Clinical use of Anti-Müllerian Hormone to Monitor Resumption of Ovarian Activity following Removal of a 4.7 mg Deslorelin Implant in Queens.

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Research Article

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Abstract

Objective: The use of deslorelin implantsto control reproduction in cats is increasing but because of its prolonged duration, cat breeders oftenrequest implant removal before the end of the treatment. Assaying Anti Mullerian Hormone (AMH) concentrations might be useful to predict time of resumption of ovarian activity in deslorelin-treated queens following implant removal. In queens a minimum of 3 weeks during increasing photoperiod after implant removal has been described for resumption of ovarian activity but no information about AMH concentrations were observed for determining ovarian activity.

Animals: Sixteen queens in whichdeslorelinimplants were surgically removed after3, 6 or 9 months (n= 6, 4 and 6 queens, respectively) were used in this study.

Procedures: A general and reproductive health check with a GnRH stimulation test were performed before the treatment.After implant removal queens were checked every 1-2 weeks withreproductive ultrasonography, a vaginal smear and blood collection to assay AMHconcentrations.

Results: AMH concentrations decreased significantly during treatment to $\leq$ 2.5+0.6 ng/ml ($p \leq 0.05$) and reached a nadir at 1.9$\pm$0.9 ($p<0.05$) one-week post-removal.Following implant removal AMH concentrations started to rise reaching a value of 4.3$\pm$1.2 ng/ml on the third week and were not different from pre-treatment levels on week 6 post-removal (5.8 ng/ml +0.9, $p \geq 0.05$).AMH values did not differ depending on duration of deslorelin treatment but were lower in adult queens ($p<0.05$).

Clinical relevance: AMH assay can be a useful tool to follow resumption of feline ovarian function following a deslorelin treatment.

Introduction

In adult females Anti-Müllerian Hormone (AMH) or Müllerian Inhibitory Substance is a dimeric glycoprotein formed by two 72 kDa subunits assembled by sulfide bridges belonging to the transforming growth factor-β (TGF-β) superfamily (Place et al. 2011). During early embryonic development Sertoli cell secretion of AMH suppresses the development of the paramesonephric duct system thus blocking the development of the female reproductive tract in male embryos (Rey 2005; Place et al. 2011). Females secrete AMH later in embryonic life following completion of Mullerian duct development (Josso 1986). In post-pubertal females AMH influences follicular growth prior to antral development by regulating the threshold of sensitivity to follicle stimulating hormone (FSH) of preantral and small antral follicles (Place et al. 2017) while, in adult females, AMH is produced by granulosa cells of primary, secondary and small antral follicles with the latter considered as the primary source of AMH in the bloodstream (Broekmans et al. 2008; Zec et al. 2011). Gonadectomy in mammalians markedly decreases serum AMH concentration which makes AMH assay as a highly specific and sensitive test for differentiating between neutered and entire status (La Marca et al. 2005; Claes et al. 2013; Axnér and Ström Holst 2015; Ciccarelli et al. 2018).

Deslorelin is a synthetic agonist of gonadotropin releasing hormone (GnRH) that causes downregulation of GnRH receptors at the level of the anterior hypophysis following an initial pituitary stimulation leading to a short-lived secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Padula 2005). Its off-label use for control of reproduction, postponement of puberty and ovulation induction has been reported in cats (Munson et al. 2001; Rubion et al. 2006; Toydemir et al. 2012; Ackermann et al. 2012; Risso et al. 2012; Goericke-Pesch et al. 2013; Pisu and Veronesi 2014; Cecchetto et al. 2017). Information on concentration of AMH in deslorelin treated queens might be useful to monitor resumption of ovarian function following implant removal. In queens, the interval between deslorelin implant removal and resumption of ovarian function is 3 weeks during increasing photoperiod and up to 7 weeks during decreasing photoperiod (Ferré-Dolcet et al. 2021a). As photoperiod may be an important factor in estrus expression, the aim of this study was to assess whether AMH assay can be a useful tool to monitor resumption of ovarian activity following a prolonged period of ovarian inactivity such as after a deslorelin treatment.

Material And Methods

Animals

Privately owned queens with a request of temporary control of reproduction were presented to the Veterinary Teaching Hospital of the University of Padua, Italy. For inclusion in the study, the queens should be sexually mature (having had at least one heat), in good general health conditions and not experiencing a diestrual phase (Progesterone< 1.0 ng/ml). The study was designed as a non-blinded and non-randomized study where queens were treated with a 4.7 mg deslorelin implant for 3-, 6- or 9-month duration. Implants were placed 1.5 cm cranial to the umbilical scar and owners were advised to restrict contact with intact tomcats during the first 2 weeks following implantation because of the possibility of heat induction. At the end of the study, if requested by the owner, the queen was surgically spayed.

Clinical checks, reproductive ultrasound and hormonal assay

Prior to treatment a clinical and reproductive exam and collection of a blood sample for hematology, biochemistry and hormone assay was performed. Serum progesterone (P4) concentration was assayed by an automated immunoassay analyzer (TOSOH; Futurlab, Limena (PD), Italy) in order to avoid enrolling diestrus queens into the study (P4 values > 1.0 ng/mL). Concentration of AMH was assayed using an ELISA technique (AMH Gen II Elisa™, Immunotech s.r.o., Prague, Czech Republic) in order to monitor resumption of ovarian activity during and after treatment with deslorelin. To rule out reproductive tract abnormalities, abdominal ultrasonography was performed prior to deslorelin treatment using a microconvex 3.5-5 MHz probe (Philips Affinity 50G; Philips, Milan, Italy).

Implant removal and follow up

Prior to implant removal at 3, 6 or 9 months after implantation, a GnRH stimulation test was performed by IM injection of a 50 µg (total volume 0.5 mL) gonadorelin dose (Fertagyl™, Intervet) two hours prior to blood sampling (for serum AMH assay) followed by a clinical and reproductive exam. Three, 6 or 9

months after treatment, implant removal was performed following pre-medication with IM mixture of 0.008 mg/kg of dexmedetomidine (Dexdomitor™; Zoetis), 2.0 mg/kg of ketamine (Imalgene 1000™; Merial) and 0.3 mg/kg of butorphanol (Dolorex™; MSD), and propofol (Proposure™; Merial) administered intravenously. Implant was removed through a 2 cm skin incision approximately 1.5cm cranial to the umbilical scar. The incision was closed with intradermal absorbable material (3/0 Monosin™, Braun). Following implant removal queens were checked every 7-14 days with a clinical exam, a vaginal smear and a blood sampling for serum AMH assay. In order to time resumption of ovarian activity owners were advised to let us know when the queen started to show behavioral estrus. Clear signs of heat or/and the presence of ≥50% of keratinized cells on vaginal cytology were considered as indicators of resumption of ovarian activity and a last blood sample for AMH assay was performed.

Ovariectomy and ovarian histology

Fourteen/16 queens were surgically spayed at the end of the study. Ovariectomy was performed by midline laparotomy. Ovaries were fixed for 48 hours in a 4% paraformaldehyde solution. A 4-mm thick section of each ovarian paraffin-embedded specimen was obtained with a rotary microtome (RM2145, Leica, S.p.a., Milan, Italy) stained with hematoxylin-eosin (Leica, autostainer XL) and evaluated under light microscope (Olympus BX-40; Olympus, Segrate, Italy).

Statistical analysis

AMH values were evaluated with a statistical package (SAS 9.4, SAS Institute Inc., Cary, NC, USA) and analyzed with a variance model with random and repeated animal effects. Time, age, treatment, stage of the estrus cycle, and some interactions (time by class of age and treatment by class of age) were included in the analysis. Time was indicated in a series from 0 to 8 and classified as “time 0” (day of implantation), “1” (implant removal), “2” (day 8 following removal), ”3” (day 15 following removal), “4” (day 22 following removal), “5” (day 29 following removal), “6” (day 36 following removal), “7” (day 43 following removal) and “8” (day 50 following removal). Animals were divided into younger or older than 12 months of age. A t-Student test compared the queens that presented estrus resumption during increasing or decreasing photoperiod.

Results

Animals

A total of 16 queens (1 Ragdoll, 1 Sacred Cat of Burman, 14 Europeans) were divided into three groups and treated with a 4.7 mg deslorelin implant which was left in situ for 3 (n=6), 6 (n=4) or 9 months (n=6). Three/16 queens were treated in anestrus while thirteen/16 queens were treated during the follicular phase. At the end of each experimental period all implants were removed fully (Ferré-Dolcet et al., 2020; Ferré-Dolcet et al., 2021). Results concerning duration of treatment time, age, body weight, presence of estrus signs, and the interactions time-age and treatment-age are reported in Table 1. At the end of the study, ovariectomy was requested for 14/16 queens, while the two remaining queens were mated the following year having a normal pregnancy and parturition.

Table 1

Signalment (cat ID, breed, age and body weight), estrous cycle stage at treatment onset, treatment duration of 3 (green), 6 (orange) and 9 months (blue), bo treatment and concentration of anti-Mullerian hormone (AMH) at treatment onset (Time 0), on the day of implant removal (Time 1), and on post-removal day 8 (Time 4), 29 (Time 5), 36 (Time 6) and 43 (Time 7) in 16 queens treated with a 4.7 mg deslorelin implant. As there was only one sample corresponding to Tir considered for statistical analysis.

ID	BREED	AGE (MONTHS)	BODY WEIGHT (KG) AT IMPLANTATION	PHASE OF ESTRUS AT IMPLANTATION	TREATMENT (MONTHS)	BODY WEIGTH (KG) AT REMOVAL	AMH (ng/mL) TIME 0	AMH (ng/mL) TIME 1	AMH (ng/mL) TIME 2	AMH (ng/mL) TIME 3	AMH (ng/ml TIME 4
1	European	24	3.0	ESTRUS	3	4.7	2.1	1.5	1.2	1.2	1.4
2	European	7	3.0	ANESTRUS	3	3.9	11.4	1.0		2.4	3.3
3	European	9	3.8	INTERESTRUS	3	4.5	7.4	4.7			8.8
4	European	9	3.4	ESTRUS	3	4.1	8.2	2.8	1.5		1.0
5	European	17	3.0	INTERESTRUS	3	3.4	7.8	2.4			2.4
6	European	7	3.0	ANESTRUS	3	4.2	11.4	4.7		4.5	7.9
7	European	7	2.8	ANESTRUS	6	3.6	8.8	3.0		6.5	
8	European	11	3.9	INTERESTRUS	6	4.2	7.2	5.0		2.7	3.3
9	European	96	3.2	ANESTRUS	6	4.3	6.2	1.3		0.70	
10	Ragdoll	29	3.2	INTERESTRUS	6	4.0	6.7	3.2		1.1	
11	European	6	2.3	ESTRUS	9	4.0	8.2	1.0		3.0	8.4
12	European	7	3.4	ANESTRUS	9	4.4	3.4	0.1		1.1	
13	European	7	3.8	ANESTRUS	9	5.4	9.0	4.5	3.9		
14	Birman	12	2.5	INTERESTRUS	9	2.8	5.6	0.9		1.5	
15	European	8	3.5	ESTRUS	9	4.7	7.8	17,0			3.8
16	European	12	3.4	ESTRUS	9	3.7	8.0	2.2	0.1		1.5

Anti-Müllerian hormone concentration

Average (mean±standard error) AMH concentrations at the beginning of the study prior to deslorelin treatment were 7.3±0.6 ng/mL and decreased to 2.5±0.6 ng/mL on the day of implant removal ($p<0.05$). When comparing AMH concentrations following implant removal with those prior to study onset, values at the third week were still lower (3.9±0.7 ng/ml, $p<0.05$) while values at 6 weeks were back to normal (5.8±0.9 ng/ml, $p\geq0.05$) (Table n. 2 and Figure n. 2). For interval n. 6 (between 29- and 43-days post-removal) there was only one sample and therefore this time class was not considered for the statistical evaluation. Queens under 12 months of age showed higher concentrations of AMH when compared to queens older than 12 months of age ($p<0.05$) throughout the study (Figure n. 3).

Return to estrus

Based on behavioral signs and vaginal smears, ovarian activity was restored from 3 to 7 weeks depending on the photoperiod where the implant was removed (increasing or decreasing photoperiod, respectively, $p<0.05$), irrespective of treatment length and queen's age. Mean AMH concentrations (mean±standard error) were 4.3±1.2 ng/mL and 5.1±0.9 ng/mL when return to estrus occurred when return to estrus occurred at 3 weeks or between 4 and 7weeks post implant removal, respectively ($p>0.05$) (Table 2 and Figure 4).

Table 2
Average concentrations of anti-Mullerian hormone (AMH) (ng/mL+ SE) in 16 queens of various breeds and ages treated for 3, 6 or 9 months with a 4.7 mg deslorelin implant.

Time	[AMH] ng/mL
0 (implant administration)	7,92±0,73[a]
1 (implant removal)	2,58±0,65[bc]
2 (day 8 post-removal)	1,92±0,92[c]
3 (day 15 post-removal)	2,15±0,71[c]
4 (day 22 post-removal)	3,91±0,71[bc]
5 (day 29 post-removal)	3,76±0,76[bc]
6 (day 36 post-removal)	*
7 (day 43 post-removal)	5,85±0,96[ab]

Ovariectomy and histologic evaluation of the ovaries

Ovariectomy was planned as soon as first post-implant removal estrus was detected. Different growth and follicular stages were detected in both ovaries of all queens. In addition, two queens presented histological evidence of corpora lutea (Figure 5).

Discussion

This is the first study describing AMH concentrations in deslorelin treated queens and the use of serum AMH assay to monitor resumption of ovarian activity in queens following reversible chemical neutering with deslorelin.

In agreement with previous studies, queens treated with a deslorelin implant during a non-follicular phase (interestrus or anestrus) showed estrous behavior of 2-5 days duration starting 1-2 days after implantation with the disappearance of these signs until the end of the treatment or early removal of the implant (Ferré-Dolcet et al. 2021a).

In mammals gonadectomy markedly decreases serum AMH concentration, therefore assaying AMH is considered a highly specific and sensitive way to differentiate intact from neutered animals (Axnér and Ström Holst 2015; Alm and Holst 2018). In Axnér & Ström Holst study (2015), all spayed queens had undetectable AMH values (<0.14 ng/mL), while the AMH serum concentration of the intact ones ranged from 1.3 to 19 ng/mL, being higher in younger animals (Axnér and Ström Holst 2015). Our results agree with Axnér & Ström Holst (2015) observations as AMH concentrations in our queens on the day of the first clinical check prior to deslorelin implantation (Time 0) was significantly higher than in the samples obtained on the day of implant removal (Time 1). The fact that AMH concentrations did not reach basal levels during deslorelin treatment may be due to the presence of primordial follicles (Zec et al. 2011) which are not inhibited by deslorelin treatment (Toydemir et al. 2012) and are able to secrete low quantities of AMH (Ueno et al. 1989b, a; Baarends et al. 1995; Durlinger et al. 2002; Gruijters et al. 2003). A gradual restoration of normal AMH concentrations was observed following implant removal in our queens, who reached pre-treatment levels at 3-6 weeks post-removal depending on photoperiod.

Queens are known to be long-day breeders with reproductive cyclicity being controlled by photoperiod through melatonin secretion (Olson et al. 1984). As noted above, some of the queens showing estrous at 3 weeks post-removal did so in the presence of AMH concentrations lower than pre-treatment concentrations (4.3±1.2 ng/mL, $p<0.05$) (Figure 4). In these queens, implant removal was performed at time of increasing photoperiod: this suggests that photoperiod a) may override AMH secretion and b) should be considered when monitoring resumption of ovarian function by means of AMH assay in deslorelin-treated queens. On the other side, the interval from implant removal to estrous behavior increased up to 7 weeks on average in queens in which the deslorelin implant was removed during decreasing photoperiod: these queens did not show estrous behavior despite having concentrations of AMH not different from those of queens showing estrus at 3 weeks (5.1±0.95 ng/mL, $p>0.05$). In both populations of queens histology showed that ovarian function had resumed fully at time of ovariectomy with tertiary follicles being present as it has been described previously (Ferré-Dolcet et al. 2021a). The presence of corpora lutea in the ovaries of two queens from the study could be due to the administration of 50 µg gonadorelin dose on the last clinical check provoking the luteinization of the dominant follicles (present at the time of estrus during consultation) as it has been previously described (Ferré-Dolcet et al. 2021b).

In conclusion, assaying AMH can give an indication of the degree of ovarian activity which may allow a clinical judgment in case of a delayed resumption of ovarian function. An AMH concentration of 4.3±1.2 ng/mL could indicate a cut-off value for determining complete restoration of ovarian activity in queens.

Declarations

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Conflicts of interest:

The authors declared no potential conflicts of interest.

Data availability statement:

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authors contribution:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lluis Ferré-Dolcet and Stefano Romagnoli.Silvia Ferro took part on the evaluation of the histology samples. Barbara Contiero performed the statistical analysis and evaluation. Tamara Badon took part on the AMH analysis on the lab.The first draft of the manuscript was written by Lluis Ferré-Dolcet and Christelle Fontaine, Donatella Gelli and Stefano Romagnoli commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval:

The present study was approved by Ethics Committee of the University of Padova (Project n. 329860).

Consent to participate:

Informed consent was obtained

Consent for publication:

Informed consent was obtained

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Figures

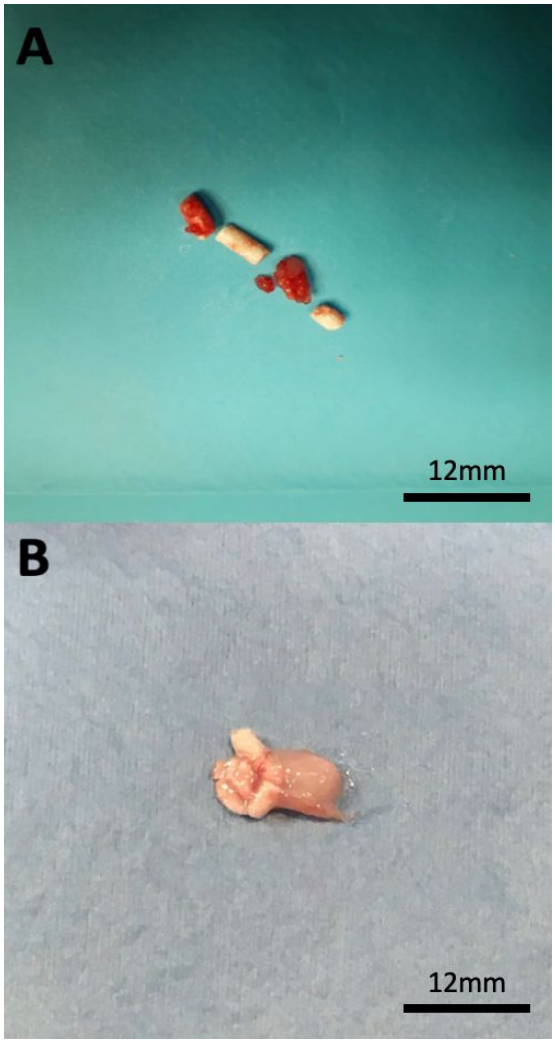

Figure 1

(a) A 4.7 mg deslorelin implant removed from a queen treated for three months embedded in abdominal fat following surgical removal. (b) A 4.7 mg deslorelin implant removed from a queen nine months following administrationruptured in several small pieces while being surgically removed.

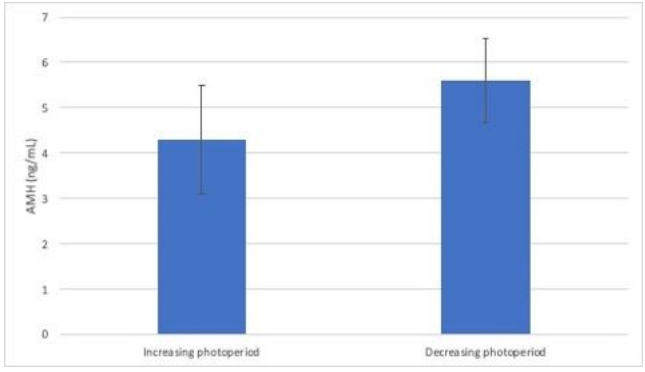

Figure 2

Average concentrations of anti-Mullerian hormone (AMH) (ng/mL + SE) in 16 queens of various breeds and ages treated for 3, 6 or 9 months with a 4.7 mg deslorelin implant. Time 0 = day of implantation; 1 = day of implant removal; 2 = day 8 post-removal; 3 = day 15 post-removal; 4 = day 22 post-removal; 5 = day 29 post-removal; 6 = day 36 post-removal; 7= day 43 post-removal. (As for period 6 there was only one sample, this interval was excluded from statistical analysis)

Figure 3

Average concentration of anti-Mullerian hormone (AMH) (ng/mL) as a function of time and age in 16 queens of various breeds and ages treated for 3, 6 or 9 months with a 4.7 mg deslorelin implant. Time 0= day of implantation; 1=day of implant removal; 2= 1-week post-removal; 3 = 2 weeks post removal; 4 = 3 weeks post removal; 5 = 4 weeks post removal; 7 = 6 weeks post removal in < 12-months subject-group and ≥ 12-months subject-group. The blue line represents queens younger than 12 months of age (n=9), while the orange one represents queens older than 12 months (n=6). For period 6 there was only one sample, therefore this interval was excluded from the statistical analysis.

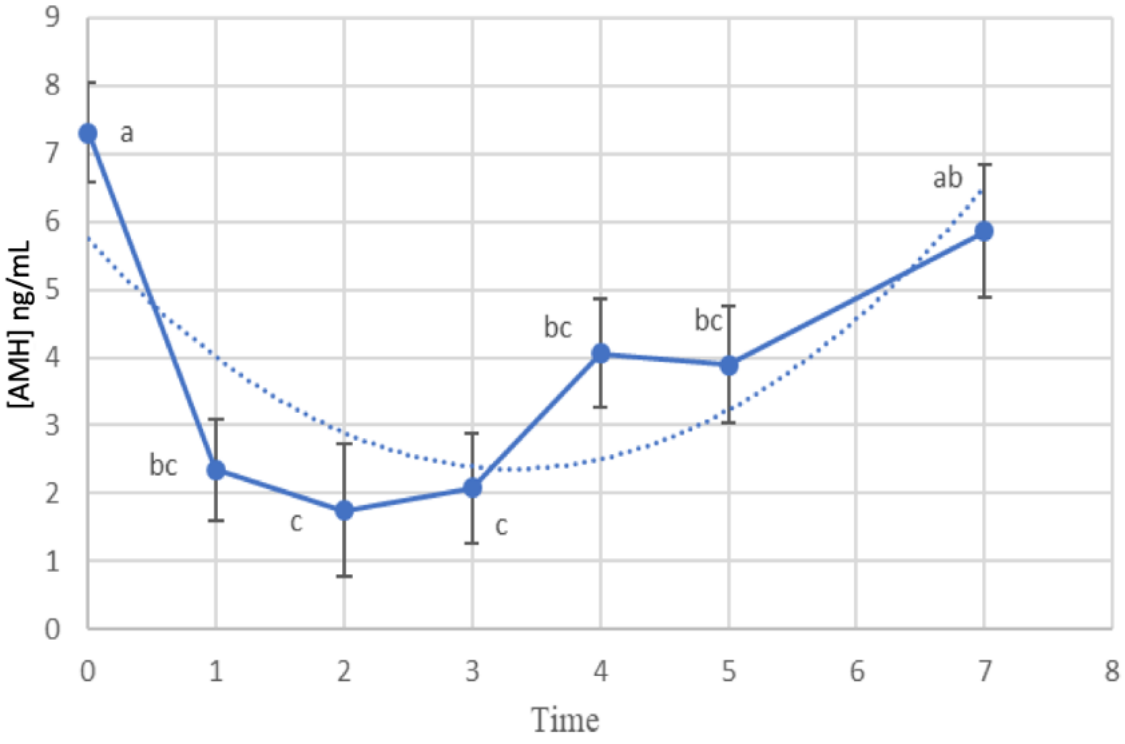

Figure 4

Mean concentrations of anti-Mullerian hormone (AMH) in 16 queens of various breeds and ages at time of return to estrus following removal of a 4.7 mg deslorelin implant. Mean AMH concentrations were 4.3±1.2 ng/mL at 3 weeks post removal (increasing photoperiod, n=7) and 5.6±0.95 ng/mL at 4-7 weeks post removal (decreasing photoperiod, n=9) (p>0.05). Despite no difference in AMH concentrations (reflecting ovarian activity in both groups of queens) estrus occurred earlier when implants were removed at time of increasing photoperiod (3 weeks post-removal) as opposed to time of decreasing photoperiod (6-7 weeks post-removal)

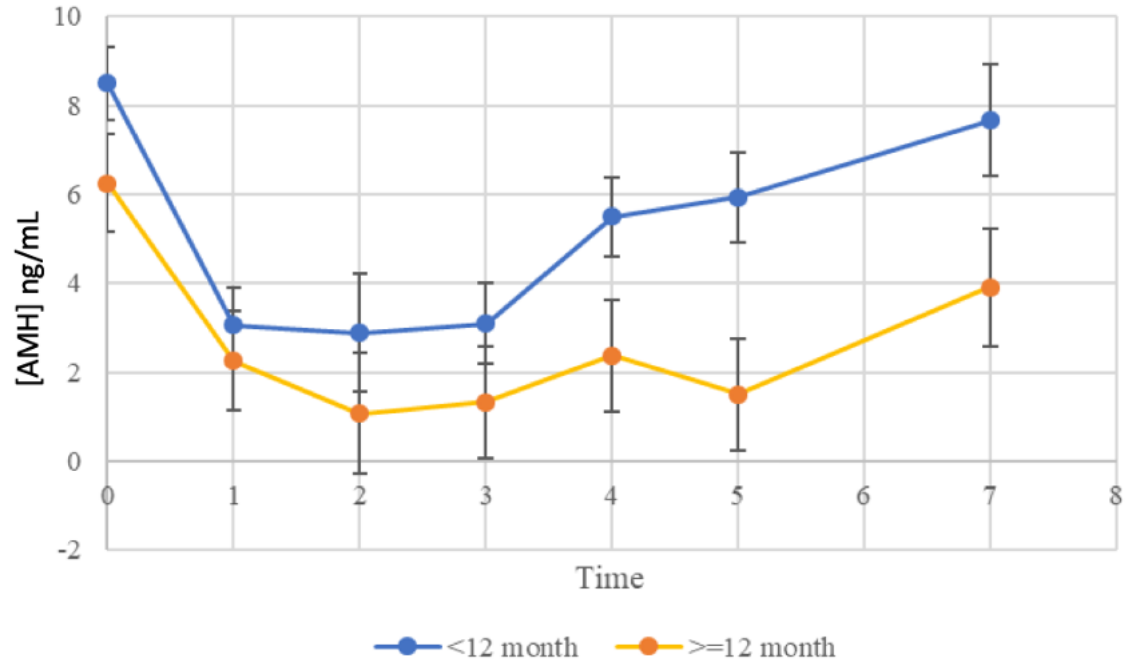

Figure 5

Ovarian histology (4x) of a queen treated with a 4.7 mg implant for nine months. Ovariectomy performed 47 days following implant removal once estrus was confirmed behaviorally and cytologically. Follicles at different stages are present: primordial follicles (black arrows), primary follicles (black arrowheads) secondary follicles (white arrow). Inset: high magnification (10x) of a mature follicle showing the cumulus oophorus surrounded by the corona radiata.