

Prediction of Equine Abortion with Measurement of Serum Magnesium Level

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ABSTRACT

Background: Magnesium (Mg) is the 4th most abundant cation in the mammalian body. Mg activates many enzymes and affects the nerve conduction, uterine contractility and other smooth muscle contractility. Mg is a calcium antagonist. Its effect on uterine smooth muscle is relaxation and tocolysis. Decrease of Mg plays an important role in labor and deficiency of Mg has been associated with various pregnancy complications in women. However, less is known about the reproductive roles of Mg in mares. This study aimed to measure and compare the maternal serum Mg concentration in 2nd trimester abortus and healthy pregnancy in Arabian mares and find the relationship of serum Mg concentration with abortus.

Materials, Methods & Results: The study groups consisted of 8 healthy foaling mares (group H) and 8 mares with spontaneous abortion within the 2nd trimester of their pregnancies (group A). In group A, Mg was analyzed in the serum samples belonging to the 1st months of pregnancies and the months in which 2nd trimester abortions occurred (the last collected sample before abortion). In group H, serum samples from the 1st months of pregnancies and samples in the pregnancy months corresponding to the abortion month of each mare in group A were analyzed. In group A, serum Mg concentration decreased from the 1st month to the abortion month (28.12 ± 1.27 µg/g and 24.54 ± 0.69 µg/g, respectively) whereas it increased from the 1st month to the matching gestational month in group H (30.43 ± 0.36 µg/g and 36.16 ± 0.66 µg/g, respectively). A significantly lower serum Mg concentration was observed in abortion month for group A (24.54 ± 0.69 µg/g) versus the matched gestational month for group H (36.16 ± 0.66 µg/g).

Discussion: Studies conducted in women have shown an association between low maternal serum Mg concentration and abortion or preterm labor. Significantly lower serum Mg concentrations of women with preterm labor versus their healthy pregnant controls were reported. Consistent with these previous reports in women, in this study a significantly lower serum Mg concentration was observed in abortion month for group A versus the matched gestational month for group H. However, it would not be right to compare serum Mg concentrations of the present study with the human studies mentioned, due to the species difference. Besides, we could not find any report evaluating the serum Mg concentration in mare abortion. Low serum Mg concentrations with preterm labor in women despite their various etiology were reported. In this study the reasons for the abortions in mares were not determined and may be various. Consistent with the previous reports in women, in the present study an association between low serum Mg concentration and abortion was found in Arabian mares (although the probably varied reasons for abortion). Rather than attributing the cause of all cases to Mg deficiency, it is more appropriate to interpret Mg as an element that participates in and reflects the stimulation process of uterine contractility caused by various abortion factors in mares. In conclusion; maternal serum Mg concentration of the abortion month in Arabian mares was significantly lower both compared to the 1st gestational months of these mares and compared to the abortion-matched months of ongoing healthy pregnancies. Further studies considering the results of this pioneering study may lead the use of serum Mg concentration as a predictor of abortion in mares thus to pave the way for applying the necessary preventions during the high-risk pregnancies.

Keywords: mare, abortion factors, preterm labor, magnesium, uterine contractility, pregnancy, spectrophotometry.

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INTRODUCTION

Magnesium (Mg) is the 4th most abundant cation in the mammalian body [28]. It has roles in metabolic regulation and in intracellular enzymatic processes [22]. Neuromuscular system, immune system, cardiovascular system, energy metabolism, nucleic acid and protein synthesis are maintained by the help of Mg [1,25,28]. Deficiency of Mg has been associated with various reproductive disorders and pregnancy complications [22]. Studies in women have shown an association between low Mg concentrations and abortion or preterm labor [1,3,6,8,15,16,19,20,26]. Astha [3], concluded that the lower serum Mg concentration reflected the tendency and initiation of preterm labor in women and serum Mg estimation in pregnancy might be a valuable marker for predicting preterm labor in women. Considerably less is known about Mg with respect to its involvement in physiological and pathological processes in mares. Pregnancy failure is a major economic problem for the equine breeding industry. The incidence of abortion in mares is usually cited as 10%. The causes of abortion can be infectious and non-infectious [11,13,21]. There are some reports on Mg concentrations in horses in association with ongoing healthy pregnancy, lactation [7], peripartum period [5,27], season [4,10], equine herpesvirus 1 infection related or unrelated abortions [18]. However, we could not find any publication comparing the serum Mg concentrations in mares with healthy pregnancies versus those who experience spontaneous abortion. This study aimed to measure and compare the maternal serum Mg concentration in 2nd trimester abortus and healthy pregnancy in Arabian mares and find the relationship of serum Mg concentration with abortus.

MATERIALS AND METHODS

Animals and study design

In order to reach the sufficient number of aborted mares, the study was initially started with the follow-up of 64 Arabian mares with positive pregnancy diagnosis, at Vocational School of Veterinary Medicine, Istanbul University-Cerrahpaşa (latitude 41° N). Mares that we started to follow-up their pregnancies had no history of any medical problem, had no medication and were clinically healthy in the previous month. Mares were ranged 4-13 years of age and 350-450 kg in weight with a body condition score 5 to 7 [12]

at the beginning of the study. All mares were kept in individual stalls (3.5 m × 3.5 m) and were allowed to exercise in the paddocks during the day (8-9 h on average). They were fed adjusted to the stage of their pregnancies with 2-4 kg of mixed feed (50 kg of oats soaked 5 h before feeding, then 15 kg of barley paste added) and roughage (alfalfa and meadow grass) 3 times a day (at 06:00, 15:00, and 23:00) and received *ad libitum* fresh water.

Finally, the study groups were retrospectively formed as 8 healthy foaling mares (group H) and 8 mares with spontaneous abortion within the 2nd trimester [29] of their pregnancies (group A). Each group A mare have a matched group H control whose sample from the month in which the group A mare aborted. While selecting H group mares, the similarity between them and A group mares in terms of age, weight and foal gender was taken into consideration. The remaining 48 mares (3 embryonic resorption, 1 1st-trimester abort, 1 preterm labor, 43 successful pregnancies) were not enrolled in this study.

Diagnosis and follow-up of pregnancies

Pregnancy diagnoses were made 14 days after the ovulation by ultrasonography¹ (MyLab One Vet) equipped with an electronic (7.5 MHz for B-mode, SV 3513 Vet) transrectal transducer¹. For each mare, the time of ovulation was accepted as the zero day of pregnancy. Follow-up examinations were performed with ultrasonography on the 25th, 35th and 60th days of pregnancy, and then monthly with transrectal palpation until the abortion or normal delivery. The abortion day of each mare were noted.

Collection of blood samples and analysis of serum Mg concentrations

Blood samples were always harvested between 09:00 a.m. and 10:00 a.m., monthly from 30th day (1st month) of pregnancy to the delivery or abortion. Blood samples were collected by jugular venipuncture into 10 mL vacuum tubes containing clot activators². The serum was separated by centrifugation at 1,814 g for 10 min at 4°C within 1 h of collection and transferred to 1.5 mL tubes for storage at -40°C. In accordance with the project plan and budget, only serum samples belonging to the 1st months of pregnancies and the months in which 2nd trimester abortions occurred (the last collected sample before abortion) were analysed in group A. In group H, serum samples from the 1st

months of pregnancies and samples in the pregnancy months corresponding to the abortion month of each mare in group A were analysed. Thus, all the samples from the 2nd sampling time were selected from the 2nd trimester of pregnancy for both groups. Serum Mg concentrations were determined by the method of spectrophotometry [17]. Mass spectrometer³ (NexION 1000 ICP) was used for Mg analysis.

Statistical analysis

The Shapiro-Wilk test was utilized in order to verify the normal distribution of the data. The SPSS 13.0 package program was used for the statistical analyses. The paired sample *t*-test was performed to compare the 2 sampling times within each group. The independent samples *t*-test was performed for the comparison of the groups with regard to the serum Mg concentrations and age. The significance level was accepted as $P < 0.05$. Results were expressed as mean and standard error (S.E.).

RESULTS

All measured and calculated parameters were normally distributed. The mean ages of the mares in group A and in group H were similar ($P > 0.05$) as being 9.12 ± 0.89 and 9 ± 1.01 years, respectively. The gender of aborted fetuses in group A and healthy foals in group

H were equally distributed (4 females and 4 males in each group). All mares in group A aborted in the 2nd trimester of pregnancy which is defined by Suthers *et al.* [29] as being between days 121 and 240. The days of the abortions belonging to the 8 mares were 122, 127, 140, 156, 189, 205, 218 and 220. Comparisons of the mean serum Mg concentrations of the groups with regard to the sampling times and comparisons of the mean serum Mg concentrations between sampling times for the same group were given in Table 1. Without exception, serum Mg concentration of each mare in group A decreased from the 1st month to the abortion month, while it increased from the 1st month to the matching gestational month in each mare of the group H.

Relative differences in Mg concentrations between the 2 evaluation points (from 1st month to the abortion month for group A and from 1st month to the matched gestational month for group H) of each mare in both groups were given in Figure 1. While all of the relative differences of the mares in group A were negative numbers, they were all positive for the mares in group H. Mean relative difference in Mg concentration of the group A was significantly lower than the mean of the group H, $(-0.11 \pm 0.04$ and 0.18 ± 0.02 respectively) [$P < 0.001$].

Table 1. Comparisons of the mean serum magnesium (Mg) concentrations.

	Serum Mg concentration (µg/g)		P-value ^c
	First month	Abortion month for group A and matched month for group H	
Group A (n=8)	28.12 ± 1.27^a	$24.54 \pm 0.69^{b,x}$	0.025
Group H (n=8)	30.43 ± 0.36^a	$36.16 \pm 0.66^{b,y}$	< 0.001
P-value ^d	ns	< 0.001	

Values are shown as the means and standard error (S.E.); ns: $P > 0.05$. ^{a,b}Differences between the means of sampling times carrying various letters in the same line are significant ($P < 0.05$). ^{x,y}Differences between the means of groups carrying various letters in the same column are significant ($P < 0.05$). ^cSignificance level of differences between sampling times for the same group. ^dSignificance level of differences between the groups for the same sampling time.

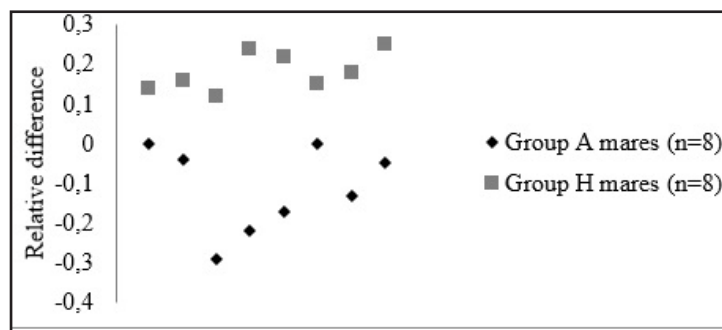


Figure 1. Relative differences in serum magnesium (Mg) concentration of each mare in the groups (from 1st month to abortion month for group A and from 1st month to matched gestational month for group H)

DISCUSSION

A description of the Mg concentration in equine abortion is lacking. In this study, we determined serum Mg concentration of mares in relation to abortion. The results were compared with those obtained for controls with uncomplicated pregnancies. The reference serum laboratory ranges for Mg in horses have been reported as 0.90-1.15 mmol/L [14] or 1.6-2.3 mg/dL [24]. The results obtained in this study were found to be higher than these reference values. The fact that we used only Arabian breed and pregnant mares in this study may have created the difference. Additionally, different methods of determining serum Mg concentrations may have an impact on the results. Berlin and Aroch [7] reported the total serum Mg concentration as 0.61-0.99 mmol/L in different breed of horses in the 3rd trimester of pregnancy. In line with our study, relatively higher results compared to the reference laboratory ranges were reported by Silva *et al.* [27], who found the serum Mg concentrations of Mangalarga Marchador mares at T-60, T-30, T-15 (days before foaling) as 1.6, 2 and 1.4 mmol/L, respectively. Horse serum Mg reference ranges to be determined by considering breed and pregnancy status may be more reliable.

Studies conducted in women have shown an association between low maternal serum Mg concentration and abortion [1,8,19] or preterm labor [6,15,16,20,26]. Borella *et al.* [8] reported serum Mg concentration of women in abortion and in successful pregnancies as 0.67 ± 0.13 and 0.80 ± 0.11 mmol/L, respectively. Shahid *et al.* [26] reported a serum Mg concentration of 1.87 ± 0.3 versus 2.10 ± 0.4 mg/dL in preterm versus term labors. The cut off point for maternal serum Mg in women was found to be 4.7 mg/dL [15] or 1.6 mg/dL [20]. It was also reported significantly lower serum Mg concentrations of women with preterm labor at gestational age 28 weeks 1 day to 32 weeks versus their healthy pregnant controls (1.64 mg/dL and 1.99 mg/dL, respectively) and preterm labor at gestational age 32 weeks 1 day to <37 week versus their healthy pregnant controls (1.82 mg/dL and 1.97 mg/dL, respectively) [3]. Consistent with these previous reports in women, in this study a significantly lower serum Mg concentration was observed in abortion month for group A versus the matched gestational month for group H. However, it would not be right to compare serum Mg concentrations of the present study with the human studies mentioned, due to the species difference. Besides, as mentioned before, we could not find any

report evaluating the serum Mg concentration in mare abortion. Silva *et al.* [27] found serum Mg concentration in mares at T-30, T-15 (days before term foaling) and T-0 (1st 6 h after term foaling) as 2, 1.4 and 1.3 mmol/L, respectively and observed a significant decrease from T-30 to T-0. Mg activates many enzymes and affects the nerve conduction, uterine contractility and other smooth muscle contractility [6,26]. Mg is a calcium antagonist. It has inhibitory effects on the activity of smooth muscle. Its effect on vascular smooth muscle vasodilation and on uterine smooth muscle is relaxation and tocolysis [2,6,23,26]. Decrease of Mg plays an important role in labor and thus also may cause premature labor in women [6,9,26]. The possible mechanism by which low Mg concentration induce uterine irritability is by inhibition of adenylyl cyclase with resultant in cytoplasmic calcium concentration [6,16]. The decrease in serum Mg level has an important role in the physiology of normal labor and may also be valuable in predicting preterm labor in women [3].

A significant decrease in serum Mg concentration was determined in preterm labor in women and this decrease was not dependent on whether the etiology for the preterm labor [16]. Low serum Mg concentrations with preterm labor in women despite their various etiology was reported [3]. In this study the reasons for the abortions in mares were not determined and may be various. Although the probably varied reasons for abortions, serum Mg concentration of each mare in group A was lower in the abortion month versus the concentration of their 1st month of pregnancy. The mean serum Mg concentrations of the mares in abortion month was significantly lower versus the mean serum Mg concentration of the 1st month of pregnancy in group A. In the present study an association between low serum Mg concentration and abortion was found in Arabian mares, but rather than attributing the cause of all cases to Mg deficiency, it is more appropriate to interpret Mg as an element that participates in and reflects the stimulation process of uterine contractility caused by various abortion factors in mares.

This study has a number of limitations - firstly: autopsies of the aborted fetuses were not performed and the reasons for abortion of each mare could not be determined; secondly: serum Mg concentrations were not evaluated just prior to term-delivery and thirdly: statistical analysis to determine a serum Mg cut-off point for the high-risk pregnancies in mares

could not be performed due to the limited number of the population. Although it has some limitations, to our knowledge, this is the 1st study to investigate the relationship between maternal serum Mg concentration and abortion in mares.

CONCLUSIONS

Maternal serum Mg concentration of the abortion month in Arabian mares was significantly lower both compared to the 1st gestational months of these mares and compared to the abortion-matched months of ongoing healthy pregnancies. Further studies are needed to determine a serum Mg cut-off point for mares, to use it as an easily applicable abortion predictor and thus to pave the way for applying the necessary preventions during the high-risk pregnancies.

MANUFACTURERS

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²Becton Dickinson Vacutainer. Berkshire, UK.

³PerkinElmer. Shelton, CT, USA.

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Ethical approval. All animal procedures were carried out in accordance with the approval of the Unit Ethical Committee at the Istanbul University-Cerrahpaşa Faculty of Veterinary Medicine (Approval number: 2020/09).

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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