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ORIGINAL ARTICLE

Anti-Müllerian hormone concentrations in female rabbits and its relation to spay status, pseudopregnancy and ovarian follicle numbers

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Abstract

Anti-Müllerian hormone (AMH), known for its role during foetal sexual differentiation, is secreted by the Sertoli cells in males and the granulosa cells in females during post-natal life. As serum AMH concentrations correlate with follicle numbers, AMH is utilized as a marker of ovarian reserve in many species. In dogs and cats, AMH is used as a diagnostic tool to determine spay or neuter status. In the available literature, no research regarding serum AMH levels in rabbits has been published yet. The objectives of the present study were to (1) measure serum AMH concentrations in female rabbits and investigate the value of AMH as a diagnostic tool to differentiate between spayed and intact does and (2) relate measured AMH levels to pseudopregnancy and ovarian follicle numbers. For AMH measurement, serum samples were obtained from sexually intact (n = 64) and spayed (n = 22) female rabbits. Spayed does were of various breeds; intact rabbits were Zika hybrid rabbits. In the intact does, AMH measurement was complemented by determination of progesterone levels, gynaecological examination and histopathological evaluation of the uterus and ovaries, including follicle counts. Serum AMH and progesterone concentrations were measured using a human-based chemiluminescence immunoassay (CLIA) and an enzyme-linked fluorescence assay (ELFA), respectively. Depending on progesterone levels, sexually intact does were classified into follicular (n = 52) or luteal phase (n = 12). Median serum AMH levels were 1.53 ng/ml (range 0.77–3.36 ng/ml) in intact and 0.06 ng/ml (range ≤0.01–0.23 ng/ml) in spayed does. AMH concentrations between the intact and spayed rabbits differed significantly and did not overlap ($p < .001$). Receiver operating characteristic (ROC) curve analysis yielded a sensitivity and specificity of 100% for a cut-off level of 0.50 ng/ml. Follicular or luteal phase had no significant influence on measured AMH levels (t = 0.061, df = 62, p = .951). While the number of secondary follicles correlated significantly with AMH concentrations (r_s = 0.410, p = .001), the number of primary or antral follicles did not (r_s = 0.241, p = .055 and r_s = 0.137, p = .281, respectively). In conclusion, a single determination of serum AMH

concentrations was adequate to distinguish spayed from intact female rabbits. Among sexually intact individuals, whether does were in follicular or luteal phase had no significant influence on measured serum AMH concentrations. The relationship between small growing follicles and AMH levels as described in other species could be partially confirmed, as secondary follicles correlated significantly with AMH.

KEYWORDS
anti-Müllerian hormone, follicle numbers, pseudopregnancy, rabbit, spay status

1 | INTRODUCTION

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein and responsible for suppression of Müllerian duct development during embryogenesis in males (Cate et al., 1986; Jost, 1947). If not suppressed by AMH, the Müllerian duct develops into the uterus, vagina and fallopian tubes (Cate et al., 1986). In males, AMH is exclusively produced by Sertoli cells of the testes (Josso, 1973). In females, AMH is predominantly secreted by granulosa cells of pre-antral and small antral ovarian follicles (Vigier et al., 1984), where it has two roles in ovarian physiology. Firstly, AMH inhibits the recruitment of primordial follicles into the pool of growing follicles, and secondly, AMH decreases the responsiveness of growing follicles to follicle-stimulating hormone (FSH). Thus, AMH is responsible for preserving the ovarian follicle pool and avoiding premature exhaustion (Durlinger et al., 2002).

In human medicine, AMH is mainly used as an indirect, non-invasive marker for the size of the ovarian reserve, as serum AMH levels correlate with the number of follicles (Van Rooij et al., 2002). Similarly, AMH concentrations correlate with the number of small growing follicles in cows, mares, bitches and hamsters (Anadol et al., 2020; Claes et al., 2016; Monniaux et al., 2012; Place & Cruickshank, 2009).

Since AMH is mainly produced in the male and female gonads, it can be used as a diagnostic tool to distinguish between intact and castrated individuals (Place et al., 2011; Themmen et al., 2016). Ovariectomy decreases serum AMH concentrations significantly in women, bitches and queens (Alm & Holst, 2018; Anadol et al., 2020; Axnér & Ström Holst, 2015; Flock et al., 2022; La Marca et al., 2005; Pir Yagci et al., 2016; Place et al., 2011; Themmen et al., 2016; Walter et al., 2019). Similarly, in bitches and queens, AMH can provide evidence for ovarian tissue in suspected cases of ovarian remnant syndrome (ORS) (Flock et al., 2022; Karakas Alkan et al., 2019; Place et al., 2011; Turna Yilmaz et al., 2015). In rabbits, as uterine adenocarcinoma is the most common tumour type, ovariohysterectomy is increasingly performed as a preventive for uterine and ovarian neoplasms (Quesenberry & Carpenter, 2011; Walter et al., 2010). Therefore, in does with unknown medical history, knowledge about spay status is essential, and AMH could be an effective diagnostic tool for spay status in these cases.

In the species of interest, knowledge about normal reference intervals and possible cycle-associated changes in serum AMH concentrations are required for its application in a clinical setting. In the bitch and the queen, significant fluctuations of serum AMH concentrations during the oestrous cycle were reported, albeit limited by breed influences and high individual variations (Flock et al., 2019; Walter et al., 2019). In horses, while one study described no significant changes in AMH levels during the oestrous cycle or pregnancy (Almeida et al., 2011), a recent study suggested increased AMH concentrations in oestrous mares compared with mares in dioestrus (Dal & Kasikci, 2020). In most studies in cattle, no significant variations of serum AMH levels throughout oestrous cycles were described (El-Sheikh Ali et al., 2013; Monniaux et al., 2010; Rico et al., 2009).

Along with felids, minks and ferrets, rabbits are induced ovulators (Senger, 2012). If fertilization does not occur after ovulation, pseudopregnancy can develop (Rubin & Azrin, 1967). Pseudopregnancy persists 16–18 days and is accompanied by increased progesterone levels of 1–2 ng/ml on day two post-ovulation, up to 12–20 ng/ml between days six and eight post-ovulation (Norris & Lopez, 2011). On Days 16–18, progesterone concentrations return to basal levels of <1 ng/ml (Browning et al., 1980). Throughout the reproductive cycle, female rabbits display periods of increased and reduced sexual receptivity lasting about 7–14 and 1–4 days, respectively (Easson, 2001; Mc Nitt et al., 2013). In periods of increased receptivity, the doe's vulva is commonly enlarged, moist and of reddish purple colour (Quesenberry & Carpenter, 2011). In periods of decreased receptivity, rabbits have bright pink vulvas with no swelling and only slight moistness (Ramirez et al., 1986). On each ovary, follicular development occurs in waves of 5 to 10 follicles. Mature follicles remain active for about 12–14 days, after which they degenerate, followed by reduced sexual receptivity (Mc Nitt et al., 2013).

The objectives of the present study were to (1) measure serum AMH concentrations in female rabbits and investigate the value of AMH as a diagnostic tool to differentiate between spayed and sexually intact does and (2) relate measured AMH levels to pseudopregnancy and ovarian follicle numbers.

2 | MATERIALS AND METHODS

2.1 | Animals and collection of specimens

The present study was conducted in accordance with the guidelines of the Protection of Animal Act and was approved by the ethics committee of the Faculty of Veterinary Medicine of the Ludwig Maximilian University (ref. no 273–July 19, 2021).

After owner agreement, blood samples of spayed rabbits ($n = 22$) were collected from does which were presented at a local veterinary practice due to dental disease and routine health check-ups. Sampling of 1 ml of blood was performed 0.4–7.2 years (mean 2.7 years) after initial spaying by puncture of the marginal ear vein. Spayed animals were lionhead ($n = 8$), lop ($n = 5$), mini lop ($n = 4$), pygmy ($n = 4$) and angora hybrid ($n = 1$) rabbits, aged 1.6–12.1 years (mean 5.6 years) and weighing 1.49–2.85 kg (mean 2.23 kg).

Specimens of sexually intact does ($n = 64$) were collected from animals after they were sacrificed due to a different authorized study (ref. no 55.2-2532.Vet 03-17-110, Bavarian Government). Following euthanasia by intravenous application of pentobarbital, 2 ml of blood was collected by cardiac puncture. Before sacrifice, all does were deemed clinically healthy. Intact rabbits were white-coloured, female Zika hybrid rabbits, aged 5.7–8.8 months (mean 7.5 months) and weighing 3.86–7.20 kg (mean 5.72 kg).

Blood samples were left to clot at room temperature and were then centrifuged at 2000 *g* for 5 min (IKA® mini G S000). Thereafter, serum was collected and stored at −20°C until measurement of hormones.

In the intact does, AMH measurement was complemented by determination of progesterone levels, gynaecological examination and histopathological evaluation of the uterus and ovaries, including follicle counts.

2.2 | Gynaecological examination

The vulva of each intact doe was examined and checked for enlargement, change of colour (pale, pink or red) and vaginal discharge. The vulva's appearance was documented photographically to ensure impartial comparability. Thereafter, mammary glands were checked for enlargement and milk production.

2.3 | Histopathological examination

Following euthanasia, the ovaries and uterus were removed, dissected free of surrounding fat and immersed in 7% buffered formalin. After fixation in formalin for at least 24 h, serial dehydration with ethanol and clearing with Xylol was performed. Specimens were then embedded into paraffin blocks and serially sectioned at 4 µm. Ovaries were cut sagittally, the uterus was cut transversely. For each ovary, one representative section was selected from the midsagittal and lateral sagittal planes, totalling three sections. In addition, a representative section from the uterus was obtained from the middle of both uterine horns and at the height of both cervices. Tissue ribbons were stained with a Giemsa and haematoxylin–eosin stain. Specimens were then examined for histopathological conditions. Further, follicle numbers and presence of corpora lutea were documented. Follicles with a single layer of cuboidal granulosa cells were regarded as primary follicles, those with multiple layers were classified as secondary follicles. Follicles with an antrum of any size were defined as antral follicles (Place & Cruickshank, 2009). Only follicles with a healthy oocyte nucleolus were recorded. Slides of the uterus were examined for epithelial hyperplasia, stromal oedema and lumen dilatation, indicating pseudopregnancy (Geyer et al., 2016). All histological slides were evaluated by a single examiner, unaware of the results from the gynaecological examination and hormone analysis.

2.4 | Hormone analysis

Serum AMH concentrations were measured at a commercial laboratory (Laboklin GmbH & Co. KG, Bad Kissingen, Germany) using a chemiluminescence immunoassay (CLIA) on a cobas E602 analyzer (Roche). The AMH assay was validated for rabbits. In the sexually intact animals, intra-assay coefficients of variation were between 1.40 and 1.47%, inter-assay coefficients of variation were between 3.70 and 4.91%. In the spayed animals, intra-assay and inter-assay coefficients of variation were 2.55 and 8.21%, respectively. Additionally, the intra-assay coefficient of variation was calculated for serial dilutions (1:2, 1:5: 1:10, 1:20) using serum of sexually intact rabbits and a physiologic salt solution as a diluting agent. The intra-assay coefficients of variation were 5.41% at 3.63 ng/ml, 6.52% at 2.15 ng/ml and 17.06% at 1.8 ng/ml. Comparison of the expected values to the measured values after dilution yielded a mean recovery rate of 107.13% (range 94.91–138.89%). Recovery of human AMH standard added to rabbit serum showed changes in optical density parallel to the AMH standard curve. Minimum and maximum detection limit of the AMH assay were 0.01 and 23 ng/ml, respectively. Measurements of serum progesterone concentrations were conducted using an enzyme-linked fluorescence assay (ELFA) (miniVidas; bioMérieux, Inc.). Sexually intact rabbits with progesterone levels <2 ng/ml were classified as non-pseudopregnant (follicular phase), whereas does with progesterone concentrations >2 ng/ml were considered pseudopregnant (luteal phase) (YoungLai et al., 1989).

2.5 | Statistical analysis

Statistical analysis was performed by using the software R v3.6.3 and SPSS 28.0.1.0 (IBM, Ehningen, Deutschland). AMH concentrations were displayed as mean ± standard derivation and median with range. Normal distribution and homogeneity of variances were confirmed by Shapiro–Wilk and Levene's tests, respectively. In the spayed and intact animals, the Shapiro–Wilk test revealed a non-normal distribution of AMH levels; therefore, the Mann–Whitney *U* test was used. In the pseudopregnant and non-pseudopregnant intact rabbits, the Shapiro–Wilk test revealed a normal distribution of AMH levels, Levene's test showed equal variance; therefore, the parametric t-test was used to check for significant differences between the groups. For correlation analysis of AMH and follicle numbers, Spearman's rank correlation coefficient was used. For analysis of differences between vulva parameters in relation to AMH, the Mann–Whitney *U* test was used. Receiver operating characteristic

(ROC) curve analysis was performed to obtain optimal AMH cut-off values and their associated sensitivity and specificity values in the sexually intact and spayed does. A p-value <.05 was defined as statistically significant.

3 | RESULTS

Median serum AMH levels in the intact does ($n = 64$) were 1.53 ng/ml (range 0.77–3.36 ng/ml) and 0.06 ng/ml (range ≤0.01–0.23 ng/ml) in the spayed does ($n = 22$; Table 1). AMH concentrations between intact and spayed rabbits differed significantly and did not overlap ($p < .001$; Figure 1). ROC curve analysis yielded a sensitivity and specificity of 100% for a cut-off level of 0.50 ng/ml. If the cut-off level was set at 0.15 ng/ml, sensitivity and specificity were 100 and 95.5%, respectively.

In the 64 intact rabbits, gynaecological examination revealed enlarged mammary glands in 17 (26.56%) and milk production in 14 (21.87%) does. Vulva colour was red in 23 (35.94%), pink in 39 (64.06%) and pale in 0 rabbits. Enlargement of the vulva was noted in 31 (48.44%) does. Vaginal discharge was observed in none. Histopathological examination of reproductive organs did not reveal any pathologic conditions. Epithelial hyperplasia, stromal oedema and lumen dilatation of the uterus was noted in 18 (28.13%) rabbits. In 12 (18.75%) does, multiple corpora lutea on the ovaries were evident. Likewise, 12 (18.75%) does had serum progesterone levels >2 ng/ml and were therefore considered in luteal phase (pseudopregnant). Fifty-two (81.25%) does had progesterone concentrations <2 ng/ml and were therefore deemed in follicular phase (non-pseudopregnant). Median progesterone concentrations of does in follicular and luteal phase were 0.30 ng/ml (range ≤0.25–1.67 ng/ml) and 16.04 ng/ml (range 4.44–28.30 ng/ml), respectively. All 12 pseudopregnant does had multiple corpora lutea in the ovaries, and epithelial hyperplasia, stromal oedema and lumen dilatation in the uterus. Progesterone levels were significantly higher if corpora lutea were present ($p < .001$). Enlargement of mammary glands and milk production was noted in 4 (33.33%) and 3 (25%) does in luteal phase, and in 13 (25%) and 11 (21.15%) does in follicular phase.

Median AMH levels of rabbits in follicular phase were 1.52 ng/ml (range 0.77–3.36 ng/ml) and 1.55 ng/ml (range 0.80–3.32 ng/ml) in does in luteal phase (Table 1). Whether does were in follicular or luteal phase had no significant influence on measured serum AMH concentrations ($t = 0.061$, df = 62, $p = .951$; Figure 2). Vulva colour or whether a vulva was enlarged showed no significant effect on measured AMH levels ($p = .401$ and $p = .337$, respectively).

In the ovaries, mean follicle numbers were 44.0 (range 14.0–113.0) for primary, 21.3 (range 5.0–46.0) for secondary and 15.2 (range 7.0–32.0) for antral follicles. The correlation of serum AMH concentrations and the number of primary or antral follicles was not significant ($r_s = 0.241$, $p = .055$ and $r_s = 0.137$, $p = .281$ respectively), whereas AMH levels correlated significantly with the number of secondary follicles ($r_s = 0.410$, $p < .001$; Figure 3).

4 | DISCUSSION

AMH is a valuable diagnostic tool in reproductive medicine. Regarding rabbits, there is no research about serum AMH levels in the available literature. To the authors' knowledge, this was the first study to measure serum AMH concentrations in female rabbits and relate them to spay status, pseudopregnancy and ovarian follicle numbers, using a human-based CLIA.

The results of the present study suggest that a single measurement of serum AMH concentrations is indicative for the absence or presence of the ovaries in rabbits. The ovaries appear to be the main source of AMH production in the doe. This is in accordance with the findings in women, bitches and queens (Anadol et al., 2020; Axnér & Ström Holst, 2015; Flock et al., 2022; La Marca et al., 2005; Pir Yagci et al., 2016; Place et al., 2011; Themmen et al., 2016; Walter et al., 2019). Therefore, AMH appears to be an excellent diagnostic tool in does with unknown medical history where spay status is unclear. All spayed rabbits had AMH levels of ≤0.07 ng/ml, except for one with 0.23 ng/ml. This doe appeared to be a statistical outlier, as the AMH concentration was about 4 standard deviations greater than the mean in the other spayed rabbits. At a cut-off value of 0.50 ng/ml, the AMH assay had a sensitivity and specificity of 100%, which was about 8-fold higher compared with the cut-off level of 0.06 ng/ml described in dogs using the same AMH assay (Walter et al., 2019). No attempt was made to confirm absence of the ovaries in the reportedly spayed does. Thus, there was a possibility some animals may not have been spayed after all, though for no rabbit symptoms of ORS were reported.

Pseudopregnancy had no significant influence on measured serum AMH levels. Neither did colour nor enlargement of the vulva. Thus, in the sexually intact rabbits, AMH appears to be a reliable tool for diagnosing the spay status regardless of luteal or follicular phase, or changes in vulva colour or size. Similarly, in bitches

Spay status	Reproductive phase	Group size	Serum AMH concentration [ng/ml]				
			Mean	SD	Median	Min	Max
Intact		64	1.67	0.64	1.53	0.77	3.36
	Follicular phase	52	1.68	0.60	1.52	0.77	3.36
	Luteal phase	12	1.66	0.82	1.55	0.80	3.32
Spayed		22	0.05	0.04	0.06	0.01	0.23

TABLE 1 Mean serum anti-Müllerian hormone (AMH) concentrations with standard deviation (SD) and median serum AMH concentrations with minimum (min) and maximum (max) values in sexually intact and spayed female rabbits.

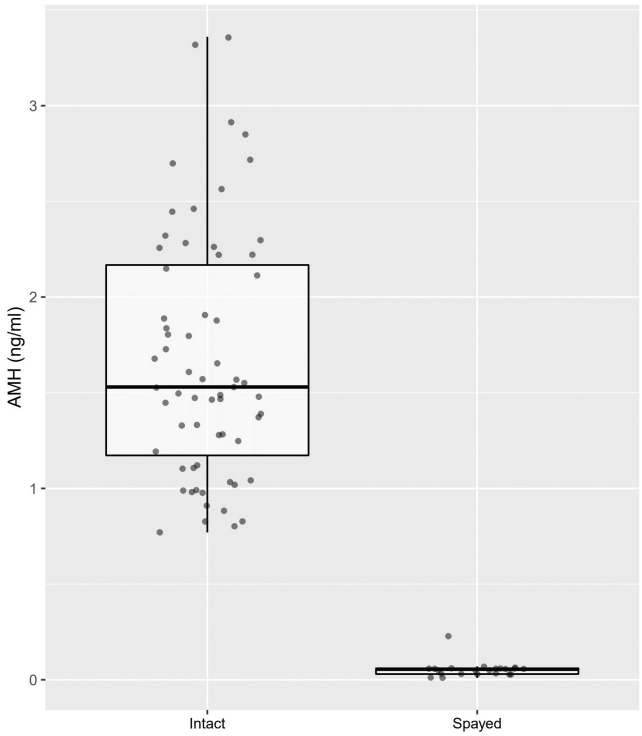

FIGURE 1 Combined dot and box plot of serum anti-Müllerian hormone (AMH) concentrations in sexually intact ($n = 64$) and spayed ($n = 22$) female rabbits. AMH levels between the intact and spayed rabbits differed significantly and did not overlap ($p < .001$).

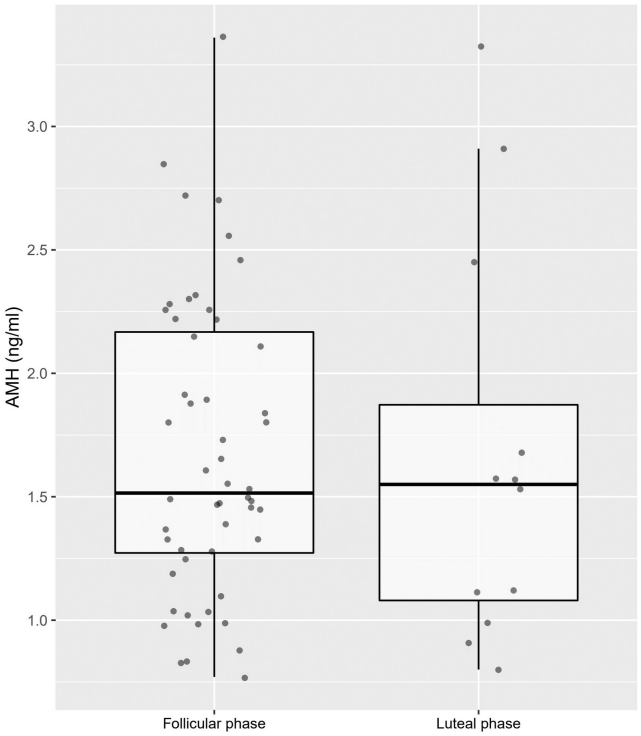

FIGURE 2 Combined dot and box plot of serum anti-Müllerian hormone (AMH) concentrations in sexually intact female rabbits in follicular ($n = 52$) and luteal ($n = 12$) phase. Whether does were in follicular or luteal phase (pseudopregnancy) had no significant influence on measured serum AMH concentrations ($t = 0.061$, $df = 62$, $p = .951$).

and queens in luteal phase, determining AMH concentrations was a reliable method for diagnosing spay status (Alm & Holst, 2018; Axnér & Ström Holst, 2015). Interestingly, in the present study, enlargement of mammary glands and milk production were noted in only 33.33 and 25% of does in luteal phase, and in 25 and 21.15% of does in follicular phase, respectively. Therefore, enlarged mammary glands and milk production were not a reliable indication of pseudopregnancy. The present study was limited by only measuring the serum AMH concentration within a single time frame of an individual rabbit. Because of possible intraindividual variations of AMH levels, obtaining multiple blood samples from the same animal throughout its reproductive cycle would have given more insight into AMH concentrations in rabbits, as significant fluctuations of AMH during the cycle could lessen the reliability of a single measurement and hamper clinical decision making. Furthermore, compared to the spayed does which were of various ages, intact rabbits were young and of similar ages. Further research is needed in intact does of advanced ages. While the time from spaying to AMH measurement was highly variable in the spayed rabbits ranging from 0.4 to 7.2 years (SD = 2.04), measured serum AMH concentrations were not as variable (SD = 0.04; Table 1). Another study limitation was that the majority of does were in follicular phase. More does in luteal phase would have made comparison of AMH levels in rabbits in luteal and follicular phase more balanced. The raise in AMH concentrations described in bitches and cows during follicular phase could not be seen in does (Brugger et al., 2011; Walter et al., 2019). Though, comparison is difficult due to the doe being an induced ovulator. As no does were gravid in the present study, it remains to be seen whether pregnancy influences AMH levels in rabbits. In horses and queens, pregnancy does not seem to influence serum AMH concentrations (Almeida et al., 2011; Flock et al., 2019).

The correlation between small growing follicles and serum AMH levels as described in cows, mares, bitches and female hamsters (Anadol et al., 2020; Claes et al., 2016; Monniaux et al., 2012; Place & Cruickshank, 2009) could be confirmed partly in the present study. While the number of secondary follicles correlated significantly with AMH levels, primary and antral follicles did not. In dioestrous bitches, while the correlation of AMH levels and secondary follicles was significant as well ($r = 0.942$, $p < .01$), serum AMH concentrations correlated negatively with antral follicle numbers ($r = -0.765$, $p < .05$) (Anadol et al., 2020). Furthermore, in hamsters, AMH levels and number of pre-antral follicles correlated significantly ($r^2 = 0.41$, $p = .0004$) (Place & Cruickshank, 2009). Whether secondary follicles are the main source of AMH production in the doe should be investigated in future studies by evaluating immunohistochemical reaction of follicles for AMH as described in mature bovine ovaries (Vigier et al., 1984). As in the present study only three sectional planes of each ovary were evaluated, some follicles might have been missed out on. More accurate follicle numbers could have been achieved by serially sectioning of the ovaries at 6 µm and evaluating every tenth section

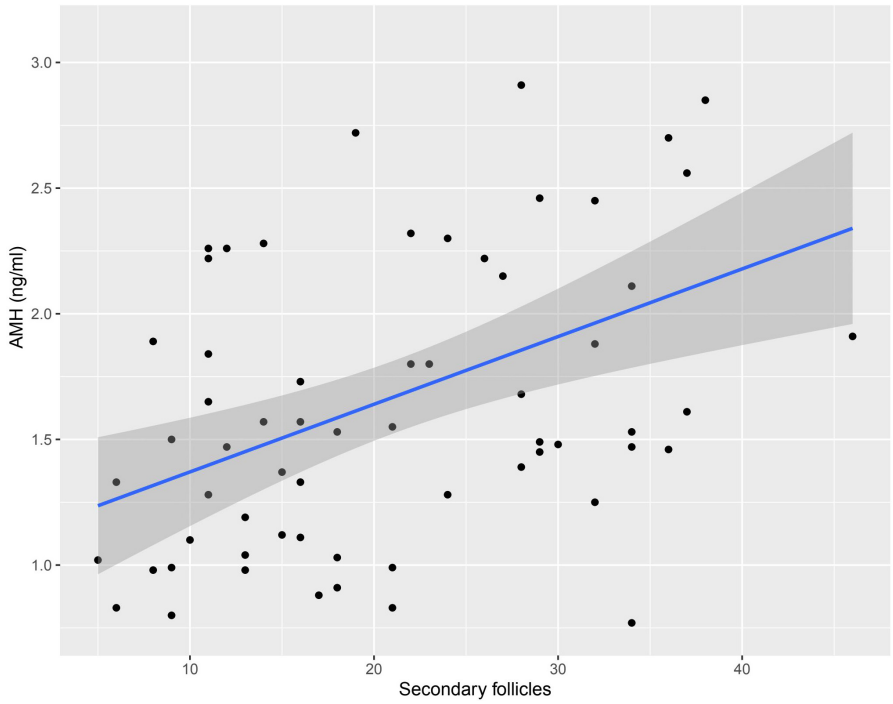

FIGURE 3 Linear regression of serum anti-Müllerian hormone (AMH) concentrations and number of secondary follicles. AMH levels correlated significantly with the number of secondary follicles ($r_s = 0.410$, $p < .001$).

as described in hamsters (Place & Cruickshank, 2009). Though, a statistical average of follicle counts can be expected by using three representative sections from each ovary. A stereological approach using a fractionator, optical dissector technique as described in the mouse ovary, might have yielded more precise follicle counts (Myers et al., 2004).

The same human-based AMH CLIA used in the present study has been described in female intact and spayed dogs and cats (Flock et al., 2022; Walter et al., 2019). Median serum AMH concentrations in the intact does (1.53 ng/ml) were about 2.4-fold higher compared to the intact bitches (0.65 ng/ml) and about 5.6-fold lower compared with intact queens (8.46 ng/ml) (Flock et al., 2022; Walter et al., 2019). Whether this represents absolute higher levels of AMH in does and queens or better cross-reactivity of rabbit and feline AMH with the assays murine anti-AMH antibodies remains to be determined. Spayed rabbits had 6-fold higher median AMH concentrations compared with spayed bitches and queens (both <0.01 ng/ml) (Flock et al., 2022; Walter et al., 2019). Interestingly, while in bitches no AMH was left in circulation 1 year after spaying (Walter et al., 2019), in the present study, even after up to 7.2 years after spaying of does, traces of AMH could still be measured. This could be due to another small source of AMH production in the doe or the erroneously detection of a different protein in the assay used. In dogs, anti-mouse antibodies can cause erroneously increased AMH levels (Bergman et al., 2019). The human-based AMH assay used in the present study was validated for rabbits by determination of intra and inter-assay coefficients of variation and by comparison of optical density parallel to the AMH standard curve. While this displayed evidence of parallelism of assay results, direct evidence of AMH measurement was not provided. Further research is needed in rabbits.

As stated in the introduction section, AMH has a wide array of diagnostic applications in veterinary medicine. With the present study we hope to promote further research regarding these applications in rabbits, for example as a diagnostic tool for ORS, assessment of fertility and gonadal disorders. In rabbits, due to the fragile consistency of the ovaries, risk of incomplete gonadectomy and ORS is present (Harcourt-Brown & Chitty, 2013). It remains to be determined whether AMH can indicate presence of ovarian tissue in cases of ORS in rabbits as described in dogs and cats (Flock et al., 2022; Karakas Alkan et al., 2019; Place et al., 2011; Turna Yilmaz et al., 2015) and whether AMH can be used as a predictor for the response to superovulation as reported in cattle, sheep and mares (Umer et al., 2019), or as a diagnostic tool for granulosa cell tumours, a neoplasia which also infrequently occurs in does (Walter et al., 2010).

5 | CONCLUSION

In conclusion, a single determination of the serum AMH concentration, using a human-based chemiluminescence immunoassay (CLIA), was adequate to distinguish spayed from intact female rabbits, making AMH an excellent marker for presence or absence of the ovaries in the doe. Consequently, the doe's ovaries appear to be the main source of AMH production. In the sexually intact rabbits, whether does were pseudopregnant or not had no influence on measured AMH levels. While the correlation of serum AMH concentrations and the number of primary or antral follicles was not significant, AMH levels correlated significantly with the number of secondary follicles.

AUTHOR CONTRIBUTIONS

Florian Böhmer contributed to acquisition of specimens from intact female rabbits, gynaecological examination, measurement of serum progesterone concentrations and writing of the manuscript;

Katharina Erber contributed to pathohistological examination and follicle counts; Anja Ewringmann contributed to acquisition of blood samples from spayed does; Ruth Klein did the AMH assay; Sven Reese and Christine Böhmer contributed to statistical analysis; Andrea Meyer-Lindenberg and Beate Walter contributed to project guidance and revising of the manuscript.

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CONFLICT OF INTEREST

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

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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