BattLab
Quality Veterinary Diagnostics
from disease to optimal health

LABOKLIN aktuell

Issue 01/2023

Breeding soundness examination and hormonal pregnancy diagnosis in horses

Photo credits: envatoelements

Reproductive fitness

Indication and sampling

Fertility disorders of the mare can be caused by bacterial colonisation of the reproductive organs. During natural covering, these bacteria can then lead to an infection of the stallion. For good fertility, uterus and cervix must be free of pathogenic or conditionally pathogenic agents.

This requires a bacteriological and possibly also a mycological culture of a swab sample taken under sterile conditions.

In mares that have a healthy foal at foot after an undisturbed birth and which show no clinical signs of a genital infection as well as in three-year-old maiden mares, it can be assumed that there are usually no pathogens in the uterus that could impair fertility. In these cases, a microbial examination is not carried out, yet it is often requested by the owner of the stallion in natural breeding.

A culture test is absolutely necessary if

- mares have not or not successfully been covered or inseminated the previous year;
- clinical signs of genital disease are present;
- mares have aborted;
- foaling and passing of the placenta were not normal;
- mares have returned to oestrus twice in the current breeding season.

Pregnancy must always be ruled out before taking a swab sample! The ideal time is when the mare is in oestrus and the cervix is open. For a reliable bacteriological result, it is very important that the swab does not come into contact with the skin, the vulvar vestibule or the vaginal mucosa. There may be pathogens in these areas that are not present in the uterus.

Batt Laboratories Ltd, University of Warwick Science Park, The Venture Centre, Sir William Lyons Road, Coventry CV4 7EZ
Tel: 0247 632 3275. Fax: 0871 750 5323. Email: admin@battlab.com.

In any case, sampling should be done with a speculum in order to obtain the best possible results.

Cervical forceps can be used to facilitate the passage through the cervical canal. When taking samples with the help of a speculum, the mucous membrane can simultaneously be assessed for signs of inflammation or deposits.

Reproductive fitness: bacteriological findings

The following bacteria are classified as pathogenic in the breeding soundness examination; here, treatment is recommended before breeding even if mares are clinically healthy:

- beta-haemolytic streptococci
- *Staphylococcus aureus*
- *Escherichia coli* var. *haemolytica*
- *Pseudomonas aeruginosa*
- *Klebsiella* spp.
- *Raoultella ornithinolytica* (formerly: *Klebsiella ornithinolytica)*
- *Actinobacillus equuli*
- *Bordetella bronchiseptica*
- *Rhodococcus hoagii* (formerly called *R. equi*)

Pathogen differentiation is done based on culture morphology and by MALDI-TOF.

If *Escherichia coli* are present and there is no haemolysis, treatment is still recommended before breeding if the bacterial count is high and they are detected in pure culture. The microbiological findings must always be assessed in connection with the clinical changes observed in the gynaecological examination.

In these cases, the attending veterinarian decides on whether to allow the mare to mate or if any measures are necessary, such as treatment or a waiting period.
If pathogens are found that are relevant for the reproductive fitness, an antibiogram is prepared using the microdilution method.
Other bacteria that are considered non-specific are listed in the findings, but no susceptibility test will be carried out.

The bacteriological examination usually takes 2 – 3 days, including the antibiogram.

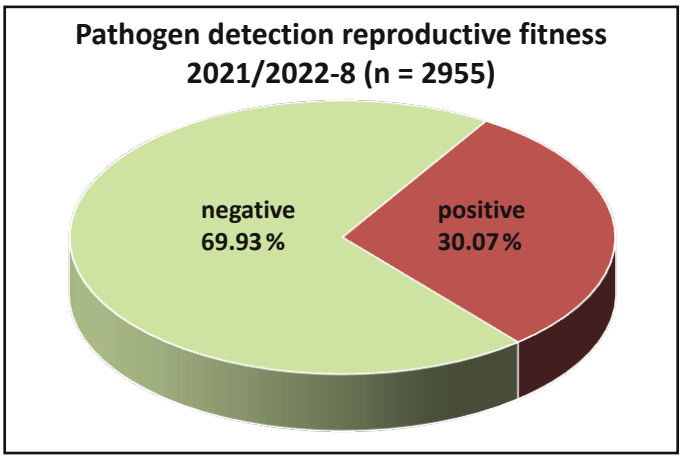

Fig. 1: Proportion of pathogenic bacteria detected in culture when testing reproductive fitness (horse) *Photo credits: Laboklin*

We analysed swabs of horses submitted in 2021 up to August 2022 for breeding soundness examination by culture. There was usually no information given about clinical signs.

Pathogens were detected in about 30% of the submitted samples. With 21.79%, beta-haemolytic streptococci were detected most frequently among the bacteria classified as pathogenic.

Fig. 2: beta-haemolytic streptococci on blood agar *Photo credits: Laboklin*

In 1.70% of the samples, there were *E. coli* without haemolysis and in 1.19% of the samples, *E. coli* with haemolytic properties were present at high levels in pure culture.

Even though *Staphylococcus aureus* was only the fourth most common pathogen detected (0.81%), it is worth mentioning that 24% of the isolates were methicillin-resistant.

Taylorella equigenitalis

Contagious equine metritis (CEM) is a notifiable venereal disease caused by the bacterium *Taylorella equigenitalis*. While infected stallions are usually asymptomatic carriers, *Taylorella equigenitalis* can lead to endometritis and fertility disorders in mares. However, inapparent infections may occur as well. According to Directive 92/65/EEC, swab samples must be taken from the mare from at least 2 sites – the clitoral fossa and the clitoral sinus. In addition to growing a culture, PCR is another suitable test method. Regardless of the method, only swabs with added charcoal (Amies transport medium) are to be used.

The culture should be started at the latest 24 hours after sampling (48 hours after sample collection when transport is refrigerated). When using PCR, there should be a maximum of 48 hours between sample collection and test preparation. Please note that a separate swab is required for each method.

In Icelandic horses, in addition to the culture test of the breeding soundness examination, it is required to do a culture to exclude *Taylorella equigenitalis* prior to breeding.

A culture test for *Taylorella equigenitalis* takes 7 days. PCR has the benefit of providing a quicker diagnosis within 1 – 3 working days.

Reproductive fitness: mycological findings

Especially if whitish coatings are found during a visual inspection of the vaginal mucosa, a fungal infection should be considered. A massive infection usually results in the uterus being severely filled with grey-mucous and cloudy secretion. The cervical mucosa and the endometrium appear dirty-dark red in colour and clearly inflamed.

The most common yeasts detected in the positive mycological cultures were of the genus *Candida*, in our sample material, *Candida parapsilosis* (3.5%) dominated in pure culture and with a high count.

Candida parapsilosis can infect skin and cornea as well as mucous membranes. In humans, it is feared as the causative agent of endocarditis after a catheter procedure.

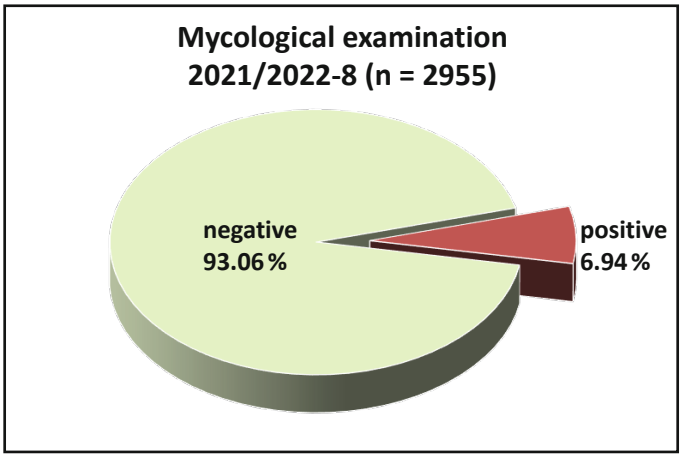

Fig. 3: *Proportion of detected yeasts and moulds in mycological culture*
Photo credits: Laboklin

A predisposing factor for an infection with yeasts is a previous long-term antibiotic treatment, with the main cause possibly being the intrauterine application of antibiotics. Semen diluents containing antibiotics as part of artificial insemination are also being discussed.

The most frequently isolated moulds were *Mucor* spp. and various Aspergillus species, but counts were usually low. If counts are low, especially in case of moulds, it should be questioned whether a positive culture is due to contamination.

Reproductive fitness: conclusion

During the breeding season, it is important to assess the breeding suitability of all mares intended for breeding. This not only includes a gynaecological examination but also a microbiological examination of cervical or uterine swabs.

Starting the breeding soundness examination early in the pre-season is recommended so that even animals diagnosed with pathogens can be put into breeding condition by taking appropriate therapeutic measures without losing valuable time. If the result of a swab sample is positive, treatment should be carried out before the animal is covered. Treatment success can be controlled at the earliest 10 days after treatment is finished by doing a new bacteriological or mycological examination. If the follow-up is normal, the next oestrus can be used for breeding. With good breeding management

and under ideal conditions, all diagnostic and therapeutic procedures are completed before the breeding season starts.

Hormonal pregnancy diagnosis

After the necessary preparation for reproductive fitness is completed, healthy mares can be inseminated or covered. Examinations accompanying the oestrus are solely of clinical nature and are carried out and documented as follicle checks by rectal palpation – possibly supplemented by ultrasound.

Pregnancy checks are also usually done by rectal palpation, supplemented by ultrasound, especially in the early stages. This is the only way to get an image of a live embryo in early gestation and to exclude e. g. twin pregnancies.

However, manual examination is not always possible in practice. In small horses/miniature breeds, refractory patients or wild and zoo animals, or if rectal lesions are likely to occur, a rectal palpation should be avoided.

In these cases, pregnancy-specific hormones can be determined, which show typical concentrations in defined stages.

We have 2 hormones available for this: pregnant mare serum gonadotropin (PMSG) = equine chorionic gonadotropin (eCG) and oestrone sulphate.

PMSG/eCG

With slight individual variations, this hormone is secreted by the endometrial cups approximately between the 35th and the 120th day of gestation. We recommend taking the sample between the 45th and the 100th day post ovulation. The highest levels of PMSG are measured between days 60 and 75. In the "peripheral areas" of the recommended time frame, there may be questionable results that need to be confirmed by follow-up examinations. If foetal resorption occurs, which can happen particularly during this period, the endometrial cups continue to produce PMSG for weeks, even though there is no longer a viable foetus present. The detection of PMSG is therefore false positive. Furthermore, the affected mares fail to show any heat during this time.

Oestrone sulphate

This hormone is produced by an intact feto-maternal unit and therefore indicates a viable foetus. Starting around the 40th day of gestation, this hormone is secreted in increasing amounts. In these early stages, however, no reliable differentiation from cyclic oestrogen secretion is possible.
We recommend the determination of oestrone sulphate from the 110th day of gestation onwards, as pregnant mares show much higher levels of the hormone at this time. In addition, mares previously found to be in foal by testing for PMSG should now be rechecked by testing for oestrone sulphate. Oestrone sulphate testing can also be done in urine.

Mares with a questionable or inconclusive test result should be re-examined after 3 to 4 weeks.

If the test result is negative in mares that have definitely been in foal for more than 110 days, there may be damage to the foetus/placenta. In such cases, a rectal palpation or ultrasound examination is essential.

Progesterone

Progesterone is the corpus luteum hormone. The test is only useful between the 18th and 21st day post ovulation – assuming the mare has a stable 21-day cycle. As the test cannot distinguish between cycle and pregnancy corpus luteum, detection of an active corpus luteum function at this time only means that the mare is not "returning to oestrus" at the expected time.

Dr Anton Heusinger and Dr. Antje Wöckener

Further reading

Tibary A, Pearson L, Fite C. Chapter 8 - Reproductive Tract Infections. In: Debra C. Sellon, Maureen T Long, Hrsg. Equine Infectious Diseases, 2nd Edition, 2014. S. 84-106.

Heusinger A, Woeckener A. Bakteriologische Untersuchungen zur Zuchthygiene bei der Stute- Enke Verlag Pferdespiegel. 2013; 4: 150-151.

Aurich C. Fortpflanzungsstörungen beim Hengst und Deckinfektionen. In: Reproduktionsmedizin beim Pferd. Aurich C, Hrsg. Stuttgart: Parey, 2005. S. 267-296.

Döcke F. Veterinärmedizinische Endokrinologie. Jena: G. Fischer, 3. Auflage, 1994.

Hoffmann B, Gentz F, Failing K. Investigations into the course of progesterone-, oestrogen- and eCG-concentrations during normal and impaired pregnancy in the mare. Reprod Domest Anim. 1996; 31: 717-723.