

# Measurement of serum anti-Müllerian hormone concentration in female dogs and cats before and after ovariectomy

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**Abstract.** Anti-Müllerian hormone (AMH), or Müllerian inhibitory substance, is a hormone that is best known for its production by fetal testes in mammals and as the inhibitor of Müllerian (paramesonephric) duct development in males. However, following the development of the Müllerian ducts into the oviduct, uterus, and upper vagina in female mammals, the ovaries produce AMH, which can be found in measureable amounts within the peripheral circulation, especially in adults. The ovaries appear to be the sole source of AMH in the circulation; therefore, it may be a useful marker in clinically relevant situations when an assessment of the presence or absence of ovaries or ovarian remnants in dogs and cats is important. To that end, a commercially available, human-based assay was evaluated for the measurement of AMH in dogs and cats. A preliminary assessment involved a single test on a set of serum samples from dogs that were submitted to a diagnostic endocrinology laboratory for other tests. Favorable preliminary results led to a more formal assessment of the assay using serum samples from dogs and cats with the presence or absence of the ovaries known by surgical confirmation. Overall, a single measurement of serum AMH concentration was highly effective at distinguishing ovariectomized from intact adult animals. In addition, the assay also accurately identified several cases of ovarian remnant syndrome.

**Key words:** Anti-Müllerian hormone; canine; feline; Müllerian inhibitory substance; ovary.

Anti-Müllerian hormone (AMH), also known as Müllerian inhibitory substance, is a protein hormone that is a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily. Anti-Müllerian hormone production and secretion by the Sertoli cells in fetal testes inhibits development of the embryonic Müllerian (paramesonephric) ducts into the oviducts, uterus, and upper vagina in male eutherian mammals. The ovaries also produce and secrete AMH, with onset occurring some time after completion of Müllerian duct development. The granulosa cells of primary, secondary, and early antral ovarian follicles produce AMH, and the early antral follicles are thought to be the principal source of AMH in the circulation.<sup>3</sup> Because the serum concentration of AMH markedly declines following bilateral ovariectomy,<sup>9</sup> the ovaries are believed to be the sole source of AMH in the circulation. Anti-Müllerian hormone can be measured throughout the estrous cycle in mice,<sup>8</sup> throughout the menstrual cycle in women,<sup>9</sup> and is detectable in hamsters during seasonal anestrus.<sup>13</sup> Because AMH can be detected in the circulation of intact female mammals in a variety of reproductive states, the present study was conducted to evaluate the potential of AMH for assessing the presence or absence of ovaries (or ovarian remnants) in conditions that are relevant to veterinary practice.

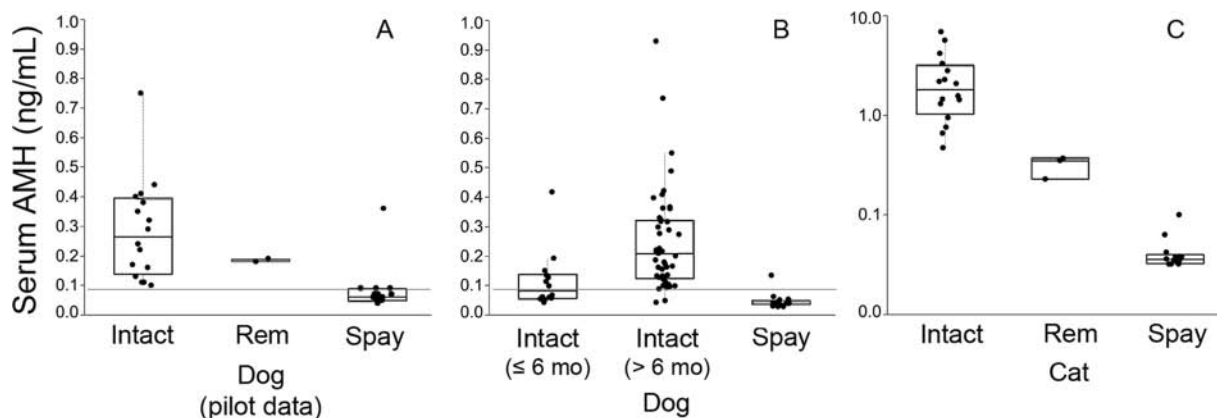
Presence or absence of the ovaries is an important consideration in shelter medicine, because the charters for most

shelters require documented evidence that an animal has been ovariectomized (OVX) before they can be adopted by the public. For example, it is estimated that millions of dogs and cats enter U.S. shelters annually (Humane Society of the United States, 2008. Pet overpopulation estimates. Available at [http://www.humanesociety.org/issues/pet\\_overpopulation/facts/overpopulation\\_estimates.html](http://www.humanesociety.org/issues/pet_overpopulation/facts/overpopulation_estimates.html)), and approximately 58% of dogs and 46% of cats had not been ovariectomized at the time they were relinquished to shelters.<sup>15</sup> A definitive determination as to which animals are OVX or intact has become more difficult with evolving changes in practice. A visible surgical scar is often not apparent when animals are ovariectomized at a very young age<sup>12</sup> or when performed as a laparoscopic procedure. Therefore, some dogs and cats may require exploratory surgery to determine the presence or absence of ovaries. These procedures add costs to the already tight budgets of shelters, and expose

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**Figure 1.** Combined dot and box plots of serum anti-Müllerian hormone (AMH) concentrations in (A) a pilot study of dogs, and (B and C) investigations of dogs and cats with surgically confirmed presence or absence of ovaries. The gray horizontal lines in panels A and B represent the cut-off AMH concentration (0.09 ng/ml) established for dogs in the pilot study. Note: AMH data in cats (C) are plotted on a log scale owing to the relatively high AMH concentrations in intact cats. Sample sizes: (A) intact = 16, spay = 15, remnant (rem) = 2; (B) intact  $\leq$  6 months of age = 14, intact  $>$  6 months of age = 49, spay = 16; (C) intact = 16, remnant (rem) = 3, spay = 13.

already disadvantaged animals to potentially unnecessary surgery. As such, a nonsurgical diagnostic test is needed to determine the reproductive status of shelter animals. Similarly, a better test is needed to assess suspected cases of ovarian remnant syndrome, as the diagnostic tests presently available have substantial drawbacks.<sup>1,4,5,11</sup>

The nonsurgical means by which the presence or absence of the ovaries or ovarian remnants can be assessed are fraught with difficulties. As mentioned above, the presence or absence of a surgical scar has become less reliable. Some facilities may perform an exploratory laparotomy on all animals relinquished because of missed diagnoses when an apparent ovariohysterectomy (OHE) scar was present. Vaginal cytology may be useful, but only if the animal presents during proestrus or estrus. Even in these cases, testing may need to be supplemented by 1 or more determinations of serum estrogen or progesterone concentrations.<sup>5</sup> Moreover, the measurement of sex steroids can yield equivocal results depending on the reproductive state of the animal.<sup>1</sup> More definitive tests that follow a stimulatory injection of gonadotropin-releasing hormone (GnRH) are costly and require multiple visits and blood samplings.<sup>4</sup> Similarly, a single measurement of luteinizing hormone (LH) was found not to be a reliable means for distinguishing between OVX and intact dogs,<sup>11</sup> and GnRH stimulation prior to the LH test has been recommended.<sup>4</sup> Abdominal ultrasonography can be informative in suspected cases of ovarian remnant syndrome, however, its effectiveness depends on the expertise of the examiner, the state of the estrous cycle at the time of the examination, and the size of the ovarian remnant.<sup>2</sup> Thus, a diagnostic test from a single blood sample for a hormone or other factor that is easily and reliably detected when the ovaries are present, or undetectable when the ovaries are

completely absent, would be a benefit to the veterinary community.

Anti-Müllerian hormone is an excellent candidate hormone because it remains detectable even in anestrus females<sup>13</sup> and disappears from the circulation after the ovaries have been removed.<sup>9</sup> In the present study, a commercially available AMH enzyme-linked immunosorbent assay (ELISA) for use with canine and feline samples was evaluated. The assay has been used successfully in a number of mammalian species,<sup>8,9,13,14</sup> but to the authors' knowledge, this is first assessment of its use in dogs and cats.

The preliminary evaluation of the human-based AMH ELISA<sup>a</sup> was performed on canine samples that had been sent to the Diagnostic Endocrinology Laboratory, Animal Health Diagnostic Center at Cornell University (Ithaca, New York) for clinical testing of hormones other than AMH. Analyses for AMH were postponed until all requested test results were finalized and reported. The residual volume of samples was stored frozen until assayed for AMH. Requisition forms were reviewed to identify an even mix of spayed ( $N = 15$ ) and intact animals ( $N = 16$ ). No attempt was made to confirm the spay status in the majority of cases; thus, there was a risk that some animals may have been misidentified as spayed or intact. However, 2 cases of ovarian remnant syndrome were confirmed by contacting the referring veterinarians. Overall, 33 samples were assayed in the first set of samples.

All serum samples were run in duplicate (20- $\mu$ l aliquots) according to the manufacturer's instructions. A serial dilution of a canine sample with an initial AMH concentration of 0.41 ng/ml was parallel to the standard curve down to 0.09 ng/ml; the intra- and interassay coefficients of variation were 3.6% and 3.9%, respectively. Data were analyzed with commercial statistical programs.<sup>b,c</sup>

The serum AMH concentration for the dogs listed as spayed was less than or equal to 0.09 ng/ml, with the exception of 1 animal with an AMH concentration of 0.36 ng/ml (Fig. 1A). This dog appeared to be a statistical outlier, as the AMH concentration was more than 3 standard deviations greater than the mean for the other dogs in the OVX group. Each of the 16 dogs listed as intact had an AMH concentration (range: 0.10–0.41 ng/ml) greater than the maximum of the spay group, exclusive of the outlier. This established the initial cut-off for AMH concentration in dogs at 0.09 ng/ml. The 2 dogs with confirmed ovarian remnant syndrome had AMH concentrations (0.18 and 0.19 ng/ml, respectively) that were well within the range for intact dogs.

To avoid the possible misidentification of OVX and intact dogs and cats, a second set of samples was obtained from animals with surgical confirmation of ovarian presence or absence. Serum samples were collected from 63 intact dogs and 16 intact cats prior to their being spayed at the Cornell University Hospital for Animals (Ithaca, NY), at Lollypop Farm, Humane Society of Greater Rochester (Fairport, NY), and the SPCA of Tompkins County (Ithaca, NY). Serum samples were collected from 16 OVX dogs and 13 OVX cats at least 9 days post OHE. Samples from post-OVX animals were collected by the veterinarians at Lollypop Farm, SPCA of Tompkins County, and the College of Veterinary Medicine, Cornell University, who had performed the OHE. An additional 3 samples from cats with confirmed ovarian remnants were included in the assay.

For dogs that were definitively determined to be OVX or intact, some overlap in the AMH concentrations between groups was found (Fig. 1B). However, this was determined to be largely due to a number of young, intact females having low AMH concentrations. Of 14 intact dogs that were 3–6 months of age, 50% had an AMH concentration greater than the established cut-off (0.09 ng/ml), whereas 93.9% (46/49) of intact dogs older than 6 months of age had AMH concentrations greater than 0.09 ng/ml. All of the OVX dogs were more than 6 months of age, and 6.2% (1/16) of them had an AMH concentration greater than the cut-off. If limited to dogs older than 6 months of age, the sensitivity (probability of correctly identifying intact dogs) of the AMH test was 93.9% (95% CI: 82.1–98.4%), and the specificity (probability of correctly identifying OVX dogs) was 93.8% (95% CI: 69.8–99.7%).

Intact cats had AMH concentrations that were an order of magnitude higher than in intact dogs. Whether this represents absolutely higher concentrations of AMH or better cross-reactivity of feline AMH with the ELISA antibody remains to be determined. In cats that were definitively determined to be OVX or intact, there was no overlap in AMH concentrations between the 2 groups (Fig. 1C). In 3 cases of feline ovarian remnant syndrome, AMH concentrations were intermediate to those of spay and intact cats. The sample sizes of cats should be enlarged before a definitive cut-off is identified.

To the authors' knowledge, this is the first investigation of serum AMH concentrations in dogs and cats. The results of the present study indicate that an assessment of serum AMH concentration can assist in determining the presence or absence of ovaries, including post-spay remnants, in adult dogs and cats. Anti-Müllerian hormone may have advantages over tests for other hormones (e.g., estradiol, progesterone, LH) for the diagnosis of ovarian remnant syndrome because AMH does not require dynamic testing and is less subject to the stage of the estrous cycle.<sup>3</sup> However, substantially more cases of confirmed ovarian remnant syndrome need to be evaluated before AMH can be recommended for routine presurgical assessment of this condition.

The majority of false-negative results in intact dogs (i.e., AMH concentration at or below the 0.09 ng/ml cut-off) were apparent in animals between 3 and 6 months of age. Given their age, these dogs were likely sampled before the onset of puberty.<sup>6</sup> Anti-Müllerian hormone has also been reported to be lower, or even undetectable, in prepubertal girls.<sup>7,10</sup> The most pronounced false-positive AMH result (Fig. 1A) was detected in a dog that was listed as spayed in the hospital record and in the records of the referring veterinarian, but documentation of the actual OHE could not be found. Thus, presence or absence of ovaries could not be firmly established in this animal with relatively high AMH.

For the purpose of assessing presence or absence of ovaries in dogs and cats, a single determination of serum AMH concentration by a human-based ELISA appears to be adequate. This semiquantitative test could be an effective diagnostic tool for clinicians. One caveat that might dissuade the use of this assay in veterinarian medicine is its high cost. To address this issue, the authors and their affiliates<sup>d</sup> are working to develop a more economical assay for diagnostic purposes in dogs and cats.

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### Sources and manufacturers

- a. DSL-10-14400, Diagnostic Systems Laboratories, Webster, TX.
- b. JMP 8.0.1, SAS Institute, Cary, NC.
- c. StatXact 9, Cytel, Cambridge, MA.
- d. Preventia LLC, Cohasset, MA.

### Declaration of conflicting interests

Dr. Place has submitted U.S. Patent Application 12/435,607, *Determination of serum anti-Müllerian hormone as a diagnostic test for spay in companion animals*. The Cornell Center for

Technical Enterprise and Commercialization has a licensing agreement with Preventia LLC, Cohasset, MA, which is developing a point of care test to evaluate AMH in dogs and cats.

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