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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

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(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 h, 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 Ref. [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 6th month of pregnancy, twice a month from the 7th to the end of 9th month of pregnancy, and then weekly until foaling. Blood samples were collected always in the morning, between 8.00 and 10.00 a.m., from the jugular vein into plain vacutainer and, after centrifugation at 1500 × 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 13th 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