ISSN: 1220-3173; E-ISSN: 2457-7618

ANTI-MÜLLERIAN HORMONE CONCENTRATIONS IN CANINE FEMALES DIAGNOSED WITH OVARIAN CYSTS

CONCENTRAȚII ALE HORMONULUI ANTI-MÜLLERIAN LA FEMELELE DIN SPECIA CANINĂ DIAGNOSTICATE CU CHIȘTI OVARIENI

Z.M. GÁL[1), *)], A.R. POP [1)], Ana HÎRUȚA[1)], Alexandra IRIMIE[1)], I.Ș. GROZA[1)]

ABSTRACT | REZUMAT

AMH (Anti-Müllerian Hormone) is crucial in dogs as it aids in assessing reproductive health, diagnosing reproductive disorders, and determining spay status, thus ensuring better management of breeding programs and overall canine health. This research aims to evaluate differences in AMH levels between healthy female dogs and female dogs with ovarian cysts. This study evaluated 131 canine females, both healthy and presenting ovarian cystic alterations, divided into 4 breed categories. The individuals included in this research were divided into two groups: the control group consisting of healthy, intact canine females and the study group that included bitches with cystic ovarian pathologies. We conducted an abdominal ultrasound examination and analysed seric progesterone levels to establish the study and control groups. AMH concentrations were assayed in order to analyse the seric levels difference between healthy females and bitches with ovarian cysts. AMH levels for each serum sample were determined using an enzyme-linked immunosorbent assay using the Canine Anti Müllerian Hormone ELISA Kit, following the manufacturer's instructions. The results of the analyses revealed that serum AMH levels increase in the context of cystic ovarian pathology. In contrast, no significant between-group changes were identified regarding the age of the individuals. There was highlighted an increased value of the AMH concentrations between the giant and large breed groups, AMH levels being positively correlated with the breed size.

Keywords: reproduction, canine, ovarian cyst, AMH, ELISA kit

Hormonul anti-Müllerian (AMH) este esențial la specia canină, deoarece contribuie la evaluarea sănătății reproductive, la diagnosticarea tulburărilor reproductive și la determinarea statutului sterilizării, asigurând astfel o mai bună gestionare a programelor de reproducție și a sănătății canine în general. Această cercetare își propune să evalueze diferențele în nivelurile de AMH între femelele din specia canină sănătoase și cățele cu chisturi ovariene. Acest studiu a evaluat 131 de femele din specia canină atât sănătoase, cât și care prezentau alterări chistice ovariene, împărțite în 4 categorii de rase în funcție de talie. Indivizii incluși în această cercetare au fost împărțiți în două grupuri, grupul de control format din cățele intacte sănătoase și grupul de studiu care a inclus femele cu patologii ovariene chistice. Acestea au fost examinate folosind ecografia abdominală și li s-au dozat nivelurile serice ale progesteronului pentru a stabili grupurile de studiu și de control. Concentrațiile de AMH au fost analizate pentru a determina diferența de niveluri serice dintre femelele sănătoase și cățelele cu chisturi ovariene. Nivelurile de AMH pentru fiecare probă de ser au fost determinate cu ajutorul unui test imunoenzimatic cu ajutorul kitului ELISA pentru hormon anti-Müllerian canin, urmând instrucțiunile producătorului. Rezultatele analizelor au arătat că nivelurile serice de AMH cresc în contextul patologiei ovariene chistice. În schimb, nu au fost identificate modificări semnificative între grupuri în ceea ce privește vârsta indivizilor. A fost evidențiată o valoare crescută a concentrațiilor de AMH între grupurile de rase gigant și de talie mare, nivelurile de AMH fiind corelate pozitiv.

Cuvinte cheie: reproducție, canină, chist ovarian, AMH, kit ELISA

Alfred Jost (1916-1991) is renowned for identifying the Müllerian inhibitor, which is now known as anti-Müllerian hormone (AMH) or Müllerian inhibiting substance (MIS). Jost clarified the debate about the process of somatic sex differentiation by demonstrating that male traits are induced in the foetus by the testicular hormones testosterone and AMH. These hormones are respectively responsible for the development of the Wolffian ducts, urogenital sinus, and external genitalia, as well as the regression of the Müllerian ducts (7). AMH is a glycoprotein classified within the transforming growth factor (TGF) β-family AMH is exclusively present in the somatic cells of the gonads, specifically in Sertoli cells in males and granulosa cells in females (8). Its primary biological function, which gives the hormone its name, is to initiate the regression of foetal Müllerian ducts in males (9).

In females, AMH is primarily expressed in preantral and small antral follicles, both in dogs and other species(13). AMH has been demonstrated to inhibit the initiation of primordial follicle growth, thereby safeguarding the ovary from premature depletion of

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
*) *Corresponding author: zoltan.gal@usamvcluj.ro*

the follicle pool (2). Moreover, AMH has been shown to counteract the stimulatory effect of follicle-stimulating hormone (FSH) on the growth of pre-antral and small antral follicles, a function that is crucial during cyclic recruitment (3). AMH is also regarded as an indirect biomarker for the total number of ovarian follicles (6). Since the ovaries are the sole source of this hormone, spaying significantly reduces AMH concentrations (18). In female dogs, AMH concentrations are known to rise during the last three weeks of anoestrus, peaking up to six days before ovulation, at which point they begin to decline (13, 21). AMH concentration measurements can be used to determine the presence or absence of ovaries in dogs and cats and, consequently, for the diagnosis of ovarian remnant syndrome (ORS) (15). Significantly elevated serum AMH concentrations can be used to distinguish granulosa cell tumours (GCT) from other ovarian pathologies in female dogs. However, slightly elevated serum AMH levels may also result from other ovarian alterations, particularly luteinized follicular cysts (20).

Functional ovarian cysts present as solitary or multiple fluid-filled structures of varying sizes, which can be unilateral or bilateral (4). They usually occur in female dogs aged 6-8 years. While the exact pathogenesis remains unclear, possible contributing factors include an insufficient LH surge, intrafollicular alterations in gonadotropin receptors, and changes in growth factors (17). The diameter of the cysts generally ranges from 0.2 to 4.0 cm, with a median of 0.5^2 cm. Despite their relatively small size, these cystic follicles can exert significant endocrine activity, potentially disrupting normal ovarian function and leading to hyperestrogenism as a severe consequence. The pathogenesis and treatment of cystic ovarian diseases in female dogs remain unclear. Consequently, early diagnosis and appropriate treatment are crucial to prevent disease progression. In a study involving ovaries from 400 female dogs, the incidence of ovarian pathology was approximately 28.8%. Of these cases, over 75% were non-neoplastic cystic structures, including luteal cysts (23.8%),parovarian cysts (22%), follicular cysts (10.5%), rete ovarii hyperplasia (10.5%), oophoritis (7.9%), rete ovarii adenomas (4.0%) and surface structure cysts (2%) (10-12).

The aim of the current study was to evaluate and compare the levels of Anti-Müllerian Hormone (AMH) between healthy female dogs and those diagnosed with ovarian cysts. This comparison seeks to determine whether AMH levels can serve as a reliable biomarker for detecting ovarian cysts in female dogs, thereby aiding in the diagnosis and management of reproductive health issues. Understanding these differences could improve veterinary diagnostic protocols and enhance treatment strategies for affected animals.

MATERIALS AND METHODS

Study design

The study included a total number of 131 bitches. The dogs were presented at a private practice clinic for a specialised examination of the reproductive system. All the bitches underwent abdominal ultrasound examination. The inclusion criteria for the study group were the presence of ovarian cysts bigger than 4.5-5 mm in diameter and for the bitches to be either in the dioestrus or anoestrus period. For the identification of the oestrus phase, progesterone dosing was used as per the routine examination protocol. The inclusion criteria for the bitches in the control group was the lack of presence of pathological structures of the ovaries and being in the same stage of the oestrus cycle (dioestrus, anoestrus) as the study group.

In order to perform the statistical analysis, the breeds were organised into the following categories: small, medium, large, and giant breeds. The small breed group included Dachshund (n=2), Miniature Bull Terrier (n=1), French Bulldog (n = 2), Pug (n=1), White Highland White Terrier (n=1), Yorkshire Terrier (n=7), Bichon Frise (n=3), Maltese (n=6), Shih Tzu (n=3), Pekingese (n=1) and small mixed breed (n=2). The breeds that form the medium breed group are: Siberian Husky (n=3), Hungarian Short-Haired Pointer (n=1), American Bully (n=10), English Bulldog (n= 1), Bull Terrier (n=1), Beagle (n=16), Lagotto Romagnolo (n=2) and medium mixed breed (n=2). In the large breed group, the following breeds were included: Rottweiler (n=2), Labrador Retriever (n=14), Cane Corso (n=2), German Shepherd (n=4), Malinois Shepherd (n=2), Akita Inu (n=1), American Akita (n=3), Alaskan Malamute (n=2) and large mixed breed (n= 1). The giant breed group includes Saint-Bernard (n= 11), Central Asia Shepherd (n=4), Romanian Bucovina Shepherd (n=1), Bernese Mountain Dog (n=5), Carpathian Shepherd Dog (n=1), Caucasian Shepherd Dog (n=8), Presa Canario (n=1) and Tibetan Mastiff (n=1). Blood samples were collected from all patients for routine blood work, progesterone, and AMH evaluation. The blood samples were centrifuged at 5000 rpm for 10 minutes and the serum was stored at −18° C until the evaluations were done. All owners provided informed written consent, granting permission for sample collection and its use in research, as well as for the anonymous publication of the results.

Evaluation of AMH

AMH concentration for each serum sample was determined using an enzyme-linked immunosorbent assay (Canine Anti Müllerian Hormone ELISA Kit, Shanghai Coon Koon Biotech Co., Ltd., Shanghai, China) following the manufacturer's instructions. The extent of enzymatic turnover of the substrate was assessed by measuring absorbance at wavelength of 450 nm.

For the calculation of Anti-Müllerian Hormone concentration, the standard concentrations provided in the manufacturer's manual and the corresponding optical density values obtained from spectrophotometric readings were used to determine the regression equation of the standard curve. This equation was used to calculate the sample concentration based on the optical density. After the standard curve was created, the equation $y = 0.0065 x + 0.1127$ was obtained. Using

this equation, the concentration values were calculated based on the optical density of each sample. As a result of the calculations, a coefficient of determination R^2= 0.9983 was obtained, which confirms the proper execution of the ELISA procedure.

Statistical analysis

Normal distribution of the obtained AMH data was based on the Kolmogorov-Smirnov test evaluation. Comparison between the control group AMH results and the study group AMH results were performed by independent samples t-test. To study the difference between the four breed categories in regards to the AMH measurements, the One-Way ANOVA test was used. Values of $p < 0.05$ were considered statistically significant. The statistical analysis was performed using IBM® SPSS Statistics version 29.0 (Armonk, NY, USA) and MedCalc® Statistical Software version 22.032 (MedCalc Software Ltd., Ostend, Belgium).

RESULTS AND DISCUSSIONS

The study group included a total of 76 canine females from 34 different breeds and 5 crossbreeds. The age ranged from 9 months to 16 years, with an average of 6 years. The control group included a total number of 55 bitches of 6 different breeds. The age ranged between 1 and 13 years, with an average of 4.5 years. The results for the statistical analysis of control group AMH results indicated that the arithmetic mean was 10.0842 ng/ml (95% CI for the mean: 9.7953–10.3731, SD: 1.0686). For the study group, the AMH results indicated that the arithmetic mean was 10.6266 ng/ml (95% CI for the mean: 10.3704–10.8828, SD: 1.1212). Based on the application of the independent samples t-test, it was noted that there was a statistically significant difference (p = 0.006) between the AMH levels in the control group and the AMH levels in the study group. These differences are illustrated in Fig. 1.

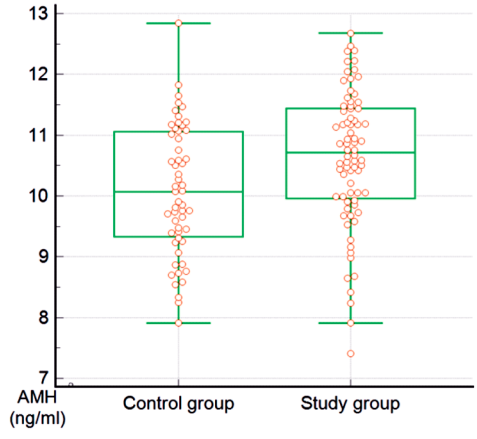

Fig. 1. AMH differences between control group and study group

Legend: the middle line in the box represents the median value, while the upper edge of the box represents the upper quartile and the lower edge of the box represents the lower quartile. The lines at the top and bottom represent the greatest value (maxi-mum) and lowest value (minimum).

Based on the linear regression equation applied to the AMH levels as a dependent parameter and age as an independent parameter, the results indicated that there isn't a correlation between age and AMH levels, neither in the control group, nor in the study group (Fig. 2).

In regards to the difference in AMH values between the four breed categories, the results have been segregated for the study group and the control group. For the study group, the Kolmogorov–Smirnov test results indicated that p = 0.200 (>0.05), which corresponds to a normal distribution of the data.

More specifically, Table 1 shows the AMH values for

Table 1

Statistical parameters for AMH values of the study group in accordance with breed category

Breed category	Lower limit 90% CI	Upper limit 90% CI
Giant	6.0502–8.3218	11.9635–14.2351
Large	8.0756–9.4262	12.2643 to 13.6149
Medium	8.0840–9.3037	12.1363 to 13.3560
Small	8.4294–9.6338	11.6724 to 12.8768

Legend: SD – standard deviation, CI – confidence interval, RI – reference interval, n – number of individuals

Table 2

Reference interval for 95% of AMH values in study group

Breed category	Lower limit 90% CI	Upper limit 90% CI
Giant	6.0502–8.3218	11.9635–14.2351
Large	8.0756–9.4262	12.2643 to 13.6149
Medium	8.0840–9.3037	12.1363 to 13.3560
Small	8.4294–9.6338	11.6724 to 12.8768

Legend: CI – confidence interval

each breed category in the study group, together with the corresponding statistical parameter, and Table 2 shows the reference interval for 95% of AMH values in the study group.

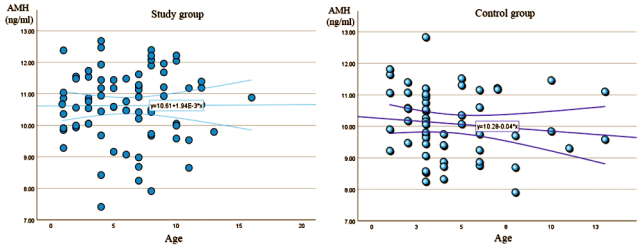

Fig. 2. Scatter plot for observing the relationship between AMH and age in the study group and control group

Legend: The two lines above and beyond the middle line represent the confidence interval of the statistical average.

In order to study whether there are any statistically significant differences between the four breed categories, the one-way analysis of variance (ANOVA) was used. The results indicated that F_stat=1.29 and p = 0.29 (>0.05), hence there are no statistically significant differences between the breed categories part of the study group. Further analysis of the Pearson correlation coefficient, as illustrated in the correlogram (Fig. 3), indicated that there were no correlations (p > 0.05) between the AMH levels of any of the breed categories part of the study group.

For the control group, the Kolmogorov–Smirnov test results indicated that p = 0.231 (>0.05), which corresponds to a normal distribution of the data.

Table 3 shows the AMH values for each breed category in the control group together with the corresponding statistical parameter, and Table 4 shows the reference interval for 95% of AMH values in the control group.

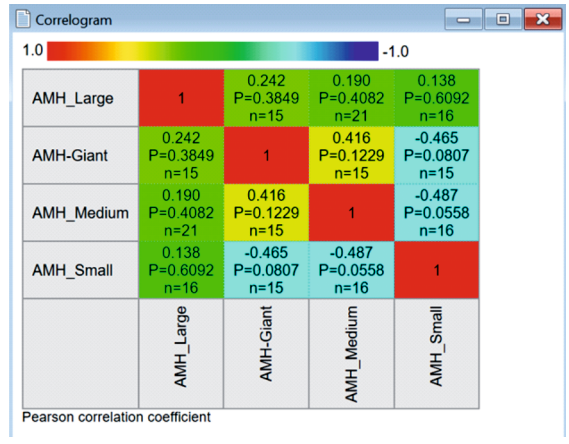

Fig. 3. Correlogram illustrating the Pearson correlation coefficient between the breed categories of the study group

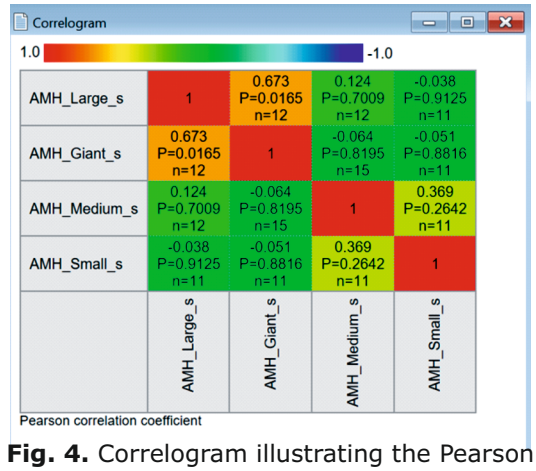

Fig. 4. Correlogram illustrating the Pearson correlation coefficient between the breed categories of the control group

Table 3

Statistical parameters for AMH values of the control group in accordance with breed category

Breed category	n	Mean ± SD	Median	Min	Max	Confidence Interval for Mean 95%	Reference interval for 95%
Giant	17	9.99 ± 0.91	9.91	8.54	11.65	(9.52, 10.46)	(8.20, 11.79)
Large	12	10.03 ± 1.47	9.73	7.91	12.84	(9.10, 10.97)	(7.15, 12.92)
Medium	15	10.43 ± 1.07	10.94	8.25	11.82	(9.84, 11.03)	(8.33, 12.54)
Small	11	9.78 ± 0.72	9.76	8.70	11.02	(9.28, 10.27)	(8.36, 11.20)

Legend: SD – standard deviation, CI – confidence interval, RI – reference interval, n – number of individuals

Table 4

Reference interval for 95% of AMH values in control group

Reference interval for 95%	Lower limit 90% CI	Upper limit 90% CI
Giant	7.5569–8.8489	11.1476–12.4396
Large	5.9076–8.4000	11.6750–14.1674
Medium	7.5225–9.1404	11.7343–13.3522
Small	7.7203–9.0079	10.5649–11.8525
Overall	7.5767–8.4028	11.7656–12.5917

Legend: CI – confidence interval

In order to study whether there are any statistically significant differences between the four breed categories, the one-way analysis of variance (ANOVA) was used. The results indicated that F_stat = 0.86 and p = 0.46 (>0.05), hence there are no statistically significant differences between the breed categories part of the control group. Further analysis of the Pearson correlation coefficient, as illustrated in the correlogram (Fig. 4), indicated that there was a positive correlation (p = 0.01) between the AMH levels of the large breed category and the giant breed category of the control group. Anti-Müllerian hormone is an ideal reproductive marker since it remains detectable in females during anoestrus and vanishes from the bloodstream following the removal of the ovaries (15).

Place et al. (15) conducted a study in which they measured the AMH concentrations between intact female dogs and spayed female dogs using an human-based AMH ELISA testing kit. The serum AMH concentration for the dogs listed as spayed was less than or equal to 0.09 ng/ml, while each of the 16 dogs listed as intact had AMH concentrations ranging from 0.10 to 0.41 ng/m (15). In comparison, the AMH concentration that was obtained in the current study for the female dogs part of the control group ranged from 7.91 to 12.84 ng/ml. The difference in results may be attributed to the fact that Place et al. (15) used a human-based AMH ELISA kit while in the current study a canine AMH ELISA kit was used.

Similarly, Alm and Holst (1) conducted a study in which they aimed to evaluate the diagnostic efficiency of detecting ovarian tissue in female dogs using an AMH assay developed for human samples and a semi-quantitative rapid immune migration LH assay designed for use in dogs. The study was conducted in 73 intact bitches and 52 spayed bitches. The AMH concentration results in intact bitches ranged from <0.1 ng/ml to 2.2 ng/ml. As previously mentioned, the AMH concentrations in the current study were higher (range 7.91–12.84 ng/ml) and this fact can be attributed to the difference in ELISA kits used. In accordance with the results in the current study, Alm and Holst (1) did not identify any association between the concentration of AMH in intact bitches and age.

Another study conducted by Walter et al. (21) aimed to investigate the secretion pattern of AMH throughout the oestrous cycle in non-pregnant female dogs. The study had a preliminary step which was conducted on 19 intact female dogs and 19 spayed females of different age and breed. AMH concentrations were measured using a chemiluminescence immunoassay on a cobas E602 analyzer using murine anti-AMH antibodies. AMH concentration in the intact female dogs ranged from 0.19 to 1.45 ng/ml. The difference between these results and the results obtained in the current study can, again, be attributed to the methodology. Additionally, similar to the results obtained in the current study, Walter et al found that there were no influences of the age on the AMH concentration (21).

An additional study conducted by Walter et al (20) aimed to determine if blood serum AMH concentration can be used to differentiate granulosa cell tumours (GCT) from other ovarian neoplasms and ovarian cysts, and to assess whether AMH can aid in the clinical diagnosis of GCT in female dogs. The study included 63 female dogs, from which 14 were without any pathological finding, 20 were identified with one or more cysts and 29 had ovarian tumours. The serum concentration of AMH was measured using a chemiluminescence immunoassay. The AMH values ranged from 0.12 to 0.99 ng/ml for the healthy female dogs, while the AMH values in the females with follicular cysts ranged from 0.11 to 2.09 ng/ml. In comparison, the AMH values in the study group from the current study ranged from 7.41 to 12.68 ng/ml. Yet again, the difference in AMH values found by Walter et al (20) and the AMH values from the current study may be attributed to the difference in methodology.

In contrast with our findings regarding the AMH concentration in relation to age, Hollinshead et al (5) observed a decrease in AMH concentrations with advancing age in female dogs, similar to the findings in humans (19). Both follicular and luteinized follicular cysts can contain thin remnant layers of granulosa cells, but luteinized follicular cysts can exhibit some grade of luteinization in some granulosa cells and thus this ovarian cyst can contribute to higher AMH concentrations in bitches (16).

However, there are limitations due to individual variations in serum AMH levels, as demonstrated in the study. AMH concentrations also fluctuate significantly during the natural oestrous cycle in bitches. Specifically, AMH levels significantly increase from late anoestrus up to six days before ovulation, then significantly decrease from early proestrus to preovulatory oestrus. Another significant decrease in AMH concentrations occurs from postovulatory oestrus to metestrus, with levels remaining low during mid-anoestrus (20).

It is worth noting that differences in AMH concentrations may also occur due to interindividual factors and the use of different immunoassay kits (14).

CONCLUSIONS

In conclusion, the statistical analysis of 131 healthy canine females and bitches with cystic ovarian pathology revealed a notable correlation between the control group (healthy females) and the study group (females with ovarian cysts). The mean AMH levels were 10.0842 ng/ml (95% CI: 9.7953–10.3731, SD: 1.0686) for the control group and 10.6266 ng/ml (95% CI: 10.3704–10.8828, SD: 1.1212) for the study group. The linear regression analysis on the relationship between the age of the bitches and the AMH levels indicated no significant correlation.

For healthy canine females (control group) across different breed categories and their AMH results, a positive correlation was observed in the giant breed group (mean AMH value 9.91 ng/ml) and large breed group (mean AMH value 9.73 ng/ml) with a p-value of 0.016. AMH levels in these groups appeared to in-

crease with the size of the breed category, unlike the other breed groups.

ACKNOWLEDGEMENTS

The authors extend their gratitude to Dr. Kalmár Zsuzsa for her assistance with the ELISA dosing technique, the Small Animal Reproduction Clinic at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, and the private practice reproduction referral clinic Quantas Repro Vet SRL in Cluj-Napoca for their help in collecting the samples.

REFERENCES

1. *Alm H., Holst B.S.,* (2018), Identifying ovarian tissue in the bitch using anti-Müllerian hormone (AMH) or luteinizing hormone (LH). Theriogenology, 106:15-20
2. *Durlinger A.L.L., Gruijters M.J.G., Kramer P., Karels B., Ingraham H.A., Nachtigal M.W., Uilenbroek J.Th.J., Grootegoed J.A., Themmen A.P.N.,* (2002), Anti-Müllerian Hormone Inhibits Initiation of Primordial Follicle Growth in the Mouse Ovary. Endocrinology, 143:1076-1084
3. *Durlinger A.L.L., Gruijters M.J.G., Kramer P., Karels B., Kumar T.R., Matzuk M.M., Rose U.M., De Jong F.H., Uilenbroek J.Th.J., Grootegoed J.A., Themmen A.P.N.,* (2001), Anti-Müllerian Hormone Attenuates the Effects of FSH on Follicle Development in the Mouse Ovary. Endocrinology, 142: 4891-4899
4. *Groza I.Ş., Bogdan L.M., Cătană R.,* (2006), Ginecologie, andrologie şi obstetrică veterinară: compendiu, (Ed.) Academiei Române, Bucharest, Romania
5. *Hollinshead F., Walker C., Hanlon D.,* (2017), Determination of the normal reference interval for anti-Müllerian hormone (AMH) in bitches and use of AMH as a potential predictor of litter size. Reprod Domestic Animals, 52:35-40
6. *Ireland J.J., Smith G.W., Scheetz D., Jimenez-Krassel F., Folger J.K., Ireland J.L.H., Mossa F., Lonergan P., Evans A.C.O.,* (2011), Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reprod Fertil Dev, 23:1
7. *Josso N.,* (2008), Professor Alfred Jost: The Builder of Modern Sex Differentiation. Sex Dev, 2:55-63
8. *Josso N., Di Clemente N.,* (1999), TGF-β Family Members and Gonadal Development. Trends in Endocrinology & Metabolism, 10:216-222
9. *Josso N., Rey R.A., Picard J.Y.,* (2013), Anti-Müllerian Hormone: A Valuable Addition to the Toolbox of the Pediatric Endocrinologist. International Journal of Endocrinology, 2013:1-12
10. *Knauf Y., Köhler K., Knauf S., Wehrend A.,* (2018). Histological classification of canine ovarian cyst types with reference to medical history. J Vet Sci, 19:725
11. *Marino G., Barna A., Mannarino C., Di Prima M.L., Zanghi A.,* (2010), Stromal cysts of the canine ovary: prevalence, diagnosis and clinical implications. Rivista ufficiale della SCIVAC, 24:9-15
12. *Maya-Pulgarin D., Gonzalez-Dominguez M.S., Aranzazu-Taborda D., Mendoza N., Maldonado-Estrada J.G.,* (2017), Histopathologic findings in uteri and ovaries collected from clinically healthy dogs at elective ovariohysterectomy: a cross-sectional study. J Vet Sci, 18:407
13. *Nagashima J., Hansen B., Songsasen N., Travis A., Place N.,*(2016), Anti-Müllerian Hormone in the Domestic Dog during the Anestrus to Oestrous Transition. Reprod Domestic Animals, 51:158-164
14. *Pir Yagci I., Pekcan M., Polat I., Kalender H., Macun H.,* (2016), Does serum anti-Müllerian hormone levels always discriminate presence of the ovaries in adult bitches? Comparison of two ELISA kits. Reprod Domestic Animals, 51:910-915
15. *Place N.J., Hansen B.S., Cheraskin J.L., Cudney S.E,Flanders J.A.,Newmark A.D.,Barry B.,Scarlett J.M.,* (2011), Measurement of serum anti-Müllerian hormone concentration in female dogs and cats before and after ovariohysterectomy. J Vet Diagn Invest, 23:524i527
16. *Rehm S., Stanislaus D.J., Williams A.M.,* (2007), Estrous cycle-dependent histology and review of sex steroid receptor expression in dog reproductive tissues and mammary gland and associated hormone levels. Birth Defects Research Pt B, 80: 233-245
17. *Sasidharan J.K., Patra M.K., Singh L.K., Saxena A.C., De U.K., Singh V., Mathesh K., Kumar H., Krishnaswamy N.,* (2021), Ovarian Cysts in the Bitch: An Update. Topics in Companion Animal Medicine, 43:100511
18. *Themmen A.P.N., Kalra B., Visser J.A., Kumar A., Savjani G., De Gier J., Jaques S.,* (2016), The use of anti-Müllerian hormone as diagnostic for gonadectomy status in dogs. Theriogenology, 86:1467-1474
19. Visser J.A., De Jong F.H., Laven J.S.E., Themmen A.P.N., (2006), Anti-Müllerian hormone: a new marker for ovarian function. Reproduction, 131:1-9
20. *Walter B., Coelfen A., Jäger K., Reese S., Meyer-Lindenberg A., Aupperle-Lellbach H.,* (2018), Anti-Muellerian hormone concentration in bitches with histopathologically diagnosed ovarian tumours and cysts. Reprod Domestic Animals, 53:784-792
21. *Walter B., Feulner H., Otzdorff C., Klein R., Reese S., Meyer-Lindenberg A.,* (2019), Changes in anti-Müllerian hormone concentrations in bitches throughout the oestrous cycle. Theriogenology, 127:114-119.