Equine reproduction: advances in artificial insemination techniques

Author : Jonathan F Pycock

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Jonathan F Pycock discusses developments in this field, from shipment delay protocols to more effective and less sperm-intensive insemination techniques

Summary

This article covers recent developments in the field of equine artificial insemination. Use of both chilled and frozen semen is discussed. For chilled semen, there is new information about what to do if the semen shipment is delayed and on maximising pregnancy rates in older mares by careful evaluation of the cervix. The number of motile sperm needed for insemination is reviewed in light of the finding that very few sperm actually reach the site of fertilisation. A new technique to improve semen quality using special filters is reported. For frozen semen, the technique of deep uterine insemination is discussed in detail. In this technique, sperm is placed at the tip of the uterine horn rather than in the uterine body. The two different methods – using a rectally-guided catheter and using a videoendoscope – are compared. Pregnancy rates using the two different techniques were found to be similar and pregnancies could be achieved with inseminating sperm numbers as low as 50 million.

Key words

Chilled semen, uterine fluid, sperm numbers, frozen semen, deep uterine insemination

IN the past 20 years, there have been enormous advances in technology in the equine

reproduction field.

Research is actively continuing in many of these areas, resulting in an array of so-called “assisted reproductive techniques” being commercially available to horse breeders. The term “assisted reproductive technique” describes any reproductive procedure apart from natural covering by a stallion. The range of techniques includes:

• collection and transfer of oocytes;

• using intracytoplasmic sperm injection;

• in vitro fertilisation;

• in vitro oocyte maturation;

• cryopreservation of oocytes, embryos and sperm;

• embryo sexing;

• sexing of sperm;

• nuclear transfer (cloning);

• artificial insemination; and

• embryo transfer.

This article will focus on recent developments in equine artificial insemination.

Growth

Artificial insemination (AI) involves introducing sperm into the reproductive tract of the mare without natural mating. During the past three decades, many national and international breed authorities throughout Europe and America have accepted AI.

In some countries, such as the Netherlands, several thousand mares are inseminated each year. Some breed restrictions apply to using AI – most noticeably, the *General Stud Book,* which regulates the thoroughbred.

It is, therefore, important to know which breed registries permit AI.

For those of us involved in the equine breeding industry, it is obvious that AI is set to play an ever-

increasing role in the future.

Two of these areas of growth will be the use of chilled semen and the technique of deep uterine insemination (DUI) when using frozen semen.

There have been studies updating our knowledge about both procedures.

What to do if semen shipments are delayed

Inseminating mares with chilled semen is a common method of breeding, allowing owners to keep their mare at home and increasing the range of stallions available.

The aim of breeding mares with chilled semen has always been to inseminate the mare zero to 24 hours before ovulation. This is achieved by daily palpation and ultrasound examinations to detect a large, soft follicle (Figure 1) and a marked endometrial oedema pattern (Figure 2).

Occasionally, mares do not ovulate as expected, or there may be problems with the courier service transporting the semen from the collection centre to the mare owner's premises or AI centre where the mare is being monitored. Previously, it was felt that because of problems with a high incidence of both pregnancy failure and infection after inseminating the sperm after ovulation, the only option was to miss the breeding cycle. The whole process of lining up the mare for insemination had to be started again. This was both costly and time-consuming.

Work from the UK looked at the breeding records for more than 150 mares inseminated with chilled semen (Newcombe and Cuervo-Aranga, 2010). It found that, contrary to popular opinion, good pregnancy rates with acceptable pregnancy losses can result from insemination with chilled semen within 16 hours of ovulation.

It was noted that it was important the mare's uterus was treated with saline flushing and an infusion of antibiotics after insemination to reduce the risk of infection developing. Repeated administration of oxytocin was also given until the uterus appeared free from fluid. This is extremely useful information for all veterinarians working with chilled semen, as it means we can often successfully inseminate the mare to obtain a viable pregnancy even if the semen arrives several hours late.

Inseminating the older mare: maximising pregnancy rates

Mares often accumulate intraluminal uterine fluid after insemination, and this can prevent the mare from becoming pregnant.

This fluid can be readily detected on ultrasound examination and graded one to four according to the degree of echogenicity (Figures 3a and 3d). The more echogenic the fluid, the more likely it is that

the fluid is contaminated with debris, including white blood cells. However, cellular fluid can appear relatively anechogenic, so care is needed in interpretation. Inspissated pus can be so echogenic that it is overlooked. The actual appearance of the fluid and the ultrasonographic appearance might not be as closely linked as was once thought.

Ultrasonographic appearance may be proportional to the size and concentration of particulate matter within the fluid, rather than the viscosity of the fluid; for example, purulent exudates can appear to be not as echogenic as expected. Air has hyperechogenic foci, and fluid with air bubbles appears cellular. Urine in the bladder can appear echogenic, despite being a watery liquid (Figure 4).

What causes this fluid to accumulate has been speculated on, and failure to drain via the cervix has been thought to be a likely cause. The cervix is vital to allow drainage, and any failure of the cervix to dilate can cause fluid accumulation. This failure of the cervix to open is particularly relevant in the case of older mares in general – and older maiden mares in particular – leading to the term “the old maiden mare syndrome”.

Often, sport or warmblood mares may not be presented for breeding until they are in their teens, and these older maiden mares can be very difficult to get in foal. Older maiden mares have an abnormally tight cervix, which fails to relax properly during heat, so that fluid is unable to drain and, therefore, accumulates in the uterus.

All too often, mare owners assume that the fertility of these mares is comparable to that of young maiden mares; one of the most important aspects of breeding an old maiden mare is to make the owner aware there is a high possibility of a problem. Research by workers in Finland confirmed that the cervix was vital in allowing this fluid to drain (Liepina et al, 2010). This means all mares must have a thorough assessment of the cervix at the time of breeding.

Sperm numbers: how many are needed?

Conventionally, mares are inseminated with a minimum of 500 million progressively motile (live) sperm. This is known as the insemination dose. For semen that is to be shipped as chilled semen, double this amount should be sent (one billion motile sperm) to ensure that there are at least 500 million motile sperm at the time of insemination. Semen should be mixed with pre-warmed (37ºC) extender immediately after collection.

A final concentration of 25 million to 50 million spermatozoa/ ml in extended semen is usually the ideal concentration for shipping chilled semen samples. This means a typical volume of 20ml to 40ml of extended semen to inseminate should be sent.

Contrary to popular belief, there is no need to warm the chilled semen before insemination. It is not known for certain if this traditional dose is more than necessary, but current thinking and clinical impressions suggest that substantially less than this number of sperm may be needed. Only very

few sperm from the millions that are inseminated make it up the uterine horn and through the oviductal papilla and on into the luminae of the oviducts. In one study, only 0.0006 to 0.0007 per cent of inseminated sperm was recovered from the flushings of mares at 18 hours after intrauterine insemination (Rigby et al, 2000).

The semen is infused by a catheter introduced via the cervix to a point half-way along the uterine body. The uterus of the mare is T-shaped and is made up of a first part called the body and then two horns (left and right) (Figure 5). Once the semen has been put in the uterus of the mare, the sperm travel up one of the horns until they reach the tip of the horn.

At the tip of the uterine horn, the sperm arrive at the entrance to the oviduct or uterine tube. This area is called the oviductal papilla or utero-tubal junction. Once sperm have passed through this junction and are in the oviduct, they can fertilise the oocyte.

How to improve semen quality

Until recently, little could be done to improve the semen of the small number of stallions with poor semen quality. In a very exciting development, workers from Brazil found that by using special filters, they could concentrate stallion sperm without the need for centrifugation of the semen sample (Alvarenga et al, 2010).

Centrifugation has been the established technique for concentrating semen samples, but damages the sperm to a greater or lesser extent, depending on the individual stallion. The centrifugation may be harmful, either by causing mechanical damage or by causing a reaction to occur in the membrane surrounding the sperm. To counter this, the special filters developed by the Brazilian group allowed the sperm to be concentrated with no damage to sperm motility and viability.

The ejaculates from eight stallions of different breeds were diluted 2: 1 (extender: semen) with a milk-based semen extender before filtering. The filters were made of a synthetic membrane that did not allow the sperm to pass through.

Other components of the ejaculate, such as seminal plasma (which can potentially be harmful to sperm quality), passed though the filters and were removed. This technique is potentially useful, especially for semen of poor quality that cannot withstand centrifugation, because concentrated sperm is much easier to freeze or chill successfully.

Insemination techniques using low sperm numbers

As stated earlier, the current thinking is that the number of sperm reaching the oviduct may be only between 100 and 1,000.

Once this discovery was made, it seemed a logical conclusion for researchers to investigate how

few sperm may be necessary to achieve fertilisation when placed directly at the tip of the uterine horn during insemination. This interest stemmed from the increase in frozen semen usage. Semen from some stallions is notoriously difficult to freeze or in very short supply, and it would be of tremendous benefit if mares could become pregnant via inseminating much lower doses of sperm.

The technique is termed low-dose insemination, and since the sperm are inseminated much further into the uterus than with conventional AI, the other term used to describe the technique is deep uterine insemination (DUI).

There are two methods for inseminating the sperm deep into the uterus. The first is to use a special catheter, which is guided up the uterine horn by placing one hand in the rectum of the mare and slowly advancing the catheter to the tip of the horn. The second method is to place an endoscope in the uterus and visualise the tip of the uterine horn. The semen can then be inseminated via a special catheter inserted down the channel of the endoscope. These two techniques will now be examined in more detail.

Deep uterine insemination using a rectally-guided catheter

The mare should be prepared for DUI in a clean, well-lit environment; stocks for restraint are essential. Light sedation may be useful in certain cases. The tail should be bandaged and tied out of the perineal region. Immediately prior to insemination, rectal examination should be performed to empty the mare’s rectum of faeces and confirm either the presence of a large follicle about to ovulate or the site of a fresh ovulation.

The inseminator must decide the side of insemination (left or right) based on this rectal examination. The vulva and perineal area should be thoroughly cleansed with very dilute antiseptic solution or mild soap. This is then thoroughly rinsed off with fresh warm water and the perineal area dried with clean, soft, disposable (paper) towels.

The inseminator should use a sterile obstetric glove (such as a glove turned inside out). In certain circumstances, a sterile surgeon’s glove should be placed over the clean rectal glove. It may be necessary to place a small amount of sterile, nonspermicidal lubricant (such as KY Jelly) on the top of the hand around the knuckles.

A special catheter is used, which is long enough (75cm) to reach the tip of the uterine horn while still having one end protrude from the vulva.

The catheters have a special rounded tip at the end (Figure 6) so that they can be advanced up the uterine horn ipsilateral to the ovary with the large follicle or fresh ovulation, without catching on the folds that line the uterus. The catheter should be held with the tip behind the fingertip and the hand brought into the vulva. The external opening of the cervix should be located with the index finger and a finger inserted into the cervical canal.

The catheter is inserted alongside the finger and gently pushed forward. It is very important that the catheter reaches into the uterine body and does not remain obstructed in the cervix. This passage through the cervix is not always easy. The hand used to introduce the catheter is then withdrawn from the vagina and placed into the rectum of the mare. The catheter can then be felt within the uterus and guided deeper into the uterus than can normally be achieved.

In fact, the catheter should be gently pushed until the tip is at the very tip of the uterine horn when it will be adjacent to the oviductal papilla. This is where the sperm should be deposited. The first straw of semen should be inserted into the catheter with the cotton plug towards the outside of the catheter. A steel plunger is used to push the straw to the tip of the catheter, where the open end lodges in the nipple-like protrusion at the end of the catheter. If more than one straw of semen is used for the insemination, the system described above provides an easy and effective way of delivering the semen by removing empty straws from the catheter without having to replace the catheter.

Using this technique, satisfactory pregnancy rates have been achieved with sperm numbers of 50 million to 100 million.

Deep uterine insemination using an endoscopic technique

It is possible to place an endoscope into the uterus of a mare through the vagina and cervix in much the same way as an insemination catheter is passed. The rectum is evacuated and the side of insemination determined, in the same way as for the rectally guided catheter technique.

The perineal area is washed and cleaned and the mare lightly sedated. A 1.6m to 2m flexible video-endoscope, with a diameter of at least 11mm, is used. The endoscope is inserted into the uterus of the mare via the cervix. Air is then passed through a channel in the endoscope into the uterus, which allows the inside of the mare’s reproductive tract to be visualised.

The operator can then gently steer the tip of the endoscope all the way up the uterus until the entrance of the oviduct into the uterus is reached. This area is termed the oviductal papilla (Figure 7).

A special narrow catheter can be passed down a central channel within the endoscope. The tip of the catheter is exposed beyond the end of the channel so it can be visualised. By carefully steering the endoscope’s tip, the catheter can be placed either very close to or touching the oviductal papilla.

Semen is then blown out of the catheter directly on to the papilla’s surface. Using this technique, acceptable pregnancy rates can be achieved using as few as five million sperm.

This represents a 100-fold decrease in the usual number of sperm needed.

By depositing the sperm so close to the site of fertilisation, the distance the sperm need to travel is reduced. In addition, exposure to the potentially hostile uterine environment is reduced. These are the two most likely reasons why sperm numbers can be so drastically reduced.

The future

The implications of DUI for the equine breeding industry need to be carefully evaluated. Obviously, to use catheters to carry out DUI is much easier and cheaper than using an endoscope. One paper looked at whether there was a difference in pregnancy rates between rectally guided or endoscopic insemination (Samper, Gomez and Sanchez, 2008). They found no difference in fertility among breeding seasons for either technique. Although non-statistically significant, there was a small, but consistent, advantage in using the endoscope with low (less than 50 million) sperm numbers.

In our practice, we routinely inseminate mares for frozen semen using the DUI technique with a catheter.

If the client wishes us to reduce the number of straws of semen used or the semen is in very short supply, then we will use the endoscope.

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