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Aspartate Aminotransferase and Gamma Glutamyl Transferase Levels in Mares with Miscarriage and Healthy Pregnancies

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ABSTRACT

***Background*:** Hormonal and physiological changes during pregnancy period have significant effects on animals' metabolisms. The purpose ofthe present study is to evaluate the variations of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels according to different gestation trimesters of the pregnant mares to assess changes of embryonic losses and abortions.

***Materials, Methods & Results*:** Blood samples from 49 throughbred mares were analysed during this study. Age ratio of the mares is 5-18 years and their body condition score (BCS; score 1 to 9) varies between 5 and 6. A total of 28 mares had given birth to healthy foals at the end of a healthy gestation period (Group C), whereas 11 mares had embryonic losses (Group E) and 10 mares had late period abortions (Group A). Following the confirmation of pregnancy, one blood sample per gestation trimester were taken (14-16 days of pregnancy for 1st trimester; 180 days for 2nd trimester, 270 days for 3rd trimester). Early embryonic losses (loss of a 16-25 days embryo) were observed in 6 of 11 mares in Group E and the 5 of 11 mares had late period embriyonic losses (loss of a 35-40 days embryo). In Group A, 6 mares had the abortion within 7th month and the remaining 4 mares had the abortion within the 8th month of pregnancy. Repeated Measures ANOVA, Kruskal-Wallis test and Mann-Whitney test were performed for statistical analysis, the mean AST level in Group C (n = 28) was higher during the 1st trimester in comparison to 2 following trimesters (respectively: $P = 0.011$; $P = 0.01$). Besides, no statistical difference was observed between 2nd and 3rd trimester regarding AST activation ($P > 0.05$). The mean GGT level in Group C was significantly decreased lower in the 3rd trimester compared to 1st and 2nd trimester (respectively: $P = 0.007$; $P = 0.009$). No statistical difference was observed between 2nd and 3rd trimester regarding GGT activation ($P > 0.05$). Among all groups (C, E, A) no significant difference was observed on AST levels ($P > 0.05$), nonetheless GGT levels had a significant rise ($P = 0.039$) in Group A in comparison to the 1st trimester levels of Group C. In Group A, there was a statistical decrease of AST during the 2nd trimester in comparison to the 1st trimester ($P = 0.001$), accompanied by a decrease of GGT activation during the 2nd trimester compared to the 1st trimester ($P = 0.009$).

***Discussion*:** The aminotransferases are catalisors that play an important role on the metabolism of amino acids and carbonhydrates. Although serum AST levels were within the reference ranges during 3 trimesters in this study, serum AST levels were determined to be decreased in the 2nd and 3rd trimesters in healthy pregnant mares compared to the 1st trimester. It is thought that these results can be obtained due to the increase in metabolic needs during pregnancy. In this study, serum GGT levels remained within physiological limits with a tendency to decrease as the pregnancy advances, in accordance with previous study results. Serum GGT levels that are used as an indicator to liver damage may vary during the pregnancy as a result of increased metabolic load. It is thought that the increase of serum GGT levels in Group A might be related to the fetal (chromosome-related) issues that may result to pregnancy losses. As a result, it is considered that serum AST and GGT levels in mares might be valuable parameters that predict embryonic loss or abortus cases.

Keywords: abortus, enzymes, serum levels, AST, GGT, embryonic loss, mares, pregnancy.

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INTRODUCTION

Hematology and serum biochemical analyses are widely used in horses to evaluate metabolical state, to identify diseases and to follow-up the ongoing treatment [9,16]. Both physiological and hormonal changes in gestating animals have notable effects on their metabolism [2,15], thereof haematological parameters and biochemical serum levels may differ in pregnant animals compared to nonpregnant animals [3,15]. Vincze *et al*. [23] have emphasized the variation of many biochemical parameters (triglycerides, urea, total protein, glucose, glutamate dehydrogenase, albumine, Aspartate aminotransferase, alkalene phosphate, creatinine) in parallel with the fetal age. [23] Aspartate aminotransferase (AST) is an enzyme present in various tissues and organs with high activation in liver [15,26], and that is mainly referred to evaluate the liver damage in clinical practice [6,14,18]. Present in many species from bacteria to mammals, gamma glutamyl transferase (GGT) is an enzyme located on the outher cell membrane with extracellular activation [19,20]. Although highly present in liver tissue, GGT enzyme in serum originates from hepatobilliar system, thus serves as an indicator of liver disfunction in clinical practice [7,11,24,25]. Also, GGT enzyme has the function to transfer amino acids through cell membranes. Companion animals have low levels of GGT in their blood, whereas the levels are raised during pregnancy due to growing protein synthesis and consumption of fetal growth [22]. The purpose of this study is to analyse serum AST and GGT levels of healthy pregnant mares in accordance with gestation trimesters and to evaluate AST and GGT activation levels in mares that had embryonic loss or abortus.

MATERIALS AND METHODS

Animals

A total of 49 Throughbred mares aging between 4 to 17 years old and having body condition scores between 5 and 6 (Body Condition Score Chart; Kentucky Equine Research, USA) were incorporated to the study. Mares were lodged in individual boxes and released to paddocs during the day. Change of food consumption depended on different gestation periods, and according to this protocol, mares were fed twice daily with concentrate food and 3 times as pregnancy progresses. The water consumption was set as *ad libitum*. The mares were divided into 3 groups according to the outcome of their pregnancy. First group was the control group (Group C) included 28 mares ages between 5-17 years with healthy pregnancy. Group E included 11 mares ages between 5-18 years that had embryonic loss, Group A included 10 mares between 5-13 years of age that aborted on their 2nd trimester of pregnancy.

Pregnancy determination by ultrasonographic

Following the ovulation (ovulation is considered as the day 0 of pregnancy), the mares were examined to ensure their pregnancy on days 14-16 with a B-mode transrectal ultrasonography[1] equipped with a linear probe at a frequency of 7.5 mHz. Following the 1st pregnancy control examination, pregnant mares underwent monthly transrectal ultrasound examinations following the 2nd (25 days of gestation) and 3rd (35 days of gestation) control examinations according to pregnancy follow-up protocols.

Blood sampling and hematological analysis

Blood samples were collected from each mare as one sample per trimester. The 1st sampling was don at 14-16 days of pregnancy (1st trimester), 2nd sampling was done at 180 days (2nd trimester) and 3rd sampling was done at 270 days of pregnancy (3rd trimester). Blood was sampled from jugular vein with blood collection needles[2]. Collected blood samples were centifuged (1500 *g*, 10 min, +4°C) to obtain blood serum[3]. AST and GGT blood serum levels of mares were analyzed on a dry biochemical analyzer[4].

Statistical analysis

All statistical analysis were run on SPSS.25[5]. In order to analyze the differences between pregnancy trimesters within the Group C (n = 28). Repeated Measures ANOVA analysis was performed and evaluated with Bonferronis comparison. If normal data distribution was not achieved while comparing Group C, Group E and Group A, Mann-Whitney U test Kruskal-Wallis analysis was performed. A *P* value less than 0.05 was considered statistically significant.

RESULTS

The labors occurred naturally in 340-350 days of pregnancy in Group C Serum AST and GGT levels in Group C according to data of 3 trimesters were shown in Table 1. The AST level was determined higher at the

1st trimester than the 2nd and 3rd trimester of pregnancy in Group C ($P < 0.05$). The mean AST level was not different between the 2nd and 3rd trimester ($P > 0.05$). The mean GGT level at the 3rd trimester in Group C was significantly lower than the 1st and 2nd trimesters ($P < 0.01$). The mean GGT levels were not different between the 1st and 2nd trimesters of pregnancy in Group C ($P > 0.05$).

In Group E, 6 mares had early embriyonic loss on day 20 and 5 mares had late embriyonic loss on day 35. In Group A, 6 Mares had abortus on their 7th month of pregnancy, while 5 mares had abortus on their 8th month. Blood samples can be collected only at once at the 1st trimester of pregnancy in Group E, whilst 2 samples were collected from the Group A mares corresponding to the 1st and 2nd trimesters of pregnancy, respectively.

The mean serum AST and GGT levels were in Group E measured as 281 ± 12.73 U/L and 20.27 ± 1.76 U/L; respectively. The abortions in Group A were observed between 190-220 days of the 2nd trimester. In Group A, the mean serum AST and GGT levels were significantly higher at the 1st trimester than the 2nd trimester ($P < 0.01$, $P < 0.01$) [Table 2].

The mean serum AST levels at the 1st and 2nd trimesters were compared between the Group C and Group A , they were failed to reach the significance in both trimesters ($P > 0.05$) [Table 3]. In Group A, the mean serum GGT level at the 1st trimester was significantly higher than Group C ($P < 0.05$) while insignificant results were obtained at the 2nd trimester ($P > 0.05$) [Table 3]. The 1st trimester of serum AST and GGT levels were compared between the groups (Table 4).

Table 1. The mean serum AST and GGT levels in pregnant mares of Group C.

Pregnancy	AST (U/L)	GGT (U/L)
1st trimester	275.14 ± 9.29[a]	19.42 ± 3.67[x]
2nd trimester	244.17 ± 7.47[b]	18.39 ± 3.47[x]
3rd trimester	244.5 ± 8.17[b]	15.89 ± 3[y]
P	$P < 0.05$	$P < 0.01$

Different letters in the same columns indicate the significant differences ([a,b]$P < 0.05$ and [x,y]$P < 0.01$).

Table 2. The mean serum AST and GGT levels in pregnant mares of Group A.

Pregnancy	AST (U/L) AST (U/L)	GGT (U/L) GGT (U/L)
1st trimester	271 ± 9.17[a]	28.8 ± 3.9[x]
2nd trimester	226.2 ± 2.01[b]	16.2 ± 0.84[y]
P	$P < 0.01$	$P < 0.01$

[a,b,x,y]Different letters in the same columns indicate the significant differences ($P < 0.01$).

Table 3. The comparison of mean serum AST and GGT levels at the first and second trimesters of pregnant mares of the Group A and Group C.

Pregnancy	Groups	AST (U/L)	GGT (U/L)
1st trimester	Group A	271 ± 9.17[a]	28.8 ± 3.9[x]
	Group C	275.14 ± 9.29[a]	19.42 ± 3.67[y]
	P	$P > 0.05$	$P < 0.05$
2nd trimester	Group A	226.2 ± 2.01[b]	16.2 ± 0.84[z]
	Group C	244.17 ± 7.47[b]	18.39 ± 3.47[z]
	P	$P > 0.05$	$P > 0.05$

[a,b,x,y,z]Different letters in the per column section indicate the significant differences ($P < 0.05$).

Table 4. The mean AST and GGT levels of the groups in the first trimester of pregnancy.

Pregnant mares	AST (U/L)	GGT (U/L)
Group C	275.14 ± 9.29[a]	19.42 ± 3.67[a]
Group E	281 ± 12.73[a]	20.27 ± 1.76[a]
Group A	271 ± 9.17[a]	28.8 ± 3.9[b]

[a,b]Different letters in the same columns indicate the significant differences ($P < 0.05$).

DISCUSSION

Variations on haematological and biochemical parameters as a result of increased metabolic needs of the mares during pregnancy shall not be considered pathology-related [8]. Gurgoze and Icen [9] stated that age distribution of mares do not have an effect on biochemical parameters of blood. This statement supports the differences between the groups of our study as not related to their wide range of age (4-17 years).

Pregnancy is a period in which metabolic needs increase and therefore changes in blood biochemical parameters are observed. The aminotransferases are catalisors that play an important role on the metabolism of amino acids and carbohydrates [15]. Activity of AST in horses is higher than in other animals [4]. Although serum AST levels were within referance ranges during 3 trimesters in this study, serum AST levels were determined to be decreased in the 2nd and 3rd trimesters in healthy pregnant mares compared to the 1st trimester ($P < 0.05$). The researchers [15] recorded a significant decrease of AST activity in the 3rd trimester related to the 1st trimester and to the 2nd trimester. Similar results between the 1st and 3rd trimesters were obtained due to the increase in the metabolic needs during pregnancy terms. In contrast with the researchers' report [15], AST activity was not statistically significant between the 2nd and 3rd trimester in this study. Conflicting results are thought to be due to the AST activity varies depending upon the breed as previously reported [15].

Serum GGT levels that indicate liver damage may vary related to the metabolic load during pregnancy. In this study, GGT levels remained within physiological range during each trimester of pregnancy with a tendency to decrease in parallel to the progression of pregnancy, in accordance with other study statements [11]. A decrease of GGT levels on the last trimester was observed in comparison to 1st and 2nd trimesters of pregnancy (respectively: $P = 0.007$ and $P = 0.009$). It is thought that the decrease in GGT during the last trimester of pregnancy might be related to increased metabolic load, as stated by the reseachers in the report [15].

Embryonic losses in mares are largely observed due to hormonal (progesterone) deficiency. Embryonic losses are not resulted from metabolic or pathologic matters such as fetal deaths (abortus). Noninfectious maternal caused embryonic losses are generally due to progesterone deficiency that leads to premature luteolysis [1,10]. In addition, as embryonic losses are observed before day 40 of pregnancy, thus no changes on the basal metabolism that reflects an extra load on the liver [21]. Therefore, changes on AST profile are not evident. Due to the same reason, in cases of infectious or noninfectious abortus cases observed past the 2nd trimester of pregnancy, it would not be significative to expect a difference on AST levels between abortus cases and the 1st trimester of pregnancy. Thus, unless event of a metabolic or infectious case, it is expected to observe no difference on maternal serum AST levels among 3 groups during the 1st trimester of pregnancy. In accordance with the statement by Milinkovic-Tur *et al*. [15], no significant difference of AST levels were observed between groups of our study during the 1st trimester.

Infectious and non-infectious abortus cases are observed in a prevalence of 5-15% by mares. Abortus cases that occur past 1st trimester of pregnancy are mostly related to fetal, endometrial defects, placental deformities, twin pregnancies, maternal stress and pathogen microorganisms [10]. According to post mortem examination aborted fetuses of Group A mares abortuses were not related to any infectious causes. One schistosoma reflexum was observed, whereas other aborted fetuses (n = 9) were not subject to fetal anomalies or placental/umbilical deformities. In line with the statement of the researchers, 9 of mares in Group A had an abortus due to uterus insufficiency or chromosomal disorders, whereas it is thought that the early period embryonic losses in Group E are due to progesterone deficiency. AST and GGT levels decreased during the 2nd trimester in comparison to the 1st trimester (respectively: $P = 0.001$ and $P = 0.009$) in line with the literature [8] in Group A. This decrease in AST and GGT levels would be difficult to unveil by the progression of pregnancy, as stated by Mariella *et al*. [13]. In our study, GGT levels in Group A had a tendency to increase in comparison to Group E ($P = 0.051$). Moreover, it was confirmed that 1st trimester GGT levels were higher in Group A even in comparison to Group C. A statistical significance was observed between Group A and Group C ($P = 0.038$). The mentioned raise of GGT levels by Group A in our study is thought to be related to the cause of embryonic losses that might be fetal (chromosomal).

Serum AST value is associated with liver pathologies caused by metabolic or infectious causes [17]. The cause of abortuses were not related to any viral or bacterial factor determined after blood analyses and examination of the aborted fetuses. There was no difference between Group C and Group A in terms of

AST levels since there was no metabolic or infectious condition in the mares.

It is stated that the GGT enzyme, in difference to other liver function enzymes has a regulator pro-oxidant function in cellular patophysiology as being part of detoxification and glutathion mecanism [5]. In the study performed by Macek *et al*. [12], the GGT activation levels of amniotic fluid by fetal chromosome defected pregnancies was measured and some variations as increases and decreases were observed. They have stated that these changes might be related to the transport mechanism by microvilli. Pregnancy losses in Group A might be considered as related to fetal chromosomal anomalies as infectious evidence was not present by both blood analysis and aborted fetus examination and no gross pathology manifestation on macroscopic examination was observed in aborted fetuses (n = 9) with the exception of 1 schistosoma reflexum (SR) case. Therefore no differences were observed in AST levels between Group C and Group A while a significant difference in serum GGT levels were determined during the 1st trimester ($P = 0.038$). In line with the previous reports [5,12], it is considered that this difference should be related to a chromosomal defect.

CONCLUSION

In this study, variation of AST and GGT activities of healthy pregnant mares were observed according to trimesters and obtained data was compared to those obtained from mares that had an abortus or an embryonic loss. As a result, it is considered that AST and GGT levels in mares could be referred to as parameters to predict embryonic losses or abortus.

MANUFACTURERS

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***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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