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Artificial Insemination in Mare

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Introduction

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 and a marked endometrial oedema pattern. Occasionally, mares do not ovulate as expected, or there may be problems with the courier service transporting the semen from the collection center to the mare owner's premises or AI center where the mare is being monitored.

Semen is collected from stallions using an artificial vagina, cooled to extend the lifespan of sperm and then shipped to the location of a mare awaiting insemination. Mares can be monitored for estrus, or heat, and semen can be ordered and scheduled to arrive before the mare ovulates an oocyte, or egg. Semen usually arrives in less than 24h by air or 48h if shipped by ground. Upon arrival, a veterinarian or trained technician can inseminate the mare prior to ovulation.

History

The horse is the first animal species for which the use of artificial insemination (Al) was recorded. Bowen (1969) cites a report from 1322 in which stallion semen was taken to inseminate mares belonging to an Arab chief. The Russian biologist, Ivanov is credited to be the first person to

develop the technique. He later organized an equine Al center and developed the improved methods of collecting, diluting and transporting the stallion's semen (Tischner, 1991).

Research specifically into artificial insemination (AI) in equids has been limited, as a number of breed societies will still not accept for registration progeny conceived in this way, but many set strict regulations, such as a limit on the number of foals that can be registered per stallion per year. In Europe, Australia, China, South Africa and the USA, equine AI is now widespread in its use.

Advantages of Artificial Insemination

1. Removal of geographical restrictions.
2. Minimization of disease transfer, both venereal and systemic
3. Reduction in injury risk both to handlers and horses. The risk is further reduced if the stallion can be encouraged to mount a dummy mare.
4. Increasing the number of mares that can be inseminated per ejaculate.
5. Improvement of native stock through semen importation.
6. Development of gene banks for future reintroduction of genetic material.
7. Breeding of difficult mares – those with physical abnormalities, especially caused by accidents, infection, poor perineal conformation, psychological problems, etc. However, care must be taken to ensure that such problems are not heritable.
8. Breeding from difficult stallions – those with physical problems, injury, infection, inadequate semen characteristics, psychological problems, etc.
9. Reduction in labor costs.
10. Semen sexing

Selection of stallion

Research studies suggest that 100-250x10^6 progressively motile sperm per insemination are optimal for conception. In other words, there are only a certain number of spermatozoa available each day therefore, care must be taken to make intervals between breeding's (in natural cover) long enough to ensure maximum conception rates. Once ejaculation frequency exceeds every other day, sperm output per ejaculate decreases. This is not to suggest, however, that are insufficient spermatozoa to impregnate a mare.

Stallion semen should be evaluated under a microscope regularly during the breeding season to document fertility. Besides frequency of ejaculation; stallion age, testes size, libido, and seasonality must also be considered when evaluating fertility. Both anabolic steroids and winter daylengths render stallions less fertile by reducing gonadotropin secretion which in turn decreases testicular size, sperm output, and libido. Older stallions produce less spermatozoa than younger

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stallions. Libido can also be reduced by overuse, misuse, and lack of teasing by an estrous mare.

Semen Collection

Methods of semen collection

1. Easiest method is the collection of dismount samples. The drips of semen are collected from the stallion after withdrawal from the mare into a sterile jar.
2. Condoms have been developed for use with horses.
3. The artificial vagina (AV).

Preparation of AV

They provide a warm, sterile lumen, surrounded by a water jacket, under some pressure, with a collecting vessel at the end, in an attempt to mimic the natural vagina. Most AVs consist of a solid outer casing with two rubber linings, an outer and an inner. The outer lining and the casing form a jacket, into which warm water and/or air is passed by means of a tap or valve.

Procedure of semen collection

The stallion is allowed to mount and the collector diverts the penis, when erect, towards the AV. The stallion should be allowed to gain intromission and enter the AV of his own free will and not have the AV forced upon him. The AV can be stabilized by being held against the hindquarters of the mare, if present, or the back of the dummy

The occurrence of ejaculation is noted, as in natural covering, by the flagging of the tail or by feeling for the contractions of the urethra and the passage of the semen along the ventral side of the penis.

After collection, the collecting vessel must be carefully removed from the AV and the semen evaluated as soon as possible. If it is not possible to carry out semen assessment immediately, it can be extended and stored at 4–5°C for up to 24 hr without appreciable reduction in its viability, and a reasonably accurate evaluation of the semen can still be obtained (Malmgren *et al.*, 1994).

Use of semen

The method used depends on the stud system and the location of the mare(s) to be inseminated. Once the semen has been evaluated, it can be considered for insemination. Semen can be used in one of four ways:

1. Used immediately undiluted to inseminate a single or possibly two mares, depending on the volume collected.
2. Diluted and used immediately for insemination into several mares.
3. Diluted and refrigerated for use over the next 72 hrs.
4. Diluted and frozen for use at a later date.

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Raw semen

The best results are obtained using undiluted semen inseminated immediately. However, such use of semen defeats two of the main objectives of AI – increasing the number of mares that can be fertilized with one ejaculate and transportation – though it may be of use as a veterinary or management aid.

Chilled semen

If semen is to be extended prior to use, it should be done so immediately. A vast array of diluents has been developed, based normally upon milk, gelatine or egg yolk plus antibiotics).

The acceptable range for a normal stallion's semen parameters.

Parameter	**Acceptable range**
Volume of sperm produced	30–250 ml
Sperm concentration	30–600 × 106 ml−1
Morphology Minimum	40–50% physiologically normal
Live: dead ratio	6.0:4.0
Motility	Minimum 40% progressively motile sperm
Longevity at room temperature	45% alive after 3 h
10% alive after 8 h	
Ph	6.9 – 7.8
White blood cells	<1500 m−1
Red blood cells	< 500 ml−1

All these extenders give acceptable fertilization rates for chilled semen. Extended semen can be stored for up to 2–3 days if extended in a ratio 2:1 (semen: extender) and cooled slowly to 4–8°C Over 4 h and kept at this temperature until use. Such treatment allows limited semen transportation and storage in a refrigerator or Equitainer.

Frozen semen

Prolonged storage can only be achieved by freezing, but the techniques of freezing horse semen is nowhere near as refined as those for cattle. Numerous extenders have been tried, most are similar to those used for chilled insemination, but with the addition of a cryoprotectant, such as glycerol. The major problem is identifying a suitable cryoprotectant that can be used to prolong the formation of ice crystals either within the sperm head, so reducing physical damage, or within the surrounding solution, reducing sperm desiccation. Glycerol is used as such an agent in cattle, but

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appears to be toxic to equine sperm. However, in the absence of any other successful agent, low concentrations of glycerol continue to be used. Detergents and a combination of sugars has also been used as cryoprotectants. In addition to variation in results with extenders, there is great variation between and within stallions, with pregnancy rates of 10–70% being reported.

Semen Dilution

The extent of semen dilution depends on the initial concentration of the sample and the motility of the sperm (Magistrini *et al.*, 1987). Insemination of diluted semen containing 100×10^6 progressively motile sperm (PMS) per insemination gives good results, but normally 500×10^6 sperm per insemination is recommended in order to allow a margin of error (PMS required = $100–500 \times 10^6$). The insemination of 800×10^6 sperm is advised when using frozen semen, in order to compensate for loss occurring during the freezing process.

Insemination Volume

The volume of inseminate varies from 10 to 30 ml for fresh semen, 10 to 60 ml for chilled and 0.5 to 5 ml for frozen (British Equine Veterinary Association, 1997). It has been suggested that volumes in excess of 100 ml or less than 0.5 ml are detrimental to conception rates.

Sperm numbers

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.

Insemination Technique

Mares are inseminated non-surgically. When using fresh or chilled semen, this should occur, as with natural service, on either day 2 or day 4 of estrus. Frozen semen requires better synchrony with ovulation, ideally to within 6 hr. Semen, both diluted and undiluted, is deposited into the uterus by means of a plastic sterile pipette, with syringe attached, or by an insemination gun, guided in through the cervix to the uterus, using the index finger. Alternatively, the pipette can be guided in through the cervix as per rectal palpation, the cervix being felt through the rectum wall. The syringe is loaded with semen held between two air bubbles and then attached to the end of the pipette. Once through the cervix, the insemination pipette is pushed into the uterus about 2 cm. When it is in place,

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the semen is slowly expelled by depressing the plunger.

Conclusion

In many parts of the world, equine AI is widespread in its use. The UK, though a leader in many aspects of the equine industry, lags behind, largely due to the failure of the Thoroughbred industry to recognize and hence register progeny conceived by AI. Until it can be persuaded that AI is an acceptable means of breeding the horse, the application of AI will be restricted to use in other breed societies. Despite this, it is evident that equine AI is here to stay and will continue to expand, opening up with it exciting opportunities in the selection and breeding of the equine species.

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