 (15-31)	1.0 ± 0.15 [a] (0.8-1.2)	90 ± 25.88 (64-138)	66 ± 10.00 (41-76)	3 ± 1.14 (1-5)	148 ± 16.59 (119-169)	3 ± 1.14 (1-5)	0.0 ± 0.05 (0-0.1)	131 ± 28.04 (93-189)	13.8 ± 2.92 (10.0-18.5)
3	72 ± 5.95 (65-86)	20 ± 4.20 (12-25)	0.9 ± 0.21 [a] (0.6-1.4)	98 ± 30.55 (69-132)	67 ± 7.68 (50-81)	3 ± 0.67 (2-4)	144 ± 21.11 (104-176)	2 ± 0.82 (1-4)	0.1 ± 0.05 (0-0.1)	135 ± 41.42 (96-240)	13.8 ± 3.11 (9.8-18.7)
4	72 ± 7.71 (55-80)	22 ± 3.13 (14-26)	0.9 ± 0.17 [a] (0.7-1.3)	86 ± 27.36 (56-111)	71 ± 9.87 (51-83)	4 ± 1.37 (3-7)	147 ± 22.38 (117-174)	2 ± 0.79 (1-3)	0.0 ± 0.05 (0-0.1)	137 ± 35.74 (88-219)	14.1 ± 2.71 (9.7-18.6)
5	74 ± 9.17 (61-91)	20 ± 3.40 (15-26)	1.0 ± 0.21 (0.7-1.4)	90 ± 26.87 (52-143)	68 ± 9.44 (50-78)	4 ± 1.52 (1-6)	146 ± 19.22 (112-172)	3 ± 1.23 (1-5)	0.0 ± 0.05 (0-0.1)	130 ± 26.29 (101-167)	14.4 ± 3.26 (9.6-15.9)
6	79 ± 9.74 (62-97)	18 ± 4.70 (13-29)	1.1 ± 0.15 (0.8-1.3)	102 ± 25.16 (54-142)	72 ± 7.59 (66-85)	3 ± 2.26 (1-8)	163 ± 30.64 (124-210)	3 ± 2.13 (1-5)	0.1 ± 0.05 (0-0.1)	129 ± 35.61 (94-222)	17.2 ± 5.59 (11.2-24.8)
6.5	82 ± 5.52[b] (76-90)	17 ± 3.75 (13-26)	1.0 ± 0.13 (0.8-1.2)	90 ± 30.46 (46-138)	68 ± 7.18 (57-81)	3 ± 1.20 (1-5)	157 ± 25.04 (115-193)	3 ± 1.40 (1-5)	0.0 ± 0.05 (0-0.1)	115 ± 25.88 (80-167)	16.0 ± 5.82 (9.2-28.6)
7	81 ± 8.33 (66-91)	16 ± 4.24 (12-22)	1.0 ± 0.17 (0.7-1.3)	81 ± 25.67 (37-117)	68 ± 7.39 (59-78)	3 ± 2.13 (1-8)	161 ± 32.90 (118-226)	3 ± 1.15 (1-5)	0.0 ± 0.05 (0-0.1)	114 ± 22.55 (91-167)	15.2 ± 4.07 (11.0-23.9)
7.5	79 ± 10.39 (68-99)	18 ± 4.22 (14-25)	1.0 ± 0.14 (0.9-1.3)	80 ± 25.56 (50-129)	66 ± 10.63 (55-85)	3 ± 1.70 (1-6)	158 ± 31.95 (109-210)	3 ± 1.08 (1-5)	0.0 ± 0.05 (0-0.1)	113 ± 19.46 (88-151)	15.7 ± 2.75 (11.2-19.6)
8	78 ± 10.52 (63-91)	18 ± 3.43 (13-23)	1.1 ± 0.18 (0.9-1.4)	83 ± 32.67 (20-135)	66 ± 10.01 (55-83)	4 ± 1.20 (2-6)	167 ± 32.54 (126-212)	2 ± 1.07 (1-4)	0.0 ± 0.05 (0-0.1)	122 ± 23.71 (94-178)	16.2 ± 2.18 (13.3-19.6)
8.5	72 ± 12.96 (51-88)	18 ± 3.71 (13-25)	1.0 ± 0.19 (0.7-1.4)	84 ± 18.91 (56-113)	61 ± 6.41 (50-76)	3 ± 1.64 (1-6)	162 ± 36.24 (115-218)	3 ± 1.70 (1-6)	0.0 ± 0.05 (0-0.1)	113 ± 21.01 (92-161)	16.4 ± 2.54 (12.2-20.2)
9	73 ± 12.93 (49-92)	20 ± 6.06 (12-28)	1.2 ± 0.11 [b] (1-1.4)	96 ± 26.28 (52-130)	69 ± 9.88 (56-88)	3 ± 1.85 (1-7)	174 ± 36.83 (122-216)	3 ± 2.13 (1-8)	0.1 ± 0.07 (0-0.2)	116 ± 24.82 (91-162)	17.7 ± 3.74 (14.2-24.5)

[a,b] denote within column significant differences with p<0.01

Month of pregnancy	**CK U/L**	**TP g/dl**	**ALB g/dl**	**UA mg/dl**	**Ca mg/dl**	**P mg/dl**
1	59 ± 31.89 (21-105)	6.6 ± 0.37 (6.1-7.1)	2.9 ± 0.31 (2.4-3.6)	0.2 ± 0.06 (0.1-0.3)	8.6 ± 2.08 (5.9-11.6)	2.8 ± 0.37 (2.1-3.3)
2	57 ± 31.71 (20-119)	6.8 ± 0.53 (6-7.5)	3.1 ± 0.22 (2.8-3.5)	0.2 ± 0.08 (0.1-0.3)	9.1 ± 2.27 (6.3-12.2)	3.0 ± 0.28 (2.5-3.5)
3	64 ± 30.40 (24-101)	6.9 ± 0.56 (5.8-7.7)	3.1 ± 0.29 (2.5-3.7)	0.2 ± 0.04 (0.2-0.3)	9.5 ± 2.71 (6.2-14.2)	3.1 ± 0.42 (2.6-4.1)
4	69 ± 29.99 (27-114)	6.9 ± 0.45 (6.3-7.6)	3.2 ± 0.40 (2.7-4.1)	0.2 ± 0.08 (0.2-0.4)	10.4 ± 2.49 (6.9-13.3)	2.9 ± 0.55 (2.0-3.7)
5	68 ± 36.06 (22-124)	6.6 ± 0.74 (5.2-7.5)	3.0 ± 0.36 (2.5-3.8)	0.2 ± 0.09 (0.1-0.3)	8.4 ± 2.49 (5-12.3)	2.9 ± 0.60 (2.3-4.0)
6	69 ± 33.31 (35-131)	6.8 ± 0.52 (6.3-7.8)	3.2 ± 0.40 (2.8-4.2)	0.3 ± 0.07 (0.2-0.4)	9.6 ± 3.19 (6.8-16.7)	3.4 ± 0.68 (2.7-4.4)
6.5	57 ± 29.21 (31-121)	6.7 ± 0.30 (6.4-7.2)	3.2 ± 0.30 (2.7-3.6)	0.2 ± 0.07 (0.1-0.3)	9.1 ± 2.73 (6-12.7)	3.2 ± 0.73 (2.2-5.0)
7	65 ± 27.89 (28-117)	6.6 ± 0.37 (6.1-7.1)	3.1 ± 0.33 (2.8-3.9)	0.2 ± 0.06 (0.1-0.3)	8.3 ± 2.17 (6.5-10.4)	3.4 ± 0.61 (2.5-4.8)
7.5	76 ± 37.02 (23-122)	6.8 ± 0.65 (5.9-8.2)	3.2 ± 0.36 (2.6-3.9)	0.2 ± 0.06 (0.1-0.3)	9.1 ± 2.20 (6.8-12.8)	3.1 ± 0.60 (1.9-4.1)
8	69 ± 35.53 (18-118)	6.9 ± 0.49 (6-7.8)	3.4 ± 0.32 (3.0-4.1)	0.2 ± 0.08 (0.1-0.4)	10.6 ± 2.88 (6.5-14.1)	3.3 ± 0.54 (2.6-4.0)
8.5	64 ± 32.67 (30-105)	6.7 ± 0.42 (5.7-7.2)	3.3 ± 0.40 (3.0-4.0)	0.2 ± 0.08 (0.1-0.4)	10.2 ± 2.35 (6.3-12.8)	3.0 ± 0.86 (2.1-4.2)
9	64 ± 26.22 (35-106)	7.4 ± 0.80 (6.2-8.8)	3.5 ± 0.37 (3.0-4.0)	0.3 ± 0.08 (0.2-0.4)	10.7 ± 2.66 (6.6-14.2)	3.2 ± 0.47 (2.5-3.8)

Weeks before foaling	GLU mg/dl	BUN mg/dl	CREA mg/dl	TRI mg/dl	CHOL mg/dl	AMY U/L	AST U/L	ALT U/L	TBIL mg/dl	ALP U/L	γGT U/L
-13 (N=2)	112 ± 40.31 (83-140)	15 ± 1.41 (14-16)	1.4 ± 0.14 (1.3-1.5)	106 ± 9.90 (99-113)	87 ± 15.56 (76-98)	3 ± 1.41 (2-4)	139 ± 2.83 (137-141)	4 ± 0.71 (3-4)	0.1 ± 0.00 (0.1-0.1)	124 ± 1.41 (123-125)	20.2 ± 0.21 (20-20.3)
-12 (N=3)	80 ± 24.75 (62-97)	15 ± 0.00 (15-15)	1.6 ± 0.07 (1.5-1.6)	127 ± 38.18 (100-154)	80 ± 9.90 (73-87)	3 ± 2.12 (1-4)	133 ± 2.08 (131-135)	2 ± 0.00 (2-2)	0.1 ± 0.07 (0-0.1)	104 ± 20.51 (89-118)	17.6 ± 1.56 (16.5-18.7)
-11 (N=5)	76 ± 8.96 (68-84)	14 ± 5.50 (9-21)	1.2 ± 0.17 (1-1.4)	108 ± 27.46 (78-138)	77 ± 9.43 (68-90)	4 ± 2.16 (2-7)	147 ± 31.18 (129-202)	5 ± 2.75 (2-8)	0.1 ± 0.06 (0-0.1)	132 ± 41.46 (88-188)	20.5 ± 9.88 (14-35)
-10 (N=8)	76 ± 11.13 (59-90)	18 ± 2.41 (15-22)	1.3 ± 0.24 (0.9-1.6)	112 ± 33.25 (41-145)	83 ± 9.09[a] (70-94)	4 ± 2.52 (1-8)	173 ± 35.30 (134-246)	4 ± 1.35 (2-6)	0.1 ± 0.11 (0-0.3)	135 ± 45.98 (88-201)	23.5 ± 7.60 (13.9-34.2)
-9	76 ± 15.72 (60-99)	19 ± 4.44 (9-23)	1.2 ± 0.19 (1-1.4)	139 ± 38.37 (86-184)	73 ± 8.72 (56-82)	4 ± 2.20 (1-7)	183 ± 55.2 (127-317)	4 ± 1.51 (2-6)	0.1 ± 0.05 (0-0.1)	128 ± 31.38 (93-196)	24.0 ± 8.06 (12.9-32.8)
-8	62 ± 19.35 (42-99)	19 ± 4.14 (14-26)	1.2 ± 0.20 (1-1.6)	115 ± 21.92 (84-130)	79 ± 9.13 (67-92)	3 ± 1.89 (1-7)	178 ± 36.33 (123-226)	6 ± 3.14 (3-12)	0.1 ± 0.07 (0-0.2)	128 ± 29.76 (109-189)	25.7 ± 9.26 (11.9-38.6)
-7	65 ± 20.02 (43-103)	20 ± 5.72 (10-27)	1.3 ± 0.16 (1.1-1.5)	115 ± 26.40 (65-155)	72 ± 8.34 (59-82)	3 ± 1.89 (1-6)	178 ± 42.44 (120-258)	5 ± 2.74 (3-9)	0.1 ± 0.09 (0-0.3)	130 ± 29.89 (87-172)	25.4 ± 7.56 (12.6-32.9)
-6	67 ± 16.39 (42-93)	21 ± 3.83 (15-28)	1.2 ± 0.12 (1-1.4)	114 ± 28.01 (68-159)	74 ± 10.14 (62-93)	3 ± 2.01 (1-7)	178 ± 45.11 (117-243)	3 ± 1.69 (1-6)	0.1 ± 0.07 (0-0.2)	129 ± 23.10 (103-166)	24.1 ± 8.50 (13.2-35.9)
-5	66 ± 20.43 (37-106)	19 ± 3.33 (14-23)	1.1 ± 0.10 (1-1.3)	96 ± 29.06 (49-131)	73 ± 11.31 (58-89)	3 ± 1.66 (1-6)	188 ± 45.40 (120-265)	4 ± 1.73 (2-7)	0.1 ± 0.05 (0-0.1)	118 ± 21.41 (85-155)	21.6 ± 6.57 (10.6-32.6)
-4	69 ± 15.51 (43-86)	18 ± 2.88 (13-22)	1.2 ± 0.18 (0.9-1.4)	108 ± 43.13 (39-171)	71 ± 7.62 (64-90)	4 ± 1.73 (1-6)	184 ± 39.86 (134-257)	4 ± 2.30 (2-8)	0.1 ± 0.05 (0-0.1)	118 ± 17.91 (96-144)	20.7 ± 5.47 (10.7-28.7)
-3	71 ± 7.69 (60-86)	20 ± 5.08 (11-28)	1.1 ± 0.16 (0.8-1.4)	99 ± 24.49 (61-130)	70 ± 12.12 (59-89)	4 ± 1.99 (1-8)	198 ± 36.88 (150-249)	4 ± 2.31 (1-9)	0.0 ± 0.05 (0-0.1)	118 ± 16.39 (90-144)	20.9 ± 6.52 (10.3-32)
-2	60 ± 11.13 (35-80)	21 ± 4.81 (10-26)	1.1 ± 0.21 (0.8-1.5)	101 ± 23.17 (69-133)	72 ± 7.67 (63-87)	4 ± 1.91 (2-8)	172 ± 32.84 (131-220)	4 ± 1.75 (2-7)	0.1 ± 0.07 (0-0.2)	119 ± 21.67 (98-145)	20.0 ± 5.63 (14-30.5)
-1	73 ± 10.90 (57-92)	19 ± 4.33 (13-25)	1.1 ± 0.23 (0.8-1.6)	104 ± 21.33 (62-132)	66 ± 7.90[b] (52-74)	4 ± 1.58 (2-6)	181 ± 26.07 (144-220)	4 ± 1.84 (2-7)	0.1 ± 0.07 (0-0.2)	112 ± 27.32 (79-152)	20.2 ± 7.79 (9.6-31.6)

Weeks before foaling	CK U/L	TP g/dl	ALB g/dl	UA mg/dl	Ca mg/dl	P mg/dl
-13 (N=2)	36 ± 7.78 (30-41)	8.2 ± 0.78 (7.6-8.7)	3.7 ± 0.99 (3.0-4.4)	0.4 ± 0.07 (0.3-0.4)	15.2 ± 3.04 (13-17.3)	4.1 ± 0.49 (3.7-4.4)
-12 (N=3)	49 ± 0.71 (48-49)	6.5 ± 0.07 (6.4-6.5)	3.5 ± 0.21 (3.3-3.6)	0.4 ± 0.2 (0.2-0.5)	10.8 ± 0.99 (10.1-11.5)	2.6 ± 0.14 (2.5-2.7)
-11 (N=5)	77 ± 55.15 (30-150)	6.9 ± 0.74 (5.9-7.8)	3.6 ± 0.32 (3.3-4.0)	0.3 ± 0.14 (0.2-0.5)	12.1 ± 3.48 (7.1-14.9)	3.4 ± 0.36 (3.1-3.9)
-10 (N=8)	51 ± 17.25 (31-81)	7.0 ± 0.75 (6.2-8.4)	3.5 ± 0.46 (2.9-4.1)	0.4 ± 0.21 (0.2-0.8)	12.2 ± 3.29 (5.6-15.8)	3.2 ± 0.99 (2.2-4.7)
-9	56 ± 20.38 (32-92)	7.6 ± 0.50 (7.1-8.6)	3.3 ± 0.39 (2.9-3.9)	0.4 ± 0.15 (0.2-0.7)	12.3 ± 3.15 (5.6-15.8)	3.1 ± 0.38 (2.4-3.7)
-8	60 ± 21.54 (36-106)	7.3 ± 0.53 (6.6-8.3)	3.5 ± 0.42 (2.9-4.1)	0.4 ± 0.16 (0.2-0.8)	13.3 ± 2.13 (7.8-15.3)	3.2 ± 0.62 (2.5-4.3)
-7	61 ± 19.37 (37-98)	7.3 ± 0.55 (6.7-8.3)	3.6 ± 0.39 (2.9-4.0)	0.4 ± 0.17 (0.2-0.7)	13.4 ± 1.09 (11.8-15.1)	3.4 ± 0.43 (2.9-4.2)
-6	54 ± 26.03 (23-101)	7.4 ± 0.64 (6.5-8.5)	3.4 ± 0.29 (3.0-3.9)	0.4 ± 0.10 (0.2-0.5)	12.9 ± 1.81 (9.8-14.8)	3.5 ± 0.55 (2.7-4.3)
-5	52 ± 21.97 (19-89)	7.2 ± 0.73 (6.1-8.5)	3.5 ± 0.38 (2.7-4.0)	0.3 ± 0.12 (0.2-0.5)	12.1 ± 2.73 (6.8-14.9)	3.1 ± 0.7 (2.2-4.3)
-4	62 ± 30.22 (27-106)	7.4 ± 0.66 (6.6-8.5)	3.4 ± 0.27 (2.9-3.7)	0.3 ± 0.07 (0.2-0.4)	12.2 ± 2.07 (8.8-14.8)	3.5 ± 0.34 (3.1-4.1)
-3	61 ± 24.82 (36-98)	7.4 ± 0.70 (6.1-8.6)	3.5 ± 0.34 (3.1-4.0)	0.3 ± 0.13 (0.2-0.6)	12.8 ± 2.02 (9.1-16)	3.7 ± 0.37 (3.0-4.1)
-2	57 ± 27.04 (36-101)	7.2 ± 0.86 (5.9-8.7)	3.5 ± 0.40 (3.1-4.0)	0.3 ± 0.10 (0.2-0.4)	12.3 ± 1.52 (9-14.3)	3.4 ± 0.42 (2.9-3.9)
-1	54 ± 21.54 (33-98)	7.2 ± 0.77 (6.3-8.9)	3.4 ± 0.46 (2.9-4.4)	0.3 ± 0.09 (0.2-0.5)	11.0 ± 2.32 (7.3-14.3)	3.2 ± 0.89 (2.6-4.6)

[a,b] denote within column significant differences with $p<0.05$

Table 1 – Serum biochemical parameters expressed as mean ± SD (min-max), obtained from the 10 Martina Franca jennies. Blood collection was performed monthly from the 1st to the 6th month of pregnancy and then twice a month from the 6th to the 9th month of pregnancy

Table 2 – Serum biochemical parameters expressed as mean ± SD (min-max), obtained by weekly blood samplings from the 10 Martina Franca jennies in the last 13 weeks before foaling. Because of the different length of pregnancy in the enrolled jennies, in the weeks in which not all the animals were sampled, the exact number of sampled animal is reported in brackets.

Biochemical analysis along pregnancy in the Martina Franca donkey

Slight changes of glucose, creatinine and cholesterol

Some similarities and differences in comparison to horse pregnancy

Some similarities and differences in comparison to adult donkeys