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Original Research Article

Anti-Müllerian hormone in feline cryptorchidism: Serum levels, tissue expression, and implications for testicular health

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ABSTRACT

Anti-Müllerian hormone (AMH) has become a pivotal subject in the study of testicular descent, maturation, integrity, and male fertility. Recent studies explored its roles and implications across various domestic species. A prominent approach involved the understanding of the modulation of AMH in reproductive disorders, including cryptorchidism. While substantial findings have been reported in dogs, ruminants, swine, and horses, data on AMH in feline cryptorchidism remains limited. Here, we aimed to bridge this gap by comparing AMH serum levels among cryptorchid, healthy intact, and castrated tomcats, employing an enzyme-linked immunosorbent assay (ELISA) kit for quantification. In addition, AMH expression in retained and descended testes was evaluated through immunohistochemistry, with positive staining quantified via pixel analysis in two distinct regions of interest: the seminiferous tubule and the interstitial space. Furthermore, tissue samples were subjected to histological evaluation and morphometric analysis, which included the calculation of seminiferous tubule areas (STA) and assessment of Johnsen scores. Thus, the relationship between AMH expression, altered testicular histology, and impaired spermatogenesis could be examined. The expression of AMH in retained and descended testes, was investigated, and the relationship between AMH expression, altered testicular histology, and impaired spermatogenesis was examined. Mean serum AMH levels differed significantly ($P < 0.001$) across the different groups being 15.35 ± 4.66 ng/mL (mean ± SD) in healthy intact tomcats ($n = 15$), 25.55 ± 2.86 ng/mL (mean ± SD) in cryptorchids ($n = 10$) and below 0.015 ng/mL in castrated cats ($n = 10$). STAs and Johnsen scores were significantly reduced in retained testes when compared to descended gonads ($P < 0.01$). Furthermore, serum AMH was negatively correlated with both the STA ($\rho = -0.725$, $P < 0.001$) and the Johnsen scores ($\rho = -0.699$, $P < 0.001$), suggesting its potential value for tissue integrity and spermatogenesis evaluation. In addition, positive immunostaining was significantly higher in retained testes ($P < 0.05$), especially in the interstitial space ($P < 0.01$), suggesting an involvement of the Leydig cells. Additionally, the increased interstitial expression was linked to the degree of tissue degeneration and the impaired spermatogenesis being negatively corelated with both Johnsen scores ($\rho = -0.309$, $P < 0.01$) and STA ($\rho = -0.208$, $P < 0.05$). Our findings underscore the potential of AMH in assessing testicular health and reveal possible interspecific differences, stressing the need for further investigation in cats.

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1. Introduction

Cryptorchidism is a congenital disorder characterized by a polygenic inheritance [1], being defined in cats as the failure of one or both testes to descend into the scrotum, remaining there by 7–8 months of age [2]. Interestingly, the incidence ranging from 1.3 to 3.8 % [3–5], makes it the most common sexual development disorder in tomcats [6]. However, in comparison to other species, less research has been conducted on its pathogenetic mechanisms and specific molecular or histopathological traits, leaving some particular aspects still unclear. In men, cryptorchidism has been associated with a series of in-tissue alterations specific to impaired spermatogenesis, testicular atrophy or even immaturity due to its link with the so-called testicular dysgenesis syndrome [7–9]. Similar structural and functional disruptions related to the lack of testicular descent have been highlighted in dogs [10], bulls [11], boars [12] and horses [13] while no data are available on retained feline testes. Cryptorchidism is considered responsible for several cellular and molecular alterations, as demonstrated in human studies, leading to certain micro-RNA and protein expression patterns [14]. In dogs, cryptorchidism was associated with clear spermatogenic arrest accompanied by severe histological alterations and lack of germ cell maturation [10]. This was explained as a consequence of the altered thermal environment characteristic to retained testes, which leads to oxidative stress [15]. Oxidative stress has the potential to produce the down-regulation of tight junction proteins leading to spermatogenetic failure [15,16]. Moreover, the exposure to higher temperatures can trigger the release of large amounts of reactive oxygen species [17], including apoptosis and autophagy of germ cells, as well as potential DNA damage in sperm cells [18]. Sertoli cells can also be damaged [15] and deprived of essential factors necessary for their maturation [19], potentially blocking their development or converting them to an immature state [20,21].

A major focus in investigating the mechanisms underlying cryptorchidism in both humans and animals has been the anti-Müllerian hormone (AMH) [22–24]. This glycoprotein, produced by Sertoli cells in males [25], was associated with crucial processes such as germ cell maturation [26] and tissue damage related to testicular degeneration or atrophy [27]. The expression of AMH is dependent on the transition of Sertoli cells to the mature stage, as demonstrated by the fluctuations in AMH levels during fetal development, puberty, and adulthood [28,29]. Moreover, AMH affects the development and function of the adult testes, being able to block the differentiation of mesenchymal cells into Leydig cells, independently decreasing the expression of steroidogenic enzymes [30]. Lately, both AMH immunoexpression and serum levels in relation to specific tissular alterations have been investigated using cryptorchid patients as study models, offering, therefore, a valuable perspective on potential AMH applications in the assessment of male fertility, gonad integrity or tumorigenesis [13,31,32]. Nevertheless, no data is currently available on the relation between the aforementioned aspects and feline cryptorchidism.

Therefore, to investigate the particularities regarding AMH in the feline model and cover the existent gap in the literature, the aims of this study were: (1) to identify and compare AMH serum levels between cryptorchid, healthy intact and castrated tomcats; (2) to consequently analyze its expression in retained and descended testes and lastly (3) to describe the relation between the latter and altered testicular histology and impaired spermatogenesis.

2. Materials and methods

2.1. Animals

To performe this study, a total of thirty-five client-owned tomcats were divided into three groups: cryptorchid tomcats, having at least one abdominal or inguinal retained testis (cryptorchid group, n = 10); healthy intact adult tomcats with both testes identified inside the scrotum (normal intact group, n = 15); and castrated adult tomcats, with gonadectomy reported at least six months before the study (castrated group, n = 10). The breed distribution of the subjects was predominantly composed of European Shorthair tomcats (n = 26), complemented by 8 British Shorthair and 1 Exotic Shorthair, with weights ranging from 3.2 to 6.8 kg. In the cryptorchid group, ages ranged from 12 to 19 months, comprising 7 unilateral inguinal cryptorchids, one unilateral abdominal cryptorchid, and 2 bilateral abdominal cryptorchids. Similarly, the age range of the intact group included individuals between 12 and 24 months, whereas the castrated group exhibited greater age variability, ranging from 12 to 84 months. All tomcats in the study, were sampled for the assessment of AMH serum levels. Individuals included in the cryptorchid and normal intact groups underwent orchiectomy, with two exceptions, one unilateral inguinal cryptorchid and one intact control, which did not receive surgery because of feline immunodeficiency seropositivity and consequent loss of owner consent. Testicular tissue from the remaining tomcats (n = 23) was stored for histological and immunohistopathological examination. Importantly, all procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Bucharest (Approval Number: EA nr. 34–03/2024), and were conditioned by owners' written consent.

2.2. Sample collection

Blood samples were obtained by puncturing either the cephalic or jugular vein and stored into plain red-top collection tubes with no additives (BD Vacutainer, Plymouth, UK). After clotting for 5 min at room temperature, the samples were centrifuged at 2500×*g* for 10 min, and the serum was preserved at −80 °C until further processing. Immediately after surgery, testicular tissue samples from the patients referred for gonadectomy (both cryptorchid and intact group) were fixed in 10 % neutral buffered formalin and embedded in paraffin blocks.

2.3. Serum AMH levels evaluation

Serum AMH levels were assessed using a previously validated [33] quantitative enzyme-linked immunosorbent assay (ELISA) kit- Canine/Feline AL-116 ELISA (Ansh Labs, Webster, TX, USA), following manufacturer instructions. Briefly, after the addition of 75 μL of the Canine AMH Assay Buffer to each coated well, 25 μL of the calibrators, controls, and serum samples was pipetted and the plate was then incubated for 60 min at room temperature on an orbital microplate shaker at 600 rpm. After extensive washing steps, 100 μL of the Antibody-Biotin Conjugate was added to each well and the microplate was incubated in the same conditions. The washed wells were further incubated with 100 μL of the Streptavidin-HRP conjugated enzyme for only 30 min. Finaly, each well was incubated 10 min with 100 μL of the TMB chromogen solution and after adding 100 μL of the stopping solution the plate absorbance was measured at 450 nm with background wavelength correction at 630 nm using PR 4100 Absorbance Microplate Reader (Bio-Rad Laboratories, Hercules, CA, USA). The intra-assay coefficient of variation was lower than 10 %. As all samples were processed in a single batch, no inter-assay coefficient of variation was computed. Detection limit of the kit was considered 0.015 ng/mL.

2.4. Histological morphometric analysis

The paraffin-embedded tissue samples were sectioned and stained with hematoxylin and eosin (HE). Sections from both testes of the bilateral cryptorchids were analyzed, while for the unilateral cryptorchids, only the retained testis was included for analysis. This decision was supported by the potential for histological alterations in the contralateral gonad as it was previously reported [10]. Therefore, only samples from intact healthy individuals constituted the control group. In this case, both testes were included in the same paraffin block, randomized sections from left and right gonads being analyzed.

In addition, twenty randomly selected seminiferous tubules, along with the neighboring interstitial space, were analyzed per section at a magnification of 400x. Image acquisition and subsequent morphometrical analysis were performed via the Leica Application Suite (LAS version 4.13, Wetzlar, Germany). The seminiferous tubule area (STA) was calculated similarly to the protocol used by Tsogtgerel et al. [13]. Thus, out of the total of twenty tubule sections, five round or nearly round seminiferous tubules were measured across their minor and major axes, and the formula for STA = π * semimajor axis * semiminor axis was utilized.

Furthermore, spermatogenic activity was assessed using a modified Johnsen score, as previously described in studies involving dogs [34,35]. Hence, a score ranging from 1 to 10 was assigned to each of the 20 examined tubule, depending on the presence of the main cell types arranged in the order of maturity, which include spermatogonia, spermatocytes, spermatids, spermatozoa, and Sertoli cells (Supplemental material 1). Results for both STA and Johnsen score were expressed by mean values per retained or descended testis.

2.5. Immunohistochemistry

From the previously stored paraffin-embedded tissue blocks, specific slides (3-aminopropyltriethoxysilane-coated) were used to receive the cut sections. The slides were subjected to deparaffinization with xylene treatment, then immersed in decreasing concentrations of ethanol for rehydration (100 %, 96 %, 50 % and 100 % H_2O). The next step was to perform the antigen retrieval in the samples, for that, the slides were kept in a box filled with distilled water and citrate buffer (pH 6.0), which was placed inside a pressure cooker containing 600 ml of distilled water. The system (cooker + slides) was microwaved (850 Watts for 12 min then 300 Watts for 10 min) and once retrieved, the box containing the slides was cooled at room temperature for 20 min. The slides were then washed with PBS for 5 min, then 200 μl of H_2O_2 was added on each section (5 min interaction), after the exposure, the slides were washed twice with phosphate-buffered saline (PBS) (5 min).

The primary antibody of choice was an anti-AMH C-terminal (anti rabbit - Abcam, Cambridge, UK) in a 1:400 dilution (antibody diluent with background reduction - DAKO Agilent – S3022832, Santa Clara, CA, USA). The slides were incubated for 30 min with the diluted primary antibody, then washed twice with PBS (5 min) and exposed to 200 μl/slide of Envision Link rabbit (DAKO Agilent – K400311-2) for 30 min. Once the incubation was completed, the slides were washed in PBS (2 × 5 min) then received 200 μl/slide of 3.3-diaminobenzidine solution (DAB- DAKO Agilent – K346811-2) and incubated for 30 min. In addition, the slides were washed in PBS for 5 min then counter stained with Hematoxylin for 10 s. To complete the process, the stained slides were then subjected to dehydration, mounted with a cover slip and stored for posterior analyzes. The negative control was performed by omitting the primary antibody, while a testis tissue sample from a prepubertal tomcat served as positive control. Unfortunately, one inguinal retained testis had to be excluded from further analysis due to storage issues that led to the lack of staining after two consecutive attempts.

Immunohistochemical data evaluation involved examining the stained tissue sections at a magnification of 400x. Five randomized captions were acquired per testis sample and analyzed using the same software as previously mentioned, the Leica Application Suite. Immunostaining quantification was performed through pixel analysis on five different randomly obtained caption per sample, employing a standardized color correction via white balance and standardized threshold. The results were expressed as percentages of reactive areas relative to the total surface of a predefined Region of Interest (ROI). The ROI was selected using a binary image editing tool. For seminiferous tubules, the ROI was defined by the total area of the analyzed tubule, while for the interstitial space, the ROI encompassed the space between two neighboring tubules. The ROI area was calculated in both situations by tracking predefined margins and applying the automated measuring tool.

2.6. Statistical analysis

The data were processed and statistically analyzed using computerized statistical software (IBM SPSS version 18.0 for Windows, NY, USA). All values were expressed as mean ± SD. The Shapiro-Wilk test was applied to evaluate the normality of the variables, with the significance level being set to $\alpha = 0.05$. Group differences were determined in non-normalized data using the Independent-Samples Mann-Whitney *U* Test and Kruskal-Wallis Test when several independent variable groups were considered. For the latter, significance values have been adjusted by the Bonferroni correction for multiple tests. Likewise, for normally distributed data, the Independent T-test and Pearson correlation tests were applied. Spearman's rank correlation coefficient was used to explore potential associations within the dataset. In order to detect differences between AMH staining quantification in both seminiferous tubules and interstitial space among the cryptorchid and healthy intact groups the Wilcoxon Signed Rank Test was used. Due to low case numbers in specific subcategories, data regarding abdominal testicular location or bilateral cryptorchid state were analyzed descriptively. Advanced analysis was conducted referring to variables falling into the categories retained or descended. Statistical significance was determined by considering P-values less than 0.05.

3. Results

3.1. Serum AMH

Mean serum AMH concentrations in the intact healthy group (n = 15) and the cryptorchid group (n = 10) were 15.35 ± 4.66 ng/mL and 25.55 ± 2.86 ng/mL (Fig. 1), respectively. All values from previously castrated tomcats (n = 10) were below the detection limit of the kit (<0.015 ng/mL). Within the cryptorchid group, subcategories were defined with mean AMH values: unilateral (24.75 ± 2.61 ng/mL, n = 8), bilateral (28.75 ± 0.64 ng/mL, n = 2), inguinal (24.09 ± 1.96 ng/mL, n

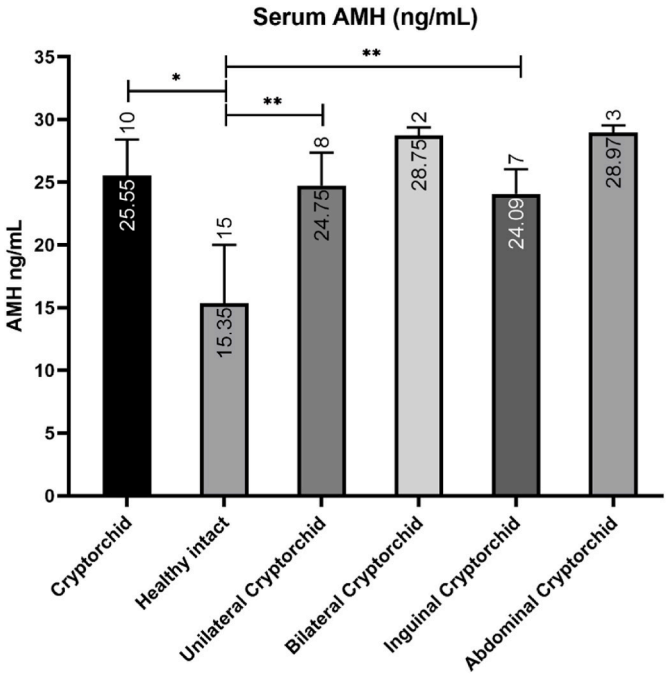

Fig. 1. AMH serum concentrations (mean ± SD) in healthy intact and cryptorchid (unilateral, bilateral, inguinal and abdominal) tomcats. Due to low number of subjects values regarding bilateral cryptorchids and abdominal cryptorchids were only presented descriptively. $P \leq 0.05$ (*), $P \leq 0.01$ (**).

= 7), and abdominal (28.97 ± 0.59 ng/mL, n = 3) (Fig. 1). The castrated group significantly differed from both the healthy intact ($P < 0.01$) and cryptorchid groups ($P < 0.001$), indicating a distinct difference in distributions. Pairwise comparisons showed also significant variation between the AMH levels specific to the intact individuals and certain subcategories such as the inguinal ($P < 0.01$) or unilateral ($P < 0.01$) cryptorchids. There was no significant correlation between the age of the intact tomcats and the AMH levels ($P = 0.228$). On the other hand, a strong negative correlation ($\rho = -0.928$) between the status of the subjects (cryptorchid, healthy intact or sterilized) and serum AMH levels ($P < 0.01$) was highlighted. This indicates that serum AMH levels decrease significantly as the status changed from cryptorchid to intact to castrated.

3.2. Histomorphometry of scrotal and cryptorchid testes and johnsen score

The STA exhibited a significant reduction in retained testes compared to descended gonads ($P < 0.001$), with mean values of 11413.08 ± 4043.50 μm^2 (n = 8) and 37569.13 ± 9576.22 μm^2 (n = 14) (Fig. 2), respectively. However, the observed variation ceased to be statistically significant ($P = 1.000$) when contrasting tubule sections from abdominally with inguinal retained testes.

For the retained testes, whether inguinal or abdominal, the most common observation was the compressed tubules with few germ cells, mainly described as containing either only Sertoli cells or spermatogonia. Therefore, the mean Johnsen score for this category was 2.83 ± 0.67 (n = 8). Conversely, the descended testes depicted mainly proof of advanced spermatogenesis with mean scores reaching 8.31 ± 1.34 (n = 14) (Fig. 3). In spite of the significant differences between the descended and retained gonads ($P < 0.001$), Johnsen scores were relatively similar when comparing the inguinal and abdominal locations ($P = 1.000$).

Strong negative correlations were found between serum AMH levels and both STA ($\rho = -0.725$, $P < 0.001$) and Johnsen scores ($\rho = -0.699$, $P < 0.001$). Moreover, larger seminiferous tubules were strongly correlated with higher Johnsen scores ($\rho = 0.804$, $P < 0.001$), being specific to the normally descended gonads ($\rho = 0.831$, $P < 0.01$).

3.3. AMH expression and quantification

The AMH expression was primarily disclosed as brown staining (Fig. 4). Positive immunostaining was significantly higher in the retained testes compared to the descended ones ($P < 0.05$). However, no significant variation was found in intratubular AMH expression between the two sample groups ($P > 0.05$). The mean reactive area within seminiferous tubules was 24.02 ± 7.66 % for sections originating from retained testicular tissue (n = 8) and 25.55 ± 7.71 % for the descended testes (n = 14). Nevertheless, analysis of interstitial AMH expression on the same sections consistently showed higher expression in the interstitium of the retained gonads (6.89 ± 5.29 % vs. 3.93 ± 3.24 %) ($P < 0.01$). Moreover, AMH reactivity was higher in the seminiferous tubules of both retained and descended testes compared to the interstitial space ($P < 0.05$). Additionally, higher AMH expression in the interstitial space correlated with lower Johnsen scores ($\rho = -0.309$, $P < 0.01$) (Fig. 5A) and reduced STAs ($\rho = -0.208$, $P < 0.05$) (Fig. 5B), further characterizing this pattern in cryptorchid gonads.

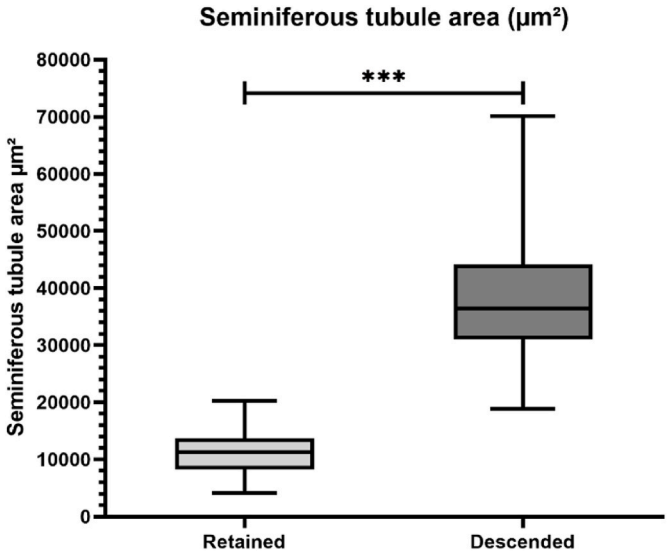

Fig. 2. Seminiferous tubule area (mean ± SD; μm^2) in retained and descended testes. Data are expressed as mean ± SD. $P < 0.001$ (***).

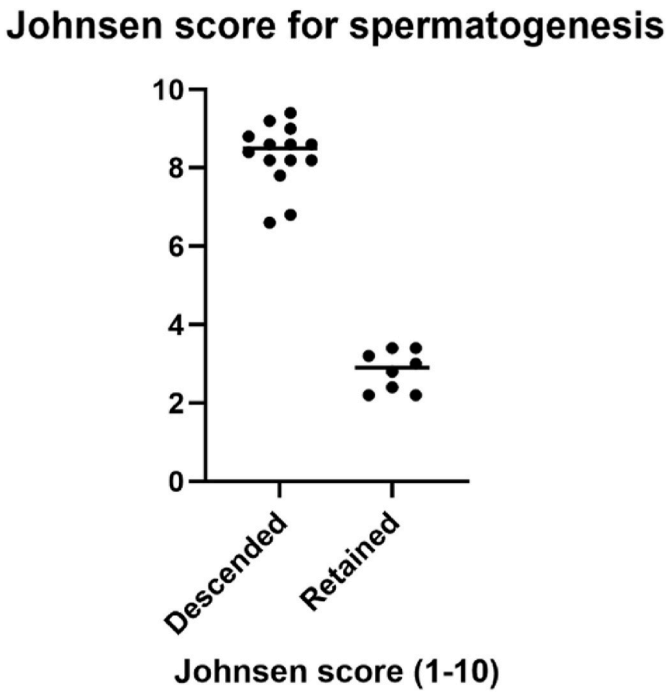

Fig. 3. Johnsen score morphometry (mean ± SD; 1–10) in retained and descended testes.

4. Discussion

In this study, we assessed AMH immunoreactivity in feline cryptorchid testes and examined its correlation with AMH serum levels, spermatogenesis, and histomorphometric characteristics.

AMH serum levels have previously been discussed in relation to the castration status of tomcats [36]. Our findings align with the available data, showing that castrated tomcats have AMH values lower than 0.015 ng/ml [36]. Conversely, our value range within the healthy intact group (15.35 ± 4.66 ng/mL) is somewhat lower but still comparable to the average AMH levels of 20.95 ± 4.97 ng/mL reported by Ferré-Dolcet et al. [37]. In addition, it is important to note that in our study, the intact healthy group had a minimum age of 12 months. This fact could explain why no significant relationship was observed between age and serum AMH in intact tomcats. The highest AMH values are indeed found in tomcats younger than 12 months [37]. In relation with the latter, our findings may suggest that AMH serum values might be lower in adult tomcats, warranting further research on age-dependent thresholds if AMH is to be used for diagnostic purposes.

Unlike in dogs, there is a lack of data concerning AMH levels in cryptorchid tomcats. Nevertheless, our results are in accordance with previous canine studies, which state that cryptorchid individuals exhibit

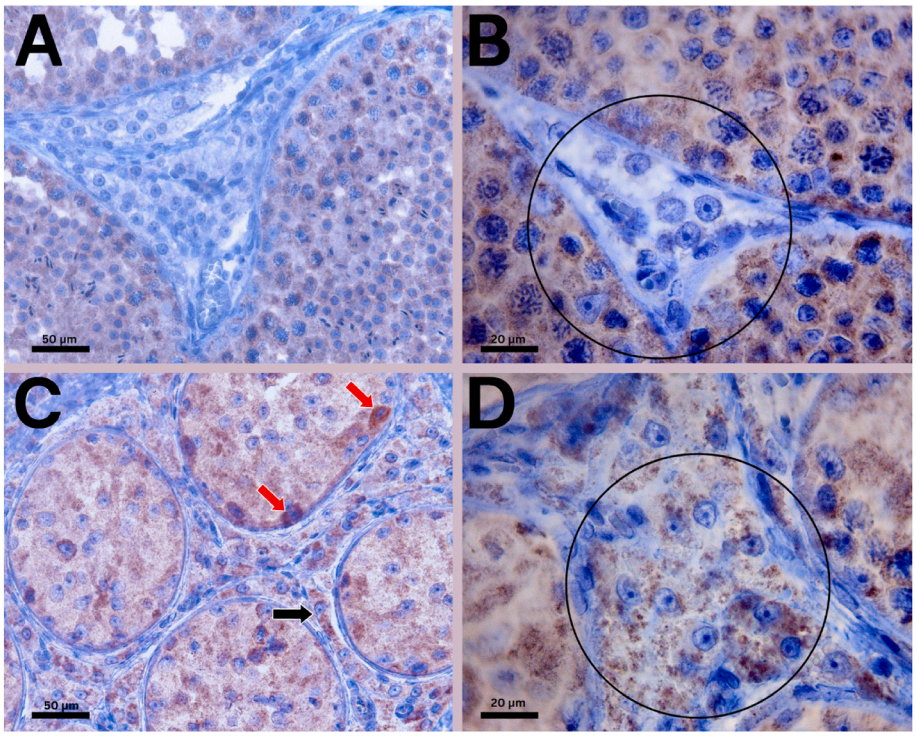

Fig. 4. The AMH immunohistochemical staining in the descended (A and B) and retained testes (C and D). Captions were obtained with different magnifications (400x magnification, A and C; 1000x magnification, B and D). Notice the difference in expression within the interstitial space (black circle) of the two categories. Arrows show intense positive staining cells: Sertoli cells (red arrow) and Leydig cells (black arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

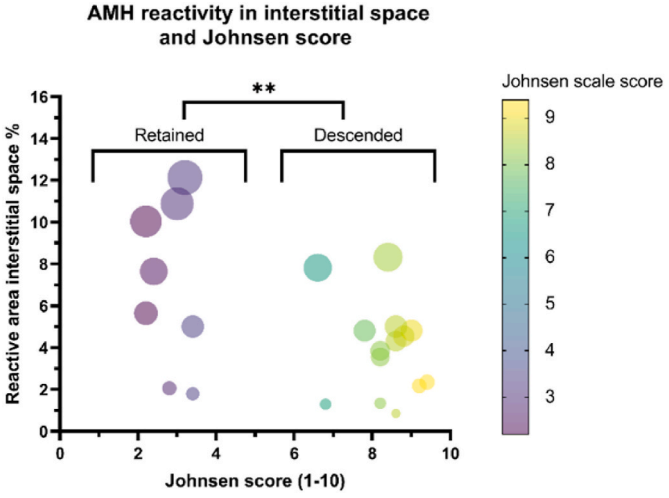

Fig. 5.A. Relation between mean interstitial space reactivity (%) and mean Johnsen score (1-10). $P \leq 0.01$ (**).

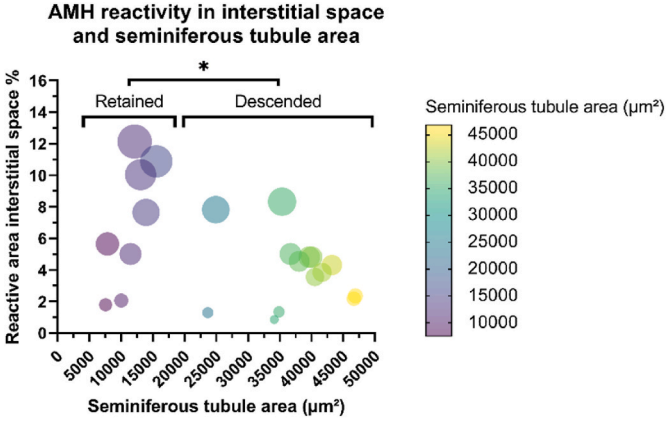

Fig. 5.B. Relation between mean interstitial space reactivity (%) and mean seminiferous tubule area (μm^2). $P \leq 0.05$ (*).

higher AMH levels compared to healthy intact or castrated dogs, regardless of the abdominal [32] or inguinal [38] location of the testes.

Histomorphometrical characteristics of cryptorchid testes were previously investigated in dogs [10,39], boars [12], bulls [11] and stallions [13]. In every instance, the most frequent histological pattern for the retained testes included degenerative events marked by reduced seminiferous epithelium and decreased tubule diameter or area. Our results were consistent with these findings, suggesting that malposition of the testis will lead to similar consequences in tomcats, causing subsequent impaired spermatogenesis. The latter was demonstrated in our study by the lower Johnsen scores associated with the retained gonads. Due to the high number of sections assigned a score of 2, the Sertoli cell-only syndrome, which is characteristic for human cryptorchidism [40], appears to be similarly specific to cryptorchid tomcats. Furthermore, a link between higher AMH serum levels and lower Johnsen scores was noted, suggesting that AMH may reveal information about fertility or sperm quality in tomcats similar to its applications in dogs [41]. Similarly, the association between higher AMH serum values and reduced STAs may qualify this assay as an indicator of poor testicular histology.

Similar to other species such as dogs [31,32] or horses [13], feline cryptorchid testes seem to reveal higher AMH immunostaining than healthy descended ones. However, our results prove a certain particularity in the tomcats as the increased expression of AMH is not attributed to the Sertoli cells, but rather to differences observed within the interstitial space. This suggests that there may be distinct mechanisms underlying AMH expression in tomcats compared to other species,

scrutinizing AMHs' cell specificity and the possible co-involvement of Leydig cells. Studies on transgenic mice have shown that AMH has the capacity to inhibit testosterone production via binding to its own receptors located on Leydig cells, by decreasing the expression of certain steroidogenic enzymes [30]. Thus, detecting a higher expression of AMH in the interstitial space may be related to a more intense binding to the receptors, resulting into a higher impact of the latter on the Leydig cell function. Moreover, the increased binding of AMH to the Leydig cell receptor may be associated with interstitial cell pathology. Research on mice has demonstrated that AMH can inhibit the proliferation of Leydig cells and promote apoptosis [42]. This suggests that the interaction between AMH and its Leydig cell receptor could play a critical role in protecting against Leydig cell tumor development. Therefore, our results on tomcat testes under neoplastic-promoting conditions, such as cryptorchidism [43,44], might also account for the infrequent occurrence of testicular tumors in tomcats [45]. Similarly, testes exposed to degenerative factors, such as gonadotoxic chemotherapy, have shown elevated AMH expression accompanied by increased apoptosis [46]. Conversely, AMH-deficient mice exhibit Leydig cell hyperplasia [47].

Earlier research on cryptorchid stallions, as documented by Ball et al. [48], revealed a decline in AMH expression correlated with degenerative changes within seminiferous tubules. Moreover, the same study found that AMH expression remained intense when tubular integrity was preserved in cryptorchid testes [48]. Interestingly, this AMH expression pattern is not consistent with the lack of variability between the intratubular expression quantified in our descended and cryptorchid samples, especially considering that the latter exhibited low Johnsen scores and reduced tubule diameters. On the other hand, higher interstitial AMH reactivity appears to be linked to the affected histological structure and impaired spermatogenesis, as larger reactive areas in the interstitial space were correlated with smaller tubules and lower Johnsen scores.

Typically, AMH is released from Sertoli cells apically into the seminiferous tubules and basally towards the interstitium and circulation [49]. However, abnormal conditions like cryptorchidism may alter AMH release dynamics or its interaction with the receptors. Therefore, we hypothesize that such abnormal conditions may trigger mechanisms that increase AMH levels in different compartments—such as blood, seminiferous tubules, or interstitial space. This rise in AMH may subsequently lead to cellular-level effects, which could translate into broader histological and functional alterations in the testes.

Despite our findings, it is important to acknowledge certain limitations of our study. The relatively small sample size, particularly within the cryptorchid group, may impact the generalizability of the results. Additionally, this limitation constrained our capacity to evaluate parameters across different subcategories. Furthermore, the age composition of the groups prevented us from assessing AMH modulation across varying age categories.

In conclusion, the present study revealed data on AMH expression in feline cryptorchid and descended testes, along with its previously uninvestigated relation to AMH serum levels and testicular tissue degeneration. Serum AMH remains a valuable tool to distinguish castrated tomcats from intact or cryptorchid individuals and may also be useful for diagnosing cryptorchidism in felines. However, to enhance the latter application, further research should focus on age-dependent thresholds for AMH serum values. The present work revealed a distinct pattern of AMH expression in tomcats, with increased immunostaining observed in the interstitial space of the retained testes, indicating possible species-specific mechanisms that may extend to a more intense effect of AMH on steroidogenesis in felines. Moreover, our findings underscore the importance of AMH in evaluating testicular health and dysfunction, particularly in the context of tissue degeneration and impaired spermatogenesis. Further research is warranted to elucidate the precise role of AMH and its cellular and receptor specificity in feline cryptorchidism.

CRediT authorship contribution statement

F.P. Posastiuc: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **G. Rizzoto:** Writing – review & editing, Resources. **N.T. Constantin:** Writing – review & editing, Investigation. **G. Nicolae:** Investigation. **K. Chiers:** Investigation. **A.I. Diaconescu:** Investigation. **A.I. Şerban:** Writing – review & editing, Investigation. **A. Van Soom:** Writing – review & editing, Supervision, Resources. **M.D. Codreanu:** Writing – review & editing, Supervision.

Declaration of interest

The authors have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2024.09.002.

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