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

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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

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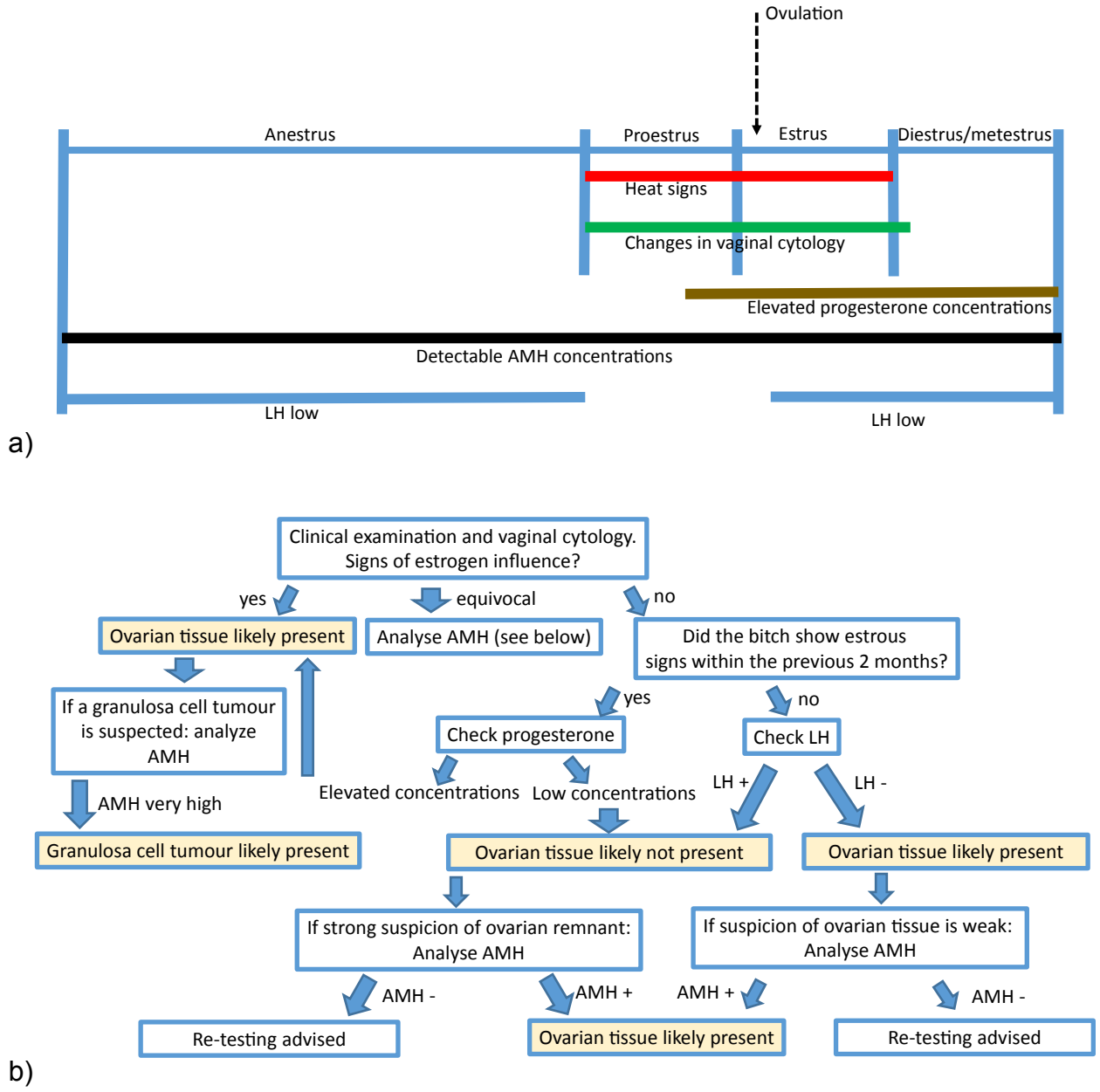

Fig. 1. **a**. Schematic drawing of possible tests for detecting ovarian tissue during the different stages of the estrus cycle. **b**. Flow chart showing steps in investigations of ovaries in bitches, based on the results of the present and other studies.

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