

Theriogenology Question of the Month

This feature is sponsored by the American College of Theriogenologists. Readers of the *JAVMA* are invited to submit contributions. Contributions should provide a learning exercise about theriogenology. A specific question should be posed for the readers. The author's answer to the question and a brief discussion should be presented. Possible topics include commonly seen problems in domestic or exotic animals. Herd problems in dairy and beef cattle, sheep, goats, horses, and exotic hoofstock, problems in kennels or catteries, or flock problems in domestic and exotic fowl also are appropriate. Please contact Dr. Craig A. Smith, Associate Editor (800/248-2862, ext 6764, or FAX 847/925-9329), for further details.

History

An 18-month-old crossbred (primarily German Shepherd Dog) female dog of unknown sexual status was examined at the veterinary medical teaching hospital of the University of Minnesota. The owners had obtained the bitch 6 months previously. Since then, the bitch had not displayed any signs of estrus.

Physical examination revealed that rectal tempera-

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ture, pulse rate, and respiratory rate were within reference ranges. The bitch weighed 18.2 kg (40 lb) and was in good body condition. Vulvar swelling or discharge was not evident, and we did not detect a palpable or visible surgical scar on the ventral midline. A specimen was collected from the vagina for cytologic examination. Because of the size of the bitch, the sample was obtained cranial to the urethral papilla at a point where the vaginal vault changes from a relatively vertical to a more horizontal position. Examination revealed noncornified vaginal epithelial cells and a few healthy-appearing WBC.

Blood samples were obtained before and 90 minutes after administration of **human chorionic gonadotropin (hCG; 300 units, IV)**. Serum was harvested, and concentrations of estradiol were determined by use of radioimmunoassay. Sensitivity of the estradiol assay was 3 to 5 pg/ml. Serum concentrations of estradiol before and after administration of hCG were 7.4 and 11.2 pg/ml, respectively. A blood sample also was collected and serum harvested for assay of **luteinizing hormone (LH)** concentration by use of a semiquantitative ELISA. Concentration of LH in serum was > 1 ng/ml.

Question

Is the bitch sexually intact? *Please turn the page.*

Answer

The ovaries have been removed in this bitch.

Discussion

Previous ovariohysterectomy may be suggested in a female dog with unknown sexual status by palpation or visual detection of a scar on the ventral midline. Detection of a scar in a bitch with an unknown history does not necessarily indicate that she has undergone ovarian removal (ovariectomy or ovariohysterectomy), because the ventral midline approach is used for virtually all abdominal surgeries in dogs. Lack of a scar suggests that the bitch is sexually intact, although it is possible that some dogs will have undergone surgery that did not leave a palpable or visible scar. Exploratory laparotomy can be used to definitively determine sexually intact status, but it is an invasive method. The ovaries of bitches are not routinely visible ultrasonographically unless pathologic ovarian conditions exist or the bitch is pregnant, precluding use of ultrasonography for determining sexually intact status in most bitches.

Estradiol release can be stimulated by administration of gonadotropins to sexually intact bitches at any stage of the estrous cycle.^{1,3} In 1 study,² administration of a gonadotropin-releasing hormone (GnRH) analogue (0.16 µg, IV) stimulated an increase in estradiol concentrations in serum of bitches within 60 to 90 minutes after injection. Sensitivity of the estradiol assay used in that study was 1.0 pg/ml. Sexually intact bitches could be differentiated from neutered bitches by assessment of maximal estradiol concentrations: all sexually intact bitches had serum estradiol concentrations > 6.1 pg/ml, whereas all neutered bitches had serum estradiol concentrations < 2.6 pg/ml. Thus, those investigators were able to correctly classify 27 of 28 bitches.

Administration of hCG also stimulates an increase in estradiol concentrations regardless of stage of the estrous cycle during which it is administered. In another study,³ 12 female dogs of unknown sexual status were administered hCG (200 or 300 U of hCG, IV). Blood samples were collected before and 90 minutes after administration of hCG. Sensitivity of the estradiol assay used was 0.2 pg/ml. The dogs were designated as sexually intact when the serum estradiol concentration was > 0.2 pg/ml (ie, greater than the sensitivity of the assay) before or after hCG administration or when the serum estradiol concentration increased substantially during the 90-minute testing period. All 12 female dogs were correctly classified, and the lowest serum estradiol concentration in the sexually intact bitches was 1.3 pg/ml, 6 times the sensitivity of the assay.

In the dog described here, the pretreatment serum estradiol concentration was 7.4 pg/ml, which was 1.5 to 2.5 times the sensitivity of the assay we used for measurement of estradiol concentrations. Even the post-treatment serum estradiol concentration of 11.2 pg/ml was only 2.2 to 3.7 times the sensitivity of the assay, suggesting a negligible difference when the sensitivity of the assay is considered during interpretation of results.

Serum concentrations of LH and follicle-stimulating hormone increase after gonadectomy in dogs.⁴ Lack of negative feedback from the gonads to the pituitary gland causes an increase in serum concentrations

of gonadotropins.⁵ In 1 study,² investigators evaluated the sexually intact status of 27 female dogs by measurement of LH concentration in serum. The LH concentrations in sexually intact female dogs ranged from 5 to 8 ng/ml, whereas LH concentrations in neutered female dogs ranged from 8 to 62 ng/ml. A commercially available semiquantitative assay^a for measurement of LH concentrations can be successfully used to determine female dogs that are sexually intact.⁶ The test can be used to differentiate serum samples containing < 1 ng of LH/ml from those containing > 1 ng of LH/ml. Because LH concentrations in serum usually are < 1 ng/ml in sexually intact bitches, except on the day of the LH peak during estrus, it may be assumed that all high values in bitches not in estrus are indicative that the ovaries were removed. It should be mentioned that the commercial LH assay is marketed for use in detecting the LH surge during estrus to maximize breeding management of bitches, rather than for use in detecting bitches in which the ovaries have been removed.

For the dog described here, serum estradiol concentrations before and after administration of hCG were higher than concentrations described in another study,² but the assay we used differed greatly from the assay used in that other study. Sensitivity of an assay is defined as the amount of hormone the assay can differentiate from a zero concentration. In the dog reported here, we used an assay with a sensitivity of 3 to 5 pg/ml, and the initial value of 7.4 pg/ml was not much greater than the sensitivity of the assay. The value of 11.2 pg/ml in the sample obtained after hCG administration cannot be considered substantially different, suggesting a lack of ovarian tissue that could respond to stimulation. Furthermore, serum LH concentration was > 1 ng/ml, again suggesting lack of ovarian tissue.

Outcome

The bitch described here was classified as neutered. The bitch is now > 2 years old and has not been in estrus, supporting our contention that she was ovariohysterectomized. Exploratory laparotomy to definitively determine ovarian removal will only be performed if the bitch requires abdominal surgery at some subsequent time.

^aStatus-LH, Synbiotics, San Diego, Calif.

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