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## DIAGNOSING THE PRESENCE OF GONADAL TISSUE - (NO) ROCKET SCIENCE?

Determining the presence of gonadal tissue in dogs and cats can be challenging when the reproductive history of the animal is unknown, especially in females during anestrus, and in males with nonscrotal testes. A combination of strategies should be used to either confirm or rule out the presence of functional gonadal tissue:

- 1) Clinical examination.
- 2) Determination of basal reproductive hormone levels and
- 3) provocative hormone tests.

During the follicular phase the demonstration of ovarian tissue is straightforward by vaginoscopy (bitch) and vaginal cytology, which can show the influence of estrogen exposure. During progression to the late follicular phase, ovulation, and luteal phase, an elevated plasma progesterone concentration indicates the presence of ovarian tissue. In contrast, the plasma progesterone and estradiol concentration in anestrus bitches are not different from those in ovariectomized bitches. In these cases provocative hormone tests are often necessary.

In tomcats, the penile spines are under direct influence of testosterone. These spines disappear within 6 weeks after castration and will reappear after administration of testosterone. Examination of the penis will therefore be a reliable method to verify testosterone influence in male cats. Rectal examination to assess prostate size can be informative about testosterone influence in male dogs.

Plasma testosterone concentration is significantly lower in castrated dogs than in intact male dogs, and the ranges do not overlap <sup>1</sup>. A single measurement of plasma testosterone concentration can therefore be used to verify the presence of testicular tissue in male dogs. In male cats, a single measurement of the plasma testosterone concentration cannot be used for this purpose, as the ranges of plasma testosterone of intact and castrated cats overlap.

In both male and female dogs, basal plasma LH and FSH concentrations increase after gonadectomy, due to the loss of negative feedback of gonadal hormones. Basal plasma FSH concentration has been shown to be higher in ovariectomized bitches than in intact anestrus bitches, and the ranges do not overlap. In contrast, the ranges of basal plasma LH and estradiol concentrations in ovariectomized bitches and intact anestrus bitches do overlap <sup>1</sup>. Based on these findings, measurement of plasma FSH concentration appears to be the most reliable means of verifying the presence of ovarian tissue during anestrus. However, in bitches with remnant ovarian tissue, the function of the hypothalamic-pituitary-ovarian axis can be altered and in this group of patients the ranges overlap.

Determination of the FSH to creatinine ratio in urine may be a useful alternative to determine the presence of functional gonadal tissue. It reflects the plasma FSH concentration over a period of several hours and therefore is expected to compensate for pulsatile FSH secretion. The FSH to creatinine ratio is much higher in gonadectomized dogs than in intact dogs, without overlapping of ranges and can be used to verify neuter status <sup>2</sup>. Unfortunately, very few routine laboratories offer/perform a canine specific FSH assay.

If clinical examination and measurement of basal plasma hormone concentrations do not enable a definitive diagnosis, provocative tests with exogenous GnRH can also be used to detect the presence of functional gonadal tissue in cats and dogs. Additionally, a GnRH stimulation test is the only reliable way to prove absence of functional gonadal tissue. After intravenous injection of a GnRH analogue, a significant rise in plasma estradiol concentration is observed in anestrus bitches and queens but not in ovariectomized animals. However, the GnRH stimulation test has the disadvantages that it is invasive, requires more than one blood sample, and is somewhat time consuming (the maximum plasma estradiol concentration after GnRH injection is only reached after 60-120 minutes) <sup>1</sup>.

Another means of verifying the neuter status of dogs is by measuring plasma anti-Müllerian-hormone (AMH) concentration. The gonads are considered to be the sole source of AMH and therefore it can be expected that AMH will be a specific indicator

of the presence of functional gonadal tissue. The plasma AMH concentration has been shown to be higher in intact dogs than in gonadectomized dogs, but with some overlapping of the ranges<sup>3</sup>. An insufficient number of animals with remnant gonadal tissue has been studied to assess firm cut-off values for this group of patients. Because of the overlapping and the large individual differences in plasma gonadotropin, estradiol, and AMH concentrations, the reliability of a single measurement of the plasma concentration of one of these hormones during anestrus may not be sufficient to verify neuter status. However, the positive predictive value of a high AMH concentration is high.

Abdominal ultrasound may be used to determine if abdominal gonads are present or not. However, the sensitivity and specificity of this technique depends on many factors, such as the level of experience of the sonographer, the specifications of the equipment used and the characteristics of the patient. Additionally, in a population of bitches with remnant ovarian tissue, abdominal ultrasound was used to verify the presence and localization of ovarian tissue and it appeared that the results of the abdominal ultrasound were not always conclusive<sup>4</sup>. Therefore, the use of abdominal ultrasound alone, cannot be used to either confirm or rule out the presence of functional ovarian tissue.

### References

1. de Gier J, Buijtels JJCWM, Albers-Wolthers CHJ, Oei CHY, Kooistra HS, Okkens AC. Effects of gonadotropin-releasing hormone administration on the pituitary-gonadal axis in male and female dogs before and after gonadectomy. *Theriogenology* 2012;77:967-78.
2. Albers-Wolthers CH, de Gier J, Oei CH, Schaeffers-Okkens AC, Kooistra HS. Validation of a noninvasive diagnostic tool to verify neuter status in dogs: The urinary FSH to creatinine ratio. *Theriogenology* 2016;86:1376-81
3. Place N, Hansen B, Cheraskin J, Cudney S, Flanders J, Newmark A, Barry B, Scarlett J. Measurement of serum anti-Müllerian hormone concentration in female dogs and cats before and after ovariohysterectomy. *J Vet Diagn Invest* 2011;23:524-7.
4. Buijtels JJCWM, 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.