# Gonadal Testing Guidelines - Dogs and Cats

## Dogs and cats

Veterinarians may be required to determine the gonadal status of animals in various circumstances including:

- After desexing surgery to confirm complete removal of gonadal tissue
- Supposedly desexed bitches and queens exhibiting signs of oestrus (confirmation of "ovarian remnant syndrome (ORS)")
- Suspected cryptorchid males
- Female cats and dogs with unknown desexed in history

**To confirm cryptorchidism:**

- Anti-Müllerian hormone (AMH)
- Testosterone
- Examination of the penile spines (cats)
- Ultrasound

**To confirm ORS or intact status in females:**

- Anti-Müllerian hormone (AMH)
- Luteinising Hormone (LH)
- Progesterone
- Vaginal cytology
- Ultrasound

## Anti-Müllerian hormone

Anti-Müllerian hormone (AMH) is produced by the follicles of a sexually mature ovary and Sertoli cells in a sexually mature testes. After complete ovariectomy or castration, levels of AMH decrease significantly. Intact females and cryptorchid males will have higher levels of AMH than completely desexed animals. A single serum AMH test can differentiate these animals. After desexing, it is recommended to wait seven days to allow serum levels to decrease before testing to confirm ovariectomy was complete. AMH is available for male or female cats and dogs. This test is also useful in differentiating mares with ovarian granulosa cell tumours from normal mares.

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## Luteinising hormone

Luteinising hormone (LH) is available for female dogs and cats (not males). In bitches and queens with functional ovarian remnants, basal LH is low due to negative feedback. Serum LH will only be elevated at the natural LH peak preceding ovulation. In ovariectomised females, LH is persistently high due to lack of negative feedback control. A single low LH value indicates that functional ovarian tissue is present. If a high value is recorded, the animal must be retested with a new sample at least two hours after original sampling. This is to differentiate a persistently high value (no functional ovarian tissue present) from the transient pre-ovulatory LH peak (functional ovarian tissue present).

Currently, there is no published literature supporting the use of the serum LH assay to distinguish fully castrated from intact male dogs or cats. However, one study has confirmed that fully castrated male dogs have higher LH levels than intact animals. Use of the LH test for male dogs is therefore off-label and not validated.

## Progesterone

Progesterone is produced by the post-ovulatory corpus luteum. Confirmation of ovarian tissue by serum progesterone therefore requires the bitch or queen to be in dioestrus or pregnant. Serum progesterone levels in anoestrus intact queens and bitches will not be different than ovariectomised animals. If random serum progesterone is less than 2 ng/ml, stimulation tests can be used to induce ovulation and progesterone production according to the following protocols:

**Queen:**

*Protocol 1:*

One to three days after onset of oestrus-like behaviour, take a baseline serum sample and submit for progesterone analysis. Inject 500 IU hCG (Chorulon, Intervet) IM. Seven days later take a second serum sample and submit for progesterone analysis.

*Protocol 2:*

Give two 0.5 ml IM doses of GnRH (Buserelin, Intervet) 24 hrs apart. Three days later take a serum sample and submit for progesterone analysis. Progesterone concentrations increase at least fivefold in ovarian remnants.

**Bitches**:

Inject hCG at 44 IU/kg or 400 IU/dog IM, or GnRH at 2 ug/kg or 50 ug/dog IM. Take a serum sample 7-14 days later and submit for progesterone analysis. The test is less reliable than in the cat, however a 7 to 14 day post injection progesterone concentration of greater than 2 ng/ml confirms ovarian remnant syndrome.

## Testosterone

Testosterone can be measured, but interpretation can be hampered by naturally low or fluctuating levels, particularly with cryptorchidism. Stimulation with GnRH or hCG may be required to demonstrate functional gonadal tissue.

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## Vaginal cytology

Cornification of vaginal cells is oestrogen-dependent and indicates cycling activity. However, this test requires the animal to be in pro-oestrus or oestrus and may need repeat testing over a week to demonstrate the increase in cornified cells. False positives can occur if the keratinised vulval epithelium is sampled rather than vaginal epithelium. False negatives can occur if the animal is not cycling at the time of sampling.

## Ultrasound for gonadal tissue

This can be performed in-house, but requires an experienced operator and sensitive ultrasound machine. Small ovarian remnants or abdominal cryptorchid testes may not be detected.

## Examination of the penile spines

In male cats, penile spines are expected to be present if there is functional testicular tissue present; spines are hormone-induced and will regress following castration.

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