Examination of mares and fillies for breeding purposes

This article provides an overview of the examination of mares and fillies for breeding purposes, with a suggested protocol for examination including: identification and history taking; physical examination; and screening for infectious reproductive diseases, potentially heritable defects and genetic diseases. Examination of the female reproductive tract is discussed based on its anatomical elements, along with guidance on assessment, indications for further investigations and conditions which may affect the future fertility of the individual. An in-depth evaluation of vulval conformation is described and quantitative assessment is illustrated using the 'Caslick's index'. The limitations of the examination are described, along with guidance on certification of the examination findings.

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The pre-breeding or 'fillies' examination is a common procedure in broodmare practice, performed either pre-breeding or before purchase or sale at public auction. The examination is designed to assess whether the filly or mare has the potential to become pregnant, carry and successfully deliver a foal at term (Pycock, 2004). In the authors' experience, clients think that this should be a quick and simple 'check'; however, in reality, this can be challenging as true fertility is only demonstrated when a viable foal is born. The veterinary surgeon should be aware of the entire range of techniques that can be used to investigate the reproductive potential of a mare, and be clear what the client's expectations are, before performing the examination. It is also important to establish what the intended breeding strategy may be: for example, is this a Thoroughbred mare that will be bred by natural cover or is this a sports horse that will be bred via artificial insemination with foals by embryo transfer? The budget the client has allowed for the future veterinary management of the mare may also have a bearing on the veterinary surgeon's opinion. Regardless of this, the examination needs to be thorough and methodical, with the results recorded in a systematic and accurate manner.

A protocol for examination would normally comprise the following procedures:

- Review of previous breeding history (if any)
- Assessment of physical condition, general health and perineal conformation
- Assessment of disease status; blood samples for serological screening of equine viral arteritis, equine infectious anaemia and clitoral swabbing to screen for *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (capsule types 1, 2 and 5) and *Taylorella equigenitalis* (contagious equine metritis organism)
- Visual examination of the vagina and cervix with a speculum
- Manual vaginal examination
- Transrectal palpation and ultrasound examination
- Visual inspection of the mammary glands
- Additional disease screening and/or genetic screening (if required)
- Collection of blood for reproductive hormone analysis (if required).

Unless the mare is repeatedly examined at all stages of the oestrous cycle certain abnormalities, such as excessive uterine fluid during oestrus, may not be detected. To increase the predictive strength of the examination the following techniques can be performed:

- Collection of endometrial samples (swab or lavage fluid) for culture with or without cytology
- Endometrial biopsy
- Hysteroscopy.

Identification and history

Accurate and detailed identification is essential and should be included in any report. Name, age, breed, colour/markings and microchip number should be checked against a valid passport. As detailed a history as possible should be collated, including:

- Present use (performance or breeding)
- Temperament
- Body condition
- Previous illness, injury or surgery

- Drug and vaccination administration (passport checked).

For mares with a previous breeding history the following information (if available) is important:

- Number of previous foals, abortions or neonatal deaths
- Any difficult foaling or gestational complications
- Last foaling date and foaling outcome
- Number of previous breedings; stallions and types of semen used
- Oestrus cycle length, duration of oestrus, intensity of oestrus signs
- Previous gynaecological examinations and/or treatments relating to sub-fertility.

Physical examination

The mare will have to be healthy and physically sound to carry a foal to term. Evaluation of the general body systems is an important part of the examination; the mare's temperament is also an important consideration. In addition, the mare should be free from any potentially heritable defects or genetic diseases that may pass to her offspring; these include:

Heritable defects:

- Parrot mouth
- Inguinal and umbilical hernia
- Laryngeal hemiplegia
- Patella luxation (ponies and miniatures)
- Congenital cataracts.

Defects that have a genetic predisposition or hereditary component:

- Wobbler syndrome
- Osteochondrosis
- Degenerative joint disease.

Genetic disease examples (note this is not an exhaustive list):

- Severe combined immunodeficiency disease and lavender foal syndrome of Arabian horses
- Hyperkalaemic periodic paralysis of Quarterhorses, Paint horses and Appaloosas
- Overo lethal white syndrome of Quarterhorses and Paint horses
- Hereditary equine regional dermal asthenia of Quarterhorses
- Congenital stationary night blindness of Appaloosas
- Junctional epidermolysis bullosa of Belgian horses
- Hoof wall separation disease of Connemaras
- Warmblood fragile foal syndrome of Warmbloods.

Note: different breed societies and registration bodies have different views on heritable traits and genetic diseases and registration eligibility; therefore the relevant breed society or stud book for the breed should be consulted if necessary.

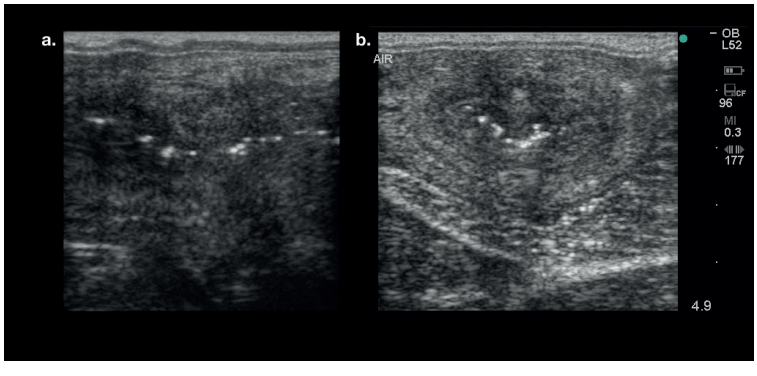

Figure 1. Transrectal ultrasound examination demonstrating (a) longitudinal view of the body of the uterus and (b) cross section of a uterine horn demonstrating hyperechoic foci indicative of air within the uterine lumen (ultrasound depth 4.9 cm).

External genitalia (perineum/vulva)

Normally the vulva provides an effective barrier to protect the uterus from ascending infection. If the vulval seal is incompetent, pneumovagina may occur and the reproductive tract can become contaminated leading to bacterial and/or fungal infection. Conformational defects compromising the vulval seal, such as an increased proportion of the vulval opening above the pelvic brim, a sunken anus and a sloping vulva, predispose to pneumovagina. Previous perineal injury as a result of foaling or external trauma may additionally disrupt the vulval seal.

Some mares make an obvious noise while walking, but in other mares the condition may be more subtle. The presence of a frothy exudate in the anterior vagina on speculum examination is pathognomic for pneumovagina. Rectal palpation of a ballooned vagina or uterus from which air can be expelled confirms the diagnosis. Real-time ultrasound examination of the uterus may reveal the presence of air represented by hyperechoic foci (*Figure 1*). Any sign of discharge from the vulva should be noted; discharge may be seen at the vulval lips or a residue may be noted on the tail hairs or hocks. Cytological and histological examination of the endometrium may demonstrate significant numbers of neutrophils and eosinophils indicative of endometritis.

The presence of any pre-existing Caslick's vulvoplasty procedure, or the likely future need for such surgery, should be documented. A vulval anterior slope of 80˚ or more to the horizontal is optimal (*Figure 2*), while an angle <50˚ is likely to result in pneumovagina (Caslick, 1937). When more than 4 cm of the vulval opening is located above the level of the pelvic brim, anterior deviation of the dorsal commissure towards the anus becomes more likely, in turn increasing the chances of pneumovagina (Pascoe, 2007). A formula for determining the 'Caslick's index' of a mare helps determine whether a Caslick's vulvoplasty is indicated (Pascoe, 1979) (*Figures 3* and *4*).

Clitoris

The clitoris should be of normal size and appearance. In some circumstances the mare may have had her clitoris removed (see bacteriological swabbing).

Vagina

The entire vagina and the external opening of the cervix should be examined visually, using a speculum with a bright light source. It is also helpful to carry out manual examination of the vagina and cervix. Vesicovaginal reflux, also known as urovagina or

Figure 2. Mare with normal perineal conformation with a vulval anterior slope of approximately 80˚. Digital palpation reveals the level of the pelvic brim, which in this case is at the level of the dorsal vulval commissure.

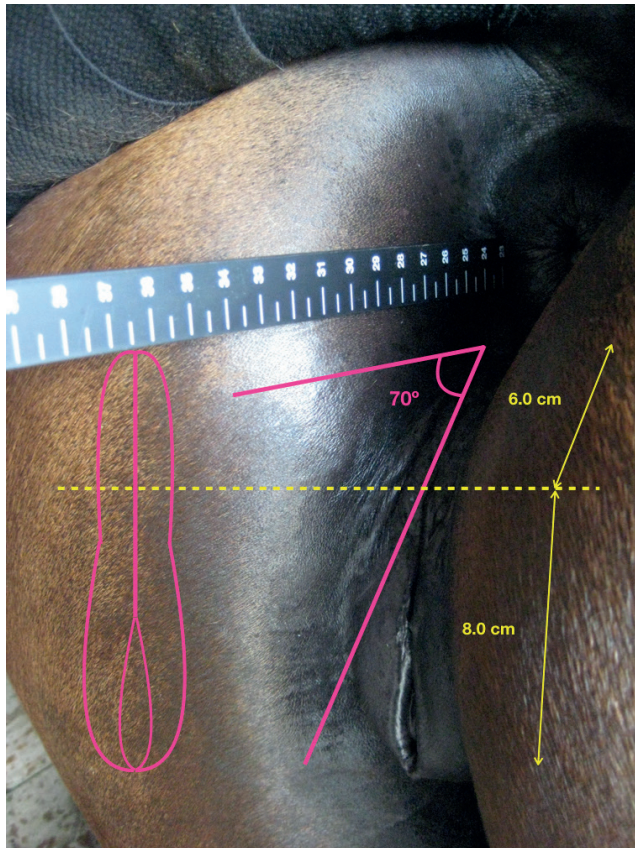

Figure 3. Poor perineal conformation of a mare, with diagrammatic overlay demonstrating the shape of the vulva, angle of inclination and measurement of the vulval opening above and below the level of the pelvic brim (dotted yellow line). Note the anus has a 'sunken' position that is 9 cm rostral to the level of the clitoris.

urine pooling, is the retention of incompletely voided urine in the vagina as a result of an exaggerated downward cranial slope of the vagina. Pneumovagina as a result of defective vulval conformation or injury also predisposes to the condition. Transient urine pooling, which is sometimes found in post-partum mares, usually resolves after post-partum uterine involution is complete. Uterine infection with an accumulation of exudate in the vagina can sometimes be confused with the condition. Vaginal bleeding from varicose veins at the dorsal vestibulovaginal junction is occasionally seen in older mares, particularly during oestrus. Treatment is not usually necessary as the varicosities usually shrink spontaneously; however, in certain mares bleeding may persist, necessitating treatment.

Manual vaginal examination of maiden mares often reveals the presence of hymen tissue, which generally breaks down with digital pressure. A complete persistent hymen can also occur, which can result in the accumulation of fluid within the vagina and uterus as a result of impairment of natural drainage. Sometimes the hymen may be so tough that it can only be breached using sharp dissection. Rarely, failure of proper fusion of the Müllerian ducts may result in the presence of bands of tissue running dorsoventrally in the vagina. They do not interfere with fertility directly but can cause discomfort during natural mating. These can be broken down manually or removed surgically.

Cervix

The cervix, while forming an important physical barrier to protect the uterus, must also relax during oestrus to allow intrauterine ejaculation of semen at coitus and drainage of uterine fluid. A cervicitis is usually associated with endometritis and/or vaginitis. Fibrosis of the cervix often occurs in older mares, particularly teenage maiden mares, and this class of mare may encounter problems with uterine drainage post mating or insemination, necessitating greater reproductive management (Pycock, 1993). Impaired cervical drainage of uterine fluid can predispose to chronic endometritis. Lacerations and/or adhesions of the cervix arise from trauma at parturition or mating. Such abnormalities may have a significant effect on fertility and therefore mares with cervical damage, other than the smallest of adhesions, should be regarded as questionable breeding prospects.

Caslick's index = distance between dorsal commissure and pelvic brim (cm) x vulval angle
= 6.0 x 70 = 420
Interpretation of results: <100 normal; 100–150 further evaluation required; >150 Caslick's vulvoplasty required

Figure 4. Example of Caslick's index with measurements from Figure 3.

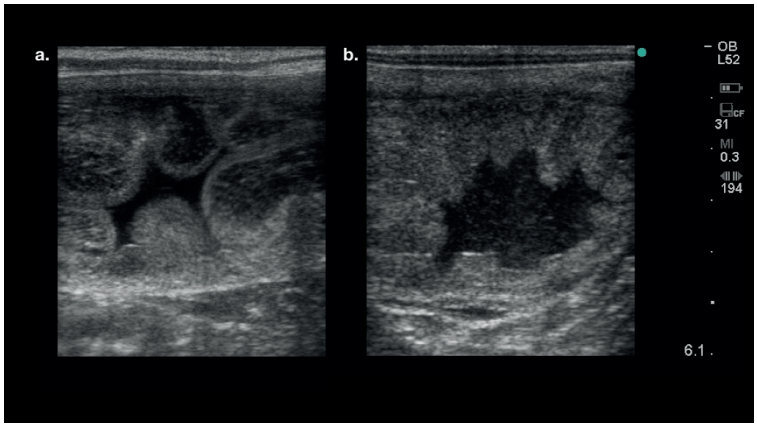

Figure 5. Transrectal ultrasound examination demonstrating two different accumulations of fluid at the uterine body-horn bifurcation. a) demonstrates a small (less than 1 cm) amount of grade 0 (scale 0-5) anechoic fluid; note the presence of grade 2 (scale 0-3) uterine oedema within the endometrial folds indicative of the mare being in oestrus or the transitional phase of the cycle. b) demonstrates approximately 2 cm of grade 2 fluid with echogenic specs; note the absence of endometrial oedema, this warrants further investigation (ultrasound depth 6.1 cm).

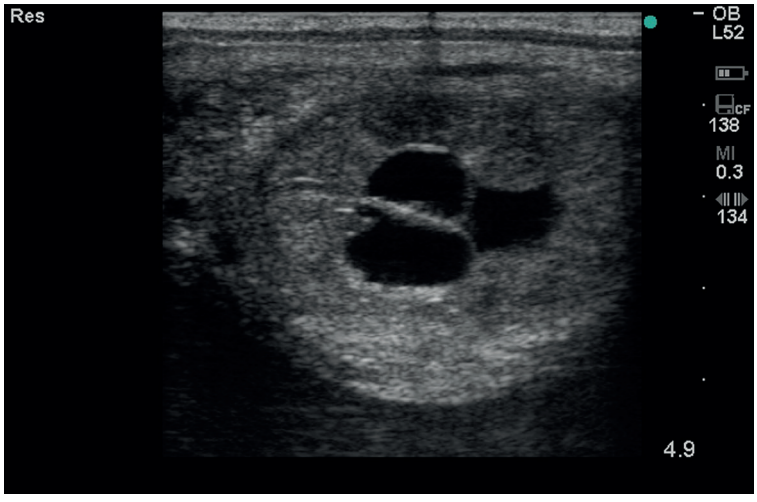

Figure 6. Transrectal ultrasound examination demonstrating three cysts in 'complex' in the uterine horn (ultrasound depth 4.9 cm).

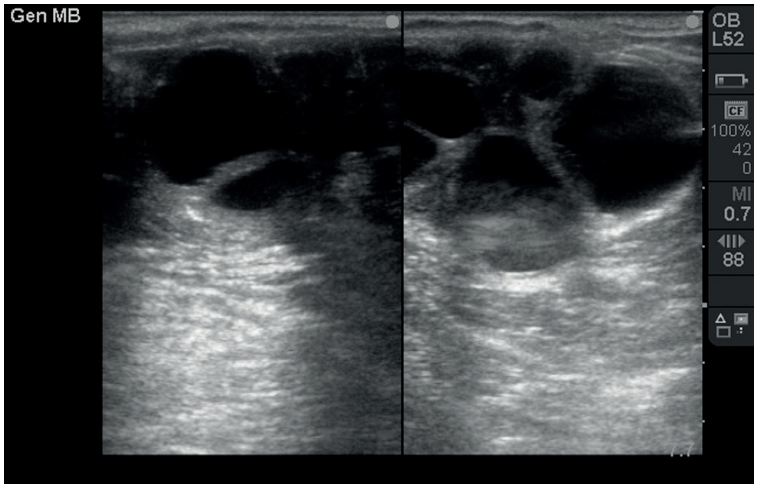

Figure 7. Transrectal ultrasound examination on 28 March demonstrating left and right ovaries of a mare consistent with being in the transitional stage of the oestrus cycle (ultrasound depth 7.7 cm).

Developmental abnormalities of the cervix have been described and these likely render the mare infertile.

Uterus

The uterus should be evaluated initially by thorough rectal palpation followed by ultrasound examination. During evaluation of the uterus any unusual shape or consistency should be noted. In particular any enlargements which may represent pregnancy, cysts, fluid accumulations, intrauterine masses or foreign bodies should be recorded. These will be further investigated during the ultrasound examination.

Detection of intraluminal uterine fluid using transrectal ultrasound imaging

Transrectal ultrasonography allows assessment of the ultrasonographic architecture of the uterus and the identification of small volumes of intrauterine fluid, which can not be palpated per rectum. In many cases, the uterine luminal fluid which accumulates before mating is sterile and contains no neutrophils (Pycock and Newcombe, 1996). The importance of these sterile fluid accumulations is that, although initially sterile, the fluid may act as a culture medium for bacteria which gain entry to the uterus at mating leading to establishment of an endometritis. The amount of fluid which should be considered significant is not clear. This is particularly true of fluid appearing during oestrus. The significance depends to some extent on when during oestrus the fluid is observed; fluid detected early in oestrus may have disappeared when the mare is further advanced in oestrus and the cervix relaxes more.

Small volumes of intrauterine fluid during oestrus do not affect pregnancy rates in contrast to mares with larger (>2 cm depth) collections of fluid (Pycock and Newcombe, 1996; Brinsko et al, 2003; *Figure 5*). In mares that are susceptible to endometritis there is an accumulation of more fluid during oestrus than in resistant mares. Intrauterine fluid during dioestrus is indicative of inflammation, and associated with subfertility (Newcombe, 1997).

Uterine cysts

Uterine cysts (lymphatic lacunae) are the most common type of uterine lesion identified in the mare. The relationship between subfertility and uterine cysts is not well established (Eilts et al, 1995). A large number of uterine cysts may reduce pregnancy rates, but some studies have concluded that there is no strong evidence to suggest that cysts adversely affect the establishment or maintenance of pregnancy. Their presence could potentially restrict early conceptus mobility thus interfering with pregnancy recognition, while later in pregnancy they could disrupt nutrient absorption by the developing conceptus (*Figure 6*).

It is difficult to determine, in a given mare, if any cysts present will have an effect on fertility however, in the authors' experiences only mares with large or multiple cysts encounter problems. It is important to note that the number of cysts usually increases with age, as does the degree of age-related degeneration of the endometrium, which will certainly have an effect on the fertility potential of the mare.

Ovaries

The ovaries should be of normal size and activity for the time of year, that is, for the expected stage of the reproductive cycle (*Figure*

7). Mares examined outside the physiological breeding season may have small and inactive ovaries, which would not be considered abnormal; however, if a mare is presented during the breeding season with such ovaries she should be considered a questionable breeding prospect or re-examination after a period of time recommended. Often the question arises of how small the ovaries can be outside the physiological breeding season and therefore how small can be judged as being normal (*Figure 8*). The answer is that this can be highly variable, with some mares continuing to have follicular activity throughout the winter, whereas others go into deep anoestrus with ovaries shrinking to the size of a kidney bean. If such inactivity is seen, then recommending the mare as a sound breeder should be made with caution. The presence of abnormally large ovaries also warrants caution and further investigation and diagnostic endocrinology may be necessary.

Diagnostic endocrinology

A diagnostic panel should include progesterone, testosterone and anti-Müllerian hormone to allow differentiation between large ovaries as a result of large and/or irregular luteal tissue, anovulatory structures including haemorrhagic anovulatory follicles, and those caused by granulosa cell tumour (Crabtree, 2011; Ball et al, 2014). Progesterone levels of greater than 2 ng/ml circulating progesterone indicates active luteal tissue and uterine oedema would normally be absent. Uterine oedema usually reflects circulating progesterone levels of less than 1 ng/ml (Pycock et al, 1995), and the degree of oedema is generally correlated with the amount of follicular development. The anti-Müllerian hormone test has become the test of choice in the diagnosis of granulosa cell tumour, and concentrations greater than 4 ng/ml seem to be diagnostic for equine granulosa cell tumours when compared to normal mare ovaries (Ball et al, 2013, 2014). The majority of granulosa cell tumours will present in the absence of active luteal tissue and the addition of testosterone may be advantageous in some cases (Crabtree, 2011).

Disease screening

The mare needs to be free from disease that may be contagious, venereally or otherwise. Ideally, potential broodmares should be screened for *Ps. aeruginosa*, *K. pneumoniae*, *T. equigenitalis*, equine viral arteritis, equine infectious anaemia and strangles so that they can fulfil even the most strict stud entry requirements; however, the client's requirements or those of the potential purchaser can be determined before the examination. As *T. equigenitalis*, equine viral arteritis and equine infectious anaemia are notifiable diseases in the UK, it is necessary to give consideration to the consequences of a positive result and informed consent gained from the owner.

Bacteriological swabbing

The three main pathogenic organisms, *Ps. aeruginosa* and *K. pneumoniae* (capsule types 1, 2 and 5) and *T. equigenitalis*, can be carried subclinically by the mare. Bacteriological swabbing of the mare's clitoris for these bacterial agents should ideally be performed before breeding (*Figure 9*). If the mare is to be bred by artificial insemination only, then such screening may not be absolutely necessary, but is still recommended. Guidelines in the Horserace Betting Levy Board Codes of Practice (https://codes.hblb.org.uk/), for the collection and processing of samples at British Equine Veterinary Association-approved laboratories, should be followed. A heavy pure growth of any bacterium warrants further investigation. The treatment regimen for positive culture of the above organisms may involve surgical removal of the clitoris (clitorectomy). In the event of a filly or mare having had a clitorectomy the authors recommend screening of the site of the removed clitoris and the endometrium for the above bacterial agents.

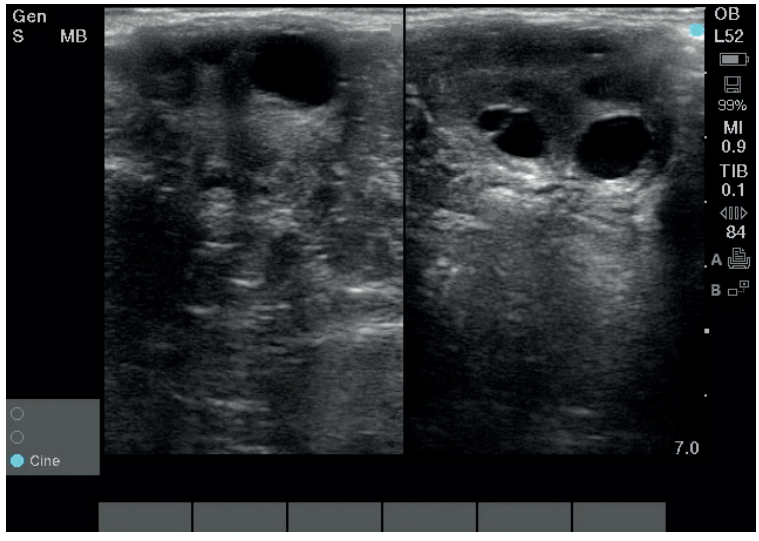

Figure 8. Transrectal ultrasound examination on 21 May demonstrating left and right ovaries of a mare consistent with seasonal anoestrus with the entire ovaries measuring approximately 2 x 3 cm, this is abnormal for this time of year and warrants further examinations/investigations (ultrasound depth 7.0 cm).

Figure 9. Taking of a clitoral swab (swab tip in the ventral clitoral fossa).

Endometrial culture and cytology

Collection of endometrial samples can be made via either trans-cervical or guarded endometrial swabs, cytobrush, or by uterine

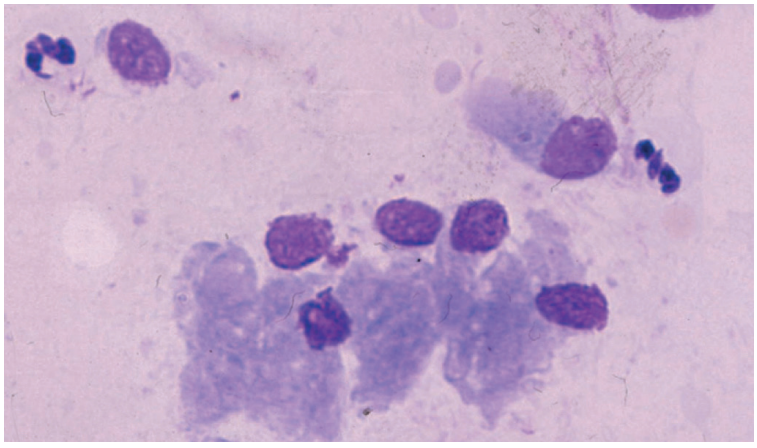

Figure 10. Cytological smear under high power stained in haematoxylin and eosin demonstrating endometrial cells with two neutrophils (middle right and top left) indicative of active inflammation.

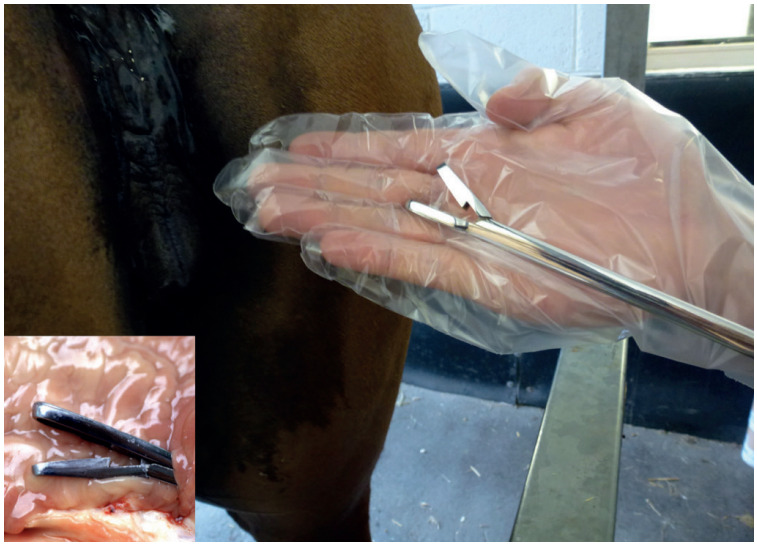

Figure 11. Preparing to perform an endometrial biopsy, and biopsy forceps against the endometrium (insert).

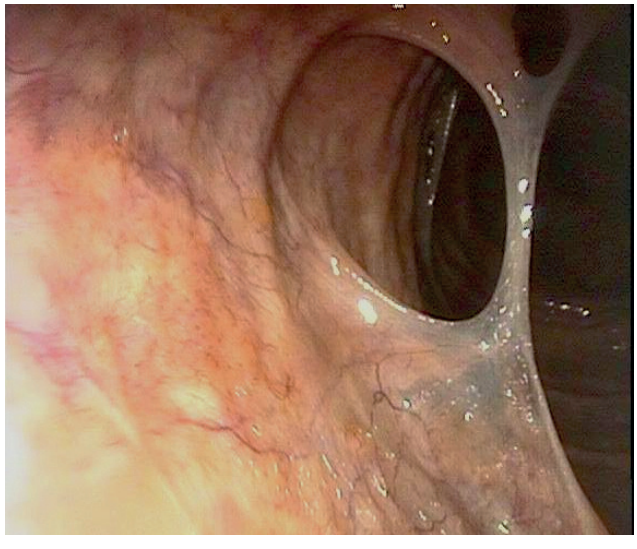

Figure 12. A uterine adhesion visible on hysteroscopy. This is an example of pathology which may influence fertility which would not have manifest unless hysteroscopy had been performed.

lavage. Positive culture results should ideally be interpreted alongside endometrial cytology results to determine the significance of any bacterial or fungal growths (*Figure 10*). Culture and cytology of lavage fluids is likely to have a greater sensitivity for endometritis than endometrial swabbing (LeBlanc et al, 2007; Overbeck et al, 2011). Ideally swabs should be collected during oestrus; however, if samples are collected during dioestrus it is recommended to administer an appropriate dose of prostaglandin to induce oestrus post sampling. Any endometritis would have to be resolved before breeding commenced. The degree of management that this would require and the prognosis for future breeding would depend on the agent(s) identified.

Endometrial biopsy

Endometrial biopsies are typically performed using Yeoman (basket-jawed) biopsy forceps, achieving tissue specimens of approximately 2 x 1 cm (*Figure 11*). Routine sampling is performed in the region of the base of the uterine horns, and single samples are usually representative of the entire endometrium. If the uterus has any abnormality on transrectal palpation or ultrasound then the veterinary surgeon should aim to take samples from both the affected area and a normal area. This may necessitate site-directed sampling using hysteroscopy. The endometrial biopsy sample should be sent to a laboratory that is experienced in evaluating such samples. Analysis of the biopsy allows assessment of the degree of inflammation (chronic infiltrative endometritis) and degenerative changes (chronic degenerative endometrial disease or endometrosis) in the endometrium and enables an objective assessment of potential fertility (Kenney and Doig, 1986; Ricketts and Alonso, 1991).

Hysteroscopy

This is usually reserved for infertility investigations, but if any of the elements of the above examination suggest that there may be an intrauterine lesion which requires further assessment then hysteroscopy can be used (*Figure 12*).

Limitations

Such an examination does not guarantee that a mare will conceive and produce a viable foal. Certain areas of the mare's reproductive tract, such as the oviducts, cannot be effectively evaluated in the pre-breeding or pre-purchase examination conditions. It is also important to note that what is determined by the examination at the specific point in time may not be true in the future. The examination should be referred to as a guide to the fertility potential of a mare and should not be referred to as an absolute measure of fertility. When it comes to reporting or certifying the results of such an examination, it is important to define what the examination actually involved and what the findings were. If appropriate, a disclaimer should be given that the results of the examination do not constitute a warranty or representation that the filly is capable of breeding or is fit for breeding purposes, simply that it has the necessary apparatus to be able to breed. The limitations of a one-off examination also need to be born in mind. If the examination is performed before purchase then the examination should either accompany a standard 'five stage' prior to purchase examination or would constitute a

'limited prior to purchase examination' and certification provided using the approved prior to purchase certificate.

Conclusions

A mare or filly that is to be used for breeding should be physically fit and healthy, free from undesirable behaviour and potentially heritable traits, free from disease, and be physically capable of breeding. These attributes can be assessed by implementing the physical examination protocol described, but one has to accept the limitations of such an examination as the true demonstration of fertility is the delivery of a live foal. The examination should be referred to as a means for selecting out obviously unsatisfactory breeding prospects and not as an absolute measure of fertility. EQ

KEY POINTS

- Understand what is being asked of you in performing the examination and what the expectations of the person(s) requesting the examination are.
- Perform the examination in a thorough and methodical manner.
- Record the results in a systematic and accurate manner.
- When reporting the results, define what the examination involved, its limitations and what the findings were.
- This examination should be referred to as a means for selecting out obviously unsatisfactory breeding prospects and not as an absolute measure of fertility.

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