DIAGNOSIS OF PREGNANCY

Rectal examination and rectal ultrasonographic examination are the most efficient and cost-effective means of diagnosing pregnancy. However, in cases that are not amenable to examination per rectum, either due to temperament or size, blood tests can provide a reliable substitute. In late pregnancy, transcutaneous ultrasonographic examination or even foetal electrocardiographic examination provide alternative means of identifying pregnancy and confirming foetal viability.

SERUM PROGESTERONE

From 18-20 days onwards, progesterone concentration in pregnant mares should remain above 6.3 nmol/L until around day 200 of gestation. However, both false positives and false negatives can occur and measurement of progesterone may therefore be unreliable in diagnosis of pregnancy or placentitis.

EQUINE CHORIONIC GONADOTROPHIN (eCG, PMSG)

Equine chorionic gonadotrophic (eCG; previously known as pregnant mare serum gonadotrophin) is secreted in large quantities by specialised cells of the foetal membranes into lymphatic sinuses of maternal endometrium during the first half of equine pregnancy. Equine chorionic gonadotrophin first appears in maternal blood 37-40 days after ovulation and concentrations rise steeply thereafter to a peak at around day 55-70, before declining steadily and disappearing completely from maternal serum between days 100 -140. The decrease in maternal serum equals the degeneration and dehiscence of endometrial cups from the endometrium.

A number of factors have been shown to influence the concentrations of eCG in mare's serum between 40 and 100 days gestation including mare size, mare parity, mare's diet, paternity of conceptus, foetal gender, twin pregnancy, degree of folding of the endometrium and the uterine environment.

False negatives: may occur from samples collected before day 35 and after day 90.

False positives: If embryonic death occurs after day 35 it does not result in regression of endometrial cups and eCG levels may remain high despite an absence of a viable foetus.

Given that detection of eCG in mare's serum gives no indication of the continued viability and presence of the foetus, follow up measurement of oestrone sulphate after day 100 is recommended when a positive eCG result is obtained.

OESTRONE SULPHATE

During pregnancy, several oestrogens are produced in high concentrations by the equine foetoplacental unit and are released into the maternal circulation. The foetal gonads of both male and female fetuses secrete large quantities of androgens, some of which are converted by the placenta into oestrone sulphate. Since oestrone sulphate is only produced by a combination of viable foetus and placenta, it is the only pregnancy endocrine test that confirms that a foetus is alive.

Oestrone sulphate can be detected from day 60, peaks at around day 150, then slowly declines (see Figure 1). Non-pregnant mares have oestrone sulphate concentrations <10 ng/ml, and a concentration of >10 ng/ml after 100 days of gestation is generally considered positive for pregnancy. More precisely, most pregnant mares have a concentration greater than 50ng/mL by 3-4 months and greater than 100ng/mL by 4-5 months.

Oestrone sulphate is not a helpful indicator of ascending placentitis or impending abortion, since it remains substantially elevated until less than 24 hours before abortion occurs (Canisso *et al* 2016).

DIAGNOSIS OF PREGNANCY

Canisso, I. F., Ball, B. A., Esteller-Vico, A., Williams, N. M., Squires, E. L. and Troedsson, M. H. (2016), Changes in maternal androgens and oestrogens in mares with experimentally-induced ascending placentitis. Equine Veterinary Journal. doi: 10.1111/evj.12556

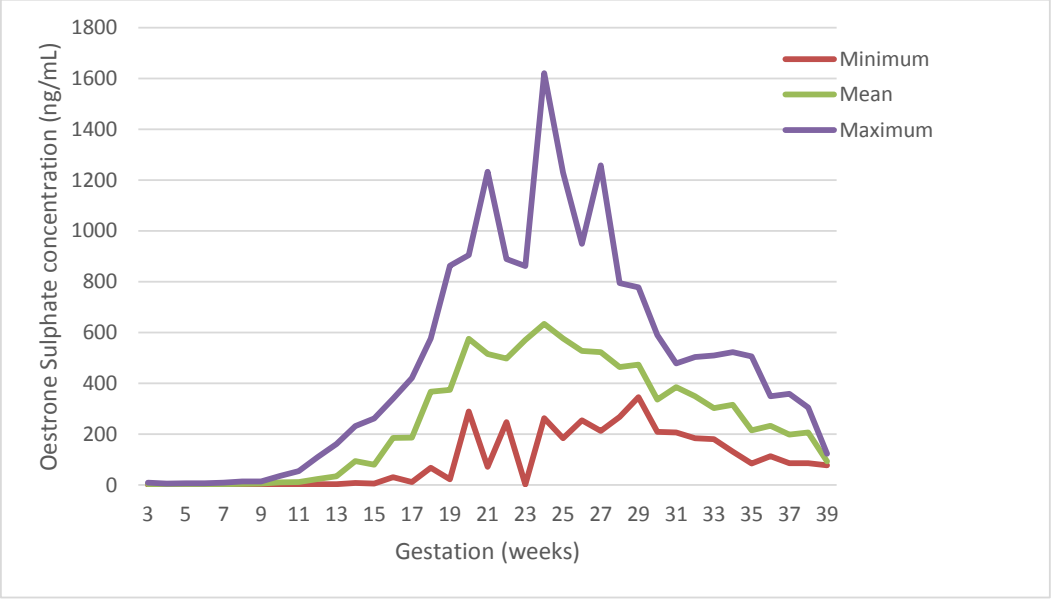


Figure 1: Minimum, mean and maximum oestrone sulphate concentrations in pregnant mares.

GRANULOSA CELL TUMOURS

Granulosa cell tumours (GCT or sex cord-stromal tumours) are the most common ovarian tumour in horses and can occur in mares of any age. They may be composed of granulosa cells alone or contain theca cells (granulosa-theca cell tumours). They are almost always unilateral, functional and benign.

CLINICAL SIGNS

The hormonal pattern is inconsistent and determines the clinical signs observed. These may include:

Oestrus cycle: Persistent, irregular or absence of oestrus

Masculinisation: stallion-like behaviour including mounting, aggressiveness, squealing, striking, and in chronic cases, increased muscle deposition, cresty neck and an enlarged clitoris.

RECTAL EXAMINATION

One ovary is normally enlarged with no ovulation fossa being palpable. GCTs are slow growing tumours but have been reported up to 40cm in diameter. The contralateral ovary is usually small, firm and inactive. If there is asymmetry in ovary size yet the smaller ovary is cycling, then a granulosa cell tumour is less likely and the enlarged ovary is more likely to have a haematoma or teratoma.

ULTRASONOGRAPHIC EXAMINATION

Granulosa cell tumours typically have a multicystic honeycomb appearance with some areas of solid tissue. A minority will have a dense homogenous appearance or will appear as a solid ovarian mass, with a single anechoic fluid-filled cyst. Haematomas or regions of necrosis within the tumour are common. The variable ultrasonographic appearance of GCTs hampers differentiation from other possible diagnoses including haematoma, cystadenoma, lymphoma and germ cell tumours.

Since clinical signs, rectal examination findings and ultrasonographic appearance are not consistent in all GCT cases they do not predict the presence of a GCT reliably. Therefore, measurement of serum hormone concentrations are recommended for diagnosis.

ANTI-MÜLLERIAN HORMONE CONCENTRATION

Serum levels of anti-Müllerian hormone (AMH) have been shown recently to be a reliable means of identifying the presence of granulosa cell tumours (GCT). In mares, AMH is produced by granulosa cells and in healthy mares serum AMH concentrations are similar throughout the oestrus cycle and pregnancy. The proliferation of granulosa cells which occurs with GCTs is associated with a marked increase in serum AMH concentration.

In a recent investigation, the diagnostic accuracy of AMH, inhibin and testosterone for the diagnosis of GCT was compared in 44 mares in which GCTs were confirmed with histopathology. Overall, the sensitivity of AMH for detection of known GCTs was 98%, compared to sensitivities of 80% for inhibin, 48% for testosterone and 84% for the combination of inhibin and testosterone (Ball *et al*. 2013). Median serum AMH in mares with histologically confirmed GCTs (n = 44) was 66.0 ng/ml (quartiles, 27.1–184.5 ng/ml). Median serum AMH in reproductively normal, non-pregnant mares (n = 94) was 0.30 ng/ml (quartiles, 0.01–0.50 ng/ml), and in pregnant mares (n = 39) the median serum AMH concentrations were 0.22 ng/ml (quartiles, 0.04–0.45 ng/ml) (Ball *et al*. 2013).

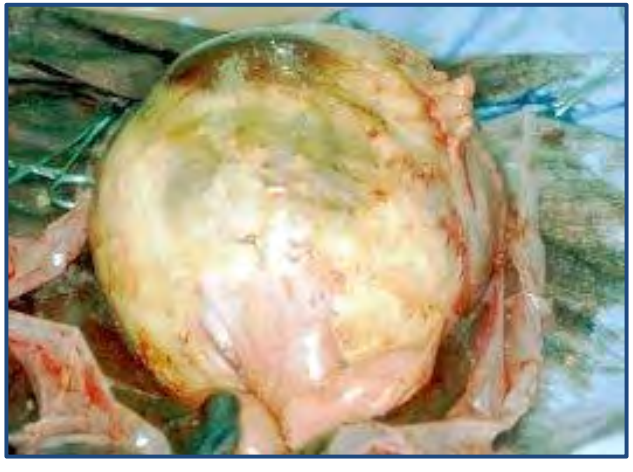
GRANULOSA CELL TUMOURS

In addition to increasing the accuracy of diagnosis of GCTs, AMH is not increased in pregnant mares, in contrast to testosterone and inhibin. Furthermore, AMH offers practical advantages, as inhibin measurement is only available commercially in the United States, resulting in a greater delay between sampling and the reporting of results and increased expense.

We recommend AMH as the test of choice for any mares suspected of having a granulosa (theca) cell tumour.

	AMH (ng/mL)	Inhibin (ng/mL)	Testosterone (nmol/L)	Progesterone (nmol/L)	
				oestrus	dioestrus
Healthy mare	<4	0.1 - 1.7	<0.5	>3	>12
GCT	>4	>0.5	>1.4	<3	

Ball, B.A., Almeida, J. & Conley, A.J. (2013) Determination of Serum Anti-Müllerian Hormone Concentrations for the Diagnosis of Granulosa-Cell Tumors in Mares. Equine Veterinary Journal 45: 199-203.



DIAGNOSIS OF CRYPTORCHIDISM

Cryptorchidism (or retention of a testis) is of importance because of associated unwanted stallion-like behaviour. Bilateral cryptorchids are usually infertile and unilateral cryptorchids have reduced sperm production. The Leydig cells of retained testicles remain capable of testosterone production and cryptorchids therefore exhibit stallion-like behaviour. Retained testicles may be at increased risk of becoming neoplastic in horses as they are in man. The condition is likely to be hereditary though the mode of inheritance may be different between stallions, as some have a high incidence of cryptorchid foals whilst others do not. Percherons, Quarter Horses, Friesians and cross-bred horses or ponies may have a higher incidence of cryptorchidism than other breeds, whilst Thoroughbreds, Arabs, Standardbreds and Morgans may be less likely to be affected.

Testicles should descend by 2 weeks of age. Failure of descent of right and left testicles occurs with equal frequency overall although the right testicle is more likely to be retained in the inguinal region (60% vs 40% in the abdomen) whereas the left is more likely to be retained in the abdomen (75% vs 25% in the inguinal region).

One in 10 horses with retained testicles have the condition bilaterally. Where the condition is bilateral, both testicles are usually retained at the same site; two thirds being intra-abdominal and the remaining third being inguinal.

Cryptorchidism may on very rare occasions (1 in 500) be a sign of intersexuality. Anorchidism and monordichism are exceedingly rare.

Stallion-like behaviour may be observed in up to 20% of geldings and is not necessarily an indication of cryptorchidism.

EXAMINATION

A diagnosis of cryptorchidism can often be made by careful examination, especially if the animal's history is known:

External palpation under sedation: Careful palpation of the scrotum and inguinal region. Beware palpation in foals as the gubernaculum is relatively large and may be mistaken for a testicle.

Internal palpation: Rectal palpation or transrectal ultrasound may identify the retained testicle.

Transcutaneous abdominal ultrasound: with experience and a co-operative patient, ultrasonography is a very accurate means of diagnosis.

ANTI-MÜLLERIAN HORMONE (AMH)

In the horse, AMH is strongly expressed in Sertoli cells of fetal, neonatal, and pre-pubertal testes, as well as cryptorchid testes, Sertoli cell tumours, and male intersex gonads. At puberty, maturation of Sertoli cells is accompanied by reduced production of AMH, however normally descended testes continue to produce some.

Claes *et al.* (2013) recently compared AMH concentrations in stallions, geldings and cryptorchids. Serum AMH concentrations were significantly higher in cryptorchid stallions (32.7 ± 5.3 ng/mL) compared with intact stallions (14.7 ± 2.4 ng/mL), and was undetectable in geldings. Thus, AMH is a reliable and specific test for the presence of testicular tissue in horses of any age. Following castration, serum AMH concentrations decline rapidly post castration (biological half-life of 1.5 days; Claes *et al.* 2011) making AMH a useful test if there is doubt over the success of a recent castration procedure. AMH has not been validated in cryptorchid donkeys at this time, but this work is being undertaken at Liphook Equine Hospital currently.

DIAGNOSIS OF CRYPTORCHIDISM

We use the same method as the published studies and recommend AMH as the test of choice for identifying cryptorchids of any age.

OESTRONE SULPHATE

In horses over 3 years of age, measurement of serum oestrone sulphate concentration is a reliable indicator of the presence of testicular tissue. In younger animals and in donkeys, cryptorchids do not reliably produce sufficient oestrone sulphate so whilst a positive result is informative, a negative result does not rule out the possibility of retained testicular tissue. When used in older horses and ponies, the test is 96% accurate in determining the presence of retained testicular tissue.

Expected Serum Oestrone Sulphate Concentrations:

Gelding	<0.02 ng/ml
Cryptorchid	0.1-10 ng/ml
Stallion	>10 ng/ml

For results between 0.05 and 1 ng/ml, further testing with a human chorionic gonadotrophin (hCG) stimulation test is recommended (see below).

RESTING TESTOSTERONE CONCENTRATION

In animals under 3 years of age, serum testosterone concentration may be measured. However, resting testosterone levels vary markedly with age and season. There is therefore the potential for overlap between horses with and without testicular tissue, with rates of misdiagnosis being up to 14% using this measurement alone. This test is not recommended because testosterone measurement following hCG stimulation is more reliable.

hCG STIMULATION TEST

The number of equivocal results is reduced by measurement of testosterone following injection of human chorionic gonadotrophin (Chorulon, Intervet). This test is recommended for all donkeys and for horses or ponies in which the results of other hormone assay measurement is equivocal.

- Measure serum testosterone concentration
- Administer 6000iu human chorionic gonadotrophin IV
- Re-measure serum testosterone concentration 24 hours later. Serum testosterone will be increased from 30 minutes to 48 hours after injection of hCG so timing of the follow-up sample is not critical.

	AMH (ng/mL)	Resting Testosterone (nmol/L)	Testosterone post hCG (nmol/L)
Gelding	<1	<0.15	<0.2
Cryptorchid	32.7±5.3	0.3 - 4.3	1 - 13
Stallion	14.7±2.4	5 - 30	

Claes, A.N., Ball, B.A., Almeida, J. & Conley, A.J. (2011). Detection of Serum Anti-Müllerian Hormone Concentrations as a Method for Diagnosis of Cryptorchidism in the Horse. In: Proceedings of the American Association of Equine Practitioners San Antonio, Texas p. 56.
Claes, A.N., Ball, B.A., Almeida, J., Corbin, J. & Conley, A.J. (2013) Serum Anti-Müllerian hormone concentrations in stallions: Developmental changes, seasonal variation, and differences between intact stallions, cryptorchid stallions, and geldings. Theriogenology 79: 1229-1235.