ANTI-MÜLLERIAN HORMONE –
A NEW TEST FOR THE DIAGNOSIS OF GRANULOSA CELL TUMOURS IN MARES

Ovarian granulosa cell tumours (GCTs) account for more than 85% of all tumours of the reproductive tract in mares and 2.5% of all equine neoplasms. Typically these tumours are benign and unilateral in nature with the contralateral ovary commonly small and inactive. GCTs are hormonally active and produce variable amounts of inhibin, oestradiol and testosterone. It is therefore common for mares to present with behavioural abnormalities including aggressiveness, stallion-like behaviour, prolonged anoestrus, or nymphomaniac behaviour. However, most mares with GCTs present with anovulatory anoestrus. GCTs display no breed predilection and have been detected in maiden, barren, pregnant and post-partum mares.

Ultrasound of the affected ovary commonly reveals an enlarged, multicystic structure of honeycomb appearance containing areas of hyperechoic tissue as seen in Figure 1. However the appearance of the tumour can be variable, presenting as a solid mass, single large fluid filled cyst or a polycystic core surrounded by a thick capsule, potentially containing focal areas of haemorrhage and necrosis (Figures 2a,b). Histologically, it is common for the ovulation fossa to be obliterated.

Clinical signs and ultrasonographic findings alone are not sufficient to confirm a diagnosis of GCT. Differentials for ovarian enlargement in the mare include neoplasia (serous cystadenoma, dysgerminoma, teratoma), physiological processes (pregnancy, haemorrhagic anovulatory follicles (HAF)), or localised pathology such as ovarian haematomas or abscessation.

The hormonally active nature of GCTs allows endocrine analysis to be used successfully as a diagnostic aid. The current standard ‘GCT panel’ quantifies serum concentrations of inhibin, testosterone and progesterone. Increased serum concentrations of inhibin (>0.7ng/mL) and/or testosterone (>50-100pg/mL), combined with decreased circulating progesterone (<1ng/mL) are suggestive of a GCT. However, serum concentrations of these 3 hormones can fluctuate depending on the stage of the oestrus cycle and stage of pregnancy. In addition, in the early stages of tumour development, levels of all 3 hormones can be within the normal range.

Anti-Müllerian Hormone (AMH) is a homodimeric glycoprotein expressed by the granulosa cells of ovarian follicles that has a role in the regulation of follicular development. AMH is highly conserved across species and has been found in all domestic mammals tested so far (horses, cattle, dogs, cats, sheep and goats). Immunohistochemistry has previously shown that AMH is expressed in granulosa cells of normal equine ovaries as well as in GCTs. Serum AMH as a biomarker has thus been investigated as a more sensitive diagnostic aid than previously measured hormones in equine GCTs.

In horses, serum AMH concentrations were first characterised by Almeida et al. (2011) using a heterologous enzyme linked
immunoassay (ELISA) in normal cyclic mares, ovariectomised mares and mares previously diagnosed with a GCT. There were no significant differences in circulating serum AMH concentrations between normal cyclic mares and pregnant mares, thus negating any confounding factors encountered by inhibin, progesterone and testosterone analysis. The concentration of AMH in ovariectomised mares was equal to or less than the limit of detection of the assay, confirming the ovarian localisation of the hormone. AMH concentrations were increased in all mares with histologically confirmed GCTs. This demonstrates significantly increased sensitivity when compared to inhibin and testosterone analysis, with levels elevated in only 73% and 45% of GCT cases respectively if these hormones are measured individually. Ball et al. (2013) conducted further studies, concluding that AMH, when measured in combination with testosterone and inhibin, achieves a sensitivity of 100%.

Canterbury Health Laboratories (Christchurch, NZ: E. info@chl.co.nz) and Vetpath Laboratory Services (Perth, WA: Vetpath.reception@vetpath.com.au) currently perform the only AMH assays available for clinical use in Australasia. Though the assays are designed for humans (NZ), and dogs and cats (WA), the manufacturers report that they also cross-react with AMH from other species including the horse. Though each assay may not have full measurement (cross reaction) of the AMH from other species it appears adequate for differentiating the elevated AMH observed in mares with GCTs compared to healthy mares. Both laboratories are currently in the process of establishing individual reference intervals for the mare and therefore it is useful to provide a clinical history with your submission, especially if you have confirmed the diagnosis of a GCT by history. It is also important to contact the relevant laboratory for specific submission requirements. Note that results are reported as AMH pmol/L, to convert to ng/mL (the unit often quoted in veterinary publications) divide the pmol/L result by 7.14.

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