

## Journal of the Hellenic Veterinary Medical Society

Vol 75, No 1 (2024)



### Anti-Müllerian Hormone Expression in the Ovarian Follicles and Factors Related to Serum Anti-Müllerian Hormone Concentrations in the Domestic Queens

A Gözer, G Dogruer, I Gokcek, T Kutlu, O Bahan, I Pir Yagci

doi: [10.12681/jhvms.33352](https://doi.org/10.12681/jhvms.33352)

Copyright © 2024, A Gözer, G Dogruer, I Gokcek, T Kutlu, O Bahan, I Pir Yagci



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

### To cite this article:

Gözer, A., Dogruer, G., Gokcek, I., Kutlu, T., Bahan, O., & Pir Yagci, I. (2024). Anti-Müllerian Hormone Expression in the Ovarian Follicles and Factors Related to Serum Anti-Müllerian Hormone Concentrations in the Domestic Queens. *Journal of the Hellenic Veterinary Medical Society*, 75(1), 6915–6924. <https://doi.org/10.12681/jhvms.33352>

## Anti-Müllerian Hormone Expression in the Ovarian Follicles and Factors Related to Serum Anti-Müllerian Hormone Concentrations in the Domestic Queens

A. Gozer<sup>1</sup> , G. Dogruer<sup>1</sup> , I. Gokcek<sup>2</sup> , T. Kutlu<sup>3</sup> , O. Bahan<sup>4</sup> , I. Pır Yagcı<sup>5</sup> 

<sup>1</sup>Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Hatay, Turkey

<sup>2</sup>Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Physiology, Hatay, Turkey

<sup>3</sup>Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pathology, Hatay, Turkey

<sup>4</sup>Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Yozgat, Turkey

<sup>5</sup>Kırkkale University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Kırkkale, Turkey

**ABSTRACT:** Anti-Müllerian Hormone (AMH) is a glycoprotein with 140 kDa molecular weight secreted by the granulosa cells, playing a crucial role in folliculogenesis. The present study aimed to reveal (1) the relationship between age, body weight, estrous cycle, and ovariectomy with the serum AMH concentrations and (2) expression of the AMH in the ovarian follicles. Serum AMH concentrations were determined by a commercial ELISA. Ovarian expression of the AMH in the granulosa cells was detected by immunohistochemistry. Twenty female cats were allocated to three groups according to the period of the sexual cycle, age, and body weight. The phase of their estrous cycle was determined according to serum estradiol 17 $\beta$  and progesterone levels. Blood samples were collected to detect serum AMH concentration on Day 0- preoperative, Day 3, and Day 10-postoperative. There was a decrease in AMH concentration on Day 3 compared to the preoperative level (Day 0) ( $P < 0.001$ ). No statistical difference in AMH concentration was found between Day 3 and 10 postoperative ( $P > 0.05$ ). Furthermore, the period of the sexual cycle, age, and body weight did not affect AMH levels ( $P > 0.05$ ). In immunohistochemical analyses, a mild AMH expression was first observed in the granulosa cells of the primary follicles, and moderate AMH expression was observed in the granulosa cells of the secondary follicles. Strong AMH expression was observed in the cumulus cells and mild AMH expression in the mural granulosa cells of the antral follicles. In conclusion, ovariectomy caused a definite decline in serum AMH concentration, and AMH expression was found in all the ovarian follicles except for primordial follicles in domestic cats.

**Key Words:** cat; ovary; folliculogenesis; antimüllerian hormone; immunohistochemistry

*Corresponding Author:*

Ahmet Gozer, Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Hatay, Turkey  
E-mail address: ahmetgozer@gmail.com

*Date of initial submission: 02-02-2023*  
*Date of acceptance: 09-09-2023*

## INTRODUCTION

Female cats are seasonal polyestrous species with induced ovulation (Bristol-Gould and Woodruff, 2006). Sexual activity depends on the day length. It starts with an increase in daylight, which causes a decrease in melatonin concentration, consequently promoting the Gonadotropin-Releasing Hormone (GnRH) secretion from the hypothalamus. GnRH provokes further Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) secretion and promotes folliculogenesis (Schäfer-Somi, 2017). Folliculogenesis is controlled by various factors, including gonadotrophins and local intraovarian peptides (Bristol-Gould and Woodruff, 2006). Several factors, such as insulin-like growth factors (IGFs), epidermal growth factors (EGFs), and transforming growth factor- $\beta$  (TGF- $\beta$ ) family members [inhibin, activin, growth differentiating factors (GDFs), bone morphogenetic proteins (BMPs)] are essential for folliculogenesis in domestic cats. These factors regulate oocyte development and granulosa cell proliferation, transformation, and steroidogenesis in ovarian follicle development (Kitiyanant et al., 2003; Phillips and Woodruff, 2004; Fujihara et al., 2014; Yıldırım et al., 2014; Kehoe et al., 2021; Perego et al., 2021).

Anti-Müllerian Hormone (AMH), also known as Müllerian inhibiting substance, is a glycoprotein with a molecular weight of 140 kDa belonging to the transforming growth factor- $\beta$  family (Cate et al., 1986; Baarends et al., 1995). Its best-known role in the male fetus was described by Jost (1953) as inhibiting the development of the Müllerian ducts consequently allowing the male genital tract development. In females, AMH is only secreted by granulosa cells of the ovarian follicles and has a significant role in folliculogenesis (Bezard et al., 1987). The critical actions of the AMH are suppression of the primordial follicle activation mediated by stem cell factor (SCF) and fibroblast growth factor-7 (FGF-7) and suppression of the FSH-dependent follicle development, consequently preventing premature depletion of ovarian follicle reserve (Durlinger et al., 2002; Nilsson et al., 2007; Lebbe and Woodruff, 2013; Hu et al., 2014).

During the last decade, many studies have investigated the role of AMH in feline reproduction. These studies have focused on the diagnostic usefulness of AMH in various reproductive diseases, such as ovarian remnant syndrome, granulosa cell tumour, and pyometra (Heaps et al., 2017; Ottosson, 2017; Flock et al., 2022a; Gozer et al., 2023), and the detection of go-

nadectomy status (Gozer et al., 2023). Moreover, the effect of age and sexual cycle on serum AMH concentration has been identified (Flock et al., 2022b; Lapuente et al., 2023). However, knowledge of the role of AMH in feline folliculogenesis is scarce (Snoeck et al., 2017; Gültiken et al., 2022). The present study was carried out to reveal or reevaluate (1) the relationship between age, body weight, estrous cycle, and ovariohysterectomy with the serum AMH concentration and (2) the expression of the AMH in the ovarian follicles of the cat.

## MATERIALS AND METHODS

The animal experiments required for the study were approved by the Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee with the decision number 2020/04-23 on 24.06.2020.

### Animals

Twenty healthy female cats that have developed at least one estrus and without any gynecological diseases were included in the present study. The age of the cats varied between 5-48 months. The average body weight of the cats was  $3.07 \pm 0.5$  kg. The cats were classified according to a) the phase of the estrous cycle: estrus (n=8), diestrus (n=4), and anestrus (n=8), b) age: <1-year-old (n=8); >1-year-old (n=12) as previously suggested by Pir Yagci et al. (2016) and c) body weight: 2-3 kg (n=10), >3 kg (n=10).

### Blood samples

Blood samples were collected from *v.cephalic-ain* serum tubes without anticoagulant to determine serum AMH, estradiol  $17\beta$  ( $E_2$ ), and progesterone ( $P_4$ ) on Day 0 (preoperative) and Day 3 and 10 (postoperative). The blood samples were centrifuged at 3000 rpm for 5 minutes, and the sera were obtained and stored at  $-20^\circ\text{C}$  until analysis (Pir Yağcı et al., 2016).

### Surgery

The cats were anesthetized by intramuscular administration of xylazine hydrochloride (1 mg/kg, Basilazin 2%, Bavet, Turkey) and ketamine hydrochloride (10 mg/kg, Ketazol 10%, Interhas, Turkey) ten minutes later. For prophylaxis, cefazolin sodium (20 mg/kg, Cefazol, 250 mg, Mustafa Nevzat, Turkey) was administered intramuscularly before the operation. Mid-line ovariohysterectomy was performed with the 3-clamp method (Santos et al., 2022). Meloxicam (0.2 mg/kg, Maksikam, Bavet, Turkey) was administered subcutaneously as an analgesic. Ovariohysterectomy operations were performed be-

tween 8 October 2020 and 1 March 2022.

### Ovary Samples

Ovaries were kept in 10% formaldehyde solution after the ovariohysterectomy until immunohistochemical and histopathological examination (Alkan-Karakaş et al., 2019).

### Detection of the estrous cycle

The concentrations of  $E_2$  and  $P_4$  were measured to determine the phase of the estrous cycle. Queens with  $E_2$  values less than 20 pg/ml were classified as anestrus,  $P_4$  values greater than 1.5 ng/ml were classified as diestrus, and  $E_2$  values above 20 pg/ml were classified as estrus (Hamouzova et al., 2017).

### Anti-Müllerian Hormone (AMH) measurement

A commercial enzyme-linked immunosorbent assay (Beckman Coulter® AMH Gen II, USA) was used for the serum AMH measurement, following the manufacturer's instructions. Samples, standards, anti-AMH biotin conjugate, and streptavidin enzyme conjugate were added to the wells coated with primary antibodies, one step at a time, and incubated as suggested by the test procedure. After each incubation, the wells were washed with a washing solution five times. After the last washing step, chromogen solution was added and incubated. At the end of the incubation, stop solution was added into wells. Wells were read with an ELISA reader (ERBA Mannheim, Lisacan) at 450 nanometers.

### Estradiol 17 $\beta$ and progesterone hormone measurements

Serum Estradiol 17 $\beta$  (Siemens, product no 10491445, Germany) and progesterone (Siemens, product no 01586287, Germany) levels were measured by direct chemiluminescent method with fully automatic immunoassay autoanalyzer using commercial reagents (Siemens, Advia Centaur XP, Germany). The measurement ranges of the  $E_2$  and  $P_4$  kits were 11.80-3000 pg/ml and 0.21-60 ng/ml, respectively.

### Histopathological examination

Formoldehyde-fixed tissue samples were dehydrated by successive immersion in 70, 80, 96, and 100% alcohol. Tissues were cleared with xylene before embedding in paraffin. Sections were cut at 4 $\mu$ m, deparaffinized in xylol, and stained with hematoxylin and eosin (H & E) after successive passage through 100, 96, 80, 70% alcohol, and water.

### Immunohistochemical examination

To demonstrate AMH expression and localization in tissues following immunostaining, the Avidin-Biotin-Peroxidase Complex (ABC) technique was performed according to the standard procedure prescribed in the commercial kit (SensiTek HRP, ScyTek Laboratories, Logan, UT®). Anti-AMH antibody (ab103233/1:50 dilution/overnight/+4 °C) was used as the primary antibody. Hydrogen peroxide (3% in methanol) was used to block endogenous peroxidase activity. Endogenous avidin/biotin blocking kit (ab64212) for endogenous biotin blocking, and proteinase K as antigen retrieval (Abcam, ab64220®) were also used. Phosphate Buffered Saline (PBS) was applied to the tissues as a negative control. 3,3'-diaminobenzidine tetrahydrochloride (DAB, ScyTek Laboratories, Logan, UT®) was used as chromogen. The samples were counterstained with hematoxylin. Follicles were classified into primordial, secondary, antral, and atretic follicles as previously described (Bristol-Gould and Woodruff, 2006; Saint-Dizier et al., 2007). Immunohistochemistry results were classified by the same specialist as 0 (no reaction), 1+ (mild), 2+ (moderate), and 3+ (strong).

### Statistical Analyses

Considering the primary hypotheses of the planned study, to determine the required minimum sample size before the study, it was calculated that it would be appropriate to be a minimum of 18 cats in the study, using the criteria of type 1 error probability ( $\alpha$ ) = 0.05, power ( $1-\beta$ ) = 0.80 and the effect size ( $f$ ) = 0.6. Before starting the significance tests, the variables were analyzed with the Shapiro-Wilk test for normality and the Levene test for homogeneity of variances. In the study, the analysis of variance of repeated measurements method was used to evaluate the serum AMH concentrations in terms of time (day 0 preoperative and day 3 and 10 postoperative). As a post hoc test, simple effects analysis with Bonferroni correction was performed. The two-way mixed ANOVA method was used to evaluate AMH levels in terms of body weight (2-3 kg and >3 kg) and age (<12 months and  $\geq$ 12 months) groups, time (0 day preoperative and 3 and 10 day postoperative) and their interaction. Simple effects analysis with Bonferroni correction was used as a post hoc test. The Kruskal-Wallis test was used to evaluate the AMH,  $E_2$ , and  $P_4$  concentrations in terms of the period of the sexual cycle (estrus, diestrus, and anestrus). The Mann-Whitney U test with Bonferroni correction was preferred as the post hoc

**Table 1.** Effect of the ovariectomy on the serum AMH concentration (ng/ml, mean  $\pm$  SEM).

AMH (n=20)	Day 0	Day 3	Day 10	P
	4.35 $\pm$ 0.18 <sup>a</sup>	0.73 $\pm$ 0.19 <sup>b</sup>	0.32 $\pm$ 0.19 <sup>b</sup>	

<sup>a,b</sup>: Different letters show statistical differences (p<0.05).

**Table 2.** Effect of the phase of the estrous cycle on serum AMH concentration and E<sub>2</sub> and P<sub>4</sub> levels (mean  $\pm$  SEM)

Hormone	Estrous Cycle Phase			P
	Diestrus (n=4)	Estrus (n=8)	Anestrus (n=8)	
AMH (ng/ml)	4.74 $\pm$ 0.82	4.15 $\pm$ 0.67	4.35 $\pm$ 0.5	0.96
E <sub>2</sub> (pg/ml)	26.66 $\pm$ 5.32 <sup>a</sup>	26.23 $\pm$ 1.75 <sup>a</sup>	13.59 $\pm$ 1.09 <sup>b</sup>	0.001
P <sub>4</sub> (ng/ml)	9.81 $\pm$ 3.08 <sup>a</sup>	0.51 $\pm$ 0.07 <sup>b</sup>	0.40 $\pm$ 0.06 <sup>b</sup>	0.007

<sup>a,b</sup>: Different letters show statistical differences (p<0.05).

**Table 3.** Serum AMH concentration in different body weights and age groups (ng/ml, mean  $\pm$  SEM)

Body weight		AMH (ng/ml)	P
		2-3 kg (n=10)	
> 3 kg (n=10)	3.70 $\pm$ 0.61		
Age	<12 months (n=8)	4.60 $\pm$ 0.85	0.534
	>12 months (n=12)	4.14 $\pm$ 0.49	

test. All statistical analyzes were performed with Stata 12/MP4 statistical package program. P<0.05 was considered the statistically significant criterion.

## RESULTS

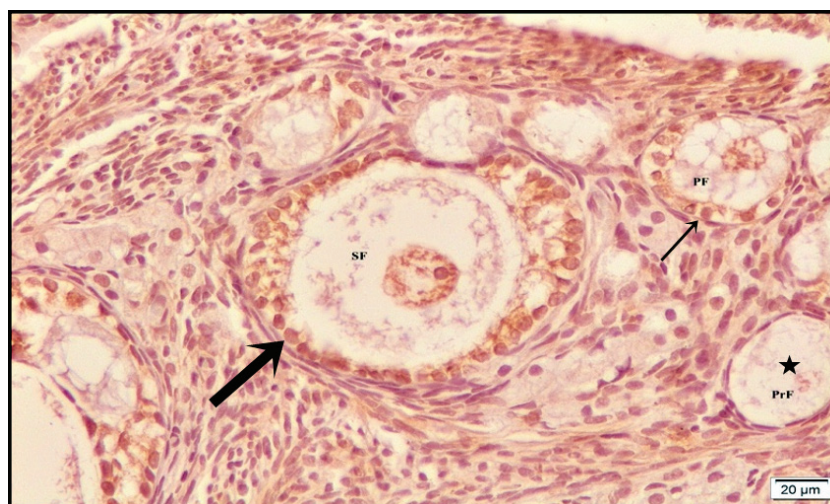
### Serum AMH concentration

In the present study, there was a significant decrease in the serum AMH concentration on the 3<sup>rd</sup> and 10<sup>th</sup> postoperative days compared to the preoperative concentration (day 0) (P<0.001). No significant differences were found between the postoperative 3<sup>rd</sup> and 10<sup>th</sup> days (Table 1). There was no significant relationship between serum AMH concentration and

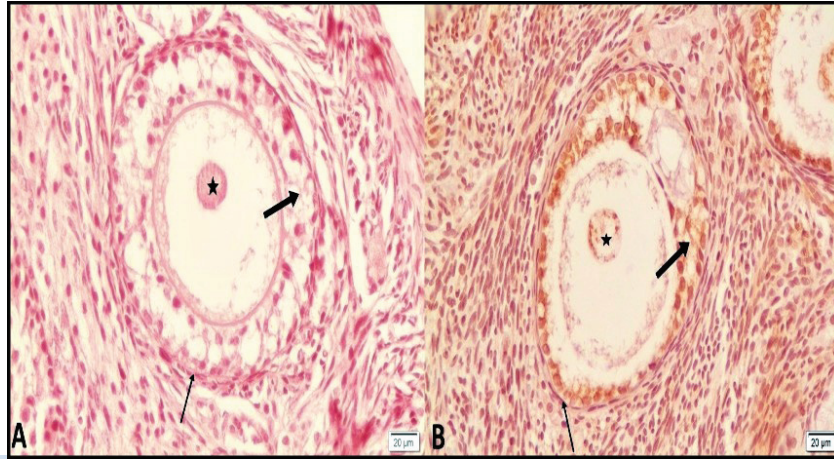
the phase of the estrous cycle (Table 2), the age, or the body weight (Table 3).

### Immunohistochemical results

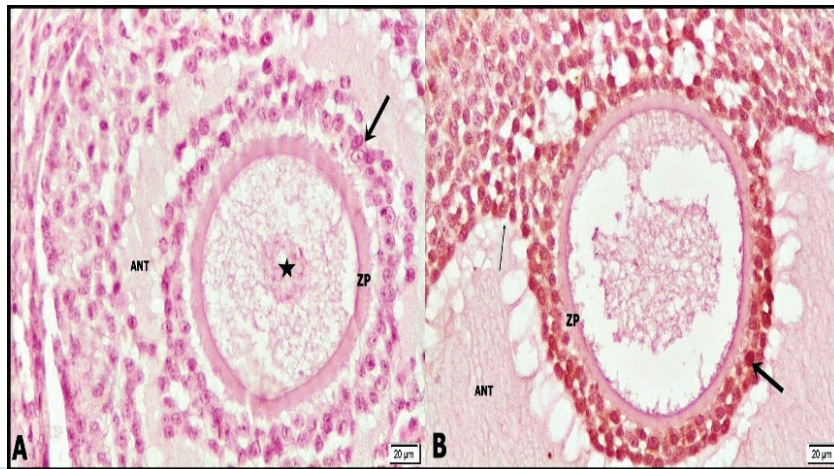
The present study detected AMH immunoreaction in all follicular stages except primordial follicles. In primary follicles, mild (+1) AMH immunoreaction was observed in the granulosa cells (Fig 1). In secondary follicles, moderate (+2) AMH immunoreactions were detected in the granulosa cells. However, no immunoreaction was observed in the thecal cells of the secondary follicles (Fig 2). In antral follicles, strong (+3) AMH immunoreaction was observed in



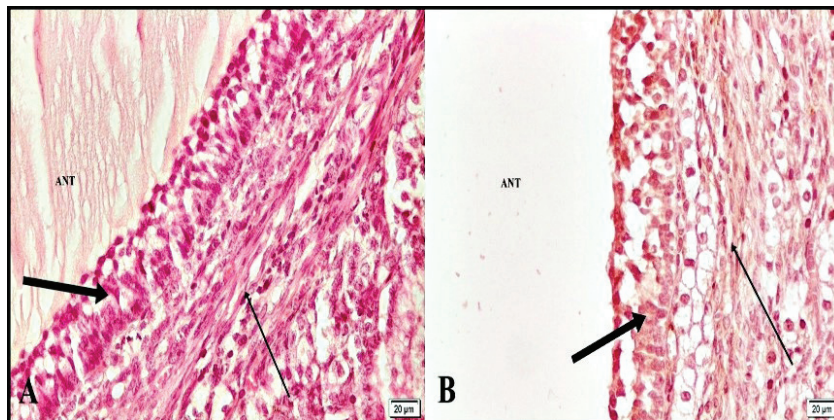
**Fig. 1.** AMH expression in the primordial, primary, and secondary follicle. Primordial follicle (PrF) (star), Primary Follicle (PF) (thin arrow), Secondary Follicle (SF) (thick arrow), 40x, IHC.



**Fig. 2.** AMH expression in the secondary follicle. A: Histopathological image of the secondary follicle; granulosa cell (thick arrow), thecal cell (thin arrow); oocyte (star) 40x, HE. B: AMH expression in the secondary follicle; granulosa cell (thick arrow), thecal cell (thin arrow); oocyte (star) 40x, IHC.



**Fig. 3.** Cumulus oophorus. A: Histopathological image of the cumulus oophorus; antrum (ANT), zona pellucida (ZP), oocyte (star) 40x, HE. B: AMH expression in cumulus oophorus; antrum (ANT), zona pellucida (ZP), in cumulus oophorus (arrow), oocyte (star) 40x, IHC.



**Fig. 4.** AMH expression in mural granulosa and theca cell. A: Histopathological image of mural granulosa and theca cell; antrum (ANT), granulosa cells (thick arrow), theca cell (thin arrow) 40x, HE. B: AMH expression in mural granulosa and theca cell; antrum (ANT), granulosa cells (thick arrow), theca cell (thin arrow) 40x, IHC.



**Fig. 5.** Negative AMH expression in corpus luteum (thick arrow); AMH expression in the antral follicle (thin arrow), antrum (ANT), the antral follicle (AF), corpus luteum (CL), 10x, IHC.

the cumulus oophorus cells (Fig 3), whereas mild (+1) AMH immunoreaction was detected in mural granulosa cells (Fig 4). No AMH immunoreaction was detected in the corpus luteum (Fig 5). In immunohistochemical examinations, the best-qualified pictures were chosen to show the AMH immunoreaction in the ovarian follicles. There were no statistical analyses performed on the ovarian expression of the AMH according to estrous cycle phase. The current study only evaluated AMH expressions of ovarian follicles via immunohistochemical methods.

## DISCUSSION

Anti-Müllerian Hormone (AMH) is a transforming growth factor- $\beta$  family member and is only secreted by the granulosa cells in the ovarian follicles (Durlinger et al., 2002). Ovariohysterectomy causes a rapid decline in serum AMH concentration (Pir Yağcı et al., 2016). Therefore, determining the presence of ovarian tissue through serum AMH measurement is a common practice in feline reproduction. Serum AMH concentration in the spayed cats varied between 0.01-0.5 ng/ml (Place et al., 2011; Axner et al., 2015; Heaps et al., 2017; Flock et al., 2022b; Gültiken et al., 2022; Gozer et al., 2023). In this study, decreasing concentrations of AMH were observed following the spaying, and postoperative Day 10 AMH concentrations in line with the previous studies (Table 1).

It has been reported that serum AMH concentration declines as the female ages (Snoeck et al., 2017). Age-related decline in the follicular reserve led to decreased serum AMH concentration (Lahoz et al., 2014; Snoeck et al., 2017; Ball et al., 2019; Fer-

ré-Dolcet et al., 2022; Lapuente et al., 2023). In our study, numerically higher serum AMH concentrations were observed in cats that were younger than one year than in cats older than 1 year. This may be attributed to the small number of animals used in the study. Serum AMH concentration of cats older than 1 year align with the ones reported by Snoeck et al. (2017). In our study, AMH concentrations in cats younger than 1 year were lower compared to those measured in other studies (Snoeck et al., 2017; Ferré-Dolcet et al., 2022; Lapuente et al., 2023). This could be the result of differences in the immunoassay, age classification, and individual differences (Flock et al., 2022b; Lapuente et al., 2023).

In cats, a definite pattern of how AMH courses in the estrus cycle has not been established yet (Lapuente et al., 2023). It has been reported that serum AMH concentration is high during anestrus and interestrus but low during estrus (Flock et al., 2022b); however, there is no difference between proestrus and estrus periods (Gültiken et al., 2022). Lapuente et al. (2023) stated that higher AMH concentration was found in the anestrus phase than in the follicular phase. Gültiken et al. (2022) reported that higher AMH concentration was observed in the pregnant cat. The current study found no statistical differences in the serum AMH concentration in different phases of the estrous cycle (Table 2). AMH concentration in different phases of the estrous cycle of cats is similar to that found in women (Hehenkamp et al., 2006), mares (Almeida et al., 2011), and goats (Monniaux et al., 2011).

In the current study, although not significant, numerically higher serum AMH concentration was-

found in the diestrus. This was unexpected since there was no immunoreaction of AMH in the corpus luteum (Fig.5). Flock et al., (2022b) stated that the median AMH concentration was 6.38 ng/ml (4.50-10.75) and 6.47 ng/ml (5.60-9.80) in metestrus and pregnant cats, respectively. Gültiken et al., (2022) also observed that mean serum AMH concentration was  $2.95 \pm 1.21$  and  $2.83 \pm 1.33$  ng/ml in 32-40 and 41-46 days of pregnancy in domestic cats. In the current study, mean AMH concentration was  $4.74 \pm 0.82$  ng/ml. Our results might be attributed to the small sample size and the individual differences, as previously reported (Flock et al., 2022b). A larger sample size may be more efficient to determine serum AMH concentration in domestic cat. However, it does not seem possible to predict how it can affect the results. It was also reported that median AMH concentration can be different among and within individual cats and high variation can be observed in a single cat throughout the estrus cycle (Flock et al., 2022b). Furthermore, a recent study by Gültiken et al. (2022) stated that the expression of AMH was observed in the feline corpus luteum and higher AMH concentration was found during pregnancy. Further investigations are needed to elucidate the relationship between AMH and corpus luteum in cat.

Previous studies have reported contradictory results regarding the relationship between the body weight and serum AMH concentration. Moy et al. (2015) stated that a negative relationship exists between obesity and serum AMH concentration. However, other studies stated no relationship between body weight and serum AMH concentration (Skałba et al., 2011; Sahmay et al., 2012). The current study found numerically higher serum AMH concentration in cats with a body weight of 2-3 kg compared to those with a body weight of  $>3$  kg ( $p=0.079$ ). However, these results should be cautiously interpreted since they might come from younger cats weighing 2-3 kg than from cats weighing  $>3$  kg. More detailed studies are needed to investigate the effect of body weight on AMH concentration in cats.

AMH expressions in the ovarian follicles were studied in different species, such as sheep, human, cow, porcine, and dog. Generally, AMH expression had development-dependent characteristics in the ovarian follicles (Bezard et al., 1987; Weenen et al., 2004; Almeida et al., 2018; Teh et al., 2018; Alkan-Karakas et al., 2019). In our study, AMH expression was first observed in the granulosa cells of the primary follicles. As the follicle size increased, stron-

ger AMH expression was encountered in the granulosa cells of the ovarian follicles in the cat (Fig 1). These results may indicate that AMH expression in the ovarian follicles have stage-dependent characteristics in the domestic cat.

In the current study, strong (3+) AMH expression was encountered in the cumulus oophorus cells (COCs), whereas mild (1+) AMH expression was detected in mural granulosa cells (Fig 3). Stronger AMH expression in the cumulus cells might result from the effect of oocyte-originated growth factors on the COCs (Convissar et al., 2017; Zhao et al., 2018). Monniaux et al. (2012) stated that oocyte-originated growth factors, such as bone morphogenetic factors (BMPs) and growth differentiating factors (GDF-9), affect the expression of AMH. In various species, it has been reported that BMP-4, BMP-6, BMP-15, and GDF-9 increase AMH mRNA expression (McNatty et al., 2005; Rico et al., 2011; Convissar et al., 2017; Zhao et al., 2018). Previous studies have illustrated that cumulus cells have greater AMH expression than mural granulosa cells (Munsterberg and Lovell-Badge, 1991; Salmon et al., 2004; Grøndahl et al., 2011). Salmon et al. (2004) indicated higher AMH expression at the oocytes collected from the preovulatory follicles co-cultured with the cumulus granulosa cells.

It has been reported that serum AMH concentration is correlated with the antral follicles count (Kevenaar et al., 2006; Ireland et al., 2008; Anadol et al., 2020; Böhmer et al., 2022) and the relationship between serum AMH concentration and follicles count have been studied in many species (Monniaux et al., 2011; Rico et al., 2009). Lapuente et al. (2023) reported that serum AMH concentration is correlated with the small antral follicles in domestic cat. In the current study, follicle counting and correlation between follicle count and AMH concentration were not performed. However, immunohistochemical results showed that AMH expression was first observed in the primary follicles (Table 5). Therefore, it can be inferred that circulating AMH may originate from the primary, secondary, and antral follicles in domestic cat. Further studies are needed to reveal the contribution of the preantral follicles to the serum AMH concentration.

## CONCLUSION

In brief, AMH can detect the ovariectomy status in domestic cat. Estrous cycle, age, and body weight



did not affect the serum AMH concentration. Primary, secondary, and antral follicles contribute to the serum AMH level. Strong AMH expressions were observed in the cumulus oophorus in the antral follicles. Anti-Müllerian Hormone expression showed development-dependent characteristics.

#### ACKNOWLEDGEMENT

This study was based on a part of the Ph.D. thesis

of the first author Dr. Ahmet GOZER and supported by Hatay Mustafa Kemal University Scientific Research Committee with Project number 20.D.019.

#### CONFLICT OF INTEREST

None declared by the authors

## REFERENCES

- Alkan-Karakaş K, Ceylan A, Alkan H, Özen D, Bayraktaroğlu AG, Kaymaz M (2019) Immunohistochemical and qPCR determination of the expression and serum level of antimüllerian Hormone in pre-pubertal, intact and ovarian remnant syndrome detected bitches. *Reprod Domest Anim* 54: 979-986.
- Almeida J, Ball BA, Conley AJ, Place NJ, Liu IKM, Scholtz EL, Moeller BC (2011) Biological and clinical significance of anti-Müllerian hormone determination in blood serum of the mare. *Theriogenology* 76(8): 1393-1403.
- Almeida FR, Costermans NG, Soede NM, Bunschoten A, Keijer J, Kemp B, Teerds KJ (2018) Presence of anti-Müllerian hormone (AMH) during follicular development in the porcine ovary. *PLoS One*, 13(7), e0197894.
- Anadol E, Gultiken N, Yarim GF, Karaca E, Kanca H, Yarim M (2020) Investigation of diagnostic use of serum anti-Müllerian hormone concentration in dioestrus and anoestrus bitches before and after ovariohysterectomy and the relationship with ovarian follicle numbers. *Pol J Vet Sci* 23(3).
- Axnér E, Holst BS (2015) Concentrations of anti-Müllerian hormone in the domestic cat. Relation with spay or neuter status and serum estradiol. *Theriogenology* 83(5): 817-821.
- Baarends WM, Uilenbroek JT, Kramer PIET, Hoogerbrugge JW, Van Leeuwen EC, Themmen AP, Grootegoed JA (1995) Anti-müllerian Hormone and anti-müllerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle, and gonadotropin-induced follicle growth. *Endocrinology* 136(11): 4951-4962.
- Ball BA, Ali HES, Scoggin KE, Riddle WT, Schnobrich M, Bradekamp E, Troedsson MHT (2019) Relationship between anti-Müllerian hormone and fertility in the mare. *Theriogenology* 125: 335-341.
- Bezard J, Vigier B, Tran D, Mauleon P, Josso N (1987) Immunocytochemical study of anti-Müllerian Hormone in sheep ovarian follicles during fetal and post-natal development. *Reprod* 80(2): 509-516.
- Böhmer F, Erber K, Ewringmann A, Klein R, Reese S, Böhmer C, Walter B (2022) Anti-Müllerian hormone concentrations in female rabbits and its relation to spay status, pseudopregnancy, and ovarian follicle

- numbers. *Reprod Domest Anim* 57(12): 1636-1643.
- Bristol-Gould S, Woodruff TK (2006) Folliculogenesis in the domestic cat (*Felis catus*). *Theriogenology*66(1): 5-13.
- Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, Donahoe PK (1986) Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. *Cell* 45(5): 685-698.
- Convissar S, Armouti M, Fierro MA, Winston NJ, Scoccia H, Zamah AM, Stocco C (2017) Regulation of AMH by oocyte-specific growth factors in human primary cumulus cells. *Reprod* 154(6): 745-753.
- Durlinger ALL, Visser JA, Themmen APN (2002) Regulation of ovarian function: The role of anti-Müllerian Hormone. *Reprod* 124: 601-609.
- Ferré-Dolcet L, Ferro S, Contiero B, Fontaine C, Badon T, Gelli D, Romagnoli S (2022) Clinical use of Anti-Müllerian Hormone to monitor resumption of ovarian activity following removal of a 4.7 mg deslorelin implant in queens. *Vet Res Commun* 1-8.
- Flock U, Fischer S, Weeger J, Reese S, Walter B (2022a) Anti-Müllerian hormone as a diagnostic tool to identify queens with ovarian remnant syndrome. *J Fel Med Surg* 1098612X221099195.
- Flock U, Reese S, Otdzorf C, Klein R, Walter B (2022b) Anti-Müllerian hormone concentrations in queens throughout the estrous cycle. *Domest Anim Endocrinol*81: 106749.
- Fujihara M, Comizzoli P, Keefer CL, Wildt DE, Songsasen N (2014) Epidermal growth factor (EGF) sustains in vitro primordial follicle viability by enhancing stromal cell proliferation via MAPK and PI3K pathways in the prepubertal, but not adult, cat ovary. *Biol Reprod*90(4): 86-1.
- Grøndahl ML, Nielsen ME, Dal Canto MB, Fadini R, Rasmussen IA, Westergaard LG, Andersen CY (2011) Anti-Müllerian hormone remains highly expressed in human cumulus cells during the final stages of folliculogenesis. *Reprod Biomed Online* 22(4): 389-398.
- Gozer A, Bahan O, Dogruer G, Kutlu T (2023) Serum antimüllerian hormone concentrations in female cats. Relation with ovarian remnant syndrome, ovarian cysts and gonadectomy status. *Theriogenology* 200: 106-113.
- Gültiken N, Yarim M, Aslan S, Gürler H, Yarim GF, Tuncay M, Schaffer-Somi S (2022). Expression of Anti-Müllerian Hormone and Its Type 2 Receptor in the Ovary of Pregnant and Cyclic Domestic Cats. *Animals*12(7): 877.
- Hamouzova P, Cizek P, Novotny R, Bartoskova A, Tichy F (2017) Distribution of mast cells in the feline ovary in various phases of the oestrous cycle. *Reprod Domes Anim*52(3): 483-486.
- Heaps LA, Scudder CJ, Lipscomb VJ, Steinbach SM, Priestnall SL, Martineau H, Garden OA (2017) Serum anti-Müllerian hormone concentrations before and after treatment of an ovarian granulosa cell tumour in a cat. *J Felin Med Surg Open Rep*3(2): 2055116917722701.
- Hehenkamp WJ, Looman CW, Themmen AP, De Jong FH, Te Velde ER, Broekman FJ (2006) Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab*91(10): 4057-4063.
- Hu R, Wang FM, Yu L, Luo Y, Wu X, Li J, Bocca S (2014) Antimüllerian Hormone regulates stem cell factor expression in human granulosa cells. *Fertil Steril*102(6): 1742-1750.
- Ireland JLH, Scheetz D, Jimenez-Krassel F, Themmen APN, Ward F, Lonergan P, Ireland JJ (2008) Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. *Biol Reprod*, 79(6): 1219-1225.
- Jost, A (1953) Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Progr Hormon Res*8: 379-413.
- Kehoe S, Jewgenow K, Johnston PR, Mbedi S, Braun BC (2021) Signalling pathways and mechanistic cues highlighted by transcriptomic analysis of primordial, primary, and secondary ovarian follicles in domestic cat. *Sci Rep* 11(1): 1-17.
- Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, Groome NP, Visser JA (2006) Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. *Endocrinol* 147(7): 3228-3234.
- Kitiyant Y, Saikhun J, Pavasuthipaisit K (2003) Somatic cell nuclear transfer in domestic cat oocytes treated with IGF-I for in vitro maturation. *Theriogenology*59(8): 1775-1786.
- Lahoz B, Alabart JL, Cocero MJ, Monniaux D, Echegoyen E, Snchez P, Folch J (2014) 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*81(2): 347-357.
- Lapuente C, Faya M, Blanco PG, Grisolia-Romero M, Marchetti C, Gobbello C (2023) Anti-Müllerian Hormone in queens: Serum concentrations and total ovarian follicle population. *Theriogenology* 119: 111-115.
- Lebbe M, Woodruff TK (2013) Involvement of androgens in ovarian health and disease. *Mol Hum Reprod*19(12): 828-837.
- McNatty KP, Smith P, Moore LG, Reader K, Lun S, Hanrahan JP, Juengel JL (2005) Oocyte-expressed genes affecting ovulation rate. *Mol Cell Endocrinol*234(1-2): 57-66.
- Monniaux D, Baril G, Laine AL, Jarrier P, Poulin N, Cognie J, Fabre S (2011) Anti-Müllerian hormone as a predictive endocrine marker for embryo production in the goat. *Reprod*142(6): 845-854.
- Monniaux D, Drouilhet L, Rico C, Estienne A, Jarrier P, Touze JL, Fabre S (2012) Regulation of anti-Müllerian hormone production in domestic animals. *Reprod Fertil Dev*25(1): 1-16.
- Moy V, Jindal S, Lieman H, Buyuk E (2015) Obesity adversely affects serum anti-müllerian hormone (AMH) levels in Caucasian women. *J Assist Reprod Genet*32(9): 1305-1311.
- Munsterberg A, Lovell-Badge R (1991) Expression of the mouse anti-mullerian hormone gene suggests a role in both male and female sexual differentiation. *Development* 113(2): 613-624.
- Nilsson E, Rogers N, Skinner MK (2007) Actions of anti-Mullerian Hormone on the ovarian transcriptome to inhibit primordial to primary follicle transition. *Reprod* 134(2): 209-221.
- Ottosson M. (2017) Anti-Müllerian hormone in canine pyometra.
- Perego MC, Bellitto N, Maylem ERS, Caloni F, Spicer LJ (2021) Effects of selected hormones and their combination on progesterone and estradiol production and proliferation of feline granulosa cells cultured in vitro. *Theriogenology*168: 1-12.
- Phillips DJ, Woodruff TK (2004) Inhibin: actions and signalling. *Growth factors*2(1): 13-18.
- Pir Yağcı İ, Polat İM, Pekcan M (2016) Evaluation of Age Related Anti-Müllerian Hormone Variations in Domestic Cat. *Kafkas Univ Vet Fak Derg*22(5): 729-732.
- Place NJ, Hansen BS, Cheraskin JL, Cudney SE, Flanders JA, Newmark AD, Scarlett JM (2011) Measurement of serum anti-Müllerian hormone concentration in female dogs and cats before and after ovariohysterectomy. *J Vet Diagn*23(3): 524-527.
- Rico C, Médigue C, Fabre S, Jarrier P, Bontoux M, Clement F, Monniaux D (2011). Regulation of anti-Müllerian hormone production in the cow: a multiscale study at endocrine, ovarian, follicular, and granulosa cell levels. *Biol Reprod*84(3): 560-571.
- Sahmay S, Usta T, Erel CT, İmamoğlu M, Küçük M, Atakul N, Seyisoğlu H (2012) Is there any correlation between AMH and obesity in premenopausal women? *Arch Gynecol Obstet*286(3): 661-665.
- Saint-Dizier M, Malandain E, Thoumire S, Remy B, Chastant-Maillard S (2007) Expression of Follicle Stimulating Hormone and Luteinizing Hormone Receptors During Follicular Growth in the Domestic Cat Ovary. *Mol Reprod Dev* 74: 989-996.
- Salmon NA, Handyside AH, Joyce IM (2004) Oocyte regulation of anti-Müllerian hormone expression in granulosa cells during ovarian follicle development in mice. *Dev Biol*266(1): 201-208.
- Santos LC, dos Anjos Cordeiro JM, da Silva Santana L, Barbosa EM, Santos BR, da Silva TQM, Silva J. F (2022) Expression profile of the Kisspeptin/Kiss1r system and angiogenic and immunological mediators in the ovary of cyclic and pregnant cats. *Domest Anim Endocrinol* 78: 106650.
- Schäfer-Somi S (2017) Effect of melatonin on the reproductive cycle in female cats: a review of clinical experiences and previous studies. *J Feline Med Surg*19(1): 5-12.
- Skalba P, Cygal A, Madej P, Dąbkowska-Huó A, Sikora J, Martirosian G, Olszancka-Glinianowicz M (2011) Is the plasma anti-Müllerian hormone (AMH) level associated with body weight and metabolic, and hormonal disturbances in women with and without polycystic ovary syndrome? *Eur J Obstet Gynecol*158(2): 254-259.
- Snoeck F, Sarrazin S, Wydooghe E, Van Soom A (2017) Age and anti-Müllerian hormone levels predict the success of in vitro maturation

- of cat oocytes. *Reprod Domest Anim*52: 98-102.
- Teh APP, Izzati UZ, Mori K, Fuke N, Hirai T, Kitahara G, Yamaguchi R (2018) Histological and immunohistochemical evaluation of granulosa cells during different stages of folliculogenesis in bovine ovaries. *Reprod Domest Anim* 53(3): 569-581.
- Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Themmen AP (2004) Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *MHR: Basic science of reproductive medicine*10(2): 77-83.
- Yıldırım K, Vural MR, Küplülü Ş, Özcan Z, Polat IM (2014) The effects of EGF and IGF-1 on FSH-mediated in vitro maturation of domestic cat oocytes derived from follicular and luteal stages. *Reprod Biol*14(2): 122-127.
- Zhao Z, Guo F, Sun X, He Q, Dai Z, Chen X, Wang J (2018) BMP15 regulates AMH expression via the p38 MAPK pathway in granulosa cells from goat. *Theriogenology* 118: 72-79