

## Anti-Müllerian Hormone

- **Differentiation: Castrated, Uncastrated or Cryptorchid**
- **Diagnosis of Granulosa Cell Tumours in Mares**

The Anti-Müllerian Hormone (AMH) is a glycoprotein that plays an important role in the sex differentiation during embryogenesis.

In male animals testosterone is responsible for the development of the Wolffian ducts, whereas AMH is needed to prevent the development of the mullerian ducts into the uterus and other mullerian structures.

In female animals AMH is not produced by the gonads during sex differentiation in early gestation, so the mullerian ducts automatically develop and the normal development of the female genitals occurs. In female animals, AMH is expressed by granulosa cells of the ovary controlling the formation of primary follicles by inhibiting excessive follicular recruitment by FSH.

### Horse

In human medicine AMH is used to diagnose Granulosa cell tumours (GCT) (Fig. 1). As GCT is the most frequent diagnosed tumour of the equine female genitals (~85% of tumours found in the reproductive organs), it was obvious to try to establish this diagnostic test for mares as well. This idea was presented by B. A. Ball et al., at the AAEP Annual Convention in November 2011, which presented equine results. They had successfully been able to establish and validate a human test also for horses.

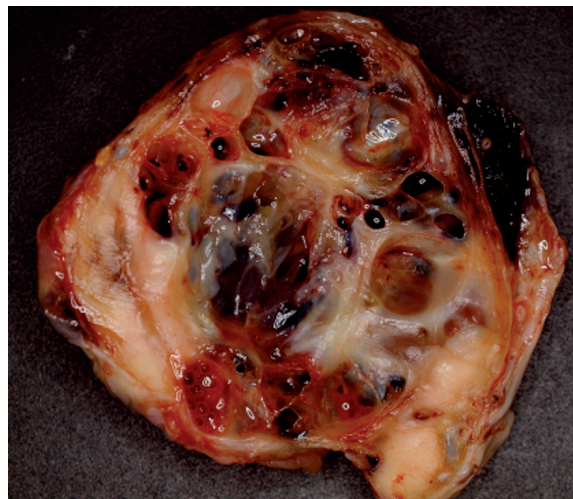


Fig.1: Granulosa cell tumour in a 9 year old mare

AMH stays the same during the reproductive cycle, but show individual differences between mares (0.22–2.94 ng/mL).

The AMH concentration is slightly higher in nonpregnant mares than pregnant mares.

Ovariectomized mares show a significantly lower AMH level (<0.1 ng/ml) and very importantly mares with granulosa cell tumours show significantly higher hormone concentration (>14.0 ng/ml) than normal mares.

For diagnosing Granulosa cell tumours the assay has a sensitivity of 95%, which is significantly higher than the other hormone tests previously used; Inhibin concentration has a sensitivity of 85%, Testosterone only 55% sensitivity. Other ovarian tumours (e.g. carcinoma, adenoma) are not detected by the AMH test.

In stallions AMH is produced by the Sertoli cells and remains high through puberty where it then declines to low levels with increasing testosterone production. Therefore stallions and cryptorchid can clearly be distinguished from castrated horses by measuring the AMH concentration (Tab. 1). AMH is in male animals a useful biomarker for active testicular tissue therefore useful to diagnose cryptorchidism. This test can even be used in young castrated animals – opposite to for instance the oestrone sulphate test.

### Dog & Cats

Neutered versus intact is very difficult to determine in female animals, especially in animals with an unknown history (e.g. stray dogs). Here the AMH test can again be useful in both bitches and female cats. The bitches should be older than 6 months when tested. The time in cycle (also anoestrus) has on the other hand no influence on the result. The necessity to perform a dynamic test is with the new AMH test available hereby eliminated!

The same applies to both male dogs and cats: AMH determination can be used to diagnose cryptorchidism. As the AMH secretion take place in the Sertoli cells, AMH concentrations above the reference value indicate presence of testicular tissue (Tab. 1).

### AMH Test & Interpretation

The AMH test is an ELISA test that (for now) is carried out once a week in our lab. Useful material is serum or lithium-heparin-plasma. The sample should be promptly centrifuged (for serum, right after coagulation) and cell-free pipetted. It is recommended to cool the sample before transportation and ship cooled if warm weather.

AMH concentration (ng/ml)		
	Castrated	Uncastrated
<b>Dog</b>		
- Female	< 0,02	> 0,5
- Male	< 0,1	> 2,0
<b>Cat</b>		
- Female	< 0,1	> 2,0
<b>Horse</b>		
- Male	< 0,1	> 2,0

Tab. 1: Normal AMH reference values for serum in different species