Plattform Besamung und Embryotransfer
Department für Kleintiere und Pferde
Veterinärmedizinische Universität Wien

Follicular development in cat ovaries: Expression of Anti-Müllerian hormone and occurrence of ovarian cysts

Ovarielle Follikelentwicklung bei Katzen:
Expression von Anti-Müller Hormon und Vorkommen von Ovarzysten

INAUGURAL DISSERTATION
zur Erlangung der Würde einer
DOCTORA MEDICINAE VETERINARIAE
der Veterinärmedizinischen Universität Wien
vorgelegt von

Mag. med. vet. Svenja Claaßen
Wien, Oktober 2023

Wissenschaftliche Betreuung: O. Univ.-Prof. Dr. med. vet. Jörg Aurich

Begutachterin: Univ.-Prof.in Dr.in med. vet. Eva Schnabl-Feichter

Contents

1. Introduction

Domestic cats are popular pets in many countries, but in comparison to other domestic animal species such as food-production farm animals, horses and dogs, research on feline reproduction is limited (Binder, 2020). This thesis is aimed at investigating the expression of Anti-Muellerian Hormone (AMH) in different ovarian structures of female cats. Furthermore, the incidence of ovarian cysts in a larger number of healthy cats presented for ovariectomy and a possible correlation between AMH and certain types of ovarian cysts was investigated.

The glycoprotein AMH has recently gained some interest as a marker for the presence of gonadal tissue in postnatal male and female animals and as a diagnostic aid to assess gonadal pathologies. This includes several interesting studies in cats (Axner and Ström Holst, 2015; Snoeck et al., 2016; Ferre-Dolcet et al., 2022; Flock et al., 2022a,b; Gültiken et al., 2022; Gozer et al., 2023; Lapuente et al., 2023). Because these findings are summarized in the introductory paragraphs of chapter 2.2., they will not be repeated here. The reader is also referred to three comprehensive reviews by Ström Holst (2017), Walter et al. (2020) and Place et al. (2022).

The following part of the introduction will focus on ovarian cysts and compare findings in different species including the cat. Ovarian cysts have been described to varying degrees in humans and animals (e.g., cattle, cats, dogs, guinea pigs) for several years. However, their etiology and pathogenicity in different animal species is still partly unclear and the identification of the different cyst types seems to be more difficult in some species than in others. In farm animals such as cattle, cysts derived from non-ovulating ovarian follicles are most evident because their presence can block ovulatory cycles for prolonged times. Other than cattle, small companion animals such as female cats and dogs are often neutered to avoid estrous behavior and unwanted pregnancies. Cysts of non-germinative origin are often diagnosed at surgery but are usually incidental findings not affecting the estrous cycle and fertility in these animals.

1.1. Cats

There are only few studies of ovarian cysts in cats. The following cyst types have been described: Wolffian or Müllerian duct cysts, cysts of the rete ovarii and luteinized and non-luteinized follicular cysts. Only the latter may potentially affect ovarian function and fertility. A combination of different cysts is possible and sometimes cysts are not classifiable. Follicular

cysts appear to be the most common type of ovarian cysts in cats (Binder et al., 2021). In a study where the authors defined ovarian cysts as fluid-filled structures > 3.5 mm in diameter in the ovaries or adjacent to the ovaries, cysts were found in 14 % of female cats; none of the cats showed clinical signs of disease or disorders of the reproductive tract (Binder et al., 2021). In an older study, 20 cats with cystic rete ovarii were described. There was no association with age, breed, or reproductive history of the cats. Clinical symptoms were not described in this study (Gelberg et al., 1984). Ovarian cysts have also been described in female cats with ovarian remnant syndrome. The incidence of cysts in these studies reached approximately 50 %, but only a smaller number of animals was studied (Gozer et al., 2023, Flock, 2022). In agreement with previous studies (Knauf et al., 2014), our research group has recently suggested that ovarian cysts in cats are mostly incidental findings without clinical relevance (Binder et al., 2021).

1.2. Dogs

Similar to the cat, ovarian cysts in the female dog are described as Wolffian or Müllerian duct cysts, - also summarized as cysts of the surface epithelium (SES) - follicular cysts, luteinized cysts, cystic corpora lutea and rete ovarii cysts (MacLachlan, 1987; Arlt and Haimerl, 2016; Maya-Pulgarin et al., 2017). Immunohistochemistry may allow some differentiation among cyst types. Both SES cysts and ovarian cysts stained positive for desmin and cytokeratin AE1/AE3. All except follicular cysts were negative for inhibin-α and only SES cysts showed a positive immune response to placental alkaline phosphatase (Akihara et al., 2007).

There is an increased incidence of ovarian cysts in bitches over six to eight years of age and cyst diameter ranged from 0.2 to 4.0 cm (Knauf et al., 2018). Because progesterone and estradiol were detectable in fluid obtained from ovarian cysts, the authors suggested that ovarian cysts inhibit ovarian function in dogs, but cause-effect relation was not analyzed. It is believed that persistent, hormonally active follicular cysts increase the risk of cystic endometrial hyperplasia in female dogs and may induce symptoms of hyperestrogenism. Although evidence based on experimental studies is lacking, in small animal practice ovarian cysts are frequently treated with GnRH analogues or hCG, and if this treatment is not successful, ovariectomy or ovariohysterectomy is recommended (Knauf et al., 2014; Sasidharan et al., 2021).

1.3. Guinea pigs

Ovarian cysts are one of the most common reproductive pathologies in female guinea pigs (Minarikova et al., 2015). A typical clinical sign frequently noted by the owners is a symmetrical alopecia over the flank region and abdomen, without pruritus or abnormal appearance of the skin (Pilny, 2014). A pear-shape appearance of the body conformation is often described. Furthermore, nonspecific clinical signs like anorexia or lethargy are reported. Animals can also be asymptomatic and cysts are an incidental finding (Pilny, 2014). Cysts can be diagnosed by transabdominal ultrasonography or in cases where they are larger also by abdominal palpation (Nielsen et al., 2003). Most frequent are cysts originating from the rete ovarii, also called serous cysts. Their prevalence ranges from 60 to over 90 % (Hong, 1980; Keller et al., 1987; Field et al., 1989; Shi, 2002; Nielsen et al., 2003) and they can vary between 5 µm and over 7 cm in diameter (Keller et al., 1987). Follicular cysts are less frequent in guinea pigs and reports on their prevalence vary (Bean, 2013; Laik-Schandelmaier et al., 2017). Ovarian cysts have been described in guinea pig from all age groups, but there seems to be a positive correlation between age of the female guinea pig, the prevalence and the size of the cysts (Keller et al., 1987; Nielsen et al., 2003; Bertram et al., 2018). Neither reproductive history (Keller et al., 1987; Nielsen et al., 2003) nor duration of estrus (Keller et al., 1987) appear to be affected by ovarian cysts in guinea pigs. Although the need for routine treatment of ovarian cysts in guinea pigs thus could be questioned, a textbook chapter author recommends ovariohysterectomy, hormonal treatment with GnRH or hCG and ultrasound-assisted percutaneous drainage of the cyst fluid (Krause, 2014).

1.4. Cattle

In large animals, ovarian cysts are the most common ovarian dysfunction of dairy cattle. Because of their high incidence and an at least transient blockade of estrous cyclicity, ovarian cysts can lead to marked economic losses for dairy farmers. The first cases of ovarian cysts or cystic ovarian follicles in cattle were reported in the 1940s (Cassida et al., 1944; Garm, 1949). Cows with ovarian cysts occasionally can show nymphomania, i.e., signs of irregular or prolonged estrus (Cassida et al., 1944; Garm, 1949), but the most common clinical sign is anestrus (López-Gatius et al., 2001). Ovarian cysts in cattle are defined as large-walled anovulatory follicle-like structures, with a diameter of more than 25 mm, which persist for six

to ten or more days in the absence of a corpus luteum (Kesler and Garverick, 1982; Silvia et al., 2002; Vanholder et al., 2006). The reported incidence of ovarian cysts in postpartum dairy cows varies between 5 and 25 % and they are divided into follicular and luteal cysts, depending on the degree of follicular wall granulosa cell luteinization (Casida and Chapman, 1951; Bartlett et al., 1986; Gaverick, 1997; Silvia et al., 2002). Occasionally they can be differentiated by transrectal ultrasound with a thicker wall in luteal compared to follicular cysts (Douthwaite and Dobson, 2000). Treatment options include GnRH analogues or progesterone releasing intravaginal devices for follicular cysts, $PGF_{2\alpha}$ for luteal cysts and ovsynch protocols which combine GnRH and $PGF_{2\alpha}$ injections irrespective of the type of cysts (e.g., López-Gatius et al., 2001; Gundling et al., 2015).

1.5. Pigs

In sows slaughtered for fertility problems, the incidence of ovarian cysts is about 10 % (Heinonen et al., 1998; Ebbert and Bostedt, 1993; Castagna et al., 2004; Szulanczyk, 2009). Follicular cysts, follicular theca lutein cysts, follicular lutein cysts and cysts of the corpus luteum have been differentiated histologically (Karveliene et al., 2007). Whereas single follicular cysts were not accompanied by pathologies of the reproductive tract, polycystic ovaries were associated with morphological changes in the oviduct and uterus, suggesting their presence as a reason for infertility (Karveliene et al., 2007; Szulańczyk, 2009). The method of choice for the diagnosis of ovarian cysts in live animals is transcutaneous ultrasonography which is also part of routine pregnancy diagnosis. In one study, ovarian cysts were defined as anechogenic structures with a smooth, thin wall and a diameter of more than 2 cm that remained visible for at least 5 days after the onset of estrus (Castagna et al., 2004). Symptoms of cystic ovaries in sows include an irregular or prolonged estrous cycle or prolonged anestrus and thus reduced fertility. The presence of ovarian cysts has a more adverse effect on pregnancy rate than on litter size (Castagna et al., 2004; Karveliene et al., 2007).

1.6. Horses

In mares, follicular cysts as develop frequently in cattle do not exist, but persistent anovulatory follicles do occur in the mare (McCue, 1998). Anovulatory follicles may contain blood and are therefore often called hemorrhagic anovulatory follicles (HAF). They result from the failure of ovulation of a preovulatory follicle, although endocrine changes typical for ovulation may occur,

such as an LH peak, an abrupt drop in estradiol and increase in progesterone concentrations (Ginther et al., 2006).

Despite the fact that the HAF fails to ovulate, it increases in diameter. Then the HAF wall thickens and becomes highly echogenic, which indicates active luteinization (Ginther et al., 2007; Cuervo-Arango and Newcombe, 2010). The incidence of hemorrhagic anovulatory follicles is approximately 5 % and 20 % of estrous cycles during the early and late phase of the horse breeding season. They are more frequent in older than in younger mares (Ginther et al., 2007). During the months with the most pronounced follicular activity (May to August) and after treatment with hormones to induce estrus and ovulation, mares have a higher risk of developing HAF (Cuervo-Arango and Newcombe, 2010). More specifically, mares treated with the synthetic $PGF_{2\alpha}$ analogue cloprostenol (CLO) are more likely to develop HAF than mares with spontaneous estrous cycles or those treated with human chorionic gonadotropin (hCG) to induce ovulation (Cuervo-Arango and Newcombe, 2009). Administration of hCG does not cause HAF formation, but it may trigger their development by inducing the molecular changes in the follicular wall associated with the LH surge (Cuervo-Arango and Newcombe, 2012).

Cysts of the surface epithelium usually arise from structural remnants of the Müllerian or Wolffian ducts. These cysts are incidental findings in a high percentage of mares and are usually not associated with reduced fertility, unless in rare cases where they mechanically interfere with ovulation or the oviductal transport of oocytes. They do not require treatment (Nielsen et al., 1976; McCue, 1998). If there is a cyst-like appearance of an ovary in the mare, however, further diagnostics including a thorough gynecological examination should be performed to rule out ovarian neoplasia (McCue, 1998).

1.7. Humans

Ovarian cysts in humans were first described by Stein and Leventhal in 1935. As in animals, in women follicular cysts, corpus luteum cysts, lutein cysts and paraovarian cysts occur. In contrast polycystic ovarian syndrome (PCOS) which is frequently diagnosed in women does not develop in domestic animals. Dermoid cysts belong to the ovarian tumors, more precisely to the teratomas (Pradhan and Thapa, 2014). The prevalence of ovarian cysts other than PCOS is reported as 15 to 20 % in postmenopausal women (Hartge et al., 2000; Dørum et al., 2005; McDonald and Modesitt, 2006). Most singular cysts in women resolve spontaneously

(McDonald and Modesitt, 2006) and the vast majority of ovarian cysts turn out to be benign alterations (Hartge et al., 2000; Dørum et al., 2005). Although the majority of ovarian cysts in women do not require therapy, complications can occur in some cases. The three classic complications of ovarian cysts in human gynecology are hemorrhage, rupture, or torsion of the ovarian cyst. These complications usually require emergency surgical management (Bottomley and Bourne, 2009). Ovaries with cystic alterations are thought to be more prone to torsion or rupture in pregnancy than in nonpregnant women (Houry and Abbott, 2001). The prevalence of PCOS in women is estimated to be 5 to 10 %, suggesting PCOS as one of the most common reproductive endocrinological disorders in women of reproductive age (Knochenhauer et al., 1998). The PCOS is characterized by irregular menstruation, hyperandrogenism, polycystic ovarian morphology and obesity (PCOS Consensus Group, 2004; Azziz, 2018). The androgen excess is often manifested by hirsutism, androgenetic alopecia and persistent acne (Franks, 2012). Also, common features in PCOS are insulin resistance and elevated serum LH concentration. In addition, PCOS is associated with an increased risk of type 2 diabetes and cardiovascular pathologies (PCOS Consensus Group, 2004).

2. Own Investigations

2.1. Aims and Hypotheses of this Thesis

It was the aim of this thesis, to determine AMH concentration in the blood of female cats presented for routine ovariectomy and to study AMH protein expression in follicles, corpora lutea and cysts of the surgically removed ovaries.

The study tested the following hypotheses:

(1) AMH is synthesized almost exclusively in the granulosa cells of the preantral and small antral follicles.
(2) Plasma AMH concentration reflects the number of ovarian follicles.
(3) Plasma AMH concentration decreases with age of the cat but is not affected by the presence or absence of corpora lutea and ovarian cysts.

2.2. Abundance of Anti-Muellerian hormone in cat ovaries and correlation of its plasma concentration with animal age, weight and stage of the estrous cycle

Svenja Claaßen, Jörg Aurich, Ingrid Walter, Camille Gautier, Christine Aurich

Theriogenology 2023, 212:30-36.

Theriogenology 212 (2023) 30–36

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Abundance of Anti-Muellerian hormone in cat ovaries and correlation of its plasma concentration with animal age, weight and stage of the estrous cycle

Svenja Claaßen [a], Jörg Aurich [b], Ingrid Walter [c,d], Camille Gautier [a], Christine Aurich [a,*]

[a] Artificial Insemination and Embryo Transfer, Department for Small Animals and Horses, Vetmeduni Vienna, 1210, Vienna, Austria
[b] Obstetrics, Gynecology and Andrology, Department for Small Animals and Horses, Vetmeduni Vienna, 1210, Vienna, Austria
[c] VetCore Facility for Research/VetBiobank, Vetmeduni Vienna, 1210, Vienna, Austria
[d] Institute of Morphology, Vetmeduni Vienna, 1210, Vienna, Austria

ARTICLE INFO

Keywords:
Cat
Ovary
AMH
Immunohistochemistry
Estrous cycle

ABSTRACT

In female animals of different species, Anti-Müllerian hormone (AMH) is produced by follicular granulosa cells and has been associated with the ovarian follicle pool. Because concentration of AMH in plasma of ovary-intact female cats is apparently more variable than previously assumed, we have analysed AMH concentration in blood of cats (n = 93) presented for routine ovariectomy and assessed ovarian histology and AMH protein expression in the surgically removed ovaries. We hypothesised that AMH is synthesized only in preantral and small antral follicles and that plasma AMH concentration reflects the antral follicle count (AFC). Corpora lutea were detected in 35% of the female cats, whereas plasma progesterone concentration was ≥1 ng/mL in 57% of the cats. Follicular cysts were present in 15 cats (16%). Positive immunostaining for AMH protein was detected in close to all primordial and antral follicles, ovarian cysts, 70% of corpora lutea and 28% of atretic follicles. Concentration of AMH in plasma averaged 6.8 ± 0.5 ng/mL (range 1.3–21.7 ng/mL). The AFC increased with increasing AMH concentration with a moderate positive correlation between AFC and AMH ($r = 0.286$, $p < 0.01$). Plasma AMH concentration was not affected by season or cats' age, weight, stage of the estrous cycle and presence of follicular cysts. In conclusion, AMH protein is expressed in all endocrine structures of the cat ovary. While AMH is a marker for the presence of ovarian tissue, its usefulness to assess ovarian function in individual female cats is of limited value.

1. Introduction

Anti-Muellerian Hormone (AMH) is a glycoprotein in the transforming growth factor-β family and has long been associated with its role for fetal sex differentiation first described in rabbits [1]. Only much later its role as a marker for the presence of gonadal tissue in postnatal male and female animals and as a diagnostic aid to assess gonadal pathologies has been described. In male animals, as in men, AMH is expressed by Sertoli cells in fetal, neonatal, prepubertal and postpubertal testes and in non-descended testes of cryptorchids (e.g., bull: [2,3]; stallion: [4,5]). In females, AMH is expressed by the granulosa cells of preantral and small antral follicles, is correlated with the ovarian follicle pool and AMH concentration in plasma allows predicting the ovarian reserve of gonadotrophin-responsive follicles in women [6–9], cows [10], does [11], ewes [12] and female cats [13]. Although AMH concentration is associated with the number of follicles and thus oocytes, its concentration is not correlated with per cycle pregnancy rate in women (reviewed by Ref. [9]). The concentration of AMH in plasma of women has been suggested to be relatively stable across the estrous cycle and among consecutive cycles [14]. More recently, however, this stability has been questioned [9]. Similarly, variations in plasma AMH concentration throughout the estrous cycle and between cycles in individual animals have been described for cats [13,15]. Serum AMH concentration was also higher in pregnant compared to cyclic cats [16] and in prepubertal female cats [13].

Irrespective of fertility, in female dogs and cats, AMH can be determined when it is desirable to assess the presence of ovarian tissue. In animals abandoned by their owners, it may be difficult to diagnose

* Corresponding author.
E-mail address: Christine.Aurich@vetmeduni.ac.at (C. Aurich).

https://doi.org/10.1016/j.theriogenology.2023.08.028
Received 9 August 2023; Received in revised form 30 August 2023; Accepted 30 August 2023
Available online 1 September 2023

whether they are already spayed. Females are also presented with the suspicion of the ovarian remnant syndrome, i.e., incomplete ovariectomy [15,17–19]. In cats, plasma AMH concentration decreases when gonadal function is downregulated with implants releasing the GnRH agonist deslorelin. In this context, determination of AMH has been suggested as a useful tool to determine resumption of ovarian activity following cessation of deslorelin effects [20].

Although AMH is increasingly determined for diagnostic purposes in female cats, its concentration in plasma of ovary-intact, healthy females is apparently more variable than previously assumed. Therefore, ovarian remnant syndrome cannot always be diagnosed accurately by AMH determination [21]. Previous studies on AMH in cats, however, often included only a limited number of animals acquired by convenience sampling. Experimental studies in cats are restricted and, in many countries, legal regulations allow such studies only with cats originating from a source explicitly approved for breeding experimental animals. If possible, descriptive studies must include a larger number of cases to allow meaningful conclusions. To gain more insight into the ovarian source of AMH and its release into the circulation, we have determined AMH concentration in blood of female cats presented for routine ovariectomy together with AMH protein expression in follicles, corpora lutea and cysts of the surgically removed ovaries. Cases were selected from all female cats presented to a large animal hospital over a one-year period. We hypothesised that (i) AMH is near-exclusively synthesized in the granulosa cells of preantral and small antral follicles, (ii) plasma AMH concentration reflects the number of ovarian follicles, (iii) plasma AMH concentration decreases with age of the animal but is not affected by the presence or absence of corpora lutea and ovarian cysts.

2. Materials and methods

2.1. Animals

For this study, both ovaries from 98 healthy cats (*Felis catus*) were collected over a one-year period at random stages of the estrous cycle. Cats were presented for routine ovariectomy at Vetmeduni Vienna, Austria. From the 98 cats, 88 came from a local animal shelter, 54 of which had already been rehomed and their new owners had been issued a voucher for a castration by the animal shelter. At the time of castration, they had been with their new owners for 8–378 days. A total of 34 cats were presented directly by the animal shelter before being rehomed and ten cats came from private owners independent from the animal shelter. According to their current owners, none of the cats had received any hormonal treatment such as progestins or GnRH agonists. Before castration, a clinical examination of the reproductive tract including transabdominal ultrasonography and collection of a blood sample for pre-surgery determination of hematocrit and total protein concentration was carried out, to ensure general health of the cat, normal development of the reproductive tract, absence of a visible pregnancy and absence of abnormalities of ovaries and/or uterus. Furthermore, recent parturition was excluded. Cats belonged to the breeds European shorthair (n = 78), European Longhair (n = 4), British Shorthair (n = 3), Persian (n = 1), Burmese (n = 1), Scottish Fold (n = 2), Sphynx (n = 3) and Maine Coon (n = 6).

Because of processing-related damage to some very small ovaries, complete histological evaluation was not possible in five cats. Therefore, 93 out of 98 cats were included into the analysis. Age of the 93 cats included for statistical analysis ranged from 4 months to 9 years (19.3 ± 18.4 months) and their weight from 1.5 to 4.7 kg (3.1 ± 0.6 kg). Out of 93 cats, four were kept together with gonad-intact males but none of these females was confirmed pregnant.

The study was approved by the Ethics and Animal Welfare Committee of Vetmeduni Vienna (ETK-020/02/2021). Because the reason for ovariectomy was not connected to our study and AMH analysis in blood plasma was performed from a routine pre-surgery blood sample, no further animal experimentation license by the competent Austrian authority was required. All owners were provided with a study information sheet and consented to participate in the study.

2.2. Surgical procedures

During preparation for surgery, a venous catheter (Vasofix Safety G22, Braun, Melsungen, Germany) was placed into one cephalic vein and one blood sample per animal was collected into an EDTA tube (S-Monovette EDTA 1.3 ml, Sarstedt, Nümbrecht, Germany). After determination of hematocrit and total protein, the remaining blood was centrifuged and the plasma aliquoted into two tubes and stored at −20 °C until determination of progesterone and AMH. Routine ovariectomy with partial resection of the uterine horns was performed via a small ventral midline incision. In case of macroscopic abnormalities of the uterus (thickening of the uterine wall in nine cats), the incision was extended, and an ovariohysterectomy was performed. The ovaries and uterus (in case of the nine ovariohysterectomized cats) were collected and fixed in 4% neutral buffered formalin immediately after surgery. After 48 h of fixation at room temperature they were placed into 70% ethanol solution.

2.3. AMH and progesterone analysis

Plasma progesterone and AMH concentrations were determined with enzyme linked immunosorbent assays (ELISA) validated for cats in our laboratory (Progesterone ELISA DE1561, Demeditec Diagnostics, Kiel, Germany; Canine AMH ELISA, AL-116, Ansh Labs, Webster, TX, USA). For the progesterone ELISA, the intra-assay coefficient of variation was 7.8%, the interassay coefficient of variation was 13.2% and the minimal detectable concentration was 10 pg/mL. For the AMH ELISA, the intra-assay variation was 5.3%, interassay variation was 19.2% and the minimal detectable concentration was 5 pg/mL.

2.4. Histopathological examination

The fixed ovaries were cut longitudinally. Several transverse sections of both uterine horns were produced where applicable. The tissue specimens were embedded in paraffin wax after preparation. Sections (3 μm thick) were excised from the resulting tissue blocks using a microtome. The sections were transferred to slides, dried overnight at 37 °C and then stained with hematoxylin and eosin. The slides were scanned with the Fritz Microscopy Slide Scanner (PreciPoint, Garching, Germany) at 20x magnification. For histologic evaluation, antral follicles, corpora lutea, atretic follicles and follicular cysts were counted on both sections of each ovary. Antral follicles were defined as fluid-filled structures with an intact follicular wall that allowed differentiation between a zona granulosa, basement membrane, theca interna and theca externa. In atretic follicles, a clear differentiation of follicular wall layers was absent, and the theca was increasingly replaced by collagenous tissue. Follicular cysts were defined as fluid-filled structures >3.5 mm in diameter and either the follicular wall covered by a monolayer of granulosa cells (n = 12) or multiple layers of luteinised theca cells (n = 3). For all ovarian structures, the mean of the counts from both ovaries was then calculated and used for statistical analyses. Cysts of the Wolffian or Muellerian ducts (n = 4 cats) and rete ovarii cysts (n = 5 cats) classified as published previously by our group [22] were recorded but not included into further analysis.

2.5. Immunohistochemistry

For immunohistochemistry, 3 μM paraffin sections were cut with the microtome. These were deparaffinized by xylol and rehydrated in a graduated alcohol series (100, 95 and 70% ethanol). The sections were then placed in distilled water and the endogenous peroxidase activity was blocked with 30% H_2O_2 in distilled water. After rinsing with tap water, the tissue sections for antigen masking were steamed for 30 min

in Tris-EDTA at a pH of 9.0 and then cooled for 20 min. The slides were rinsed in PBS and treated for the protein block with 1.5% Normal Goat Serum (Sigma Aldrich, Vienna, Austria) in PBS for 30min (150 μl goat serum in 10 ml PBS). After blockage, the sections were incubated overnight at 4 °C with a primary rabbit polyclonal AMH antibody (1:5000; Genetex, Hsinchu City, Taiwan, cat. no. GTX129593). Simultaneously, IgG controls (1:15000) were performed. After incubation with the primary antibody, slides were rinsed in PBS for 2 × 5 min and incubated with a second antibody (BrightVision Poly-HRP-*anti*-rabbit, ImmunoLogic, Duiven, The Netherlands) for 30 min. After rinsing with PBS, the samples were incubated with DAB solution (Quanto, Richard Allan Scientific, Kalamazoo, MI, USA; TA-125-QHDX) for 5 min and then washed with distilled water. The slides were counterstained with hematoxylin, then rinsed with aqua fontis, thereafter dehydratated with 96% and 100% ethanol and finally with xylol. Slides were covered with DPX (Fluka, Buchs, Switzerland) and cover glass. All stains were positive and all IgG controls negative. All immunohistochemistry evaluations were made by a single observer and AMH staining was classified as either positive or negative and no attempt was made to score staining intensity in case of positive staining. Specificity of antibody binding was confirmed by Western blot in the authors' laboratory as described recently [16].

2.6. Statistical analysis

Data were analysed with the SPSS statistics software (version 28, IBM-SPSS, Armonk, NY, USA). Data for AMH were not normally distributed (Kolmogorov-Smirnov test) and therefore non-parametric tests were used throughout. The AMH concentration in different groups of cats was compared by Kruskal-Wallis test (>two groups) or Mann-Whitney test (two groups). To analyse correlations between AMH concentration in plasma and antral follicle count, Spearman's coefficient of variation was calculated. The number of cats in different age groups with plasma progesterone concentration ≥1 ng/mL and <1 ng/mL was compared by chi^2 test. All values given are means and standard error of mean. A p-value <0.05 was considered statistically significant.

3. Results

One or more corpora lutea were detected on histology in 32% of the cats, whereas plasma progesterone concentration was ≥1 ng/mL in 55% of the cats (Fig. 1a). The percentage of cats with plasma progesterone concentration ≥1 ng/mL increased with increasing age (Fig. 1b). None of the cats was visibly pregnant using abdominal ultrasonography. Follicular cysts were present in 15 out of 93 cats and the average number of cysts in these cats was 3.3 ± 3.1 (±SD; range 1–13) per cat. Representative structures determined on cat ovaries are shown in Fig. 2.

Positive immunostaining for AMH was present in close to all primordial, primary, secondary and tertiary follicles and in all follicular cysts, but only in 28% of atretic follicles. Out of the corpora lutea, 79% stained positive for AMH (Fig. 3).

In all follicles, AMH immunostaining was restricted to granulosa cells and in antral follicles was evident in granulosa cells of the follicular wall and cumulus oophorus (Fig. 4a and b). Immunostaining was, however, negative in the majority of atretic follicles (Fig. 4c). Lutein cells in most corpora lutea stained positive for AMH, but immunostaining was visually less intense than in follicular granulosa cells and not always evenly distributed within the corpus luteum (Fig. 4d). In the 15 follicular cysts, AMH protein expression was restricted to a monolayer of cells covering the wall of the cyst (n = 12; Fig. 4e) or multiple layers of luteinized cells (n = 3; Fig. 4f).

For all 93 female cats, the mean AMH concentration in plasma was 6.8 ± 0.5 ng/mL (median 5.5 ng/mL). The concentration of AMH ranged from 1.3 to 21.7 ng/mL with the highest concentration in a 4-months-old cat. Out of the 93 cats, 39 (41.9%) had a plasma AMH concentrations <5 ng/mL, 78 (83.9%) a plasma AMH concentrations <10 ng/mL. The distribution of plasma AMH concentration in all 93 cats is summarized in Fig. 5. The antral follicle count (average of two sections from both ovaries) ranged from 1.3 to 35.5 (7.1 ± 0.5) and was 20 in the 4-months-old cat with the highest AMH concentration. Antral follicle count increased with increasing AMH concentration in plasma (Fig. 6a) and there was a significant positive correlation between antral follicle count and plasma AMH concentration in cats (Fig. 6b).

The concentration of AMH in plasma did not differ among female cats of different age groups, different weight and included into the study at different times of the year (Fig. 7a–c).

Concentration of AMH in plasma of cats was not affected by stage of the estrous cycle when cats were assigned to the follicular and luteal phase either by the presence or absence of corpora lutea on histology or by progesterone concentration ≥1 ng/mL or <1 ng/mL (Fig. 8a and b). Plasma AMH concentration did also not differ significantly between cats with and without follicular cysts (p = 0.41; Fig. 8c).

4. Discussion

The results of this study indicate that in cat ovaries, AMH is synthesized not only by preantral and small antral follicles but also by luteal cells after ovulation and formation of corpora lutea. Whereas AMH protein expression in cat preantral and antral follicles has been reported previously [16] and is in agreement with findings in other species (e.g., dog: [23]; cow: [24]; horse: [4]; pig: [25], human: [26]), AMH protein expression in corpora lutea is not a general finding across species. Its expression after ovulation in luteal cells has been considered a phenomenon unique to the polyovulatory pig [25] whereas luteal AMH protein expression has been excluded in the mono-ovulatory horse [4]. Recently, an expression of AMH in interstitial cells of cat corpora lutea at mid pregnancy has been described but no interpretation of this finding was given [16]. The present study provides the first evidence for AMH

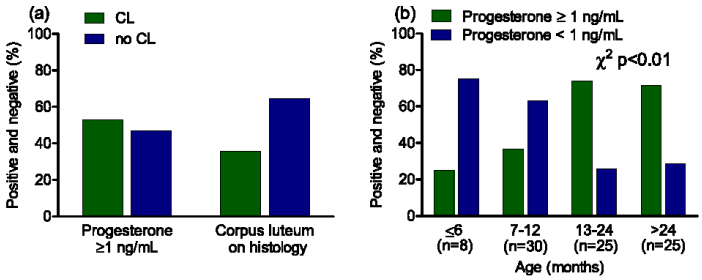

Fig. 1. (a) Percentage of cats with and without corpora lutea determined by histology or by plasma progesterone concentration (≥1 ng/mL CL and <1 ng/mL no CL); (b) percentage of cats with plasma progesterone concentration ≥1 ng/mL and <1 ng/mL by age group (total number of cats included into analysis n 93)

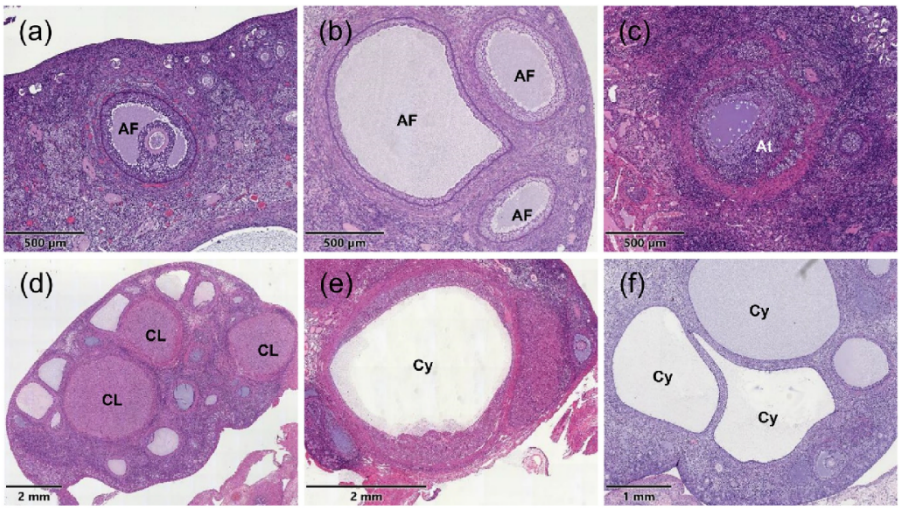

Fig. 2. Hematoxylin and eosin staining of representative cat ovaries with different structures: (a) small antral follicle (AF) with oocyte (b) multiple antral follicles (AF) (c) atretic follicle (At) (d) multiple corpora lutea (CL) and (e) luteinized cyst (Cy) and (f) multiple follicular cysts (Cy).

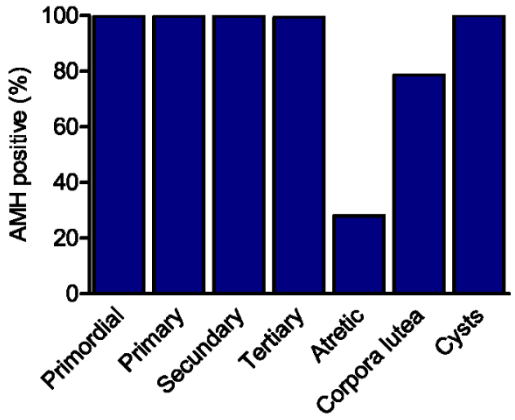

Fig. 3. Percentage of primordial, primary, secondary, tertiary and atretic follicles, corpora lutea and follicular cysts staining positive for AMH in cats (total number of cats included into analysis n 93).

expression in corpora lutea of non-pregnant cyclic cats, although with our experimental design, non-detected early pregnancy in a small percentage of these cats cannot be ruled out. Most interestingly, AMH was expressed only in 80% of the corpora lutea and not always evenly throughout an individual corpus luteum. Because of our experimental design, the exact day of ovulation and thus age of the corpora lutea could not be determined. If the expression of AMH in cat luteal tissue is anyhow related to corpus luteum age can only be speculated. It can be assumed that AMH synthesis may continue after ovulation but gradually cease in the aging corpus luteum. The physiological function of luteal AMH is at present not clear. Based on its expression in the corpus luteum of cyclic sows, it has been suggested that AMH contributes to regulation of the cyclic recruitment of small antral follicles and prevents premature exhaustion of the ovarian follicular reserve [25]. Although AMH is not expressed in the corpus luteum of women, the hormone inhibits the gonadotrophin-induced expression of the steroidogenic enzyme CYP19A1 aromatase and thus exerts an inhibitory role on early stages of follicular development [27]. In the female cats from our study, AMH expression was markedly reduced only in atretic follicles which is in agreement with findings in cows [24].

Similar to follicles, AMH expression occurred also in granulosa cells and luteinized cells of follicular cysts, indicating that these are capable of producing AMH. Although the term ovarian cyst is widely used in clinical small animal reproduction, we have recently suggested that ovarian cysts in cats are mostly incidental findings without clinical relevance [22]. Such structures in cats may more resemble (hemorrhagic) anovulatory follicles in mares which delay the next estrus [28, 29] than follicular cysts in cattle which can be the cause of a prolonged anestrus (e.g., Refs. [30,31]). This interpretation is supported by the unchanged AMH expression in cysts in the cats of our study when compared to physiological ovarian follicles. With regard to AMH protein expression, in horse ovaries strong AMH immunolabelling was observed in granulosa cells of growing follicles, whereas only faint expression was detected in granulosa cells of dominant follicles [32]. Although to the best of our knowledge, AMH expression has not been studied in horse anovulatory follicles, cats apparently differ from mares with a continuous AMH expression in growing large follicles.

Concentration of AMH in plasma of ovary-intact female cats varied markedly among animals, but AMH was always detectable and well above the minimal detection threshold of our assay. In healthy cows, it has been suggested that AMH concentrations are to some extent characteristic for the individual animal [24] and the same may be true for female cats. In contrast to cats, however, 40% of postpartum cows had undetectable plasma AMH concentrations not associated with obvious ovarian pathologies [33].

When cats were grouped by antral follicle count, AMH concentration was higher in cats with more antral follicles compared to those with fewer antral follicles detected on histology. There was, however, only a loose linear correlation between AMH concentration in plasma and antral follicle count. Although at first glance, our data may only partially agree with findings in other species, it must be considered that although

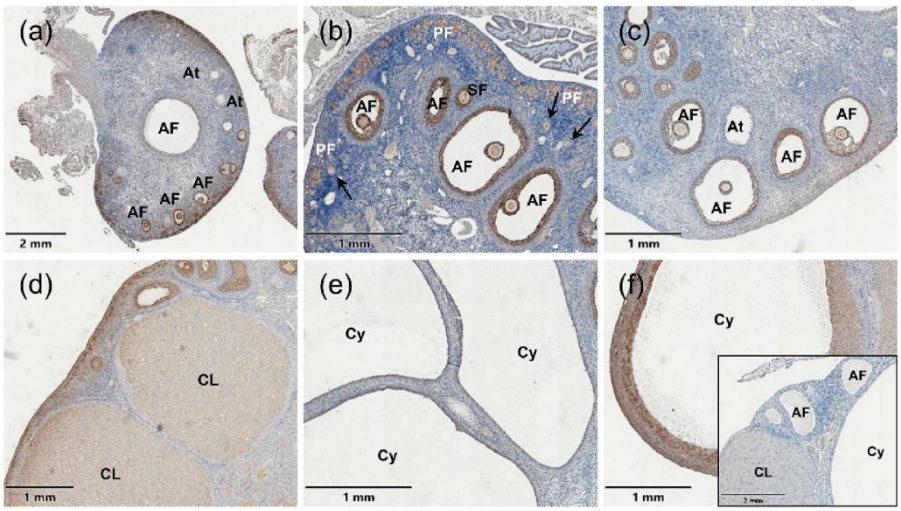

Fig. 4. Immunohistochemical staining for AMH: (a,b,c) ovaries with multiple positively stained primordial (PF), primary (black arrows), secondary (SF) and antral follicles (AF) and non-stained atretic follicles (At), (d) positively stained corpora lutea (CL) besides positively stained follicles and (e) follicular cysts with a single layer of positively stained cells covering the wall of the cyst (Cy), (f) lutein cyst with positively stained cells (Cy) and inserted negative control with corpus luteum (CL), antral follicles (AF) and a cyst (Cy).

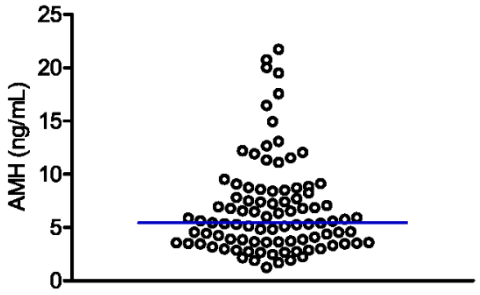

Fig. 5. Distribution of individual AMH concentration and their median (solid blue line) in plasma of all cats included into the study (n 93). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the oldest cats in our study were 9 years old, only 10 were older than 3 years. With a life expectancy in cats of over 15 years [34], old cats were thus underrepresented in our study. In horse mares, an overall positive correlation between antral follicle count and plasma AMH concentration exists but varied by mare age with a strong correlation in older mares, a moderate correlation in middle-aged mares and no correlation in young mares [35]. Our findings do not exclude that the relationship between AMH and antral follicle count varies across age groups also in cats.

Previous studies have reported higher plasma AMH concentration in younger and particular in prepubertal cats compared to older animals [13,15,17,20]. The same has been demonstrated in female dogs [36,37], mice [38] and horses [35]. Puberty in cats often occurs when they are between four and six months old [39]. Because prepubertal cats are rarely presented for ovariectomy at the authors' institution, it is possible that increased plasma AMH concentrations in cats of this young age group were missed in our study. Although statistical significance was not reached, plasma AMH concentration in cats up to one year of age, however, tended to be higher than in older cats.

The concentration of AMH in plasma of cats was not affected by the presence or absence of corpora lutea which is in agreement with previous studies in women [14] and pigs [40] and horses [35]. Some variations in plasma AMH concentration throughout the estrous cycle and between cycles in individual animals have been described previously for cats [13,15] but based on the larger cat population with individual cats only included once cannot be confirmed in the present study. Concentration of AMH in plasma was not affected by the presence or absence of follicular cysts which was to be expected because of unchanged AMH protein expression in these apparently anovulatory structures follicles and ovarian cysts.

For a long time, cats have been considered a species with mating-induced ovulations only, but recently, regularly occurring spontaneous ovulations have been described in cats based on ovarian histology [41]. Based on plasma progesterone concentration, the percentage of cats with active luteal tissue was even higher in the present study. Because only two sections per ovaries were accessed, apparently not all corpora lutea present were detected by histological examination. None of the cats was visibly pregnant and – except for four females - contact to tomcats was excluded by the owners. Our findings thus confirm and extend previous studies on spontaneous ovulations or luteinisation of follicles in female cats.

5. Conclusion

The AMH protein is expressed in granulosa and luteal cells and thus in all functional structures of the cat ovary. Therefore, in ovary-intact, postpubertal cats, AMH concentrations do not differ among stages of the estrous cycle or between cats with and without ovarian cysts. While AMH clearly is a marker for the absence of ovarian tissue in cats, its usefulness to assess ovarian function in non-spayed cats is of limited value.

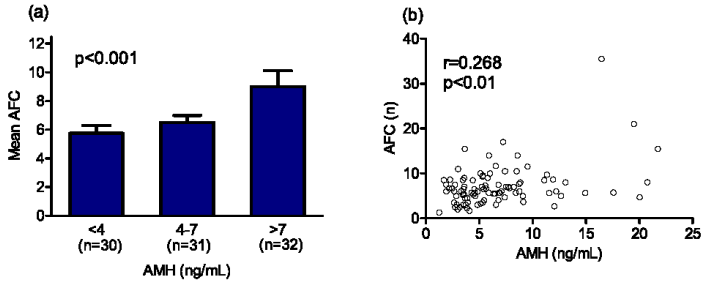

Fig. 6. (a) Mean antral follicle count (AFC) per ovary in cats with different AMH concentration in plasma and (b) correlation between AFC and plasma AMH concentration (n 93 cats).

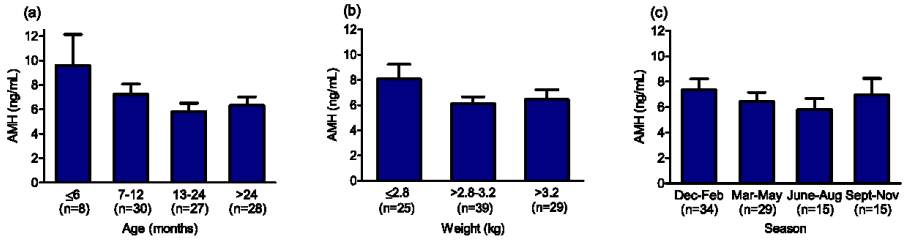

Fig. 7. Concentration of AMH in plasma of cats (n 93) grouped by (a) age and (b) weight and (c) ovariectomized at different times of the year.

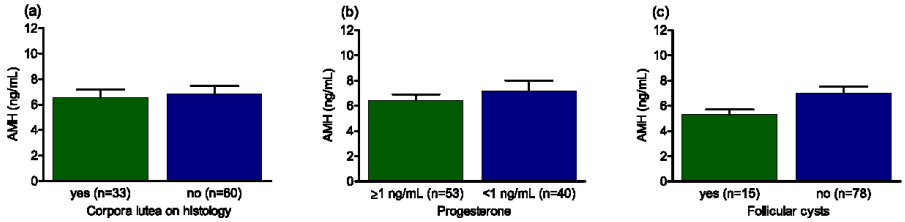

Fig. 8. Concentration of AMH in plasma of cats (a) with and without corpora lutea on histology, (b) plasma progesterone concentration ≥1 ng/mL and <1 ng/mL and (c) with and without follicular cysts determined by histology.

Funding

The study was supported by an International Academic Partnership grant from the Polish National Agency for Academic Exchange (NAWA).

CRediT authorship contribution statement

Svenja Claaßen: Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Jörg Aurich:** Conceptualization, Formal analysis, Investigation, Methodology, Funding acquisition, Writing – original draft, and revision. **Ingrid Walter:** Methodology, Validation, Formal analysis. **Camille Gautier:** Methodology, Validation, Formal analysis. **Christine Aurich:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

None of the authors has any conflict of interest to declare.

Acknowledgements

The authors are grateful to Julia Maderner for expert technical assistance with AMH analysis and to Dr. Peter Wohlsein, Department of Pathology, University of Veterinary Medicine Hannover, Germany for help with histopathological classifications.

References

[1] Jost A. The age factor in the castration of male rabbit fetuses. Proc Soc Exp Biol Med 1947;66:302–3.

[2] Rota A, Ballarin C, Vigier B, Cozzi B, Rey R. Age dependent changes in plasma anti-Mullerian hormone concentrations in the bovine male, female, and freemartin from birth to puberty: relationship between testosterone production and influence on sex differentiation. Gen Comp Endocrinol 2002;129:39–44.

[3] Scarlet D, Aurich C, Ille N, Walter I, Weber C, Pieler D, Peinhopf W, Wohlsein P, Aurich J. Anti-Muellerian hormone, inhibin A, gonadotropins, and gonadotropin receptors in bull calves after partial scrotal resection, orchidectomy, and Burdizzo castration. Theriogenology 2017;87:242–9.

[4] Ball BA, Conley AJ, Grundy SA, Sabeur K, Liu IKM. Expression of anti-Müllerian hormone (AMH) in the equine testis. Theriogenology 2008;69:624–31.

[5] Scarlet D, Wulf M, Kuhl J, Köhne M, Ille N, Conley AJ, Aurich C. Anti-Müllerian hormone profiling in prepubertal horses and its relationship with gonadal function. Theriogenology 2018;117:72–7.

[6] Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update 2011;17:46–54.

[7] Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 2011; 95:170–5.

[8] Rosen MP, Johnstone E, McCulloch CE, Schuh-Huerta SM, Sternfeld B, Reijo-Pera RA, Cedars MI. A characterization of the relationship of ovarian reserve markers with age. Fertil Steril 2012;97:238–43.

[9] Cedars MI. Evaluation of female fertility - AMH and ovarian reserve testing. J Clin Endocrinol Metab 2022;107:1510–9.

[10] Rico C, Fabre S, Medigue C, di Clemente N, Clement F, Bontoux M, Touzé J-L, Dupont M, Briant E, Rémy B, Beckers J-F, Monniaux D. Antimullerian hormone is an endocrine marker of ovarian gonadotropinresponsive follicles and can help to predict superovulatory responses in the cow. Biol Reprod 2009;80:50–9.

[11] Monniaux D, Baril G, Laine AL, Jarrier P, Poulin N, Cognieánd J, Fabre S. Anti-Mullerian hormone as a predictive endocrine marker for embryo production in the goat. Reproduction 2011;142:845–54.

[12] Torres-Rovira L, Gonzalez-Bulnes A, Succu S, Spezzigu A, Manca ME, Leoni GG, Sanna M, Pirino S, Gallus M, Naitana S. Predictive value of antral follicle count and anti-Müllerian hormone for follicle and oocyte developmental competence during the early prepubertal period in a sheep model. Reprod Fertil Develop 2014;26: 1094–106.

[13] Lapuente C, Faya M, Blanco PG, Grisolia-Romero M, Marchetti C, Gobello C. Anti-Müllerian hormone in queens: serum concentrations and total ovarian follicle population. Theriogenology 2023;197:111–5.

[14] Streuli I, Fraisse T, Pillet C, Ibecheole V, Bischof P, de Ziegler D. Serum Anti-müllerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. Fertil Steril 2008;90: 395–400.

[15] Flock U, Reese S, Otzdorff C, Klein R, Walter B. Anti-Müllerian hormone concentrations in queens throughout the estrous cycle. Domest Anim Endocrinol 2022;81:106749.

[16] Gültiken N, Yarim M, Aslan S, Gürler H, Yarim GF, Tuncay M, Inal S, Schäfer-Somi S. Expression of anti-Müllerian hormone and its type 2 receptor in the ovary of pregnant and cyclic domestic cats. Animals 2022;12:877.

[17] Axner E, Ström Holst B. Concentrations of anti-Müllerian hormone in the domestic cat. Relation with spay or neuter status and serum estradiol. Theriogenology 2015; 83:817–21.

[18] Themmen APN, Kalra B, Visser JA, Kumar A, Savjani G, de Gier J, Jaques S. The use of anti-Müllerian hormone as diagnostic for gonadectomy status in dogs. Theriogenology 2016;86:1467–74.

[19] Alm H, Ström Holst B. Identifying ovarian tissue in the bitch using anti-Müllerian hormone (AMH) or luteinizing hormone (LH). Theriogenology 2018;106:15–20.

[20] Ferré-Dolcet L, Ferro S, Contiero B, Fontaine C, Badon T, Gelli D, Romagnoli S. 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 Comm 2022; 46:917–24.

[21] Gozer A, Bahan O, Dogruer GG, Kutlu T. Serum antimüllerian hormone concentrations in female cats. Relation with ovarian remnant syndrome, ovarian cysts and gonadectomy status. Theriogenology 2023;200:106–13.

[22] Binder C, Reifinger M, Aurich J, Aurich C. Histopathological findings in the uteri and ovaries of clinically healthy cats presented for routine spaying. J Feline Med Surg 2021;23:770–6.

[23] Karakas Alkan K, Ceylan A, Alkan H, Ozen D, Bayraktaroglu AG, Kaymaz M. Immunohistochemical and qPCR determination of the expression and serum level of anti-Müllerian hormone in pre-pubertal, intact and ovarian remnant syndrome detected bitches. Reprod Dom Anim 2018;54:979–86.

[24] Rico C, Medigue C, Fabre XS, Jarrier P, Bontoux M, Clement F, Monniaux D. Regulation of anti-Müllerian hormone production in the cow: a multiscale study at endocrine, ovarian, follicular, and granulosa cell levels. Biol Reprod 2011;84: 560–71.

[25] Almeida FRCL, Costermans NGJ, Soede NM, Bunschoten A, Keijet J, Kemp B, Teerds KJ. Presence of anti-Müllerian hormone (AMH) during follicular development in the porcine ovary. PLoS One 2018;13:e0197894.

[26] Weenen C, Laven JS, Von Bergh AR, Cranfeld M, Groome NP, Visser JA, Kramer P, Fauser BCJM, Themmen APN. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004;10:77–83.

[27] Sacchi S, D'Ippolito G, Sena P, Marsella T, Tagliasacchi D, Maggi E, Argento C, Tirelli A, Giulini S, La Marca A. The anti-Müllerian hormone (AMH) acts as a gatekeeper of ovarian steroidogenesis inhibiting the granulosa cell response to both FSH and LH. J Assist Reprod Genet 2016;33:95–100.

[28] Ginther OJ, Gastal EL, Gastal MO, Beg MA. Incidence, endocrinology, vascularity, and morphology of hemorrhagic anovulatory follicles in mares. J Equine Vet Sci 2007;27:130–9.

[29] Cuervo-Arangoa J, Newcombe JR. Ultrasound characteristics of experimentally induced luteinized unruptured follicles (LUF) and naturally occurring hemorrhagic anovulatory follicles (HAF) in the mare. Theriogenology 2012;77:514–24.

[30] Kesler DJ, Garverick HA. Ovarian cysts in dairy cattle: a review. J Anim Sci 1982; 55:1147–59.

[31] Peter AT, Levine H, Drost M, Bergfelt DR. Compilation of classical and contemporary terminology used to describe morphological aspects of ovarian dynamics in cattle. Theriogenology 2009;71:1343–57.

[32] Claes A, Ball BA, Troedsson MHT, Curry TE, Squires EL, Scoggin KE. Molecular changes in the equine follicle in relation to variations in antral follicle count and anti-Müllerian hormone concentrations. Equine Vet J 2016;48:741–8.

[33] Schwarzmann L, Marchand A, Knutti B, Bruckmaier R, Bollwein H, Scarlet D. Effects of postpartum diseases on antral follicle count and serum concentration of Anti-Müllerian hormone in dairy cows. Anim Reprod Sci 2023;255:107291.

[34] Montoya M, Morrison JA, Arrignon F, Spofford N, Charles H, Hours M-A, Biourge V. Life expectancy tables for dogs and cats derived from clinical data. Front Vet Sci 2023;10:1082102.

[35] Claes A, Ball BA, Scoggin KE, Esteller-Vico A, Kalmar JJ, Conley AJ, Squires EL, Troedsson MHT. The interrelationship between anti-Müllerian hormone, ovarian follicular populations and age in mares. Equine Vet J 2015;47:537–41.

[36] Hollinshead FK, Walker C, Hanlon DW. 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 Dom Anim 2017;52(Suppl 2):35–40.

[37] Hill JR, Wilkinson J, Mallyon J, Anderson ST. Low sensitivity of a test for anti-Mullerian hormone to assess presence of ovaries in prepubertal bitches. Austr Vet J 2018;96:356–9.

[38] Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BMN, de Jong FH, Groome NP, Themmen APN, Visser JA. Serum Anti-Müllerian Hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 2006;147: 3228–34.

[39] Welsh CP, Gruffydd-Jones TJ, Murray JK. The neuter status of cats at four and six months of age is strongly associated with the owners' intended age of neutering. Vet Rec 2013. https://doi.org/10.1136/vr.101362.

[40] Almeida J, Ball BA, Conley AJ, Place NJ, Liu IKM, Scholtz EL, Mathewson L, Stanley SD, Moeller BC. Biological and clinical significance of anti-Müllerian hormone determination in blood serum of the mare. Theriogenology 2011;76: 1393–403.

[41] Binder C, Aurich C, Reifinger M, Aurich J. Spontaneous ovulation in cats - uterine findings and correlations with animal weight and age. Anim Reprod Sci 2019;209: 106167.

3. Concluding Discussion and Perspectives

This concluding discussion will only briefly repeat and summarize the interpretation of the results from the publication in this dissertation. Instead, based on our findings, perspectives for future research on reproduction of cats are proposed.

Domestic cats are popular pets in many countries, but as mentioned in the introduction, far less research is done on cats, including reproduction, compared to other domestic animal species such as farm animals, horses and dogs (Binder, 2020). Reasons for the low number of experimental studies in cats are an often uncooperative behavior and the unwillingness of owners to allow their animals to participate in controlled studies. This unwillingness could be caused, among other things, by the frequent difficulties in catching and transporting the cats (Mariti et al., 2016; Pratsch et al., 2018). In addition, legal regulations in many countries only allow experiments on cats when the animals originate from a source expressly approved by the competent authorities for the breeding of experimental animals. Exemptions from these rules are extremely difficult to justify. Strict rules on the approval of studies also apply to experimental animals.

Veterinary research on reproduction in cats, with the exception of case reports, will greatly benefit from scientific approaches that do not require authorization for animal experimentation. In Austria, it is required by law that all cats that regularly free-range outdoors must be neutered unless they are registered as breeding animals (*2. Tierverhaltungsordnung* 2004, amended 2016). In Germany, in many municipalities and cities gonadectomy is also mandatory for free-range cats. This allows access to the reproductive organs of female and male cats without requiring experimental intervention.

The results of this study show that AMH in the ovaries of cats is synthesized not only by preantral and small antral follicles, but also by the preovulatory follicles, i.e., larger follicles, and by luteal cells after ovulation and the formation of corpora lutea. To the best of our knowledge, the present study is the first to provide evidence of AMH expression in the corpora lutea of non-pregnant, cyclic cats. So far, AMH expression in the corpus luteum of non-pregnant animals, has only been detected in pig ovaries (Almeida et al., 2011). Pigs and cats are both polyovulatory species. Expression of AMH protein has also been detected in

preovulatory follicles in both species. In contrast, in the monovulatory horse mare, AMH protein expression is restricted to small antral follicles (Ball et al., 2008).

Our results suggest that AMH synthesis in cat follicles continues after ovulation but to address the hypothesis that AMH expression in luteal tissue is a specific finding in polyovulatory species, further research is needed. The experimental design of our current study could, for example, be applied to the polyovulatory rabbit. In the present study, interestingly, AMH was expressed only in 79 % of the corpora lutea and not always uniformly within in a single corpus luteum. Because of our experimental design, the exact day of ovulation and thus the age of the corpora lutea could not be determined. One may hypothesize that AMH synthesis gradually declines in the aging corpus luteum. This could be addressed in studies with ovariectomies performed in cats at defined days after either induced or spontaneous ovulations. The physiological function of luteal AMH is currently unclear and needed to be addressed in more complex experimental studies.

Similar to follicles and corpora lutea, AMH expression was also evident in granulosa cells and luteinized cells of follicular cysts, suggesting that they are also capable of producing AMH. Although the term ovarian cyst is widely used in the clinical reproduction of small animals, we have recently suggested that ovarian cysts in cats are mostly random findings with no clinical relevance (Binder et al., 2021). Cystic follicular structures in cats may more resemble hemorrhagic anovulatory follicles in mares which delay the next estrus (Ginther et al., 2007; Cuervo-Arango and Newcombe, 2012) than follicular cysts in cattle which can be the cause of a prolonged anestrus (e.g., Kesler et al., 1982; Peter et al., 2009). This interpretation is supported by the unchanged AMH expression in the follicular cysts compared to the physiological ovarian follicles of the cats in our study.

To classify ovarian cysts more easily and to better assess their incidence in female cats, further studies on cat ovaries are needed. A possibility for a better differentiation between different ovarian cyst types could be immunohistochemistry like already performed in dogs. In dogs, it was possible to differentiate types of cysts by different staining with antibodies against placental alkaline phosphatase, S100, inhibin α, desmin and cytokeratin AE1/AE3. In some cases, it was even possible to classify cysts for which this was not possible based on histology alone (Akihara et al., 2007).

In female dogs, an increased risk of ovarian cysts has been described when animals were six to eight years old (Knauf et al., 2018). Depending on the dog breed this is about 70 % of their life expectancy. With a life expectancy over 15 years in the cat (Montoya et al., 2023), the animals in our study had not reached such a high age than the dogs in study by Knauf et al. (2018). The oldest cat in the present study was nine years old and had thus only reached 60 % of her life expectancy. Therefore, further studies in older cats are necessary to determine a potentially increased incidence of ovarian cysts in older animals. A problem for such studies is the fact that the majority of cats are spayed at a young age due to legal requirements or because requested by their owners.

In some studies, ovarian cysts were defined strictly by size, but the range for a definition of cysts by size varies considerably and in dogs ranges between 0.2 and 4.0 cm in diameter (Knauf et al., 2018). Therefore, a determination of cysts by size alone should be avoided and a histological evaluation should be performed: With increasing size, however, the probability of ovarian cyst versus preovulatory follicles increases.

In our study, the AMH concentration in plasma of ovary-intact female cats varied markedly among individual animals. AMH was always detectable and well above the minimal detection threshold of our assay but was not affected by the presence or absence of follicular cysts. This is in agreement with a similar AMH protein expression in these ovarian follicles and cysts.

When cats were grouped by antral follicle count, AMH concentrations were higher in cats with more antral follicles than in cats with fewer antral follicles, but there was only a loose linear correlation between plasma AMH concentration and antral follicle count. Plasma AMH concentrations were not affected by the presence or absence of corpora lutea, consistent with previous studies in women (Streuli et al., 2008), pigs (Almeida et al., 2011) and horses (Claes et al., 2015). Some variation in AMH plasma concentration during the estrous cycle and between cycles in individual animals has been described previously for cats (Flock et al., 2022; Lapuente et al., 2023) but based on a larger cat population this could not be confirmed in the present study. Each cat, however, was included only once and thus only a single AMH result is available per animal. Further studies with repeated blood sampling in female cats are therefore justified. Such studies would allow establishing endocrine profile throughout the cat estrous cycle. Combined with ultrasound examination, it could then be determined if ovarian cysts can be diagnosed by ultrasound, whether they – as we hypothesize - resolve

spontaneously or luteinize after ovulation or if there are cases ovarian cysts associated with pathological changes and clinical symptoms.

In conclusion, our study indicates that AMH is clearly a marker for the presence of ovarian tissue in cats but its usefulness in assessing ovarian function in non-spayed individual cats is of limited value. Regarding the etiology, clinical relevance and, if necessary, therapy of ovarian cysts, science-based knowledge in cats is still limited. Overall, our study adds to the current knowledge on ovarian cysts and AMH in non-pregnant female cats. Our results, however, also raise new questions and offer some perspectives for future research in feline reproduction. Such studies may advance veterinary reproduction in one of our most beloved pets. New findings in domestic cats may also be applied to wild felids and thus provide reproductive support in wildlife conservation programs.

4. Summary

Svenja Claaßen (2023)
Follicular development in cat ovaries: Expression of Anti-Muellerian hormone and occurrence of ovarian cysts

In female animals of many species, Anti-Muellerian hormone (AMH) is produced by follicular granulosa cells and therefore has been associated with the ovarian follicle pool. In this study, we have analyzed plasma AMH concentration in female cats (n = 93) presented for routine ovariectomy and assessed ovarian histology and AMH protein expression in the surgically removed ovaries. We hypothesized that AMH is synthesized only in preantral and small antral follicles and that plasma AMH concentration reflects the antral follicle count (AFC). Corpora lutea were detected in 32 % of the cats, whereas plasma progesterone concentration was ≥ 1 ng/mL in 55 % of the cats. Follicular cysts were present in 15 cats (16 %). Positive immunostaining for AMH protein was detected in close to all primordial and antral follicles, in all ovarian cysts, 79 % of corpora lutea and 28 % of atretic follicles. Contrary to our hypothesis, AMH in the ovaries of cats is synthesized not only in preantral and small antral follicles, but also preovulatory follicles and corpora lutea. Synthesis of AMH in cats thus continues after ovulation but the physiological function of luteal AMH remains unclear. The unchanged AMH expression in granulosa cells and luteinized cells of follicular cysts further supports the interpretations that most ovarian cysts in cats are incidental findings without clinical relevance.

Concentration of AMH in plasma averaged 6.8 ± 0.5 ng/mL. When cats were grouped by AFC, AMH concentrations were higher in cats with more antral follicles than in cats with fewer antral follicles, but there was only a loose linear correlation between plasma AMH concentration and AFC ($r = 0.286$, $p < 0.01$). Plasma AMH concentration was not affected by season, age, weight, stage of the estrous cycle and presence of follicular cysts.

In conclusion, AMH protein is expressed in all endocrine structures of the cat ovary. Our study confirms that AMH is a marker for the presence of ovarian tissue in cats but its usefulness in assessing ovarian function in non-spayed individual cats is of limited value. Our study adds new information on ovarian cysts and AMH in female cats but regarding the etiology and relevance of ovarian cysts in cats, science-based knowledge is still limited.

5. Zusammenfassung

Svenja Claaßen (2023)
Ovarielle Follikelentwicklung bei Katzen: Expression von Anti-Müller-Hormon und Vorkommen von Ovarzysten

Bei weiblichen Tieren vieler Spezies wird das Anti-Müller-Hormon (AMH) von follikulären Granulosazellen produziert und ist daher mit dem ovariellen Follikelpool assoziiert. In dieser Studie haben wir die Plasma-AMH-Konzentration von weiblichen Katzen (n = 93) analysiert, die einer routinemäßigen Ovariektomie unterzogen wurden und Ovarialhistologie und AMH-Proteinexpression in den operativ entfernten Ovarien untersucht. Den Untersuchungen lag die Hypothese zugrunde, dass AMH nur in präantralen und kleinen antralen Follikeln synthetisiert wird und dass die AMH-Konzentration im Plasma die Anzahl antraler Follikel (AFC) widerspiegelt. Corpora lutea wurden bei 32 % der Katzen nachgewiesen, während die Progesteronkonzentration im Plasma bei 55 % der Katzen ≥ 1 ng/ml betrug. Bei 15 Katzen (16 %) traten follikuläre Zysten auf. Eine positive Immunfärbung für AMH-Protein wurde in allen primordialen und antralen Follikeln, in allen Ovarialzysten, in 79 % der Corpora lutea und in 28 % der atretischen Follikel nachgewiesen. Entgegen unserer Hypothese wird AMH in den Eierstöcken von Katzen nicht nur in präantralen und kleinen antralen Follikeln synthetisiert, sondern auch in präovulatorischen Follikeln und Corpora lutea. Die Synthese von AMH bei Katzen geht also nach der Ovulation weiter, aber die physiologische Funktion des lutealen AMH bleibt unklar. Die unveränderte AMH-Expression in Granulosazellen und luteinisierten Zellen von Follikelzysten unterstützt die Interpretation, dass die meisten Ovarialzysten bei Katzen Zufallsbefunde ohne klinische Relevanz sind.

Die AMH-Konzentration im Plasma betrug durchschnittlich 6,8 ± 0,5 ng/ml. Wenn die Katzen nach dem AFC gruppiert wurden, waren die AMH-Konzentrationen bei Katzen mit mehr Antralfollikeln höher als bei Katzen mit weniger Antralfollikeln, aber es bestand nur eine leichte lineare Korrelation zwischen der AMH-Konzentration im Plasma und dem AFC ($r = 0,286$, $p < 0,01$). Die AMH-Konzentration im Plasma wurde nicht durch die Jahreszeit, das Alter, das Gewicht, die Phase des Sexualzyklus und das Vorhandensein von Follikelzysten beeinflusst.

Zusammenfassend wird AMH in allen endokrinen Strukturen des Katzen-Ovars exprimiert. Unsere Studie bestätigt, dass AMH ein Marker für das Vorhandensein von Ovarialgewebe bei

Katzen ist, aber seine Verwendbarkeit bei der Beurteilung der Ovarialfunktion nicht-kastrierter Katzen ist nur von begrenztem Nutzen. Unsere Studie liefert neue Informationen über Ovarialzysten und AMH bei weiblichen Katzen, aber bezüglich der Ätiologie und Relevanz von Ovarialzysten bei Katzen sind wissenschaftlichen Erkenntnisse noch begrenzt.

6. References (Chapters 1 and 3)

Akihara Y, Shimoyama Y, Kawasako K, Komine M, Hirayama K, Kagawa Y, Omachi T, Matsuda K, Okamoto M, Kadosawa T, Taniyama H. 2007. Immunohistochemical evaluation of canine ovarian cysts. J Vet Med Sci 69:1033-7.

Almeida J, Ball BA, Conley AJ, Place NJ, Liu IK, Scholtz EL, Mathewson L, Stanley SD, Moeller BC. 2011. Biological and clinical significance of Anti-Müllerian hormone determination in blood serum of the mare. Theriogenology 76:1393-403.

Arlt SP, Haimerl P. 2016. Cystic ovaries and ovarian neoplasia in the female dog - a systematic review. Reprod Domest Anim 51 Suppl 1:3-11.

Axnér E, Ström Holst B. 2015. Concentrations of Anti-Müllerian hormone in the domestic cat. Relation with spay or neuter status and serum estradiol. Theriogenology 83:817-21.

Azziz R. 2018. Polycystic ovary syndrome. Obstet Gynecol 132:321-336.

Ball BA, Conley AJ, MacLaughlin DT, Grundy SA, Sabeur K, Liu IK. 2008. Expression of Anti-Müllerian hormone (AMH) in equine granulosa-cell tumors and in normal equine ovaries. Theriogenology 70:968-77.

Bartlett PC, Ngategize PK, Kaneene JB, Kirk JH, Anderson SM, Mather EC. 1986. Cystic follicular disease in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology, and economic impact. Prev Vet Med 4:15–33.

Bean AD. Ovarian cysts in the guinea pig (Cavia porcellus). 2013. Vet Clin North Am Exot Anim Pract 16:757-76.

Bertram CA, Müller K, Klopfleisch R. 2018. Genital Tract Pathology in Female Pet Guinea Pigs (Cavia porcellus): a Retrospective Study of 655 Post-mortem and 64 Biopsy Cases. J Comp Pathol 165:13-22.

Binder C, Reifinger M, Aurich J, Aurich C. 2021. Histopathological findings in the uteri and ovaries of clinically healthy cats presented for routine spaying. J Feline Med Surg 23:770-776.

Binder C. 2020. Uterine responsiveness to progesterone and histopathological findings in cyclic domestic cats, Dr. med. vet. thesis, Vetmeduni Vienna, Austria.

Bottomley C, Bourne T. 2009. Diagnosis and management of ovarian cyst accidents. Best Pract Res Clin Obstet Gynaecol 23:711-24.

Casida LE, Chapman AB. 1951. Factors affecting the incidence of cystic ovaries in a herd of Holstein cows. J Dairy Sci 34:1200–5.

Cassida LE, McShan WH and Meyer RK 1944. Effects of an unfractionated pituitary extract upon cystic ovaries and nymphomania in cows. J Anim Sci 3:273–82.

Castagna CD, Peixoto CH, Bortolozzo FP, Wentz I, Neto GB, Ruschel F. 2004. Ovarian cysts and their consequences on the reproductive performance of swine herds. Anim Reprod Sci 81:115-23.

Claes A, Ball BA, Scoggin KE, Esteller-Vico A, Kalmar JJ, Conley AJ, Squires EL, Troedsson MH. 2015. The interrelationship between Anti-Müllerian hormone, ovarian follicular populations and age in mares. Equine Vet J 47:537-41.

Cuervo-Arango J, Newcombe JR. 2009. The effect of hormone treatments (hCG and cloprostenol) and season on the incidence of hemorrhagic anovulatory follicles in the mare: a field study. Theriogenology 72:1262-7.

Cuervo-Arango J, Newcombe JR. 2010. Risk factors for the development of haemorrhagic anovulatory follicles in the mare. Reprod Domest Anim 45:473-80.

Cuervo-Arango J, Newcombe JR. Ultrasound characteristics of experimentally induced luteinized unruptured follicles (LUF) and naturally occurring hemorrhagic anovulatory follicles (HAF) in the mare. 2012. Theriogenology 77:514-24.

Dørum A, Blom GP, Ekerhovd E, Granberg S. 2005. Prevalence and histologic diagnosis of adnexal cysts in postmenopausal women: an autopsy study. Am J Obstet Gynecol 192:48-54.

Douthwaite R, Dobson H. 2000. Comparison of different methods of diagnosis of cystic ovarian disease in cattle and an assessment of its treatment with a progesterone-releasing intravaginal device. Vet Rec 147:355-9.

Ebbert W, Bostedt H, 1993. Cystic degeneration in porcine ovaries – first communication: morphology of cystic ovaries, interpretation of the results. Reprod Dom Anim 28:441–50.

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 46:917-24.

Field KJ, Griffith JW, Lang CM. 1989. Spontaneous reproductive tract leiomyomas in aged guinea-pigs. J Comp Pathol 101:287-94.

Flock U, Fischer S, Weeger J, Reese S, Walter B. 2022. Anti-Müllerian hormone as a diagnostic tool to identify queens with ovarian remnant syndrome. J Feline Med Surg 24:e168-e174.

Flock U, Reese S, Otzdorff C, Klein R, Walter B. 2022. Anti-Müllerian hormone concentrations in queens throughout the estrous cycle. Domest Anim Endocrinol 81:106749.

Franks S. 2012. Animal models and the developmental origins of polycystic ovary syndrome: increasing evidence for the role of androgens in programming reproductive and metabolic dysfunction. Endocrinology 153:2536-8.

Garm O. 1949. A study of bovine nymphomania. Acta Endocrinol 3, Suppl 3:1.

Garverick HA. 1997. Ovarian follicular cysts in dairy cows. J Dairy Sci 80:995-1004.

Gelberg HB, McEntee K, Heath EH, 1984. Feline cystic rete ovarii. Vet Pathol 21:304-7.

Ginther OJ, Gastal EL, Gastal MO, Beg MA, 2006. Conversion of a viable preovulatory follicle into a hemorrhagic anovulatory follicle in mares. Anim Reprod 3:29-40.

Ginther OJ, Gastal EL, Gastal MO, Beg MA, 2007. Incidence, endocrinology, vascularity, and morphology of hemorrhagic anovulatory follicles in mares. J Equine Vet Sci 27:130-9.

Gozer A, Bahan O, Dogruer G, Kutlu T. 2023. Serum Anti-Müllerian hormone concentrations in female cats. Relation with ovarian remnant syndrome, ovarian cysts and gonadectomy status. Theriogenology 200:106-13.

Gültiken N, Yarim M, Aslan S, Gürler H, Yarim GF, Tuncay M, İnal S, Schäfer-Somi S. 2022. Expression of Anti-Müllerian hormone and its type 2 receptor in the ovary of pregnant and cyclic domestic cats. Animals 12:877.

Gundling N, Drews S, Hoedemaker M. 2015. Comparison of two different programmes of ovulation synchronization in the treatment of ovarian cysts in dairy cows. Reprod Domest Anim 50:893-900.

Hartge P, Hayes R, Reding D, Sherman ME, Prorok P, Schiffman M, Buys S. 2000. Complex ovarian cysts in postmenopausal women are not associated with ovarian cancer risk factors: preliminary data from the prostate, lung, colon, and ovarian cancer screening trial. Am J Obstet Gynecol 183:1232-7.

Heinonen M, Leppävuori A, Pyörälä S. 1998. Evaluation of reproductive failure of female pigs based on slaughterhouse material and herd record survey. Anim Reprod Sci 52:235-44.

Hong CC. 1980. Spontaneous papillary cystadenocarcinoma of the ovary in Dunkin-Hartley guineapigs. Lab Anim 14:39-40.

Houry D, Abbott JT. 2001. Ovarian torsion: a fifteen-year review. Ann Emerg Med, 38:156-9.

Karveliene B, Zilinskas H, Riskevicience V. 2007. Post-mortem examination of sows genital organs culled for reproductive disturbances and immunohistochemical studies on ERalpha and PR A receptors in the anoestral sows uterus. Reprod Domest Anim 42:275-81.

Keller LS, Griffith JW, Lang CM. 1987. Reproductive failure associated with cystic rete ovarii in guinea pigs. Vet Pathol 24:335-9.

Kesler DJ, Garverick HA. 1982. Ovarian cysts in dairy cattle: a review. J Anim Sci 55:1147-59.

Knauf Y, Bostedt H, Failing K, Knauf S, Wehrend A. 2014. Gross pathology and endocrinology of ovarian cysts in bitches. Reprod Domest Anim 49:463-8.

Knauf Y, Köhler K, Knauf S, Wehrend A. 2018. Histological classification of canine ovarian cyst types with reference to medical history. J Vet Sci 19:725-34.

Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. 1998. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 83:3078-82.

Krause KJ. 2014. Ferrets, rabbits, and rodents: clinical medicine and surgery. Can Vet J 55:365.

Laik-Schandelmaier C, Klopfleisch R, Schöniger S, Weiffenbach G, Staudacher M, Aupperle H. 2017. Spontaneously arising tumors and tumor-like lesions of the cervix and uterus in 83 pet guinea pigs (Cavia porcellus). J Comp Pathol 156:339-51.

Lapuente C, Faya M, Blanco PG, Grisolia-Romero M, Marchetti C, Gobello C. 2023. Anti-Müllerian hormone in queens: Serum concentrations and total ovarian follicle population. Theriogenology 197:111-5.

López-Gatius F, Santolaria P, Yániz J, Rutllant J, López-Béjar M. 2001. Persistent ovarian follicles in dairy cows: a therapeutic approach. Theriogenology 56:649-59.

MacLachlan NJ. 1987. Ovarian disorders in domestic animals. Environ Health Perspect 73:27-33.

Mariti C, Bowen JE, Campa S, Grebe G, Sighieri C, Gazzano A. 2016. Guardians' perceptions of cats' welfare and behavior regarding visiting veterinary clinics. J Appl Anim Welf Sci 19:375-84.

Maya-Pulgarin D, Gonzalez-Dominguez MS, Aranzazu-Taborda D, Mendoza N, Maldonado-Estrada JG. 2017. Histopathologic findings in uteri and ovaries collected from clinically healthy dogs at elective ovariohysterectomy: a cross-sectional study. J Vet Sci 18:407-14.

McCue PM. 1998. Review of ovarian abnormalities in the mare. Proc Ann Conv AAEP 44:125-33.

McDonald JM, Modesitt SC. 2006. The incidental postmenopausal adnexal mass. Clin Obstet Gynecol 49:506-16.

Minarikova A, Hauptman K, Jeklova E, Knotek Z, Jekl V. 2015. Diseases in pet guinea pigs: a retrospective study in 1000 animals. Vet Rec 177:200.

Montoya M, Morrison JA, Arrignon F, Spofford N, Charles H, Hours MA, Biourge V. 2023. Life expectancy tables for dogs and cats derived from clinical data. Front Vet Sci 10:1082102.

Nielsen SW, Misdorp W, McEntee K. 1976. Tumors of the ovary. Bull World Health Organ 53:203-15.

Nielsen TD, Holt S, Ruelokke ML, McEvoy FJ. 2003. Ovarian cysts in guinea pigs: influence of age and reproductive status on prevalence and size. J Small Anim Pract 44:257-60.

PCOS Consensus Workshop Group. 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41-7.

Peter AT, Vos PL, Ambrose DJ. 2009. Postpartum anestrus in dairy cattle. Theriogenology 71:1333-42.

Pilny A. 2014. Ovarian cystic disease in guinea pigs. Vet Clin North Am Exot Anim Pract 17:69-75.

Place NJ, Hansen BS, Cheraskin JL, Cudney SE, Flanders JA, Newmark AD, Barry B, Scarlett JM. 2011. Measurement of serum Anti-Müllerian hormone concentration in female dogs and cats before and after ovariohysterectomy. J Vet Diagn Invest 23:524-7.

Pradhan P, Thapa M. 2014. Dermoid cyst and its bizarre presentation. J Nepal Med Assoc 52:837-44.

Pratsch L, Mohr N, Palme R, Rost J, Troxler J, Arhant C. 2018. Carrier training cats reduces stress on transport to a veterinary practice. Appl Anim Behav Sci 206:64–74.

Sasidharan JK, Patra MK, Singh LK, Saxena AC, De UK, Singh V, Mathesh K, Kumar H, Krishnaswamy N. 2021. Ovarian cysts in the bitch: an update. Topics Companion Anim Med 43:100511.

Shi F, Petroff BK, Herath CB, Ozawa M, Watanabe G, Taya K. 2002. Serous cysts are a benign component of the cyclic ovary in the guinea pig with an incidence dependent upon inhibin bioactivity. J Vet Med Sci 64:129-35.

Silvia WJ, Hatler TB, Nugent AM, Laranja da Fonseca LF. 2002. Ovarian follicular cysts in dairy cows: an abnormality in folliculogenesis. Domest Anim Endocrinol 23:167-77.

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, Suppl 2:98-102

Streuli I, Fraisse T, Pillet C, Ibecheole V, Bischof P, de Ziegler D. 2008. Serum anti müllerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. Fertil Steril 90:395-400.

Ström Holst B. 2017. Diagnostic possibilities from a serum sample-Clinical value of new methods within small animal reproduction, with focus on Anti-Müllerian hormone. Reprod Domest Anim 52, Suppl 2:303-9.

Szulańczyk K. 2009. Histological changes within ovarian cortex, oviductal and uterine mucosa in case of ovarian cysts presence in sows. Folia Histochem Cytobiol 47:99-103.

Vanholder T, Opsomer G, de Kruif A. 2006. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reprod Nutr Dev 46:105-19.

Walter B. 2020. Anti-Müllerian hormone in dogs and cats reproduction. Reprod Domest Anim 55, Suppl 2:26-31.

Danksagung

Ich möchte mich an dieser Stelle ganz herzlich für die unschätzbare Unterstützung bedanken, die ich während meines Doktoratsstudiums und meiner klinischen Arbeit erhalten habe.

Ganz besonders gilt mein Dank Frau Prof. Christine Aurich und Herrn Prof. Jörg Aurich, die nicht nur meine Doktorarbeit, sondern auch meine klinische Ausbildung maßgeblich gefördert und geprägt haben. Ihre Expertise und ihr Engagement haben mich in vielerlei Hinsicht bereichert und ihre Unterstützung war unverzichtbar für meinen akademischen und beruflichen Fortschritt. Die Möglichkeit, von ihnen zu lernen und mit ihnen zusammenzuarbeiten, war eine einzigartige Gelegenheit, mir neue Perspektiven zu eröffnen.

Ebenso möchte ich mich bei meinen Kollegen der Plattform Besamung und Embryotransfer und der Klinik für Geburtshilfe, Gynäkologie und Andrologie bedanken. Im Verlauf unserer Arbeit sind sie nicht nur zu verlässlichen Arbeitspartnern, sondern auch zu engen Freunden geworden. Ihre fachliche Unterstützung und Zusammenarbeit, ohne die dieses Projekt nicht möglich gewesen wäre, hat mich in meiner Arbeit immer ermutigt.

Ein großer Dank gebührt meiner Familie, insbesondere meinen Eltern, die mich auf meinem Weg immer gefördert und bestärkt haben. Ihr Vertrauen in mich war eine unerschütterliche Quelle der Motivation.
Von den Menschen, die mir auf diesem Weg immer Mut gemacht haben, mich aber leider nicht bis zum Ziel begleiten konnten, möchte ich meine Großeltern Heinz und Christa, meinen Großvater Willi und meinen Nachbarn Norbert in besonderer Erinnerung behalten.

Abschließend möchte ich mich bei der *Polish National Agency for Academic Exchange* (NAWA) für die finanzielle Unterstützung meiner Studie bedanken.