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Determination of anti-Müllerian hormone levels in female Awassi sheep of different ages

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ABSTRACT:

The present study aimed to determine the serum Anti-Müllerian Hormone (AMH) concentrations in female Awassi sheep across different age groups. For this purpose, a total of 87 female Awassi ewes were classified according to their age into four groups: Group I (n=16) (3–4 months); Group II (n=22) (6 months); Group III (n=25) (9–10 months); and Group IV (n=24) (≥24 months). A single blood sample was collected from all animals for the measurement of serum AMH levels, which were subsequently determined using a commercially available AMH ELISA kit. The medium, minimum, and maximum serum AMH concentrations for Group I, Group II, Group III, and Group IV were found to be 316.79 (88.01-1094.36) pg/mL, 493.62 (224.67-1242.55) pg/mL, 528.98 (342.52-880.78) pg/mL, and 394.23 (205.01-706.54) pg/mL, respectively. AMH levels in ewes belonging to Group II and Group III were significantly higher compared to those in Group I and Group IV ($p<0.05$). Conversely, no significant difference in AMH levels was observed between Group I and Group IV ($p>0.05$). Similarly, no significant difference was found between the AMH concentrations of Group II and Group III ($p>0.05$). In conclusion, the present study showed that serum AMH concentrations can vary according to different age groups in female Awassi sheep.

Farklı yaşlarda bulunan İvesi ırkı koyunların anti-müllerian hormon seviyelerinin belirlenmesi

ÖZET:

Bu çalışmada, farklı yaş gruplarındaki dişi İvesi koyunlarında serum Anti-Müllerian Hormon (AMH) konsantrasyonlarının belirlenmesi amaçlanmıştır. Araştırma materyalini toplam 87 baş İvesi koyunu oluşturmuş ve hayvanlar yaşlarına göre dört gruba ayrılmıştır: Grup I (n=16; 3–4 aylık), Grup II (n=22; 6 aylık), Grup III (n=25; 9–10 aylık) ve Grup IV (n=24; ≥24 aylık). Tüm hayvanlardan tek seferlik kan örneği alınmış ve serum AMH düzeyleri ticari bir ELISA kiti kullanılarak analiz edilmiştir. Gruplara ait ortanca (minimum–maksimum) serum AMH konsantrasyonları sırasıyla Grup I'de 316,79 (88,01–1094,36) pg/mL, Grup II'de 493,62 (224,67–1242,55) pg/mL, Grup III'te 528,98 (342,52–880,78) pg/mL ve Grup IV'te 394,23 (205,01–706,54) pg/mL olarak belirlenmiştir. İstatistiksel analizler sonucunda, Grup II ve Grup III'teki hayvanların serum AMH düzeylerinin Grup I ve Grup IV'e kıyasla anlamlı derecede yüksek olduğu saptanmıştır ($p<0,05$). Buna karşın, Grup I ile Grup IV arasında ve Grup II ile Grup III arasında AMH konsantrasyonları bakımından istatistiksel olarak anlamlı bir farklılık belirlenmemiştir ($p>0,05$). Sonuç olarak, bu çalışma İvesi koyunlarında serum AMH konsantrasyonlarının farklı yaş gruplarına göre değişiklik gösterebileceğini ortaya koymuştur.

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1. INTRODUCTION

Anti-Müllerian Hormone (AMH) is a glycoprotein belonging to the transforming growth factor-beta (TGF-β) superfamily, with a molecular weight of approximately 140 kDa. In males, AMH is produced by the Sertoli cells in the testes, where its primary function is the inhibition and regression of the Müllerian ducts, which are essential for the formation of the female genital tract (1). In females, AMH is secreted by the granulosa cells of developing follicles after sexual differentiation. It plays a crucial role in regulating the activation and growth of preantral follicles (2). Specifically, within the ovine ovary, AMH has been detected at high concentrations in antral follicles, particularly those measuring 1–3 mm (3). It is well-established that AMH expression decreases as follicular size increases (4) and becomes undetectable in atretic follicles (5). Furthermore, studies investigating AMH's role in ovine granulosa cells have demonstrated its capacity to modulate follicular progression. Exogenous AMH administration was shown to suppress the development of primordial follicles. Conversely, suppressing AMH production in granulosa cells was reported to enhance cell proliferation, reduce apoptosis, and increase estradiol secretion (6).

In sheep, relationship between AMH concentration and pregnancy rates have been reported (7). A positive correlation has been established between AMH levels and ovulatory response, indicating that ewes with higher circulating AMH concentrations exhibit superior ovulation rates (8). The utility of AMH measurement extends to the identification of donor ewes for embryo transfer programs. Research has demonstrated that a higher yield of embryos can be obtained from five-week-old lambs characterized by elevated AMH values (9). Furthermore, in studies involving Romanov sheep, ewes with higher basal AMH levels were reported to have a greater number of offspring (7). This association between AMH and reproductive success is further supported by observations in subfertile animals. Ewes exhibiting low reproductive performance have been shown to have significantly reduced counts of primordial and antral follicles, as well as lower AMH serum concentrations, when compared to ewes with normal fertility (10). Collectively, these findings underscore AMH's value as a reliable, non-invasive biomarker for assessing ovarian reserve and predicting prolificacy and reproductive potential in sheep.

Studies conducted in small ruminants consistently indicate that breed is a significant factor influencing circulating AMH concentrations (11, 12). Regarding the effect of age, high variability in AMH levels has been primarily reported during the prepubertal period (13-15). However, in ewes older than one year, age appears to have no significant impact on serum AMH concentrations (11, 16-17). Collectively, these findings demonstrate that both breed and developmental stage (particularly prepubertal status) significantly influence AMH concentrations in small ruminants. Consequently, determining species-specific and breed-specific reference values for AMH in sheep of various ages remains a critical area of investigation to improve the clinical utility of this biomarker.

The Awassi sheep breed, originating in Jordan, is a robust and widely distributed livestock population raised across several countries, including Türkiye, Syria, and Iraq (18). This breed is highly valued for its triple-purpose production (meat, milk, and wool) and is characterized by its notable resilience to diseases, resistance to poor nutrition, and high adaptability to challenging environmental conditions. In particular, the Awassi breed accounts for approximately 35% of the total sheep population in Mediterranean region of Türkiye (19). Despite the breed's economic importance, a review of the current literature revealed a significant gap concerning the age-dependent changes in AMH levels within the Awassi breed. Given the critical role of AMH as a biomarker for ovarian reserve and its known variation with developmental stage in other breeds, this study aims to test the hypothesis that serum AMH levels will significantly vary with age in female Awassi sheep. To address this gap, blood AMH concentrations will be investigated in female Awassi sheep categorized into distinct age groups: 3–4 months, 6 months, 9–10 months, and animals older than 24 months.

2. MATERIAL AND METHODS

Study Animals and Location

This investigation was conducted on a total of 87 healthy female Awassi sheep during the non-breeding season. The animals were sourced from a private sheep farm located in the Hatay, Türkiye. The sheep were managed under a system of free-range grazing. To examine the effects of age on serum AMH levels, the Awassi sheep were categorically divided into four age groups: Group I: ewe lambs aged 3–4 months (n=16); Group II: ewe lambs aged 6 months (n=22); Group III: ewe lambs aged 9 – 10 months (n=25); Group IV: Non-pregnant ewes, multiparous and aged ⩾24 months (2-4 years old) (n=24).

Blood Sample Collection

For the determination of serum AMH concentrations, a single blood sample was collected from all animals via the jugular vein (*v. jugularis*). Following collection, the blood samples were immediately placed under cold chain conditions and transported to the laboratory within one hour. The collected blood samples were subsequently centrifuged at 3000x g for 5 minutes to separate the serum. The resulting serum aliquots were stored at −20°C until further analysis.

Anti-Mullerian Hormone (AMH) Analysis

The quantitative determination of AMH concentration in serum samples was performed with commercial ELISA test (ESH0110, FineTest Sheep AMH, China). Serum AMH assays demonstrated a sensitivity of 9.375 pg/mL, with intra- and inter-assay CVs of 4.23–5.02 % and 4.97–5.46 %, respectively (measuring range: 15.625–1000 pg/mL).

Statistical Analyses

Statistical analyses were performed to determine significant differences in AMH levels across the four distinct age groups. The normality of data distribution was assessed using the Kolmogorov-Smirnov test, and the homogeneity of variances was evaluated using Levene's test. As the data failed to satisfy the assumptions required for parametric testing, non-parametric methods were utilized for group comparisons. Differences among the age groups were therefore assessed using the Kruskal-Wallis H test. When a significant overall effect was detected, Dunn's post hoc test was applied for multiple pairwise comparisons. All statistical procedures were executed using the SPSS software (Version 23). For all analyses, the level of statistical significance was set at $p<0.05$.

3. RESULTS

The median, minimum, and maximum serum AMH concentrations (pg/mL) were quantified across the four age groups of Awassi sheep, yielding respective average values of 316.79 (88.01-1094.36) pg/mL for the 3–4-month-old lambs, 493.62 (224.67-1242.55) pg/mL for the 6-month-old lambs, 528.98 (342.52-880.78) pg/mL for the 9–10-month-old lambs, and 394.23 (205.01-706.54) pg/mL for the non-pregnant, parous ewes aged ≥24 months (2-4 years old). Analysis of the data revealed a wide distribution of individual AMH values within each category, spanning 88.01−1094.36 pg/mL in the 3–4 month group, 224.67−1242.55 pg/mL in the 6 month group, 342.52−880.78 pg/mL in the 9–10 month group, and 205.01−706.54 pg/mL in the mature ewes (≥24 months); notably, one sample from the 3–4 month group had undetectable AMH levels and was therefore excluded from the subsequent statistical evaluation (Table 1).

Table 1: The median, minimum, and maximum serum anti-Müllerian hormone (AMH) concentrations (pg/mL) in female Awassi ewes across different age groups.
Tablo 1: *Farklı yaş gruplarındaki dişi İvesi koyunlarında ortanca, minimum ve maksimum serum anti-Müllerian hormon (AMH) konsantrasyonları (pg/mL).*

	Groups	Median	Minimum	Maximum	*P*-value
AMH (pg/mL)	3-4 months	316.79 [b]	88.01	1094.36	0.001
	6 months	493.62[a]	224.67	1242.55	
	9–10 months	528.98[a]	342.52	880.78	
	≥24 months	394.23[b]	205.01	706.54	

**Different superscripts (a, b) in the same column indicate significant differences among the groups (P<0.05)*
Aynı sütundaki farklı üst simgeler (a, b), gruplar arasında anlamlı farklılıklar olduğunu gösterir (P<0.05).

4. DISCUSSION AND CONCLUSION

Anti-Müllerian Hormone serves as a critical biomarker for assessing ovarian reserve in females across various species. In sheep, AMH has demonstrated significant utility in predicting ovulatory response (8), selecting donor animals for embryo transfer procedures (9), and estimating overall prolificacy (7). Numerous factors influence circulating AMH levels, with age and breed being particularly prominent variables. It is well-established that AMH concentrations typically decline with advancing age due to the natural depletion of the ovarian reserve. Concurrently, significant variations in AMH levels have been reported due to breed differences (11,14,20). This study specifically investigated the effect of age on AMH levels in the Awassi breed, a prevalent and economically significant sheep breed in Türkiye. To achieve this, blood samples were collected from distinct age defined developmental groups: 3–4-month-old ewe lambs, 6-month-old ewe lambs, 9–10-month-old ewe lambs, and ewes aged over 2 years. The findings from this investigation indicated that the mean serum AMH levels in the 3–4-month-old ewe lambs were significantly lower compared to the 6-month-old and 9–10-month-old ewe lambs. However, the AMH concentrations in the youngest group (3–4 months) were observed to be statistically like those of the oldest group (≥ 24 months).

Anti-Müllerian Hormone levels are generally detectable in prepubertal lambs starting from approximately two months of age. Previous studies, such as that by Lahoz et al. (13), successfully detected AMH levels in about 93% of 3.6-month-old ewe lambs, with concentrations ranging from 0 to 590 pg/mL. Consistent with this, in the current study, AMH levels were undetectable in the blood of a single lamb within the 3–4-month-old age group. However, the specific trajectory of AMH changes during the prepubertal phase appears to be breed-dependent. For instance, in Rasa Aragonesa ewe lambs, AMH concentrations were reported to increase, peaking around 3–4.5 months of age, before beginning a decline by six months of age (20). Similarly, in the dairy Sarda sheep breed, serum AMH concentrations peaked between 2 and 5 weeks of age and began decreasing by six weeks (14). In contrast, a study on Dorper ewe lambs found no statistically significant difference in AMH values between 3, 4, and 5 months of age (15). In the present study on female Awassi sheep, statistically higher AMH levels were obtained in the 6-month-old and 9–10-month-old ewe lambs compared to the 3–4 month-old ewe lambs. Crucially, no statistically significant difference was detected between the 6-month and 9–10-month-old groups. When considering both the findings of this study and the existing literature, the consensus is that AMH concentrations in sheep are subject to considerable variation based on specific age (developmental stage), emphasizing the need for breed-specific reference data.

The observed finding that serum AMH levels were significantly higher in the 6-month-old and 9–10-month-old lamb groups compared to the 3–4 month-old group is likely linked to changes in the number and size of AMH-producing follicles. In Romanov sheep, AMH production is known to be highest in follicles measuring 1−3 mm in diameter (3). Conversely, the intrafollicular AMH concentration is reported to decrease as follicular size increases, with markedly lower production detected in follicles greater than 3−5 mm in diameter and in those undergoing atresia (3).

This pattern aligns with documented ovarian activity during the prepubertal period. Studies examining ovarian follicle dynamics in Suffolk×Western White Face ewe lambs have noted an increase in the number of follicles ≥3 mm in diameter around 3.5−4 months postpartum, followed by a decrease around 4−4.5 months, and a subsequent increase again between 5.5−6 months (21). Another investigation specifically reported that the number of 2−3 mm diameter follicles increased between 3−3.5 months, declined between 6−7 months, increased again between 7−8 months, and then showed a further decreasing trend after 8 months of age (22). Furthermore, an increase in antral follicle counts, especially around six months postpartum, has been previously documented in Rasa Aragonesa ewe lambs (23). In light of this background, the elevation of AMH levels observed in the 6-month and 9–10-month-old Awassi lambs relative to the 3–4-month-old group is hypothesized to result from a physiological surge in the population of 2−3 mm diameter antral follicles during this period. However, confirming this relationship necessitates further detailed investigations, ideally incorporating supplementary techniques such as high-resolution ultrasonographic examinations of the ovaries.

In the present study, the 6-month-old and 9–10-month-old ewe lambs exhibited significantly higher serum AMH concentrations compared to the mature ewes aged over 2 years (≥24 months). This decline in AMH concentrations in the oldest group is consistent with the anticipated reduction in ovarian reserve associated with advancing age, a well-documented phenomenon across mammals. However, a notable finding was that the mean AMH concentrations in the youngest group (3–4-month-old ewe lambs) were statistically similar to those observed in the ≥24-month-old ewes. This non-linear pattern suggests that individual biological variation and dynamic shifts in follicular waves during the peri-pubertal period significantly influence AMH levels, potentially confounding simple age-based trends. Lahoz et al. (24) similarly reported strong variability between 3 and 6 months in Rasa Aragonesa ewe lambs, noting that plasma AMH levels increased during the 3–4.5-month period, declined around 6 months, and then remained at levels similar to the 6-month-old sheep at 19 months of age. They emphasized that substantial variation exists within the 3–6-month period, making repeated measurements necessary to accurately characterize an animal's AMH status.

The current investigation has several acknowledged limitations. Specifically, the study did not determine the pubertal status of the lambs under one year of age. Confirmation of puberty typically requires repeated measurement of plasma progesterone levels to confirm the occurrence of at least one estrous cycle (20). For the Awassi breed, puberty is generally reached around 7.5 months (ranging from 4 to 15 months), with lambs born in winter reaching puberty by nine months of age (18). Identifying pubertal animals is essential for a precise interpretation of AMH changes relative to reproductive maturity. Furthermore, while AMH levels were determined across different ages, integrating the AMH values detected at specific ages with subsequent pregnancy rates following first insemination would validate the predictive utility of AMH as a biomarker for fertility in the Awassi breed.

In conclusion, this study successfully established the serum AMH levels in female Awassi sheep across distinct ages. The data reveal that AMH concentrations are lower in 3–4-month-old lambs, peak in 6-month-old lambs, and stabilize in 9–10-month-old lambs. This profile suggests a different pattern of AMH fluctuation compared to some previously studied sheep breeds, supporting the hypothesis that breed differences significantly modulate AMH dynamics. Given the high individual variation observed (consistent with other studies), it is concluded that comprehensive studies employing larger sample sizes are warranted to establish robust, breed-specific reference ranges for AMH in Awassi sheep, thereby optimizing the clinical application of this biomarker.

Conflict of Interest

The author declared that there is no conflict of interest.

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Authors' Contributions

Motivation/Concept: Ahmet GÖZER
Design: Ahmet GÖZER, Gökhan UYANIK
Control/Supervision: Ahmet GÖZER, Gökhan UYANIK
Data Collection and /or Processing: Ahmet GÖZER, Gökhan UYANIK
Analysis and /or Interpretation: Ahmet GÖZER, Gökhan UYANIK
Literature Review: Ahmet GÖZER, Gökhan UYANIK
Writing the Article: Ahmet GÖZER, Gökhan UYANIK
Critical Review: Ahmet GÖZER, Gökhan UYANIK

Ethical Approval

This study was granted ethical clearance by the Hatay Mustafa Kemal University Local Ethics Committee on Animal Experiments (Approval Date: 27/11/2025, Decision Number: 2025/11-06).

REFERENCES

1. Umer S, Zhao SJ, Sammad A, Weldegebriall Sahlu B, Pang Y, Zhu H. AMH: could it be used as a biomarker for fertility and superovulation in domestic animals?. Genes. 2019;10(12): 1009.
2. Mossa F, Jimenez-Krassel F, Scheetz D, Weber-Nielsen M, Evans ACO, Ireland JJ. Anti-Müllerian Hormone (AMH) and fertility management in agricultural species. Reproduction. 2017;154(1):R1-R11.
3. Monniaux D, Drouilhet L, Rico C, Estienne A, Jarrier P, Touzé JL, et al. Regulation of anti-Müllerian hormone production in domestic animals. Reprod Fertil Dev. 2012;25(1):1-16.
4. Cizmeci SU, Kivrak MB, Takci A, Dinc DA, Coskun B. Evaluation of hormonal protocols for induction of synchronized estrus on reproductive indices in Kangal-Akkaraman ewes during the outbreeding season. Small Rumin Res. 2022; 216:106787.
5. Seifer DB, Merhi Z. Is AMH a regulator of follicular atresia?. J Assist Reprod Genet. 2014;31(11):1403-7.
6. Xu H, Cai Y, Yang H, Zhang C, Liu W, Zhao B, et al. AMH regulates granulosa cell function via ESR2/p38-MAPK signaling pathway in sheep. Commun Biol. 2025;8(1):824.
7. Turgut AO, Koca D. Anti-Müllerian hormone as a promising novel biomarker for litter size in Romanov sheep. Reprod Dom Anim. 2024;59(8):e14692.
8. Xu Q, Wang C, Wang L, Feng R, Guo Y, Feng S, et al. Correlation analysis of serum reproductive hormones and metabolites during multiple ovulation in sheep. BMC Vet Res. 2022;18(1):290.
9. McGrice H, Kelly JM, Kleemann DO, Kind KL, Hampton AJ, Hannemann P, et al. Plasma anti-Müllerian hormone concentration as a predictive endocrine marker for selection of donor lambs to improve success in juvenile in vitro embryo transfer programs. Reprod Fertil Dev. 2020;32(4):383-91.
10. Cushman RA, Yake HK, Snider AP, Lents CA, Murphy TW, Freking BA. An extreme model of fertility in sheep demonstrates the basis of controversies surrounding antral follicle count and circulating concentrations of anti-Müllerian hormone as predictors of fertility in ruminants. Anim Reprod Sci. 2023; 259:107364.
11. Šterbenc N, Mrkun J, Petročnik Š, Sterniša M, Pipan MZ. Comparative analysis of anti-müllerian hormone concentration in two ındigenous Slovenian sheep breeds. Animals. 2025;15(9):1332.
12. Kara U, Koca D. Comparative analysis of serum anti-Müllerian hormone (AMH) levels in sheep: the role of genetic background and physiological status. Arch Anim Breed. 2026;69(1): 37-44.
13. Lahoz B, Alabart JL, Monniaux D, Mermillod P, Folch J. Anti-Müllerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. BMC Vet Res. 2012;8(1):118.
14. Torres-Rovira L, Succu S, Pasciu V, Manca ME, Gonzalez-Bulnes A, Leoni GG, et al. Postnatal pituitary and follicular activation: a revisited hypothesis in a sheep model. Reproduction. 2016;151(3):215-25.

15. Neves ACC, Prado OR, Blaschi W, Barreiros TRR, Deiss L, Lahoz B, et al. Anti-mullerian hormone as a predictive endocrine marker for selection of White Dorper ewe lambs at prepubertal age. Small Rumin Res. 2023;221:106932.

16. Acharya M, Burke JM, Ashworth AJ,Rorie RW. Relationship of anti-Mullerian hormone to reproductive traits in Katahdin ewes bred in late spring or fall. Adv Reprod Sci. 2020;8:48-56.

17. Turgut AO, Koca D. Serum Anti-Müllerian Hormone Levels during Estrus and Diestrus Phases of the Estrous Cycle and its Possible Effect on Fertility in Cross-bred Hamdani Sheep. Pak Vet J. 2024;44(1).

18. Talafha AQ, Ababneh MM. Awassi sheep reproduction and milk production. Trop Anim Health Prod. 2011;43(7):1319-26.

19. Kandemir Ç, Taşkın T. Türkiye'de Koyun Irklarının Mevcut Durumu ve Geleceği: Karadeniz Bölgesi. Yüzüncü Yıl Üniv Fen Bil Enst Derg. 2022;27(1):101-12.

20. Lahoz B, Alabart JL, Cocero MJ, Monniaux D, Echegoyen E, Sánchez P, et al. Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of BMP15 genotype: an endocrine predictor to select the best donors for embryo biotechnologies. Theriogenology. 2014;81(2):347-57.

21. Bartlewski PM, Beard AP, Cook S, Rawlings NC. Ovarian activity during sexual maturation and following introduction of the ram to ewe lambs. Small Rumin Res. 2002;43(1):37-44.

22. Bartlewski PM, Beard AP, Rawlings NC. Ultrasonographic study of antral follicle development during sexual maturation in ewe lambs. Small Rumin Res. 2006;63(1-2):189-98.

23. Kinder JE, Day ML, Kittok RJ. Endocrine regulation of puberty in cows and ewes. J Reprod Fertil Suppl. 1987;34:167-86.

24. Lahoz B, Alabart JL, Monniaux D, Cocero MJ, Folch J. Relationship between plasma AMH at prepuberty and adulthood in sheep. In: 29. Scientific Meeting AETE. 2013.