Theriogenology 106 (2018) 15–20

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Identifying ovarian tissue in the bitch using anti-Müllerian hormone (AMH) or luteinizing hormone (LH)

Helene Alm [a, b], Bodil S. Holst [c, d, *]

[a] Tyresö Djurklinik, Öringevägen 1, SE-13549 Tyresö, Sweden
[b] Anicura Regional Animal Hospital Bagarmossen, Ljusnevägen 17, 128 48 Bagarmossen, Sweden
[c] Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, SE-750 07 Uppsala, Sweden
[d] Centre for Reproductive Biology in Uppsala (CRU), Box 7054, SE-750 07 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 24 March 2017
Received in revised form
19 September 2017
Accepted 19 September 2017
Available online 27 September 2017

Keywords:
Anti-Müllerian hormone
LH
Spay
Ovary
Ovarian remnant

ABSTRACT

Reliable methods for determining whether or not a bitch has ovarian tissue present are needed for cases with unknown neutering status. Vaginal cytology consistent with heat is indicative of functional ovarian tissue. Other methods are required when the bitch is not presented in suspected heat. Progesterone can be analyzed during 2 months after suspected heat. During other stages, assays for the analyses of anti-Müllerian hormone (AMH) and luteinizing hormone (LH) have been used. The AMH assay is expected to give detectable concentrations (positive) in bitches with ovarian tissue, and the LH assay should give negative results in intact bitches, except during the pre-ovulatory LH peak. The aim of the present study was to study the diagnostic efficiency for detecting ovarian tissue in bitches using an AMH assay developed for human samples, and a semi-quantitative rapid immune migration (RIM™) LH assay developed for use in dogs. An AMH concentration of ≥0.1 μg/L, and an LH concentration of ≤1 μg/L, was set as the cut-off for presence of ovarian tissue. Client or staff owned bitches were included (N = 125). There were 73 intact bitches that were classified as being in heat (N = 25); in luteal phase (N = 12); or in anestrus (N = 36), and 52 spayed bitches that showed no clinical signs of estrogen influence. In total 64 of the 73 intact bitches (88%) were correctly identified using AMH, and 70/73 (96%) intact bitches were correctly identified using the LH assay. Excluding bitches in heat, the corresponding figures were 42/48 (88%) for AMH and 48/48 (100%) for LH. Of the 52 spayed bitches, 51 (98%) were correctly identified using the AMH assay and 49 (94%) were correctly identified using the LH assay. In this population, the predictive value of a positive AMH for intact bitches was 98%, and of a negative AMH for spayed bitches was 85%. Excluding bitches in heat, the predictive value of a negative LH test for intact bitches was 94%, and the predictive value of a positive LH test for identifying spayed bitches was 100%. It was concluded that analyses of AMH and LH are useful for detecting ovarian tissue in bitches, but that low concentrations of AMH may be obtained in intact bitches, classifying them as spayed. For LH, bitches in suspected estrus should not be tested to avoid the pre-ovulatory LH-surge, that otherwise may cause intact bitches being incorrectly identified as spayed.

1. Introduction

There are several situations when it is desirable to be able to determine whether or not ovarian tissue is present in a bitch. The bitch may have been abandoned by her owner, and without a medical history it is difficult to know whether or not she is spayed. In other cases spayed bitches are presented with the suspicion of the ovarian remnant syndrome, ORS, usually because of signs associated with estrous behavior, but occasionally because of other signs, such as abdominal pain [1]. If the bitch is presented in heat, the diagnosis is usually straight forward. Vaginal cytology that is consistent with proestrus, estrus or metestrus is indicative of estrogen influence and thus of functional ovarian tissue (Fig. 1a). Rare causes of estrogen influence, such as exposure to exogenous estrogen treatments or excessive estrogen production by the adrenals, should preferably be ruled out. If the bitch has been showing

* Corresponding author. Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, SE-750 07 Uppsala, Sweden.
E-mail addresses: helene@tyresodjurklinik.se (H. Alm), Bodil.strom-holst@slu.se (B.S. Holst).

https://doi.org/10.1016/j.theriogenology.2017.09.028
0093-691X/

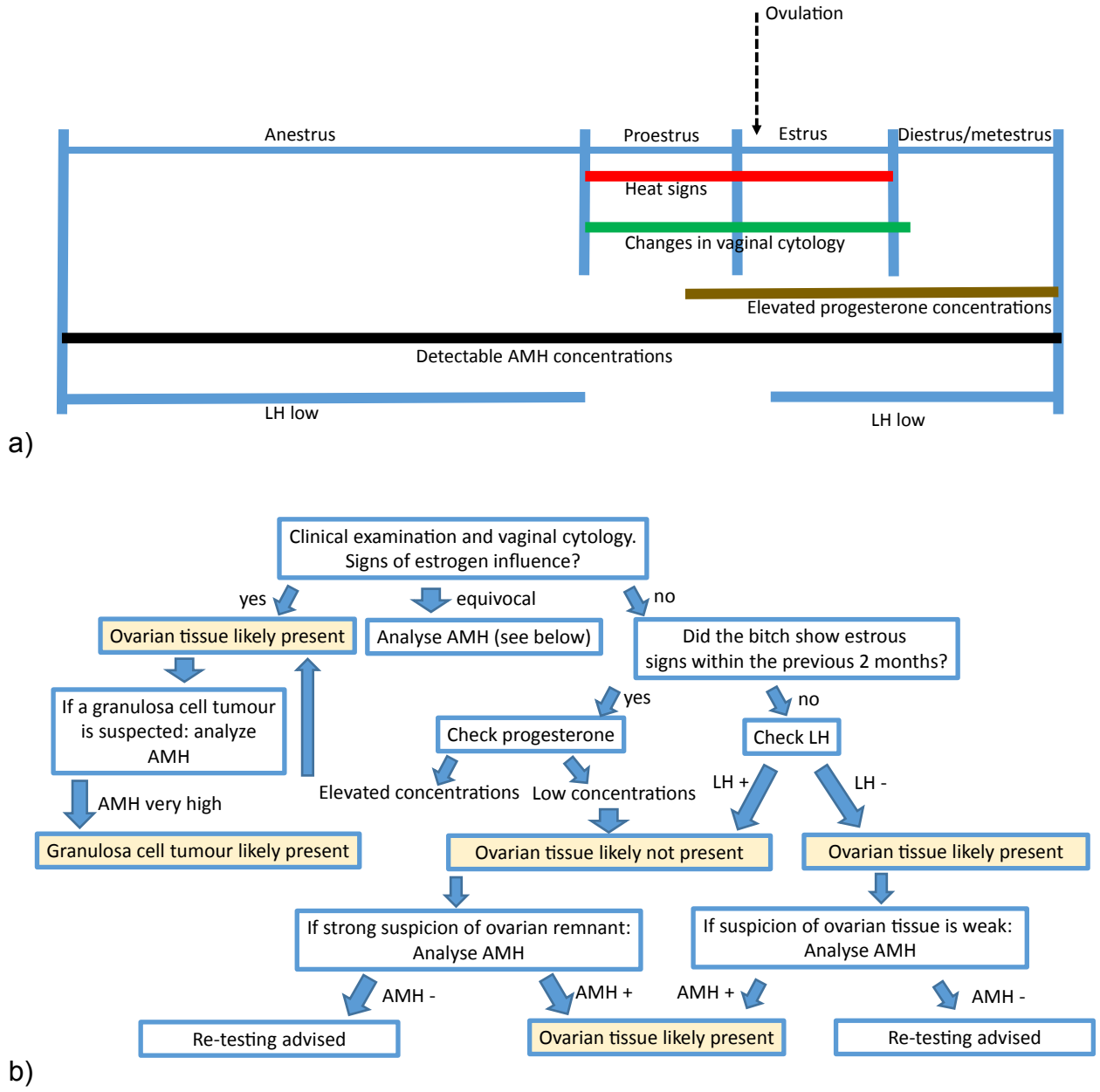

Fig. 1. **a**. Schematic drawing of possible tests for detecting ovarian tissue during the different stages of the estrus cycle. **b**. Flow chart showing steps in investigations of ovaries in bitches, based on the results of the present and other studies.

signs of estrus, and is presented within 2 months thereafter, serum progesterone can be analyzed, and increased concentrations indicate presence of luteal tissue and thus ovaries or ovarian remnants [2] (Fig. 1a).

When it is not known if the bitch has shown clinical signs of estrus, or more than two months have passed since she showed such signs, investigations may be challenging. In cases of ORS, the time from surgery to appearance of the first signs of heat has been described to vary between one month and eleven years [3,4], longer for bitches with neoplastic changes of the remnants [3]. Methods for visualizing the remnants, such as ultrasonography, are not always reliable [3,4]. Although most often found at the region of the ovarian pedicles, more often for the right ovary than left ovary [3], ovarian structures have also been found in the omentum, making them difficult to find even with laparotomy [4]. Therefore, blood tests to confirm the diagnosis are needed. One hormone that has been used for determining neutering status is luteinizing hormone (LH). LH is secreted in a pulsatile manner by the pituitary in response to gonadotrophin-releasing hormone, GnRH. Low concentrations of LH are present throughout the cycle of the bitch, with dramatic increases during the preovulatory surge. Transient pulsatile increases, lasting 50–110 min, can be seen every 1–8 h throughout the cycle, increasing in frequency during late anestrus [5,6]. Ovarian hormones, mainly estradiol, provide negative feedback on the LH secretion and in ovariectomized bitches the LH concentrations are elevated, although secretion is still pulsatile [5,7]. Analysis of serum concentrations of LH has previously been reported to be highly sensitive in finding spayed bitches, but less

specific, erroneously categorizing intact bitches as spayed. This has been suggested to be caused either by the transient pulsatile increases, or, if the bitches are analyzed during heat, by the preovulatory LH surge [8].

The anti-Müllerian hormone (AMH) has also shown promising results for determining presence of ovarian tissue in both cats and dogs [9–12] (Fig. 1a). AMH was first discovered as the agent causing regression of the Müllerian tubes in the male fetus, but has subsequently been found to be present in both males and females [13]. In the female dog, as in other species, AMH is secreted by the granulosa cells of pre-antral and antral stage follicles in the ovary after puberty [10,14]. AMH inhibits initiation of primordial follicle growth [15] and also the stimulatory effect of FSH on growth of preantral and small antral follicles [16], important during cyclic recruitment. AMH has a structure that has been well preserved through evolution and thus is similar between different species [20]. Enzyme-linked immunosorbent assays (ELISAs) developed for humans have been possible to use in several species, including dogs [9,14,17,18]. Canine-specific assays have been developed, some showing promising [10,11] and others less promising [19] results for predicting presence of ovarian tissue. Other potential indications for measuring serum AMH concentrations in dogs is e.g. selection of breeding bitches, because of a relationship between AMH concentration and litter size [20], prediction of estrus, because of elevated concentrations 8–9 days before the LH-surge [14], and diagnosis of Sertoli and granulosa cell tumors [18,21].

The aim of the present study was to study the diagnostic efficiency for detecting ovarian tissue in bitches using an AMH assay developed for human samples, and a semi-quantitative rapid immune migration (RIM™) LH assay developed for use in dogs.

2. Material and methods

The study was approved by the Uppsala Ethical Committee of Animal Experimentation (C 136/13). All owners gave spoken or written consent.

Blood samples were collected at four different clinics or animal hospitals in Sweden: Tyresö Animal Clinic, Anicura Regional Animal Hospital Bagarmossen, Anicura Animal Hospital Albano and the University Animal Hospital, Swedish University of Agricultural Sciences (SLU), Uppsala.

2.1. Dogs

125 client or staff owned bitches were included, 52 spayed and 73 intact bitches. One sample per bitch was included. Common breeds were: Labrador retriever (N = 11), Springer spaniel (N = 11), Staffordshire bullterrier (N = 7), Portuguese water dog (N = 6), Alsatian (N = 6), Miniature schnauzer (N = 5), and Standard poodle (N = 5). For other breeds the number of individuals were 4 or less. 13 dogs were mixed breed. The median age of all bitches was 6.8 years (inter-quartile range, IQR, 3.5–9 years, range 0.5–13 years) and their median weight was 20.2 kg (IQR 9.5–28.7 kg, range 2.6–60.2 kg). For the spayed bitches, median age was 9.0 years (IQR 6.0–10.5 years, range 2–13 years) and median weight 21.0 kg (IQR 8.5–27.0 kg, range 2.6–60.2 kg). Intact bitches had a median age of 5.0 years (IQR 3.0–7.5 years, range 0.5–11 years) and median weight 17.7 kg (IQR 10.0–29.1 kg, range 3.7–47.0 kg).

The 73 intact bitches were classified as in heat if they had a bloody vaginal discharge (N = 25) and in luteal phase if sampled within two months after the discharge had ceased according to the owner (N = 12). The bitches were classified as in anestrus if they showed no clinical signs of heat and their last heat was more than 2 months ago (N = 36). None of the spayed bitches showed any clinical signs of estrogen influence.

2.2. Sampling

Blood samples were collected in a routine manner from the cephalic vein. Sample time was not standardized, and samples were collected during different times of the day. After clotting, the samples were centrifuged and the serum was stored frozen at −20 °C for maximum three months and then at −70 °C for maximum a year, until analysis.

2.3. Hormone analyses

AMH and LH were analyzed in all samples.

For analysis of AMH, an enzyme-linked immunosorbent assay (AMH Gen II Elisa, Beckman Coulter) was used according to manufacturer instructions, as described previously [18]. A concentration as high as or higher than the lowest standard point; ≥ 0.1 μg/L, was set as the limit for presence of ovarian tissue [21].

A semi-quantitative rapid immune migration (RIM™) assay (Witness®LH, Synbiotics Corporation, San Diego, CA, USA) was used for analyzing LH. The assay uses gold-conjugated antibodies to give a visual line in the presence of LH. According to the manufacturer, a line in the test area that has an intensity that is similar to or greater than the control line is considered positive, with LH concentrations being >1 ng/mL (1 μg/L) (Fig. 2). A positive result was regarded indicative of ovariectomy in bitches that were not in heat. The LH tests were blinded and all tests were performed by one of the authors (BSH).

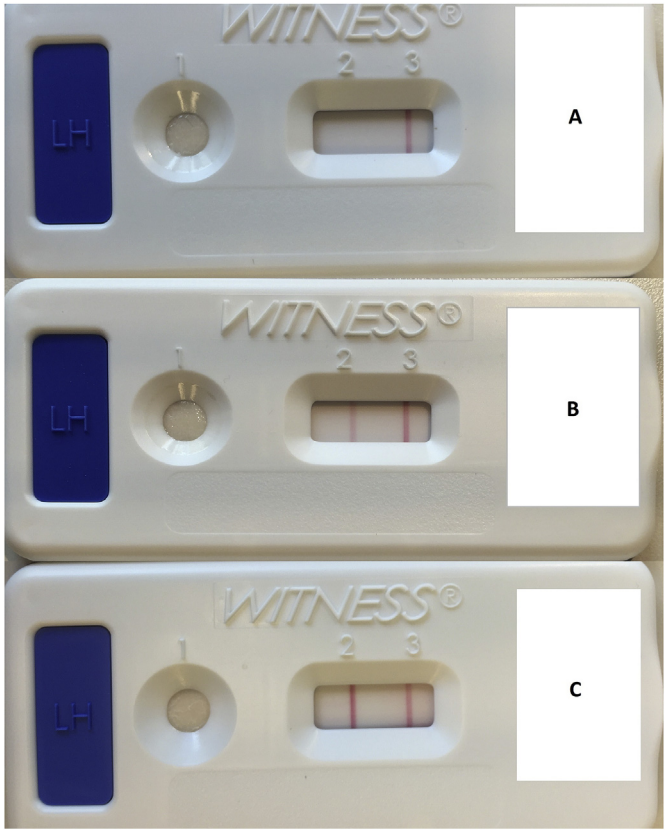

Fig. 2. Results from a semi-quantitative rapid immune migration (RIM™) LH assay developed for use in dogs. A: A negative test, with no visible test line (2) and a positive control line (3). B: A negative test, with a test line (2) weaker than the control line (3), C: a positive test with a test line (2) similar to or, as in this case, stronger than the control line (3).

2.4. Statistical analysis

Descriptive data were calculated using Minitab 17. For intact bitches, a linear regression model was fitted with LogAMH as response, age and weight as continuous predictors and estrus cycle as categorical predictor. Sensitivity was calculated as the number of bitches categorized as intact by the test divided by the total number of intact bitches. Specificity was calculated as the number of bitches categorized as spayed by the test divided by the total number of spayed bitches. The positive predictive value (PPV) was calculated as the number of intact bitches correctly identified by the test divided by the total number of bitches categorized as intact based on the test result, and the negative predictive value (NPV) was calculated as the number of spayed bitches correctly categorized by the test divided by the total number of bitches categorized as spayed based on the test result.

3. Results

In total 64 out of the 73 intact bitches (88%) were correctly identified using AMH (Table 1), and 70/73 (96%) (Table 2) were correctly identified using the LH assay. Excluding bitches in heat, the corresponding figures were 42/48 (88%) for AMH (Table 1) and 48/48 (100%) for LH (Table 2).

Of the 52 spayed bitches, 51 (98%) were correctly identified using the AMH assay and 49 (94%) were correctly identified using the LH assay. In one of the samples there was hemolysis, excluding this bitch 50/52 (96%) of the spayed bitches were correctly identified using the LH assay. The AMH concentration in the spayed bitch with a detectable AMH concentration was low (0.1 μg/L). The two spayed bitches without hemolysis that were incorrectly identified with the LH assay had both been spayed for more than five years.

In this population, with 58% intact bitches (48% intact when excluding those in heat), the predictive value of a positive AMH for intact bitches was 98%, and of a negative AMH for spayed bitches was 85%. The predictive value of a negative LH for intact bitches was 96%, and of a positive LH for spayed bitches 94%. Excluding bitches in heat, the predictive value of a positive AMH for intact bitches was 98%, and the predictive value of a negative AMH for spayed bitches was 89%. For LH, excluding bitches in heat, the predictive value of a negative test for intact bitches was 94%, and for a positive test it was 100% for identifying spayed bitches. The concentration of AMH in intact bitches was neither associated with age or weight, nor with estrus cycle. The median AMH concentration was 0.4 μg/L stages, ranging from <0.1 μg/L to 2.2 μg/L.

4. Discussion

In the present study of bitches during different stages of the estrus cycle and of spayed bitches, both AMH and of LH had a high sensitivity for determining presence of ovaries. Excluding bitches in estrus, AMH correctly identified 88% and LH 100% of the intact bitches.

AMH concentrations in intact bitches were neither associated with age or weight, nor with the estrus cycle. This is in contrast to a previous study, showing a decreasing AMH concentration with age of the bitches [20], as has been shown in other species such as people [22] and mice [23]. An association between AMH concentrations and dog weight has recently been reported, describing significantly lower concentrations in giant breeds [20]. The lack of association between AMH concentrations and age or weight in the present study may be due to a limited variation of age and weight. That there was no significant relationship between AMH concentrations and estrus cycle is in accordance with a previous study [19]. A transient increase in AMH associated with an increasing number of pre-antral and small antral follicles has been described by Nagashima and co-workers [14]. However, although concentrations were elevated for one to two weeks, there were large variations between individual bitches concerning both baseline and peak values, possibly contributing to the lack of effect of estrus cycle in the present study.

When investigating whether ovaries or not are present in a bitch, a thorough clinical examination and vaginal cytology is an important first step to determine if the bitch is showing signs of estrogen influence. Such signs may be swelling of the vulva, a vaginal discharge and presence of cornified vaginal cells (superficial cells). If a clinical and cytological investigation is clearly indicative of estrogen influence, this indicates presence of ovarian tissue. In less clear cases, further tests may help in the decision making (Fig. 1b). If testing is performed within 2 months of a period of suspected signs of estrus, progesterone can be analyzed. Testing for LH is both sensitive and specific, but bitches potentially in heat should not be tested, as the pre-ovulatory LH-peak during this phase may lead to misleading results. In the present study, 24% of bitches in heat (6/25) had a positive LH result, potentially leading to a misinterpretation of the ovarian status. Bitches in heat were suggested to contribute to the high number of “false positive“ bitches in a previous study on LH for determining ovarian status [8]. In the present study, excluding bitches in heat, no intact bitches were wrongly classified (positive) with the LH analysis. No measures (such as standardized sampling times) were undertaken to avoid LH pulses, and the results are thus representative of the clinical situation. The reason for the difference between the two studies is not known. Possible causes include different populations (the proportion of bitches in heat was not known in the previous study) and differences between the LH tests. There is a certain degree of subjectivity in the interpretation of the test line, especially when deciding if the line is similar (but slightly weaker) in intensity (and the test result thus is positive), or if the line is clearly

Table 1
Results for 125 serum samples that were tested with a commercial test for AMH to distinguish between ovariectomized and intact bitches. Results when excluding bitches in heat (N = 100) within parentheses.

Ovarian status	Result of AMH analysis		Total
	AMH pos (≥0.1 μg/L)	AMH neg (<0.1 μg/L)	
Intact (Not in heat)	64 (42)	9 (6)	73 (48)
Spayed	1	51	52
Total (Not in heat)	65 (43)	60 (57)	125 (100)

AMH pos: indicative of ovarian tissue present, AMH neg: indicative of ovariectomized.
SE for detecting ovarian tissue: 64/73 = 88% SP for detecting ovariectomy: 51/52 = 98% PPV: 64/65 = 98%, NPV: 51/60 = 85%.
Excluding bitches in heat: SE: 42/48 = 88%, SP: 51/52: 98% PPV: 42/43: 98%, NPV: 51/57: 89%.

Table 2
Results for 125 serum samples that were tested with a commercial test for LH to distinguish between ovariectomized and intact bitches. Results when excluding bitches in heat (N = 100) within parentheses.

Ovarian status	Result of LH analysis		Total
	LH neg (≤1 μg/L)	LH pos (>1 μg/L)	
Intact (Not in heat)	70 (48)	3 (0)	73 (48)
spayed	3	49	52
Total (Not in heat)	73 (51)	52 (49)	125 (100)

LH neg: indicative of ovarian tissue, LH pos: indicative of ovariectomized.
SE: 70/73 = 96%, SP 49/52 = 94% NPV: 70/73: 96%, PPV: 49/52: 94%.
Excluding bitches in heat: SE: 48/48 = 100%, SP: 49/52 = 94% NPV: 48/51 = 94%, PPV: 49/49 = 100%.

weaker, corresponding to a negative test result. In cases when this decision is difficult to make, a repeated sampling can be advised. A new sampling can also be recommended if the test result is positive and it is not clear whether the bitch was sampled during heat or not. The AMH and LH tests can be combined, using a positive AMH as a reliable means of identifying intact bitches and a positive LH as a reliable test for identifying spayed bitches (if bitches in heat are excluded).

A drawback of the present study was that the studied bitches were either intact or spayed, with no case of ORS included. It has previously been described that the LH concentrations are significantly higher in bitches with ORS than in anestrous bitches, although lower than in spayed bitches, and it has been suggested that this may be due to a lower sensitivity of the negative feed-back by ovarian hormones [4]. Higher LH concentrations than in anestrous bitches have also been described in bitches with neoplastic changes of the ovarian remnant, often a granulosa cell tumor [24], and it has been suggested that a high concentration of gonadotropins contributes to the development of GCT [4]. How these bitches will be categorized will depend on the cut-off of the specific LH test, but it is possible that the number of "false positive" LH tests (categorizing bitches with ovarian remnants as spayed) will be higher in bitches with ORS than in intact bitches. If a granulosa cell tumor is suspected, analysis of AMH is recommended, as these tumors give rise to high AMH concentrations [21].

After spaying, the LH concentration increases quickly, followed by a sharp drop by the tenth week after spaying. After that, it rises slowly until week 42 when it reaches a plateau [25]. The median LH concentration in spayed bitches is 30-fold higher than the concentration in anestrus bitches [26]. Interestingly, three spayed bitches tested negative for LH. One of these samples was hemolytic, with a line clearly weaker than the control line. This test result is in agreement with the recommendation of the manufacturer not to use hemolyzed or lipemic samples. The other two spayed bitches that tested negative had been spayed for many years. In bitches with experimentally induced hypothyroidism, the lack of feed-back from thyroid hormones initially caused an increase in thyroid stimulating hormone (TSH), but the high TSH concentration declined with time [27]. Such a loss of pituitary response to the lack of negative feed-back could potentially be a cause of the negative LH result in the two bitches with negative results, but, on the other hand, bitches that had been ovariohysterectomized >7 and > 10 years ago have previously been shown to have high LH concentrations [8]. There may be individual variations between bitches, and the background to the negative LH results in these two spayed bitches is not known.

Using the AMH assay, one of the 52 spayed bitches had a detectable AMH concentration, just on the cut-off level (0.1 μg/L). That bitch showed no clinical signs of estrogen influence, and was positive for LH, and it is therefore not likely that she had an unknown ovarian remnant. Cases with increased AMH concentration in spayed bitches were also described by Place and co-workers [9]. The cause is not known, but it may be due to assay interference. Assay interference due to heterophilic antibodies, causing falsely high AMH concentrations, has been described in a human patient [28], and has also been suspected in bitches [21]. The number of intact bitches (12%) testing negative for AMH was higher than the number of spayed bitches with a falsely high AMH concentration. It is important to realize that AMH concentrations can be low in intact bitches using this assay. Results from AMH analyses, especially if negative, should preferably be interpreted in conjunction with other data. Non-detectable AMH concentrations in intact bitches were also found by Place et al., using an assay developed for human samples [9]. The assay kit for human AMH used in the present study was in a previous report found to be better than a canine AMH kit for detecting canine AMH [19]. Another canine AMH assay has shown more reliable results, with generally higher AMH concentrations [10,11,20]. Due to the low concentration of AMH in bitches, a more sensitive assay adapted to the species may thus be valuable. It is worth noting that using a canine AMH assay, bitches with ORS were correctly diagnosed, with high AMH concentrations, in a previous study [11]. Storage is a factor that may affect AMH results. It has been shown that when human whole blood is stored in room temperature, the concentrations of AMH may increase, about 30% in 3.5 days [29], but this has not been described in canine samples [20].

5. Conclusion

Analysis of AMH and LH is useful for detecting ovarian tissue in bitches but the results must be interpreted with understanding of the variations during the cycle. For AMH, low concentrations may be obtained in intact bitches, leading to incorrectly classifying them as spayed. For LH, it is important that bitches are not tested during heat, to avoid intact bitches being wrongly classified as spayed.

Acknowledgements

We thank clients and colleagues who kindly let us take blood samples from their dogs for this study and the veterinary nurses who did most of the work in sampling the dogs. This research did not receive any specific grant from any funding agencies in the public, commercial, or not-for-profit sector. The research was supported by the Swedish University of Agricultural Sciences.

References

[1] Parker K, Snead E. Atypical presentation of ovarian remnant syndrome in a dog. J Am Anim Hosp Assoc 2014;50:e1–5.
[2] Sangster C. Ovarian remnant syndrome in a 5-year-old bitch. Can Vet J 2005;46:62–4.
[3] Ball RL, Birchard SJ, May LR, Threlfall WR, Young GS. Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007). J Am Vet Med Assoc 2010;236:548–53.
[4] Buijtels JJ, de Gier J, Kooistra HS, Naan EC, Oei CH, Okkens AC. The pituitary-ovarian axis in dogs with remnant ovarian tissue. Theriogenology 2011;75:742–51.
[5] Concannon PW. Biology of gonadotrophin secretion in adult and prepubertal female dogs. J Reprod Fertil Suppl 1993;47:3–27.
[6] Kooistra HS, Okkens AC, Bevers MM, Popp-Snijders C, van Haaften B, Dieleman SJ, et al. Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrus in beagle bitches. Biol Reprod 1999;60:65–71.
[7] Olson PN, Mulnix JA, Nett TM. Concentrations of luteinizing hormone and follicle-stimulating hormone in the serum of sexually intact and neutered dogs. Am J Vet Res 1992;53:762–6.
[8] Lofstedt RM, Vanleeuwen JA. Evaluation of a commercially available luteinizing hormone test for its ability to distinguish between ovariectomized and sexually intact bitches. J Am Vet Med Assoc 2002;220:1331–5.
[9] Place NJ, Hansen BS, Cheraskin JL, Cudney SE, Flanders JA, Newmark AD, et al. Measurement of serum anti-Mullerian hormone concentration in female dogs and cats before and after ovariohysterectomy. J Vet Diagn. Invest 2011;23:524–7.
[10] Themmen AP, Kalra B, Visser JA, Kumar A, Savjani G, de Gier J, et al. The use of anti-Mullerian hormone as diagnostic for gonadectomy status in dogs. Theriogenology 2016;86:1467–74.
[11] Turna Yilmaz O, Toydemir TS, Kirsan I, Gunay Ucmak Z, Caliskan Karacam E. Anti-Mullerian hormone as a diagnostic tool for ovarian remnant syndrome in bitches. Vet Res Commun 2015;39:159–62.
[12] Axner E, Strom Holst B. Concentrations of anti-Mullerian hormone in the domestic cat. Relation with spay or neuter status and serum estradiol. Theriogenology 2015;83:817–21.
[13] Lee MM, Donahoe PK. Mullerian inhibiting substance: a gonadal hormone with multiple functions. Endocr Rev 1993;14:152–64.
[14] Nagashima JB, Hansen BS, Songsasen N, Travis AJ, Place NJ. Anti-Mullerian hormone in the domestic dog during the anestrus to oestrous transition. Reprod Domest Anim 2016;51:158–64.
[15] Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 2002;143:1076–84.

[16] Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 2001;142:4891–9.
[17] Gharagozlou F, Youssefi R, Akbarinejad V, Mohammadkhani NI, Shahpoorzadeh T. Anti-Mullerian hormone: a potential biomarker for differential diagnosis of cryptorchidism in dogs. Vet Rec 2014;175:460.
[18] Holst BS, Dreimanis U. Anti-Mullerian hormone: a potentially useful biomarker for the diagnosis of canine Sertoli cell tumours. BMC Vet Res 2015;11:166.
[19] Pir Yagci I, Pekcan M, Polat IM, Kalender H, Macun HC. Does serum anti-Mullerian hormone levels always discriminate presence of the ovaries in adult bitches? Comparison of two ELISA kits. Reprod Domest Anim 2016;51:910–5.
[20] Hollinshead FK, Walker C, Hanlon DW. Determination of the normal reference interval for anti-Mullerian hormone (AMH) in bitches and use of AMH as a potential predictor of litter size. Reprod Domest Anim 2017;52(Suppl 2):35–40.
[21] Holst BS. Diagnostic possibilities from a serum sample-Clinical value of new methods within small animal reproduction, with focus on anti-Mullerian hormone. Reprod Domest Anim 2017;52(Suppl 2):303–9.
[22] Cui L, Qin Y, Gao X, Lu J, Geng L, Ding L, et al. Antimullerian hormone: correlation with age and androgenic and metabolic factors in women from birth to postmenopause. Fertil Steril 2016;105. 481–485.e1.
[23] Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, Groome NP, et al. Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 2006;147:3228–34.
[24] Buijtels JJ, de Gier J, Kooistra HS, Kroeze EJ, Okkens AC. Alterations of the pituitary-ovarian axis in dogs with a functional granulosa cell tumor. Theriogenology 2010;73:11–9.
[25] Reichler IM, Pfeiffer E, Piche CA, Jochle W, Roos M, Hubler M, et al. Changes in plasma gonadotropin concentrations and urethral closure pressure in the bitch during the 12 months following ovariectomy. Theriogenology 2004;62:1391–402.
[26] Beijerink NJ, Buijtels JJ, Okkens AC, Kooistra HS, Dieleman SJ. Basal and GnRH-induced secretion of FSH and LH in anestrous versus ovariectomized bitches. Theriogenology 2007;67:1039–45.
[27] Diaz-Espineira MM, Mol JA, van den Ingh TS, van der Vlugt-Meijer RH, Rijnberk A, Kooistra HS. Functional and morphological changes in the adenohypophysis of dogs with induced primary hypothyroidism: loss of TSH hypersecretion, hypersomatotropism, hypoprolactinemia, and pituitary enlargement with transdifferentiation. Domest Anim Endocrinol 2008;35:98–111.
[28] Cappy H, Pigny P, Leroy-Billiard M, Dewailly D, Catteau-Jonard S. Falsely elevated serum antimullerian hormone level in a context of heterophilic interference. Fertil Steril 2013;99:1729–32.
[29] Fleming R, Fairbairn C, Blaney C, Lucas D, Gaudoin M. Stability of AMH measurement in blood and avoidance of proteolytic changes. Reprod Biomed online 2013;26:130–2.