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The Correlation Between Anti-Müllerian Hormone Concentrations and Reproductive Parameters in Different Age Groups in Purebred Arabian Mares

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Abstract

The objectives of this study were to examine correlations of serum anti-Müllerian hormone (AMH) levels with age, ovarian function and fertility performance in purebred Arabian mares and to assess the potential use of AMH concentrations as a fertility indicator for the selection of breeder animals. For this purpose, thirty-six non-lactating purebred Arabian mares with no previous fertility problems constituted the material of the study. The animals were assigned to groups according to their age: Group I (aged 4-8 years), group II (9-18 years) and group III (19-25 years). Mean serum AMH concentrations were significantly higher (P<0.001) in group II (0.873±0.096 ng/mL) than in groups I (0.466±0.051 ng/mL) and III (0.347±0.068 ng/mL). Furthermore, serum AMH concentration was positively correlated with the daily increase in ovarian follicle diameter as well with mares with higher conception rates. It was also negatively correlated with anovulation and number of mating per conception. In summary, a strong relationship was found between peripheral AMH concentrations and fertility performance in purebred Arabian mares.

Keywords: *Mare, Anti-Müllerian hormone, Age, Reproductive performance*

Farklı Yaş Guruplarındaki Safkan Arap Kısraklarda Anti Müllerian Hormon Konsantrasyonları Ile Reprodüktif Parametrelerin Korelasyonu

Öz

Bu çalışmanın amacı, safkan Arap kısraklarda serum anti-Müllerian hormon (AMH) seviyelerinin yaş, ovaryum fonksiyonları ve fertilite performansı ile korelasyonlarını incelemek ve AMH konsantrasyonlarının damızlık hayvan seçimi için fertilite göstergesi olarak potansiyel kullanımını değerlendirmektir. Bu amaçla çalışmanın materyalini herhangi bir fertilite problemi bulunmayan ve laktasyonda olmayan 36 Safkan Arap kısrak oluşturdu. Hayvanlar yaşlarına göre 3 gruba ayrıldı: Grup I (4-8 yaş), grup II (9-18 yaş) ve grup III (19- 25 yaş). Ortalama serum AMH konsantrasyonları, grup II'de (0.873±0.096 ng/mL) grup I (0.466±0.051 ng/mL) ve III'e (0.347±0.068 ng/mL) göre anlamlı derecede yüksek bulundu (P<0.001). Kısraklarda serum AMH konsantrasyonunun, günlük ovaryum folikül çap artışıyla ve gebelik oranları ile pozitif korelasyon gösterirken anovulasyon ve gebelik başına düşen aşım sayısı ile negatif korelasyon gösterdiği görüldü. Özetle, safkan Arap kısraklarda periferik AMH konsantrasyonları ile fertilite performansı arasında güçlü bir ilişki tespit edildi.

Anahtar sözcükler: *Kısrak, Anti-Müllerian hormon, Yaş, Reprodüktif performans*

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INTRODUCTION

The anti-Müllerian hormone (AMH) is a homodimeric glycoprotein belonging to the transforming growth factor beta family and is secreted only from the gonads in both males and females [1,2]. In addition to causing regression of the Müllerian ducts during embryonic development, it is also involved in regulating development of primordial follicles [3-5]. AMH contributes to maintenance of oocyte reserves by reducing the sensitivity of developing follicles to follicle-stimulating hormone (FSH) and thereby limiting the number of actively developing follicles [3,6]. Measuring the AMH level has a particularly wide range of uses in women, including determination of the ovarian reserve, which is the number of functional follicles, monitoring the transition into the menopausal period, diagnosis of polycystic ovarian syndrome and granulosa cell tumours and determination of low ovarian response and prognosis in in vitro fertilization applications [2]. Furthermore, it has been reported that AMH can be also used for the diagnosis of ovarian remnant syndrome in bitches [7,8] and granulosa cell tumours in mares [3,9,10] as well as a fertility indicator in ewes [11], cows [12] and bitches [13].

In mares, AMH is secreted by the granulosa cells of preantral and small antral follicles [3,14] and its plasma concentration was found to be positively correlated with the number of follicles having a diameter of 6-20 mm [14]. AMH is secreted at a lower level by follicles with a diameter greater than 30 mm [3] and its plasma concentration increases with higher numbers of developing follicles [15]. Although serum AMH concentrations may show individual variations among mares, these concentrations reportedly do not vary with the different stages of the estrous cycle or pregnancy [3,9].

Anti-Müllerian hormone concentrations decrease with the ageing process in mice [16], bitches [13] and women [17]. In older mares in which the follicular stage is prolonged, the occurrence of ovulation-related problems is significantly increased [18]. It has been reported that the number of antral follicles were decreased in senile mares (>19 years old), which causes plasma AMH concentrations to be lower than those of middle-aged mares (aged 9-18 years) [14]. To date, although there are some studies that investigated the influence of AMH on fertility performance in mares, the correlations between reproductive parameters and AMH levels and age are not yet clearly understood [14,19,20].

The aim of this study was to examine the correlation of serum AMH concentrations with age and certain reproductive parameters during the spring transition and in particular, to investigate the feasibility of using AMH values as a fertility indicator for breeder animal selection in purebred Arabian mares, which have a relatively short breeding period and are of very high economic value in the northern hemisphere.

MATERIAL and METHODS

Ethics

In this study, the Ethics Committee report (Approval number: 2016/20) was received before application in accordance with the directions of the Dollvet Inc. Experimental Animals Local Ethics Board.

Animals

The subjects of this study were 36 non-lactating Purebred Arabian mares, which were assessed as not conceiving in the previous breeding season. These mares were referred to the Animal Hospital of Harran University, Faculty of Veterinary Medicine between February 10-June 30, 2017 and were confirmed as having no apparent fertility problems upon gynecological clinical examination (ultrasonographic examination of uterus and ovaries and inspection of cervix, vagina and vulva). The animals were assigned to three groups according to their age: Group I (young animals aged 4-8 years, n=12), group II (middle-aged animals aged 9-18 years, n=12) and group III (senile animals aged 19-25 years, n=12).

AMH Assay

Between the 10th and 15th of February, blood samples were taken from the *V. jugularis* into dry tubes and were allowed to clot. Once coagulated, the samples were centrifuged at 3000 rpm for 15 min. The sera were stored at -20°C until analyzed. Serum AMH levels were determined by the ELISA (DRG Instruments ELISA Mat 2000) using a commercial kit (Beckman Coulter, AMH Gen II, USA). All serum assays were performed in duplicate. The lower and upper limits of detection were 0.375 ng/mL and 150 ng/mL, respectively. The respective intra- and inter-assay coefficients of variations were <8% and <10%, respectively. The standard curve range was 0.07-22.5 ng/mL, and the limit of detection value of the ELISA kit was <0.1 ng/mL.

Ultrasonographic Examinations

During the breeding season (February 15-June 30), repeated daily ultrasonographic examinations (SIUI, CTS-800, linear probe, 5 MHZ, Guangdong, China) were made until ovulation occurred. These examinations provided information on the stages of the sexual cycle, daily development of ovarian follicles, single or multiple ovulation status and the presence of anovulatory follicles. Mares with a follicle diameter greater than 35 mm and showing a positive response to stallion teasing were mated every other day until ovulation with stallions known to have normal fertility parameters by andrological examination. In mares that were detected to have ovulated, the first pregnancy examinations were performed on the 15th day post-ovulation. Mares that were found to have conceived were further examined on days 30 and 45. The mares that displayed a healthy gestation at the third examination were considered to be definitively

Table 1. *Mean AMH concentrations (ng/mL) and reproductive outcomes of the mares*

Group	Age (year) (X±Sx)	AMH (ng/mL) (X±Sx)	DIFD (%) (X±Sx)	FDO (mm) (X±Sx)	NIMC (number) (X±Sx)	CR (%)
I	6.08±1.12	0.466±0.05[a]	2.52±0.14[a]	42.72±0.80	1.62±0.26	10/12 (83.3)[ab]
II	12±2.32	0.873±0.096[b]	2.89±0.29[a]	43.69±0.93	1.20±0.20	11/12 (91.67)[b]
III	20.84±2.78	0.347±0.068[a]	2.11±0.06[b]	44.12±0.65	2.00±0.29	5/12 (41.67)[a]
P value		0.001	0.002	0.779	0.168	0.013

AMH: *Anti-Müllerian hormone,* ***DIFD:*** *Mean daily increase in follicle diameter,* ***FDO:*** *Follicle diameter at the time of ovulation,* ***NIMC:*** *Number of mating per conception,* ***CR:*** *Conception rate, [a,b] Means with different superscripts in the same column show statistically significant differences*

pregnant. Throughout this period, mares that suffered embryonic and early fetal death were also recorded.

Statistical Analyses

Statistical analysis of the data was performed with the Statistical Package for the Social Sciences (SPSS for Windows; version 22.0, USA) program. The normal distribution of the variables and analytical methods were examined using visual (histogram and probability plots) and Kolmogorov-Smirnov/Shapiro-Wilk tests. Descriptive analyses were given as means±standard error for normally dispersed and normally non-dispersed variables. The homogeneity of variances was determined by the Levene test. The data were analyzed by a one-way ANOVA test because age, AMH and follicle diameter at the time of ovulation were normally distributed. These parameters were compared between the groups using the Kruskal-Wallis test because it was determined that the mean daily increase in follicle diameter and number of mating per conception did not show a normal distribution. Two-handed comparisons were made using the Mann-Whitney U test and assessed using the Bonferroni correction. P values <0.05 were considered as statistically significant results. Comparisons were done with the post-hoc Tukey test, when the difference between the groups was significant. Correlation coefficients and statistical significance were calculated by the Spearman test for correlations between AMH and some reproductive parameters that were not normally distributed or between AMH and ordinal variables. The normal distributions with AMH (age and follicle diameter at the time of ovulation) were calculated by Pearson's test. P value of <0.05 was used to establish statistical significance.

RESULTS

Serum AMH concentrations, mean daily increase in follicle diameter, follicle diameter at the time of ovulation, number of mating per conception and conception rate in the different study groups are shown in *Table 1*. The mean serum AMH concentrations were 0.466±0.051 ng/mL in the young mares (group I), 0.873±0.096 ng/mL in the middle-aged mares (group II) and 0.347±0.068 ng/mL in the senile mares (group III). The mean serum AMH concentration calculated for all the mares used in the study was 0.515±0.324 ng/mL. The differences in mean serum AMH concentrations between groups I and II (P<0.01) and between groups II and III (P<0.001) were statistically significant while the difference between groups I and III was insignificant (P>0.05). With respect to the daily follicle diameter increase among the groups, the difference between groups I and II was not significant, while the differences between groups I and III, and between groups II and III, were statistically significant. Anovulation and embryonic mortality rates for groups I, II and III were 1/12, 0/12, 2/12 and 2/12, 0/12, 2/12, respectively and there was no statistical significance among the groups.

Table 2.*Correlation of Anti-Müllerian Hormone values with age of the mares and reproductive outcomes*

Reproductive Outcomes	Anti-Müllerian Hormone	
	r	P
Age of mares	-0.243	0.153
Daily increase in follicle diameter	+0.463	0.013
Ovulated follicular diameter	-0.104	0.564
Multiple ovulation	-0.047	0.787
Anovulation	-0.440	0.007
Conception	+0.608	0.000
Number of mating per conceptions	-0.562	0.001
Embryonic death	-0.072	0.675

In the present study, it was ascertained that in purebred Arabian mares, serum AMH concentrations were significantly and positively (+) correlated with daily increase in follicle diameter (r=+0.463) and conception rate, and were negatively (-) correlated with the anovulation rate (r=-0.440), number of mating per conception (r=-0.562) and follicle diameter at the time of ovulation (r=-0.104) *(Table 2)*.

DISCUSSION

Almeida et al.[9] reported the mean serum AMH concentrations in cyclic, pregnant, and ovariectomized mares were 0.96±0.08 ng/mL, 0.72±0.05 ng/mL and 0.06±0.003 ng/mL, respectively. These authors reported that there were no differences between pregnant and cyclic mares or between the different stages of the estrous cycle for AMH concentration. Vernunft et al.[15] reported that plasma AMH concentrations ranged between 0.6±0.04 and 1.2±0.05

ng/mL, depending on the number of developing follicles in the ovaries. In the present study, the mean serum AMH concentrations measured in purebred Arabian mares were 0.466±0.051 ng/mL in young animals aged 4-8 years, 0.873±0.096 ng/mL in middle-aged animals aged 9-18 years, and 0.347±0.068 ng/mL in senile animals aged 19-26 years. The AMH concentrations measured in the different age groups in this study showed that the AMH levels of group II, composed of middle-aged mares, not only fell within the AMH range previously reported by Vernunft et al.[15], but also displayed similarity to the concentrations measured by Almedia et al.[9] in cyclic mares. The levels of AMH measured by both these groups being similar to the concentrations detected in middle-aged mares in the present study was attributed to these authors having used cyclic animals with no reproductive health problems. Furthermore, in this study the lowest number of mating per conception and the highest conception rate were also detected in group II which displayed AMH concentrations similar to those reported in previous studies. In addition, in the present study, the lowest conception rate and the highest number of mating per conception were detected in the senile mares in group III which had the lowest AMH concentrations. Claes et al.[14] reported the mean number of antral follicles as 13.6 in senile mares, 25.5 in young mares and 27.8 in middle-aged mares. In parallel with these numbers, these authors reported the mean plasma AMH concentrations as 0.21 ng/mL in senile mares, 0.47 ng/mL in middle-aged mares and 0.29 ng/mL in young mares. In the present study, similar to the results reported by Claes et al.[14], serum AMH levels were highest in the middle-aged mares and lowest in the senile mares. Furthermore, when assessed together with the results reported by Claes et al.[14], the lowest conception rate and the highest number of mating per conception in the senile mares, together with their reduced serum AMH levels, were considered to be also related to the reduced number of antral follicles found in these animals.

In the present study, the maximum follicle diameter at the time of ovulation was largest in the senile mares (group III) and was smallest in the young mares (group I). These data are in agreement with those reported by Ginther et al.[21]. Likewise, consistent with the results of Ginther et al.[21], in the present study, the daily increase in follicle diameter was found to be lowest in the senile mares and highest in the middle-aged mares. These findings support the hypothesis of Ball et al.[3], who proposed that AMH plays an important role in follicular recruitment and selection of the dominant follicle.

In this study, it was ascertained that the serum AMH concentrations measured during the spring transition period in mares were positively correlated with the daily increase in follicle diameter and conception rate in the breeding season and were negatively correlated with the anovulation rate and number of mating per conception. The lowest serum AMH concentrations were measured in the senile mares (group III). Furthermore, in that group the lowest daily increase in follicle diameter and conception rates were positively correlated with AMH concentrations; as well as with the highest anovulation rate and number of mating per conception, which were negatively correlated with AMH concentrations.

It was reported that, in parallel with reduced gonadal functions and ovarian reserves in older women, AMH concentrations were also decreased [17]. In females, the major source of AMH is the granulosa cells of early antral follicles [2,3]. Korkmaz et al.[13] indicated that the number of granulosa cells of preantral follicles shows a striking decrease with advanced age in bitches. Granulosa cells, which physically support oocyte development and provide the necessary micro-environment for this process, are capable of active differentiation [5]. In the present study, a decrease was observed in the AMH concentrations of purebred Arabian Mares with advanced age, which was associated with the increased number of mating per conception and anovulation rate and decreased conception rate, all of which may be attributed to possibly decreased numbers of preantral granulosa cells occurring with ageing. The lowest number of antral follicles detected in senile mares by Claes et al.[14] supports this suggestion. In a previous study [14], it was reported that there is a relationship between peripheral AMH concentrations and age which directly affects the fertility outcomes of mares. In contrast to the report by Ball et al.[3] and Hanlon et al.[20] declared that there was no relationship between AMH and fertility in mares. In contrast, this relationship was seen clearly in our study with purebred Arabian Mares: when they were allocated to young, middle and older ages, peripheral AMH concentrations were correlated with fertility outcomes.

In conclusion, serum AMH concentrations were highest in middle-aged mares (aged 9-18 years) and lowest in senile mares (aged 19-26 years). According to our results, AMH is positively correlated with the daily increase in follicle diameter, follicle diameter at the time of ovulation and conception rates in mares. It was also negatively correlated with the number of mating per conception. Therefore, AMH is a reliable parameter for selection of Purebred Arabian Mares undergoing breeding and routine reproductive examinations as an indicator of their fertility.

Declaration of Conflicting Interests

The authors(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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