Evaluation of serum anti-Müllerian hormone (AMH) and equine chorionic gonadotrophin (eCG) concentrations in pregnant mares in relation to foetal sex

Melih Uçmak[1], Özge Turna[1], Zeynep Günay Uçmak[1], Asuman Erzengin[2]

[1]Istanbul University - Cerrahpaşa, Faculty of Veterinary Medicine,
Department of Obstetrics and Gynaecology, Avcılar, Istanbul, Türkiye
[2]Istanbul University - Cerrahpaşa, Institute of Graduate Studies, Avcılar, Istanbul, Türkiye

Received October 22, 2021
Accepted December 13, 2022

Abstract

The aim of this study was to investigate the foetal sex related difference and progression in maternal serum anti-Müllerian hormone (AMH) and equine chorionic gonadotrophin (eCG) concentrations during different points of time in pregnant Arabian mares. The study groups formed by 12 healthy male offspring- and 12 healthy female offspring-foaling mares, designated as group MFM and group FFM, respectively. Peripheral blood samples were collected on the day of natural mating and then monthly until the 6th month (mo) of gestation. Serum AMH was measured in all serum samples; eCG was measured in samples collected from 2 to 5 months of gestation. Serum AMH concentrations of group FFM at mo 4 and mo 5 (3.89 ± 0.49 ng/ml; 2.89 ± 0.32 ng/ml), were significantly higher than in group MFM (2.11 ± 0.46 ng/ml; 1.87 ± 0.32 ng/ml), ($P < 0.05$). The mo of gestation (mo 1–6) had no effect on serum AMH concentrations of either group MMF or FFM ($P > 0.05$). Serum eCG concentrations of group FFM at mo 2 (359.73 ± 41.51 mIU/ml), were significantly higher than in group MFM (255 ± 21.18 mIU/ml) ($P < 0.05$). Group-time interaction for eCG concentrations at mo 2–4 was non-significant ($P > 0.05$). Concentrations of serum AMH showed no relationship with corresponding eCG levels at mo 2–4 ($P > 0.05$). Individual variations in AMH and eCG concentrations and the inability to determine a cut-off point for determination of foetal sex make these hormones unlikely candidates for determining foetal sex in the mare.

Horse, pregnancy, sex determination, hormones

There is an increasing interest in foetal sex determination in the horse breeding industry. The breeders' preferences for foal sex depend on their individual considerations and choices. While males are preferred for thoroughbred racing and Western riding reining horses, the opposite is true for Polo sports and Western riding cutting horses. Knowing the foetal sex provides many advantages to the breeders. They can determine commercial strategies, make sales or decisions, improve breeding management, determine insurance coverage and limits, etc. It is also important for customers buying a pregnant mare to know the sex of the fetus (Holder 2000; Bucca 2005; Aurich and Schneider 2014). Currently, there are a limited number of methods available for determining the sex of the foetus in pregnant mares. Sex can be determined by detecting the location of the genital tubercle with trans-rectal ultrasonography between days 59 and 68 of pregnancy and by trans-abdominal ultrasonographic imaging of the primary sex organs of the foetus between days 100 and 220. This technique requires a B-mode real time portable scanner equipped with appropriate transducers and a specialist (Bucca 2005; Aurich and Schneider 2014). It has been previously reported that sex determination is made by identification of foetal DNA in foetal fluid collected by amniocentesis with the aid of polymerase chain reaction (PCR) (Kamimura et al. 1997) or in blood plasma (Moura de Leon et al. 2012). However, in addition to the laboratory requirements for both techniques (Kamimura et al. 1997; Moura de Leon et al. 2012), amniocentesis also presents a risk of abortion (Kamimura et al. 1997).

Address for correspondence:
Zeynep Günay Uçmak
Faculty of Veterinary Medicine
Department of Obstetrics and Gynaecology
34320 Avcılar, Istanbul, Türkiye

Phone: 0-212-4737070 / 17316
E-mail: zeynep.gunayucmak@iuc.edu.tr
http://actavet.vfu.cz/

Sexual differentiation of equine embryonic gonads occurs around day 40 (Merchant-Larios 1979). Two different testicular hormones have a role in foetal masculinization; while testosterone enables the development of the Wolffian ducts, the urogenital sinus and external genitalia, anti-Müllerian hormone (AMH) causes regression of the Müllerian ducts. AMH is not secreted from the female foetal gonads, and thus the oviduct, uterus, and vagina are formed (Rodolfo 2005). Despite this clear knowledge in foetal development, there are limited number of studies about the possible effect of sex-related difference on maternal AMH concentrations in women (La Marca et al. 2005; Lutterodt et al. 2009; Empey et al. 2012; Shaukat and Shahid 2018) and in cows (Stojsin-Carter et al. 2017). In addition, the results of these studies are not consistent with each other. Lutterodt et al. (2009) and La Marca et al. (2005) reported that foetal sex did not affect maternal serum AMH concentrations of women and attributed this to the hypothesis that AMH could not cross the placental barrier and was not secreted from the placenta. However, in a later study (Wang et al. 2009), it was proven that AMH has autocrine/paracrine production in the human endometrium. Empey et al. (2012) reported that they found higher AMH concentrations in women carrying male foetuses at 11–15 weeks of pregnancy, whereas Shaukat and Shahid (2018) found higher serum AMH concentrations in women carrying female foetuses in the first trimester of pregnancy. Both studies (Empey et al. 2012; Shaukat and Shahid 2018) suggested the hypothesis that the maternal ovaries may respond to the foeto-placental signal in a dimorphic pattern related to foetal sex.

Equine chorionic gonadotrophin (eCG), formerly known as pregnant mare serum gonadotrophin (PMSG), begins to be secreted by special trophoblast cells that form endometrial cups on days 36–38 of pregnancy in mares, reaching the peak values between days 50–75, and declining to undetectable serum values until the day 150 in parallel with the degeneration of the endometrial cups (Cole and Hart 1930; Allen 1969; Allen 2001). To our knowledge, there is only one abstract that investigates the effect of foetal sex on maternal serum eCG concentration in mares, and these researchers found higher eCG concentrations in early pregnancy in mares carrying female foetuses (Mönke and Franz 1985).

The aim of the present study was to investigate the foetal sex-related difference and progression in maternal serum AMH and eCG concentrations during different points of time in pregnant mares.

Materials and Methods

All animal procedures were carried out in accordance with the approval of the Animal Experiments Local Ethics Committee at the Istanbul University (Approval Number: 2018/43).

Animals and experimental groups

The preliminary materials of the study were 40 Arabian mares within the age range of 6–10 years that had at least one previous uncomplicated parturition and came from the horse farm owned by the FEME Livestock and Agriculture Industry Import Export Limited Company. Following natural mating in the breeding season, pregnancies in these mares were diagnosed by trans-rectal ultrasonography (Esaote MyLab One Vet; Esaote Pie Medical, Genova, Italy) equipped with a 7.5 MHz linear probe on day 22 (Sertich 1997). Monthly control examinations as ultrasonographic (existence of the embryo and foetal heart beat) and rectal palpation were performed in mares with positive pregnancies until parturition. Mares whose pregnancies were negative or which had embryonic or foetal loss during their pregnancies were excluded from the study. This study was started with 40 mares against the risk of failure to develop pregnancy, embryonic or foetal loss, abortion, the mare being sold outside the horse farm and also to ensure that groups of mares carrying male and female foetuses were formed equally. Based on the project plan and budget, the total number of mares included in the study was limited to 24. The final two groups of the study consisted of 12 healthy male and 12 healthy female offspring foaling mares: group MFM and FFM, respectively.

Sample collection and serum analysis

Peripheral blood samples were collected by the jugular vein puncture on the day of the natural mating (just before copulation) and then monthly until the 6th month (mo) of gestation. Following transport and 2 h of rest at room temperature to favour clotting, samples were centrifuged at 1,500 × *g* for 15 min at 4 °C. Serum was separated and stored in Eppendorf tubes at −80 °C until further analyses. Haemolysed samples were excluded

from the study as recommended by the manufacturer. Serum AMH concentrations of the mares belonging to the time points of pre-mating and 1–6 mo of pregnancy were measured in duplicate with an equine specific enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (AL-115, Ansh Labs, Webster, TX, USA). The intra- and inter-assay coefficients of variation were 5.79 (n = 6) and 8.71% (n = 6), respectively. Serum eCG concentrations of the mares at 2–5 mo of pregnancy were measured in duplicate with an ELISA kit according to the manufacturer's instructions (MBS 701410, MyBiosource, San Diego, USA). The intra- and inter-assay coefficients of variation were 7.82 (n = 4) and 10.41% (n = 4), respectively.

Statistical analysis

SPSS 13.0 package programme was used for the statistical analysis. A general linear model (GLM) analysis was performed to compare groups MFM and FFM in terms of serum AMH concentrations at various sampling times. The model included the groups as between the subject effect and AMH concentration on the day of natural mating as covariate. Independent sample *t*-test was applied to compare the groups at sampling times for serum eCG concentrations. Independent sample *t*-test was also used to compare the ages of the groups and the change and relative change of AMH concentrations of the groups between mo 1–4 and mo 1–5. Repeated measures analysis of variance (ANOVA) was used to compare the time-dependent changes in serum AMH and eCG concentrations of each group and the sampling times were evaluated as within subject effect. Pearson's correlation test was used to analyse the correlation between serum AMH and eCG concentrations at mo 2–4. Data are given as mean ± standard error of the mean (SEM). Significance was set at $P < 0.05$.

Results

In the study, we started with 40 Arabian mares due to the risk management mentioned earlier; six mares did not conceive while 34 mares conceived by natural mating during the breeding season. Of these 34 mares, two were sold out of the horse farm (no further contact), two aborted and 30 gave birth to healthy foals. Data from only 24 healthy foaling mares (12 MFM and 12 FFM) were used adhering to the project planning and budget. In group MFM, five serum samples belonging to three mares (n = 2 from mo 2, n = 1 from mo 3, n = 1 from mo 4, n = 1 from mo 5) and in group FFM, 5 serum samples belonging to 4 mares (n = 2 from mo 3, n = 2 from mo 4, n = 1 from mo 5) were excluded due to haemolysis. The exclusion of haemolysed serum samples in the study decreased the number in some comparisons also depending on the statistical model used. While in GLM analysis and in independent sample *t*-test, haemolysed serum samples simply reduced the number of the corresponding month, only the mares with full rank datasets were used in repeated measures ANOVA. Mean ages of the groups MFM and FFM were 8.3 ± 0.43 and 8.25 ± 0.33 years, respectively, and no significant difference was observed between the groups ($P > 0.05$).

Serum AMH concentrations of the groups obtained on the day of natural mating were similar ($P > 0.05$), 3.22 ± 0.38 ng/ml for MFM and 3.96 ± 0.49 ng/ml for FFM. Serum AMH concentrations of group FFM at mo 4 and mo 5 (3.89 ± 0.49 ng/ml, n = 10; 2.89 ± 0.32 ng/ml, n = 11) were significantly higher than those of group MFM (2.11 ± 0.46 ng/ml, n = 11; 1.87 ± 0.32 ng/ml, n = 11), ($P < 0.05$). The mo of gestation (mo 1–6) had no effect on serum AMH concentrations of either group MMF or FFM ($P > 0.05$), (Fig. 1). Change in AMH concentrations of the groups between mo 1–4 and mo 1–5 were not significantly different ($P > 0.05$) but the change in the negative direction was numerically greater in group MFM, (Fig. 2A). The relative changes between mo 1–4 and mo 1–5 normalized to the level at mo 1 in serum AMH were not significantly different between the groups ($P > 0.05$), but the relative change of group MFM between mo 1–5 normalized to the level at mo 1 in serum AMH had a strong tendency towards the negative direction ($P = 0.06$), (Fig. 2B).

Serum eCG concentrations of group FFM at mo 2 (359.73 ± 41.51 mIU/ml, n = 12) were significantly higher than in group MFM (255.22 ± 21.18 mIU/ml, n = 10), ($P < 0.05$). Serum eCG was not detected at mo 5. Group-time interaction for eCG concentrations at mo 2–4 was not significant ($P > 0.05$), (Fig. 3).

Concentrations of serum AMH were unrelated to the corresponding serum eCG concentrations at mo 2–4 ($P > 0.05$).

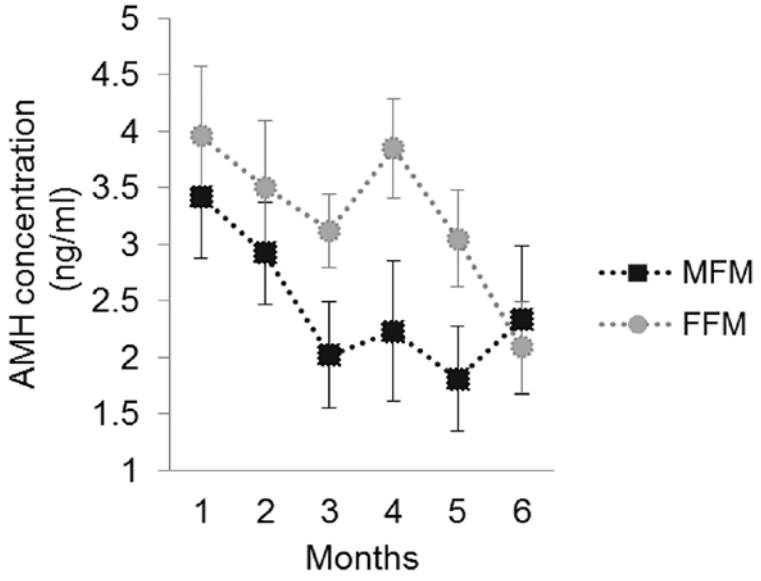

Fig. 1. The effect of gestation month on the mean serum anti-Müllerian hormone concentrations in groups of male offspring foaling mares (n = 9) and female offspring foaling mares (n = 8)*

AMH - anti-Müllerian hormone; MFM - male offspring foaling mares, FFM - female offspring foaling mares
*Since the statistical method used, repeated measures ANOVA, requires full rank datasets, only animals with full rank datasets (n = 9 for MFM and n = 8 for FFM) were used to generate these data.

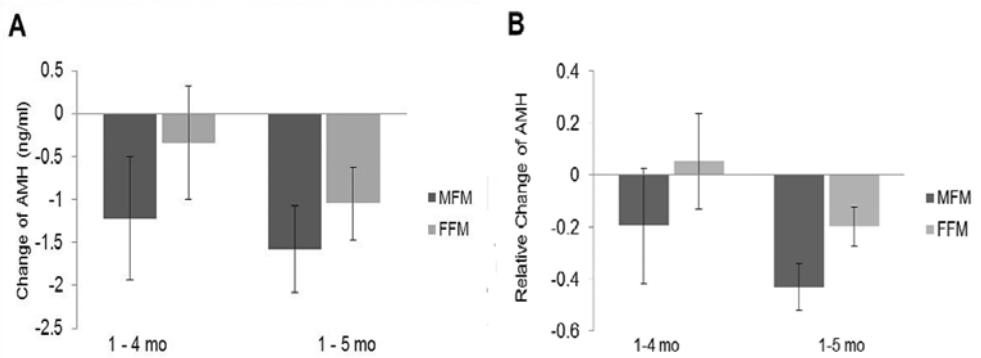

Fig. 2. A) Change B) Relative change of serum anti-Müllerian hormone concentrations in groups of male offspring foaling mares and female offspring foaling mares between months 1–4 and 1–5

AMH - anti-Müllerian hormone; MFM - male offspring foaling mares, FFM - female offspring foaling mares; mo - month

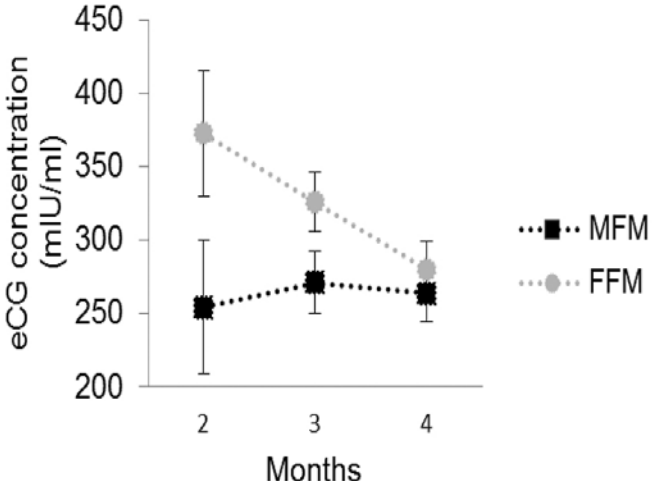

Fig. 3. The effect of gestation month on the mean serum equine chorionic gonadotrophin concentrations in groups of male offspring foaling mares (n = 9) and female offspring foaling mares (n = 8)*

eCG - equine chorionic gonadotrophin; MFM - male offspring foaling mares, FFM - female offspring foaling mares
*Since the statistical method used, repeated measures ANOVA, requires full rank datasets, only animals with full rank datasets (n = 9 for MFM and n = 8 for FFM) were used to generate these data.

Discussion

Sex related differences and progression in maternal serum AMH and eCG concentrations in pregnant mares are presented in this study.

Uliani et al. (2019) reported that serum AMH concentrations of mares aged 5–15 years were similar and significantly higher than those < 5 years old, and serum AMH concentrations in mares > 25 years of age were significantly lower than in younger mares. Almeida et al. (2011) reported on the individual variability in AMH concentrations. In the present study, mares of both MFM and FFM groups were aged 6–10 years, and no significant difference was observed between the mean ages of the groups. Besides, since the effect of AMH concentration on the day of natural mating was found significant, it was included in the GLM analysis as a covariate. Korkmaz et al. (2020) reported mean serum AMH concentrations in young, middle aged, and senior mares as 0.466 ± 0.051, 0.873 ± 0.096 and 0.347 ± 0.068 ng/ml, respectively. Almeida et at. (2011) reported mean serum AMH concentrations of cyclic mares and pregnant mares as 0.96 ± 0.08 and 0.72 ± 0.05 ng/ml, respectively. In our study, mare serum AMH concentrations determined at all evaluation points (Fig. 1) were different and higher than the findings mentioned above, but similar to two studies (Scarlet et al. 2018; Uliani et al. 2019) in which the same commercial kit (Equine specific ELISA kit, AL-115) was used.

There are studies with conflicting results regarding the effects of foetal sex on serum/plasma AMH concentrations in women. Lutterodt et al. (2009) and La Marca et al. (2005) reported that the sex of the foetus had no impact on the maternal serum AMH concentrations of women. Empey et al. (2012) reported that they found higher AMH concentrations in women carrying male foetuses at 11–15 weeks of pregnancy than those carrying female foetuses. On the other hand, Shaukat and Shahid (2018) found higher AMH levels in women carrying a female foetus in the first trimester of pregnancy compared to women carrying a male foetus. They also found that pre-term babies had lower AMH concentrations compared to full-term babies, and mothers who had pre-term labour had

higher AMH concentrations compared to mothers with full-term births. A study conducted in cows (Stojsin-Carter et al. 2017) reported that carrying a female or male foetus did not have any effect on maternal plasma AMH concentration in the same gestation period. To our knowledge, this is the first study investigating the foetal sex-related difference and progression in serum AMH concentrations of pregnant mares. Consistent with Shaukat and Shahid (2018), serum AMH concentrations of group FFM in this study at mo 4 and 5 were significantly higher than those in group MFM.

During the embryonic sex differentiation period, AMH is secreted from the testicles of male foetuses but not from female gonads (Rodolfo 2005). AMH was detected in the serum of second-trimester male foetuses but undetected in the serum of female foetuses; even in immunohistochemical staining, the testes were strongly stained for AMH, whereas female gonads were not stained (Lutterodt et al. 2009). It has been reported that AMH, a glycoprotein with a molecular weight of 140 kDa (Di Clemente et al. 2003), cannot cross the placenta, and AMH concentrations in male foetuses cannot affect the maternal circulation (Lutterodt et al. 2009). Our study's finding that the AMH concentration in group MFM was lower than in group FFM at mo 4 and 5 supports this view. It has been reported that AMH and AMHRII mRNA expressions were observed in the human placenta and foetal membranes regardless of sex, and male foetal membranes were stained more intense in immunohistochemical staining (Novembri et al. 2015). With these findings, Novembri et al. (2015) hypothesized that the placenta and foetal membranes may play a role in the regulation of AMH. Recently, a miRNA-based communication between the placenta and foeto-maternal compartments was defined (Chang et al. 2017), and the role of miRNA in the regulation of AMH was reported (Lei et al. 2010). We hypothesize that this AMH regulation mechanism in which placenta and foetal membranes may play a role, can work in such a way that AMH is high in the maternal blood circulation when it is low in the foetal blood circulation and low in the maternal blood circulation when it is high in the foetal blood circulation. The issue has not been clarified yet and further molecular studies are needed.

In seven of the eight studies that McCredie et al. (2017) reviewed, AMH concentrations were reported to decline with the progressing pregnancy of women. Köninger et al. (2013) reported in their cross-sectional study (n = 450) that the AMH concentrations of pregnant women dropped significantly from the first to the second trimester while this decrease was non-significant in their longitudinal cohort study (n = 15). Almeida et al. (2011) evaluated AMH concentrations of mares monthly throughout their pregnancy and reported no effect of time on it. In line with Almeida et al. (2011), in the present study, the mo of gestation (mo 1–6) had no effect on serum AMH concentrations of either group MFM or FFM. However, there was a non-significant decline in AMH concentrations in the groups from mo 1 to 3 of pregnancy (Fig. 1) as was found in the longitudinal cohort study of Köninger et al. (2013). Negative effects of maternal adiposity and haemodilution on AMH (Nelson et al. 2010; McCredie et al. 2017) are unlikely to be seen in the early stages of pregnancy in mares. Decreased follicular activity has been reported between days 50 and 140 of gestation in mares (Squires et al. 1974). With the contribution of the secondary corpus luteum formed by the influence of eCG in mares, a second peak is formed in the progesterone concentration after day 40 of gestation, and this concentration remains high until day 90 (Squires and Ginther 1975). In this study, the most likely cause for the non-significant decrease in AMH concentration between mo 1 and 3 of gestation in mares may be that increased progesterone negatively affects the follicle-stimulating hormone (FSH) and partially suppresses follicular activity.

Human chorionic gonadotrophin (hCG) secreted from the young trophoblast cells of the placenta in women, peaks at 56–58 days of gestation and decreases to the lowest level at 18 weeks (Braunstein et al. 1976). Concentrations of hCG in maternal serum of women

carrying a female foetus in the third trimester of pregnancy were found to be significantly higher than those carrying a male foetus (Haning et al. 1989; Steier et al 1999; Gol et al. 2005). Although there are many studies (Haning et al. 1989; Steier et al 1999; Gol et al. 2005) evaluating concentrations of hCG secreted from the placenta in women in relation to the foetal sex, we could only find one source in literature (Mönke and Franz 1985) in which the concentrations of eCG secreted from the placenta in mares were evaluated in relation to the foetal sex. The findings of the present study that the serum eCG concentrations of group FFM at mo 2 were significantly higher than those of group MFM, are consistent with the findings of Mönke and Franz (1985). Further studies are needed to explain how eCG secretion or metabolism is affected by foetal sex in early gestation of mares. It has been reported that the peak of eCG concentration in mares occurs between 50–85 days after ovulation, and it was no longer detected in the circulation on day 150 (Wilsher and Allen 2011). The non-significance of group-time interaction for mo 2–4 in this study may be due to the fact that our evaluation points were widely spaced (monthly) and that we could not catch the onset and peak of the secretion.

Lutterodt et al. (2009) reported no correlation between the concentrations of AMH and hCG in pregnant women. Similarly, in the present study, no correlation was found between serum AMH and eCG concentrations in the groups of mares at mo 2–4.

To our knowledge, this is the first study investigating the difference and progression of AMH concentration in pregnant mares in relation to foetal sex. However, individual variations in AMH and eCG concentrations and the inability to determine a cut-off point for determination of foetal sex make these hormones unlikely candidates for determining foetal sex in the mare. We found significant sex-related differences in the AMH concentrations in mo 4 and 5 of pregnancy and in the eCG concentrations in mo 2^{d} of pregnancy in mares, which may provide clues to future molecular studies that will explain the regulation mechanisms of these hormones during pregnancy.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by The Scientific and Technological Research Council of Turkey, Tübitak-1002 Short Term Funding Program (grant no. 1190673).

The authors are also grateful to Prof. Dr. Bülent Ekiz for the statistical analysis, FEME Manager Veterinarian DVM Ömer Mehmet Erzengin for his support.

References

Allen WR 1969: The immunological measurement of pregnant mare serum gonadotropin. J Endocrinol **32**: 593-598

Allen WR 2001: Fetomaternal interactions and influences during equine pregnancy. Reproduction **121**: 1470-1626

Almeida J, Ball BA, Conley AJ, Place NJ, Liu IKM, Scholtz EL, Mathewson L, Stanley SD, Moeller BC 2011: Biological and clinical significance of anti-Müllerian hormone determination in blood serum of the mare. Theriogenology **76**: 1393-1403

Aurich C, Schneider J 2014: Sex determination in horses-current status and future perspectives. Anim Reprod Sci **146**: 34-41

Braunstein GD, Rasor J, Danzer H, Adler D, Wade ME 1976: Serum human chorionic gonadotropin levels throughout normal pregnancy. Am J Obstet Gynecol **126**: 678-681

Bucca S 2005: Equine fetal gender determination from mid to advanced gestation by ultrasound. Theriogenology **64**: 568-571

Chang G, Mouillet JF, Mishima T, Chu T, Sadovsky E, Coyne CB, Parks WT, Surti U, Sadovsky Y 2017: Expression and trafficking of placental microRNAs at the feto-maternal interface. Faseb J **31**: 2760-2770

Cole HH, Hart GH 1930: The potency of blood serum of mares in progressive stages of pregnancy in effecting the sexual maturity of the immature rat. Am J Physiol **93**: 57-68

Di Clemente N, Josso N, Gouedard L, Belville C 2003: Components of the anti-Müllerian hormone signaling pathway in gonads. Mol Cell Endocrinol **211**: 9-14

Empey R, Santillan D, Santillan M, Tyler E, Hunter S, Smith E, Stegman B 2012: The influence of fetal sex on patterns of change in anti-Mullerian hormone during pregnancy. Proc Obstet Gynecol **2**: 4
Gol M, Güçlü S, Demir A, Erata Y, Demir N 2005: Effect of fetal gender on maternal serum human chorionic gonadotropin levels throughout pregnancy. Arch Gynecol Obstet **273**: 90-92
Haning RV, Curet LB, Poole K, Boehnlein LM, Kuzma DL, Meier SM 1989: Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol. Am J Obstet Gynecol **161**: 1549-1553
Holder RD 2000: Fetal sex determination in the mare between 55 and 150 days gestation. AAEP Proceedings **46**: 321-324
Kamimura S, Nishiyama N, Ookutsu S, Goto K, Hamana K 1997: Determination of bovine fetal sex by PCR using fetal fluid aspirated by transvaginal ultrasound-guided amniocentesis. Theriogenology **47**: 1563-1569
Korkmaz Ö, Emre B, Polat İM, Zonturlu AK, Pir Yağcı İ, Pekcan M, Gürler Ş, Akkuş T, Çebi Şen Ç 2020: The correlation between anti-müllerian hormone concentrations and reproductive parameters in different age groups in purebred Arabian mares. Kafkas Univ Vet Fak Derg **26**: 53-57
Köninger A, Kauth A, Schmidt B, Schmidt M, Yerlikaya G, Kasimir-Bauer S, Kimmig R, Birdir C 2013: Anti-Mullerian-hormone levels during pregnancy and postpartum. Reprod Biol Endocrinol **11**: 60
La Marca A, Giulini S, Orvieto R, De Leo V, Volpe A 2005: Anti-Müllerian hormone concentrations in maternal serum during pregnancy. Hum Reprod **20**: 1569-1572
Lei L, Jin S, Gonzalez G, Behringer RB, Woodruff TK 2010: The regulatory role of Dicer in folliculogenesis in mice. Mol Cell Endocrinol **315**: 63-73
Lutterodt M, Byskov AG, Skouby SO, Tabor A, Andersen CY 2009: Anti-Müllerian hormone in pregnant women in relation to other hormones, fetal sex and in circulation of second trimester fetuses. RBMOnline **18**: 694-699
McCredie S, Ledger W, Venetis CA 2017: Anti-Müllerian hormone kinetics in pregnancy and post-partum: a systematic review. RBMOnline **34**: 522-533
Merchant-Larios H 1979: Ultrastructural events in horse gonadal morphogenesis. J Reprod Fertil Suppl **27**: 479-485
Moura de Leon PM, Campos VF, Dellagostin OA, Deschamps JC, Seixas FK, Collares T 2012: Equine fetal sex determination using circulating cell-free fetal DNA (ccffDNA). Theriogenology **77**: 694-698
Mönke U, Franz A 1985: Investigations on the sex ratio of horse embryos and its importance for PMSG production in mares. Anim Breed Abstr **53**: 7359
Nelson SM, Steward F, Fleming R, Freeman DJ 2010: Longitudinal assessment of antimüllerian hormone during pregnancy-relationship with maternal adiposity, insulin, and adiponectin. Fertil Steril **93**: 1356-1358
Novembri R, Funghi L, Voltolini C, Belmonte G, Vannuccini S, Toricelli M, Petraglia F 2015: Placenta expresses anti-Müllerian hormone and its receptor: Sex-related difference in fetal membranes. Placenta **36**: 731-737
Rodolfo R 2005: Anti-Müllerian hormone in disorders of sex determination and differentiation. Arq Bras Endocrinol Metab **49**: 26-36
Scarlet D, Wulf M, Kuhl J, Köhne M, Ille N, Conley AJ, Aurich C 2018: Anti-Müllerian hormone profiling in prepubertal horses and its relationship with gonadal function. Theriogenology **117**: 72-77
Sertich P 1997: Pregnancy evaluation in the mare. In: Youngquist RS (Ed.): Current therapy in large animal theriogenology. WB Saunders Company, Philadelphia. First edn, 898 p.
Shaukat A, Shahid A 2018: Maternal anti-mullerian hormone levels can predict fetal gender and pregnancy outcome. Gynecol Obstet **8**: 83
Squires EL, Garcia MC, Ginther OJ 1974: Effects of pregnancy and hysterectomy on the ovaries of pony mares. J Anim Sci **38**: 823-830
Squires EL, Ginther OJ 1975: Follicular and luteal development in pregnant mares. J Reprod Fertil Suppl **23**: 429-433
Steier JA, Myking OL, Bergsjø PB 1999: Correlation between fetal sex and human chorionic gonadotropin in peripheral maternal blood and amniotic fluid in second and third trimester normal pregnancies. Acta Obstet Gynecol Scand **78**: 367-371
Stojsin-Carter A, Costa NN, De Morais R, De Bem TH, Costa MP, Carter TF, Gillis DJ, Neal MS, Ohashi OM, Miranda MS, Meirelles FV, Favetta LA, King WA 2017: Fetal sex alters maternal anti-Mullerian hormone during pregnancy in cattle. Anim Reprod Sci **186**: 85-92
Uliani RC, Conley AJ, Corbin CJ, Friso AM, Maciel LFS, Alvarenga MA 2019: Anti-Müllerian hormone and ovarian aging in mares. J Endocrinol **240**: 147-156
Wang J, Dicken C, Lustbader JW, Tortoriello DW 2009: Evidence for a Müllerian-inhibiting substance autocrine/paracrine system in adult human endometrium. Fertil Steril **91**: 1195-1203
Wilsher S, Allen WR 2011: Factors influencing equine chorionic gonadotrophin production in the mare. Equine Vet J **43**: 430-438