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Artificial insemination in the horse

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Summary — According to early records, horses were inseminated artificially long before other large domestic animals. By the end of the 19th century, there were reports from several European countries of artificial insemination being used in mares but it was in Russia, in the first decade of the present century, that the technique was first developed for serious commercial use. Between the 2 world wars, there was extensive use of AI not only in the Soviet Union but also in a number of central European and Balkan countries. After 1945, AI in Europe declined in parallel with the use of the horse as a farm animal. By 1962, it was estimated that the total number of mares being inseminated artificially throughout the world was 750 000 of which 80% were in China. At that time, the number of cows being bred by AI had risen to over 58 million. There are a number of reasons, apart from the obvious commercial ones, why AI has proved less popular for horses than for cattle. There is wide variability in survival of spermatozoa from individual stallions following freezing and thawing. Only 30% of a group of stallions regularly produced semen that retained good motility following freezing. In addition, although the mechanics of insemination are easy in the mare, oestrus detection in the absence of a stallion can be difficult. Therefore, because the length of oestrus varies considerably and the relationship between it and ovulation is much less precise than in other large domestic animals, it can often take considerable experience to determine the optimum time for insemination especially using preserved semen that may only have a short survival time in the mare's reproductive tract. Many stud book authorities have also been opposed to the use of AI. There are good reasons for this opposition on the part of the Thoroughbred where an international racing and breeding industry is involved, but it has not necessarily been appropriate for other breeds. Nevertheless, the result has been to delay widespread acceptance of AI and has meant that research into methods of preserving stallion spermatozoa has lagged behind that of other species. In recent years, however, more breeders of horses have come to realize the advantages to be gained from the use of artificial breeding techniques with the result that many breed societies, with the exception of those controlling the Thoroughbred, will now admit for registration foals born following AI.

artificial insemination / horse

Résumé — L'insémination artificielle chez les chevaux. *Selon des anciennes données, les juments ont été inséminées artificiellement longtemps avant les autres espèces animales domestiques de grande taille. À la fin du 19e siècle, il était mentionné dans plusieurs pays Européens que l'IA était utilisée, mais c'est en Russie, dans la première décade de ce siècle, que la technique s'est développée pour une utilisation commerciale sérieuse. Entre les deux guerres mondiales, l'IA était largement utilisée, non seulement en Union Soviétique, mais aussi dans plusieurs pays d'Europe Centrale et Balkanique. Après 1945, l'IA en Europe a diminué parallèlement avec l'utilisation des chevaux comme animaux de ferme. En 1962 il était estimé que le nombre total de juments insémi-*

nées artificiellement dans le monde était de 750 000 dont 80% en Chine. Dans le même temps, le nombre de vaches mises en reproduction par IA s'élevait à plus de 58 millions. Il existe un certain nombre de raisons, mises à part les raisons commerciales, pour lesquelles l'IA équine s'est avérée moins populaire que chez les bovins. On observe une variabilité importante dans la survie des spermatozoïdes d'un étalon à l'autre après congélation et dégel. Seulement 20% des étalons produisent régulièrement de la semence qui présente une bonne motilité après congélation. De plus, bien que la réalisation de l'IA soit aisée chez la jument, la détection de l'oestrus en absence d'étalon peut s'avérer difficile. Par conséquent, puisque la durée de l'oestrus varie de façon importante et parce que la relation entre celui-ci et l'ovulation est beaucoup moins précise que pour les autres espèces domestiques, il est souvent nécessaire d'avoir une sérieuse expérience pour déterminer le moment optimum de l'IA, particulièrement lorsqu'on utilise de la semence conservée qui n'a qu'une durée de survie limitée dans le tractus génital femelle. Beaucoup de responsables de livres généalogiques ont également été opposés à l'utilisation de l'IA. Il y a de bonnes raisons pour cette opposition du coté des Pur Sang où des entreprises internationales de course et de sélection sont concernées, mais ce n'est pas forcément justifié pour les autres races. Néanmoins le résultat a été de retarder l'acceptation large de l'IA, ce qui a conduit au fait que la recherche sur les méthodes de conservation de la semence d'étalon est restée loin derrière les autres espèces. Ces dernières années toutefois, un nombre croissant d'éleveurs de chevaux se sont mis à réaliser quels avantages pouvaient être retirés de l'utilisation des techniques de reproduction artificielle, ce qui a eu pour résultat que beaucoup d'organisations de sélection, sauf celles contrôlant les Pur sang, vont maintenant admettre d'enregistrer les poulains nés à la suite d'IA.

insémination artificielle / cheval

INTRODUCTION

The horse is the first animal species for which the use of artificial insemination (AI) is recorded. Bowen (1969) cites a report from 1 322 in which stallion semen was taken to inseminate mares belonging to an Arab chief. However, it was not until the end of the 19th century that the technique began to be considered seriously and most of the early reports, both from Europe and America, involved horses. Initially, it was thought of mainly as a method of controlling venereal disease or treating sterility in mares and it was not until the first decade of this century that it began to be used as a means of breed improvement. The Russian biologist, Ivanov, working in the Russian royal stud, is credited with being the first person to develop the technique for widespread use. In 1903, he organized an equine AI centre and developed and improved methods of collecting, diluting and transporting stallion semen (Tischner, 1991). After the first World War, AI was being practised extensively in horses in both the Soviet Union and several other Central European countries with reports of 300 to 500 mares a year being inseminated with semen from a single stallion. Following the second World War, however, there was a general fall in the number of horses being inseminated artificially both in the Soviet Union and the West.

In contrast, interest grew rapidly in the use of AI in other species of domestic animals and soon the number of cattle being inseminated artificially far outstripped the number of mares being impregnated in the same way. Bowen (1969) reports that, by the early 1960s, 58 millon cattle worldwide were being bred using AI compared with an estimated 750 000 horses, 80% of which were in China.

There is a number of reasons why AI has been slow to gain the same sort of universal acceptance by horse owners as it has by farmers rearing cattle. One factor is the conservatism of many equine breeders who have been unwilling to adopt new methods and have not had the commercial pressure that caused the farming community, initially with great reluctance, to accept AI and, eventually, embryo transfer in cattle. This hesitant attitude has been compounded by the stud book authorities that regulate equine breeds who were equally reluctant to accept changes in breeding techniques. Opposition to the use of artificial methods has been strongest amongst the breeders of Thoroughbreds and it is still not possible to register any Thoroughbred born as a result of AI or embryo transfer. There are good reasons why this should be so in a complex, international industry that is devoted to breeding horses to race against each other anywhere in the world. In these circumstances, with the huge amounts of money involved, not only in breeding but also in racing and gambling, it is important to have rules that ensure fairness and are applied universally. In addition, there are reasonable grounds for fears that widespread use of AI would distort both the genetic and the financial structure of the Thoroughbred industry.

These restrictions, however, are not necessarily appropriate for other equine breeds but, until recently, there has been a tendency for their stud book authorities and for breeders in general to adopt the attitudes of the Thoroughbred community. This has been especially true of the UK where, both in terms of its financial importance and the number of animals involved, the Thoroughbred industry dominates horse breeding and tends to set the standards that others follow. As a result, the use of AI for horse breeding in Britain has been particularly slow to gain acceptance.

The fact that the economic pressure on farmers and horse breeders is very different has been emphasized by the progressive decline, throughout the 20th century, in the use of the horse as an agricultural or draught animal. An important consequence of this is that far less research has been devoted to artificial breeding methods in horses than in cattle and this, in turn, has fostered the (largely false) impression that, due to the physiology of the mare or the characteristics of stallion spermatozoa, the species was less suited to the application of these techniques.

Before going on to consider AI in horses in detail, it will be helpful to describe briefly some of the special features of the anatomy and physiology of the equine genital tracts in order to understand the effects these will have on the way AI is practised in horses.

ANATOMY AND PHYSIOLOGY OF THE EQUINE GENITAL TRACT

The mare

The cervix of the mare is ≈ 8 cm in length and is much less muscular and tortuous than that of a cow. It is possible, without too much difficulty, to force a finger through the cervix at any time of the oestrus cycle or even during pregnancy. In oestrus, it softens and becomes progressively more relaxed until, close to ovulation, 3 or more fingers can be inserted through it (Greenhoff and Kenney, 1975). The duration of oestrous can vary markedly and, compared with other large domestic mammals, is relatively long and bears a much less precise temporal relationship to ovulation than, for example, it does in the cow. Ginther (1979) reported the mean length of oestrus in a group of mares as 6.2 ± 2.3 d but, within this group, there were wide individual variations. In 69% of the mares, ovulation occurred some time in the last 48 h of oestrus but 14% did not ovulate until 1 or more d after the end of oestrus. An added difficulty in establishing when to mate a mare is that, without the presence of a stallion to stimulate her, oestrus is often difficult to detect and, as a result, selection of the optimum time for insemination can present breeders with considerable problems.

The stallion

For the stallion, mating is a relatively rapid process with ejaculation normally achieved after 5–11 thrusts of the penis in the mare or artificial vagina, following which the stallion ejaculates his semen in 5–10 jets (Tischner *et al,* 1974). While the stallion is thrusting, there is a marked engorgement of the glans penis which acts as a seal within the vagina creating an increase in pressure. It is thought that the effect of this is to cause the softened cervix to be forced open. The first 3 jets of the ejaculate contain > 80% of the spermatozoa and are produced at high pressure while the stallion is at maximum engorgement and thrusting hard with his urethral process in close apposition to the cervix. The result is that the sperm-rich fraction is deposited directly into the uterus. Subsequent jets, consisting predominantly of accessory gland secretions (Mann, 1975), arrive as detumescence is beginning and the stallion is no longer thrusting hard and are, therefore, deposited mainly in the vagina and thus never come in contact with the spermatozoa.

SEMEN COLLECTION

Early attempts at semen collection relied on a sponge inserted in the vagina of a mare. Although this was effective, it was wasteful of spermatozoa and resulted in contamination of the semen by vaginal secretions and bacteria. Sponges were later replaced by condoms which were popular for a time and enabled a complete ejaculate to be collected but had considerable drawbacks. They were liable to burst in operation (with the risk of the teazer mare becoming pregnant) and were difficult to fit on an excited stallion or to retrieve from his penis after ejaculation. They also resulted in semen samples contaminated by bacteria and other deposits contained in penile smegma (Nishikawa, 1959). Although they are still used occasionally to obtain semen from stallions reluctant to accept other methods, they have generally been superseded by the artificial vagina (AV) as the preferred way of collecting semen. With an AV, the whole ejaculate is available and, with care, the sample can be relatively uncontaminated. In fact, using an open-ended AV to collect only the sperm-rich fraction of the ejaculate, it is possible to obtain not only a highly concentrated sample but also one that is sterile and free from bacterial contamination (Tischner and Kosiniak, 1986). Use of an electro-ejaculator has also been tried, but the results have generally been unsatisfactory: although the technique may result in the production of some seminal fluid, it seldom elicits a full ejaculate containing spermatozoa.

Semen collection from a stallion using an AV is generally a simple process. An occasional horse may be reluctant to accept the device at first but most can quickly be trained to ejaculate without difficulty. Collection is usually carried out with the stallion mounting a teazer mare that is in oestrus but, again, the majority can be trained to use a dummy although, in some cases, the presence of a mare in the collecting area is necessary to stimulate a stallion's interest.

STALLION SEMEN

Compared with human or bull spermatozoa, those of a stallion, when maintained outside the genital tract, are fragile, easily damaged and short-lived unless immediately inseminated or mixed with a suitable diluent. This is in marked contrast to the survival of the spermatozoa once they are deposited in the mare. Normal stud practice is to mate a mare only once every 48 h while she remains in oestrus. For the majority of stallions, more frequent mating is unlikely to result in any improvement in conception rate although a longer mating interval is often accompanied by reduced fertility. However, there is good evidence that spermatozoa from some stallions survive as long as 7 d in a mare (Woods *et al,* 1990). In contrast, the unfertilized oocyte after ovulation appears to have a much shorter survival time. Woods *et al* (1990) noted that conception rates began to fall if insemination occurred more than 6 h after ovulation and that, if insemination was carried out more than 24 h after ovulation, there was little chance of a pregnancy developing. The time needed for stallion spermatozoa to capacitate *in vivo* has not yet been established.

INSEMINATION OF MARES

As a result of the relaxation of the mare's cervix during oestrus, AI of a horse is much easier than it is in species where spermatozoa are normally deposited in the vagina during mating and the cervix remains closed, making it difficult to insert a catheter into the uterus. However, because in the mare spermatozoa are normally deposited directly into the uterus in large numbers during natural mating, it appears that it is necessary to use a relatively large insemination dose during AI to ensure conception. For example, using fresh semen in cattle, the insemination of 2.5×10^6 motile sperm into the uterus will achieve satisfactory conception rates (Macmillan and Watson, 1977) and, even when using frozen semen, 12.5×10^6 motile sperm are generally considered adequate. In contrast, Pickett *et al* (1987) observed that, even under ideal conditions, at least 100×10^6 motile sperm are necessary for maximum reproductive efficiency in the mare. Where conditions are less than ideal, they recommend an insemination dose of 500×10^6 motile spermatozoa.

EQUINE STUD PROCEDURES

In the past, when there was widespread use of the horse as an agricultural animal, it was common practice for a stallion to be "walked out" to cover the mares in his area. A different circular route would be walked each working day of the week with mares gathering at prearranged rendezvous. Often > 20 km would be covered on the circuit with a stallion serving as many as 15 mares along the route. With a stallion visiting an area only once a week, this had the drawback that mating often did not coincide with the optimum time to ensure conception. There was also the risk that any infectious disease would be widely disseminated.

Nowadays, it is much more common for the mare to visit the stallion. This can be done in 2 ways with the mares either "walking in" to the stallion stud for a short visit on the day of mating or for the mare to reside at the stallion stud until she has been confirmed as being in foal.

Walking in

With this method, all the mare management is the responsibility of the mare owner who will assess oestrus and is responsible for ensuring that the horse is mated at the optimum time. This has a number of limitations. It is difficult to control the spread of infection and, in the absence of a teazer stallion, it may be difficult to as-certain when the mare should be taken to be covered. It also necessitates transporting the mare who, in many cases, will be accompanied by a young foal. For this reason, it is important for the stallion to be close by, especially in cases where the mare does not ovulate as expected and needs to be mated a second or third time.

Resident

On many studs, especially the majority of top class Thoroughbred studs, it is the practice for mares to arrive well in advance of covering. In fact, in most cases, foaling mares arrive before foaling. This practice avoids the need to transport a very young foal, infection can be controlled and the mare's oestrus cycle monitored so that she is covered close to ovulation. If ovulation does not occur, it is usually the practice to give a repeat covering 48 h after the previous one. This method has the disadvantage of being costly for the mare owner and needs extensive facilities at the stallion stud.

If used responsibly, AI has the potential to improve fertility and to overcome many of the limitations of conventional horse breeding methods. It can also help reduce the cost of producing foals by improving efficiency on studs.

ADVANTAGES OF ARTIFICIAL INSEMINATION IN HORSES

In those equine breeds for which the use of AI is now allowed, the technique offers a number of advantages:

– transport of animals is eliminated and the risks of passing infection between mare and stallion are greatly reduced;

– to the mare. Pregnancies can be established in animals that are either too injured, lame or nervous to be mounted safely by the stallion or are susceptible to endometritis following natural mating;

– to the stallion. The risks of being kicked or otherwise injured during mating are greatly reduced. In addition, the use of AI enables more mares to be bred to any one stallion and also conserves his sexual energy thereby improving fertility;

– to the stud. It offers big savings in time and labour: with the use of split ejaculates, several mares can be inseminated following a single semen collection. Quality control is also improved: the insemination dose for each mare can be monitored so that she receives adequate spermatozoa to ensure maximum reproductive efficiency;

– semen can be stored and transported following collection.

PRESERVATION OF EQUINE SEMEN

The birth of the first foal to be produced as a result of insemination of a mare with frozen stallion semen was reported in 1957 by Barker and Gandier, who used spermatozoa recovered from the epididymis. Success with stallion semen frozen following collection by ejaculation was not achieved until 1966 (Nagase *et al,* 1966). Because of poor pregnancy rates and lack of interest by many horse breeding stud book authorities, there was little incentive for research and the development of the technique since these initial successes has been much slower than in the breeding of other large domestic animals.

However, in recent years, there has been a rapid increase in interest in the use of frozen semen by horse breeders as they have become aware of the benefits to be gained from being able to store and transport semen. As a result, there has been an increase in research and acceptable conception rates are now being reported from a number of countries. In spite of this, the fact remains that the only country to have made extensive use of frozen stallion semen is China where it is being used to inseminate more than 40 000 mares each year with a 68% conception rate (Tischner, 1991). Experience from research in other countries suggests that there is great variability between stallions in the extent to which fertility is retained following freezing with perhaps less than one third of a population of stallions producing semen with good fertility following the freezing/thawing cycle. In all cases, when compared with fresh semen, both the fertility of spermatozoa and their survival time in the mare's genital tract are reduced following freezing. In spite of the drawbacks, increasing use is now being made of frozen semen for horse breeding and, provided stallions are carefully evaluated and selected, satisfactory pregnancy rates can be achieved.

To overcome some of the limitations of frozen semen, many horse breeders have resorted to chilling semen as a way of preserving its life outside the genital tract. It has been shown that if semen is mixed with a suitable extender (*eg* milk/glucose/antibiotics) immediately after collection and then slowly cooled to 5 °C, sperm from a majority of normal, fertile stallions will retain the ability to fertilize ova for at least 24 h and, in some cases, up to 72 h. Extensive use is now being made in a number of countries of semen treated in this way and, in experienced hands, excellent conception rates are being achieved. Transport containers have been developed which cool the semen and hold it at 4–6 °C for 2–3 d, enabling semen to be transported considerable distances between studs.

When using transported semen, whether frozen or chilled, mare management at the receiving end is critical to ensure that insemination takes place at the optimum time. Chilled semen has a limited life and, if it is to be transported far, must be ordered some time in advance of requirement while frozen/thawed semen must be inseminated as close to ovulation as possible because of the limited survivial time of the spermatozoa following thawing. It is here that the rather loose relationship between oestrus and ovulation in the mare presents difficulties and, for this reason, considerable expertise is necessary if the best results are to be achieved.

CONCLUSION

As horse breeders throughout the world become more aware of the advantages to be gained from AI, the use of the technique which they largely ignored for many years is now becoming increasingly common in horse breeding. In many breeding programmes, AI has now superseded natural mating as the usual way of achieving conceptions and, with improved methods of storage and transport, this trend will continue. However, due to the special requirements of horse breeders, the nature of equine AI will be very different from the way the technique is practised in agriculture. Instead of being used predominantly as a method of breed improvement with the widespread and almost exclusive use of a small pool of carefully selected males, the emphasis is likely to be on the advantages the technique offers to stud management. In addition, by using transported semen, the costly and potentially hazardous transport of animals will be reduced and breeders will have a much wider choice of matings. However, with an increased potential for disseminating disease and for a single male to sire many more offspring than by natural mating, it is important that breed societies and national authorities establish careful guidelines for stallion selection, the practice of AI and the training of those involved in its use.

REFERENCES

Barker CAV, Gandier JCC (1957) Pregnancy in a mare resulted from frozen epididymal spermatozoa. *Can J Comp Med Vet Sci* 21, 47-51

Bowen JM (1969) Artificial insemination in the horse. *Equine Vet J* 1, 978-110

Ginther OJ (1979) *In: Reproductive Biology of the Mare. Characteristics of the Ovulation Season.* McNaughton and Gunn Inc, Ann Arbor, MI, ch 6,133-165

Greenhoff G, Kenney RM (1975) Evaluation of reproductive status of non-pregnant mares. *J Am Vet Med Assoc* 167, 449-458

Macmillan KL, Watson JD (1977) Factors influencing AB conception rates. VII. Interactions involving semen fertility levels, sperm dose rates and technician ability. *NZ J Exp Agric* 5, 11-19

Mann T (1975) The biochemistry of stallion semen. *J Reprod Fertil* 23, 47-52

Nagase H, Soejima S, Niwa T, Oshida H, Sagara Y, Ishizaki N, Hoshi S (1966) Studies on the freezing storage of stallion semen. I. Fertility results of stallion frozen semen in concentrated pellet form. *Jpn J Anim Reprod* 12, 48-51

Nishikawa Y (1959) *Studies on Reproduction in Horses.* Japan Racing Assoc, Tokyo, 208-209

Pickett BW, Squires EL, Mc Kinnon AO (1987) Influence of insemination volume and sperm number on fertility. *In: Procedures for Collection, Evaluation and Utilization of Stallion Semen for Artificial Insemination.* Colorado State Univ Anim Reprod Lab Bull No 03, ch 7

Tischner M (1992) Artificial insemination in horses, a review of the current *status quo. Equine Vet Educ* (in press)

Tischner M, Kosiniak K (1986) Bacterial contamination of stallion semen collected by 'open' artificial vagina. *Vlaams Diergeneesk Tijdschr* 55, 90-94

Tischner M, Kosiniak K, Bielanski W (1974) Analysis of the pattern of ejaculation in stallions. *J Reprod Fertil* 41, 329-335

Woods J, Bergfelt DR, Ginther OJ (1990) Effects of time of insemination relative to ovulation on pregnancy rate and embryonic-loss rate in mares. *Equine Vet J* 22, 410-415