cpdsolutions
transferring knowledge through excellence in training

Equine Reproduction

Mini Series

Session Two: Stallion Reproduction

Madeleine Campbell BVetMed MA (Oxon) MA (Keele) Phd DipECAR DipECAWBM MRCVS
European and RCVS Recognised Specialist in Equine Reproduction

CPD Solutions: Stallion Reproduction.

Dr Madeleine Campbell
BVetMed (Hons) MA(Oxon) MA(Keele) PhD DipECAR Dip ECAWBM (AWSEL) MRCVS

Hobgoblins Stud and Equine Reproduction Centre

LEARNING OBJECTIVES

- Health test requirements for the breeding stallion
- How to take the necessary samples to fulfil health test requirements
- Training the stallion for artificial semen collection
- Processing semen for fresh and chilled use
- Common reproductive problems of stallions seen in practice- diagnosis and treatment

I: Venereal diseases of stallions which may be transmitted in semen:

Venereal diseases are those diseases which are transmitted during coitus or contact with the genitalia. All of the bacterial and viral venereal diseases which can be transmitted during natural coitus can be transmitted in fresh, chilled and frozen semen and it is therefore crucial that the HBLB Codes of Practice are adhered to under ALL breeding circumstances.

Bacterial venereal diseases of the horse are Contagious Equine Metritis Organism, *Klebsiella pneumoniae* capsule types 1,2 and 5 and *Pseudomonas aeroginosa.* For full details of each of these diseases see the current HBLB Codes of Practice. All stallions should be swabbed according to the protocol laid down in the HBLB Codes each year, and all semen should be accompanied by a certificate stating that these swabs have been taken and tested for Contagious Equine Metritis Organism, *Klebsiella pneumoniae* capsule types 1,2 and 5 and *Pseudomonas aeroginosa* with negative result.

The main viral disease which is transmitted during coitus and in all types of semen is Equine Viral Arteritis virus. In March 2006 BEVA, the BEF and the BHS issued a joint briefing document on EVA. Equine Viral Arteritis (EVA) is a very serious viral disease of horses. EVA is currently a relatively common disease in Europe and other parts of the world but is relatively rare in the UK. However, the risk of it increasing in prevalence within the UK, with potentially very serious welfare and economic effects, has increased over recent years due to the increased international movement of horses for breeding and competition purposes. Read the HBLB Codes of Practice for full details of the disease and the various ways in which it is transmitted. The aim of current control measures is to prevent the spread of EVA within the UK horse population. Because horses which have EVA and are capable of passing the disease onto other horses do not necessarily show clinical signs of disease, control and prevention is based upon blood testing to show which animals have the disease and therefore pose a risk to other animals. Stallions which have been infected with EVA have the potential to shed the EA virus in their semen despite being completely free of clinical signs of disease. For this reason, it is crucial that all stallions are either tested free of EVA according to the HBLB Codes, or are tested negative, vaccinated against EVA with a primary course and then re-tested to prove that their positive titre to EVA antibodies is due to vaccination and not due to exposure to the disease. There is currently no way of distinguishing a positive titre due to vaccination from a positive titre due to infection, and for that reason accurate record keeping is essential. Vaccinated stallions should receive boosters in line with the manufacturer's recommendations. Note that in recent years there have been issues with the supply of the one commercially available vaccine. In cases of an unexpected positive serology result for EVA in a stallion, Defra should be notified. In this circumstance or when there is no accurate record of vaccination history it is necessary to test aliquots of semen for the presence of the EA virus.

II: Paperwork accompanying semen to be inseminated

The 2007 HBLB Codes of Practice contained for the first time a checklist for those inseminating equine semen. The semen should be accompanied by paperwork specifying the name of the stallion, the date on which the semen was collected, confirming the health status of the semen and specifying the progressive motility, the concentration of the semen, the dilution rate in extender and the insemination volume / dose per mare. In addition to the requirements laid out in the Codes, Veterinary Surgeons receiving semen from abroad should also note that original health test paperwork must accompany the shipment, and that the seals on the container must be kept in case of Defra inspection.

III: Semen assessments

The following parameters are routinely measured at the time of semen collection. Normal values are given in brackets.

(i) Total volume (15 – 100 ml)
(ii) Total motility (>50%)
(iii) Progressive motility (>50%)
(iv) Morphology (minimum 50% morphologically normal live sperm cells; no more than 15% of any one deficit)
(v) Concentration (100-800 million sperm cells/ml of semen)

Common abnormalities of motility include no motility and lack of progressive motility including circling.
Common morphological defects of sperm cells include detached heads; kinked tails and the presence of proximal cytoplasmic droplets.
The most important aspect of a semen evaluation is the ability to distinguish between genuine abnormalities which relate to spermatogenesis or sperm maturation and abnormalities caused by the handling of the semen which do not reflect genuine sub-fertility.

In practical circumstances when the veterinary surgeon is receiving semen to inseminate (rather than processing semen for shipping), the semen evaluation will be less comprehensive. In the case of chilled semen the insemination dose is typically between 10 and 60 mls. In the case of frozen semen the number of straws should be specified e.g. 8 straws / insemination dose. Although it may on occasion be necessary or desirable to measure the concentration of semen which is being inseminated, it is more normal in practice to assess only the total and progressive motility of a semen sample. All samples should be warmed to 38 °C and held at that temperature for assessment. For chilled semen, one may choose to inseminate the majority of the semen dose and then to retain a little for assessment, or to remove a little for assessment before insemination. It is not necessary to warm the entire insemination dose of chilled semen before insemination. For frozen semen, when insemination volumes are typically very small, it is normal to perform the insemination and then to use the small volume which is left in the catheter / straws for assessment.

It is accepted that each mare needs a minimum insemination dose of 500 million progressively motile morphologically normal sperm of chilled semen to have a reasonable chance of conceiving. Progressive motility should exceed 50% at the time of insemination. It is the industry norm to provide at least 600 million sperm cells in one insemination dose of frozen semen, and for that semen to have a progressive motility of at least 30%. If the inseminating veterinary surgeon, having assessed the semen and read the paperwork, believes that these standards are not being met he/she should contact the stallion manager and inform the mare owner.

IV Semen collection (including training the stallion for semen collection)

.
There are various models of AV available – this author routinely uses the Missouri model. AVs should be filled so that the lumen is c 38° C, and lubricated with a non-spermicidal lubricant, wearing a long glove. Novice stallions may not necessarily ejaculate the first time they mount the dummy. Semen should be filtered either during or immediately after collection to remove the spermicidal gel portion of the ejaculate.

Semen can be collected from stallions for use in artificial insemination using a real mare, a 'dummy' mare, or 'ground collection'. If a real mares is being used, she should either be in behavioural oestrus, or to be ovariectomised. Ovariectomised mares have historically been treated with oestrogen to make them display signs of oestrus. There is no oestrogen currently licensed for use in horses in the UK. The use of a real mare exposes both stallion and mare to injury from kicking or biting, and the person collecting the semen is of necessity situated between the two horses. Collection of semen using 'ground collection' (i.e. when the stallion mounts neither a real mare nor a dummy, but is collected from whilst standing on the ground) is useful in some cases of orthopaedic or neurological disease which prevent the stallion from mounting and ejaculating. However, it is not well tolerated by all stallions and is always dangerous for the semen collector and stallion handler. Collection using a dummy mare is generally the safest and preferable method.

Dummy mares are commercially available. They should be situated under cover, in an area with adequate space, and with a non-slip, disinfectable flooring. Dummy mares should be disinfected between stallions. Safety is paramount – everyone involved in the collection process should wear a hard hat, a back protector and shoes which provide protection. Everyone should be aware in advance of where they should go should they become endangered. The stallion should be suitably restrained e.g. using a Chiffney bridle and long lead rope (not a lunge line), and the stallion handler should be experienced. The stallion is trained to mount the dummy by placing the teaser mare (usually in oestrus) on the far side of the dummy and allowing the stallion to tease her over the dummy. With patience, the stallion will become aroused and will start to try and reach the mare by jumping onto the dummy. Some stallions become frustrated (and dangerous) during the training process – multiple short training sessions are more rewarding and safer than fewer long sessions. When the stallion has mounted the dummy, the person handling the artificial vagina (AV) moves forward on the left side of the dummy, and deflects the stallion's penis into the AV. The stallion handler should also be on the left side of the dummy.

V Common reproductive problems of stallions seen in practice- diagnosis and treatment

(i) Lack of libido

Lack of libido can limit a stallion's breeding career, and becomes rapidly frustrating for all involved, including the stallion himself. In stallions as in the males of other species, Gonadotrophin Releasing Hormone (GnRH) from the hypothalamus promotes release of the luteinising hormone (LH) from the anterior pituitary gland, and LH stimulates the Leydig cells of the testes to secrete testosterone. Testosterone is important for spermatogenesis, and also plays a role in sexual behaviour. However, the relationship between plasma testosterone levels and libido is not straightforward, with some recent research suggesting that higher plasma testosterone levels are primarily a reflection of competitive breeding effort rather than the cause of a stallion's libido.

Lack of libido is commonly encountered in practice. Successful treatment of lack of libido in stallions requires a very careful history taking, appreciation of the likely underlying cause, and appropriate, sympathetic, and patient management. In the course of undertaking this process, one must never forget the safety risks to personnel and horses. Lack of libido may be consistent, or erratic. It frequently has a psychogenic cause, which may be associated with pain. Treatment in the first instance should revolve around identifying and treating underlying causes of the lack of libido (e.g, pain due to an orthopaedic problem); retraining and adjusting management practices. Pharmacological treatment of lack of libido should be a last resort – see references.

McDonnell, S. M. (2011). Pharmacological manipulation of ejaculation. In: Equine Reproduction. McKinnon, A.O., Squires, E.L., Vaala, W.E and Varner, D.D. Wiley Blackwell**.** 1413-1417

McDonnell, S. M., Kenney, R.M., Meckley, P.C and Garcia, M.C. (1985). Conditioned suppression of sexual behaviour in stallions and reversal with diazepam. Physiol Behaviour **34**(6): 951-956.

(ii) ‘Accumulator stallions’

In stallions which do not ejaculate frequently, sperm may accumlulate in the (anatomically long) vas deferens. This typically results in an ejaculate with a very high concentration of very poor quality when the stallion is collected from for the first time in a while – detached sperm heads and ‘rafts’ of dead cells folating across the microscope slide are common. One often sees such a picture at the beginning of the breeding season. Treatment is frequent collection from the stallion until the ejaculate normalises itself – this may take up to 10 days of daily collections. In some stallions it is necessary to collect from them regularly to avoid this ‘accumulator’ problem, even if semen orders are infrequent.

(iii) Urospermia

Urospermia occurs as a result of the common pathway through the urethra for semen and for urine. The pathogenesis is not well understood, but essentially the sphincter at the neck of the bladder which ought to close tightly during ejaculation fails to do so sufficiently, and so urine “leaks” and contaminates the ejaculate. This failure may be related to Neurological dysfunction e.g. Cauda equine syndrome or EHV 1 infection, or might reflect urinary incontinence. Typically, the ejaculate is a large volume, has a yellow colouration, an unpleasant odour (ammonia), and poor sperm motility, with an unusually swift decline in motility following collection. The pH of the ejaculate may be more alkaline than normal (normal = 7.7). When the ejaculate is tested with a BUN reagent test strip the strip turns from yellow to green within 10 seconds. Calcium carbonate crystals may be visible on microscopic examination of the ejaculate. Fertility rates using AI (particularly shipped semen) are poor. When natural cover is being used poor fertility / high rates of post-breeding endometritis in the mares which have been covered may be the only clues. Treatment of urospermia includes encouraging the stallion to urinate before collection; possible use of diurectics, prompt extension of the ejaculate to 25 million / ml, and often centrifugation of the ejaculate, removal of the (urine-containing) supernatant, and re-extension of the sperm pellet.