

Improving Reproductive Efficiency:
Hysteroscopic and Deep Horn Insemination in the Mare

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Take Home Message

Hysteroscopic insemination of the uterine tube and deep horn insemination are non-surgical assisted reproduction techniques that are used to breed mares with a reduced dose of semen. Pregnancy rates are highest when: appropriate doses of semen are used, fertile stallions are selected, mares resistant to endometritis are bred, and the procedures are performed quickly, cleanly, and atraumatically.

Introduction

Reproductive efficiency in the equine industry is problematic because overall rates are low and it creates significant economic loss. Breeders are facing challenges such as: the life expectancy of the mare, the availability of the stallion, a decreased return on their investment, increased veterinary costs, and lost income from open mares or late foaling mares. Pregnancy rates and foaling rates are lower than they should be. According to the Jockey Club only 55% of all Thoroughbred mares that are bred foal. An evaluation of mares bred versus foals registered in Alberta suggests that in this province we have a similar level of reproductive efficiency. There have been reports of new assisted reproductive techniques, such as low dose insemination using a hysteroscopic approach, or via deep horn insemination. Questions often asked include under what circumstances will they help increase reproductive efficiency and profitability of breeding? What are these new assisted reproductive technologies?

Low dose insemination techniques in the mare include hysteroscopic insemination and deep horn insemination. Low dose insemination techniques may have application when: there is a limited amount of semen, the semen is very costly, epididymal spermatozoa are used, sex-selected spermatozoa are used, the stallion is subfertile, or other management procedures have failed to produce acceptable fertility



Limited amounts of semen

Limited amounts of semen are available from stallions with excessive books, or below average sperm output, or deceased stallions where there is no opportunity to replenish the stored amount. Injuries or disease that decrease the number of spermatozoa produced influence the number of mares a stallion may breed. Examples of conditions that will interfere with a stallion's spermatozoal output include: testicular torsion or inguinal hernia requiring unilateral castration, testicular tumors, breeding injuries, or chronic diseases such as kidney disease, laminitis, or peritonitis.

Costly semen

Frozen semen is often sold by the breeding dose without a guarantee of fertility. In these cases when the frozen semen is very expensive, but of good quality, more than one mare may be inseminated using a single dose of semen if low dose insemination technology is used. Alvarenga published a report where he obtained the same pregnancy rate using 2 straws of frozen semen delivered through a hysteroscopic approach, as when 8 straws were used with conventional AI. This type of approach increases reproductive efficiency.

Epididymal spermatozoa

Epididymal spermatozoa are a pool of spermatozoa that are in the final stages of maturation or are in storage prior to ejaculation. Epididymal spermatozoa are generally collected from stallions that are gelded, terminally ill, or deceased. The spermatozoa are often flushed out of the vas deferens and collected from the tail of the epididymides. The number of mature spermatozoa obtained from this method is low.

Sex selected spermatozoa

A cell sorting machine called a flow cytometer has been adapted to separate live fluorescently spermatozoa by their sex chromosome content from many different species, including horses. The x and y spermatozoa each have a different fluorescent marker and the spermatozoa are sorted into 3 pools: x chromosome bearing, y chromosome bearing, and undetermined. The process of spermatozoal sex-selection is inefficient and only a small percentage of the total spermatozoa sorted are available for insemination, with the current technology only 2.5 million spermatozoa / instrument per hour may be sorted requiring low dose insemination techniques. A dose of 5 million total sex-selected spermatozoa is often used. The reason an owner would pursue the use of sex selected spermatozoa is because there is a sentimental or economic benefit to having either a filly or colt. Many horse breeders feel that certain traits are carried in the male or female line. The price a pregnant mare brings is influenced by the sex of the foal she is carrying. Mares that are examined in early pregnancy by ultrasound examination to determine the sex of the fetus, prior to sale, bring a higher price at auction.

Subfertility

There are many causes of subfertility in stallions. Stallions that have a lower than 30% per cycle pregnancy rate are not usually commercially viable. When considering using a subfertile stallion the mare owner should be aware that some causes of subfertility are heritable. There are stallions that genetically are inferior in terms of semen quality and will pass this characteristic along. The subfertile stallions that are best selected are ones that had a previously successful breeding career and some event has been identified that caused their fertility to decline (founder or colic for example). The types of subfertility that are helped by low dose insemination techniques remain to be defined. The most likely stallions to be helped are those with low numbers of otherwise normal motile spermatozoa. Stallions with severe motility and morphology issues may not benefit from low dose insemination. Enrichment techniques to isolate the most vigorous spermatozoa from subfertile stallions and the removal of potentially toxic seminal plasma needs to be further investigated to determine if it will increase pregnancy rates.

Other management procedures have failed

There are cases where conventional breeding management has failed to produce acceptable pregnancy rates from certain stallions. In these cases the number of spermatozoa used with low dose insemination techniques needs to be tailored to each individual stallion. Generally improved breeding management, conventional artificial insemination, is tried first and is then followed by deep horn insemination or hysteroscopic insemination.

Low dose insemination procedures

Greek or Latin roots are often used in medical terminology. The Greek root "hyster" means uterus. Words with this Greek root in our language today include hysterectomy, and hysterical (believed to come from the uterus during the pain of child birth). The first half of the word "hyster" means uterus, and the second half of the word oscopy means to have a look inside. Therefore, hysteroscopy means to have a look inside the uterus. Hysteroscopic insemination is performed by introducing endoscopic equipment into the uterus, allowing direct visualization of the uterotubal opening. A small catheter is then passed through the working channel of the endoscope and the sperm cells are deposited onto the uterotubal opening. The uterotubal opening is where spermatozoa enter the oviduct (called a uterine tube in horses). The uterine tube connects the ovary with the uterus. It is the site of fertilization. Hysteroscopic insemination has been reported in horses as a method used to reduce the number of sperm cells required for conception. Pregnancies have been achieved using the hysteroscopic technique with 1% of the conventional insemination dose. Most authors report that this technique shows an advantage over conventional artificial insemination (AI) when the spermatozoal number is below 10 million spermatozoa and volume is low. At the time that the hysteroscopic insemination technique was developed, the flow cytometer was adapted to separate live fluorescently labeled equine spermatozoa by their sex chromosome content. Hysteroscopic insemination has been used to deliver sex - selected spermatozoa. This technique allows fresh, cooled, and frozen - thawed sex - selected spermatozoa to be used to achieve pregnancies. Table 1 lists recent reports on this technique.

Deep horn insemination is an artificial insemination technique where a flexible insemination pipette is passed vaginally through the cervix and is then directed using a rectal approach into the tip of the uterine horn on the side of the dominant follicle. The concept is that deposition of the spermatozoa closer to the junction of the uterus and oviduct (called a uterine tube in the horse) will increase the chance of conception with a reduced dose of spermatozoa, or improve the chance of conception in a stallion with suboptimal fertility. The advantage to this tool is that it does not require expensive equipment to perform. One of the difficulties in assessing the usefulness of the technique is that there is little scientific agreement on the dose of motile, or motile and normal spermatozoa needed for conception in the mare when using fresh, cooled, or frozen spermatozoa. There appear to be large differences between stallions in the number of spermatozoa needed for good conception rates. The key is that each stallion is likely to have an optimum dose that is affected by the way the spermatozoa are processed. There are a few reports that deep horn insemination improves the fertility if a low number of spermatozoa are used in a small volume while others refute that it is helpful. The opening to the uterine tube is very small and it is located along the curve at the end of the uterine horn. Rectally directed deposition onto the uterotubal papilla is therefore not possible. The rectum of the mare is emptied. The mare is prepared as for routine artificial insemination and the side of the preovulatory follicle determined. A flexible AI pipette is used. The pipette is passed through the cervix through a vaginal approach and into the uterine body. The inseminator's hand is introduced into the rectum and the end of the pipette identified and directed into the desired uterine horn. The location may be confirmed by feel or by ultrasound. Some inseminators include an elevation in the end of the uterine horn after deep horn insemination to help coat the end of the uterine horn with spermatozoa, other authors sedate the mares prior to performing the procedures. Table 2 lists recent reports on this technique.

Preparation of the Mare

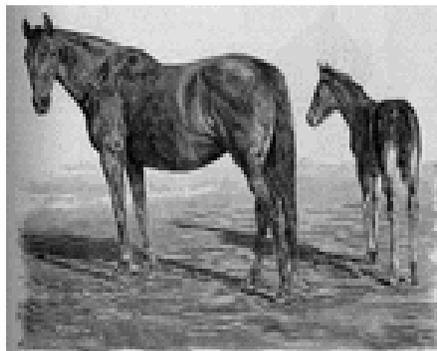
Mares are restrained in a tie stall or stocks, the rectum is emptied of manure, and the side of the preovulatory follicle(s) determined. The mare's tail should be wrapped and the perineum washed with mild soap and rinsed with water. Veterinarians usually use sedative and analgesic drugs prior to performing low dose insemination. Dosages are determined and adjusted based on the body size, breed, and temperament of the mare, generally mares are administered 0.01 - 0.02 mg/kg dormosedan and 0.01 - 0.02 mg/kg butorphanol IV. Additional amounts of these drugs are occasionally needed to stop excess motion of the mare. Adequately sedated mares are essentially unaware the procedures are being performed. A rope may be used around the front of the mares to keep them positioned with their hindquarters near the tailgate of the stocks or tie stall, because sedated mares relax as their head drops they tend to lean forwards. The presence of blood and serum products in the uterus are believed to be detrimental to fertility. Rough technique will lead to bruising and inflammation of the uterus with has the potential to interfere with conception and should be avoided. These procedures should take less than 10 minutes to perform.

Preparation of the Spermatozoa

There is some debate as to whether the removal of seminal plasma, or processing the spermatozoa to enhance the number of fertile spermatozoa is beneficial for low dose insemination. Nie et al., 2003 evaluated the use of 3 processing techniques: percoll, sephadex glass wool, and nothing. Percoll is a substance that selects spermatozoa based on their density and tends to remove sperm with abnormal head shapes; and sephadex glass wool, a system that removes membrane damaged spermatozoa. These authors showed that with normal stallions the additional processing did not improve fertility. They were using 25 million total spermatozoa. The benefit to processing may only be realized when fewer spermatozoa are inseminated or when there are higher percentages of damaged or abnormal spermatozoa.

Summary

Deep horn insemination and hysteroscopic insemination are low dose insemination techniques with specific applications in equine reproduction such as the use of sexed fresh semen, sexed frozen semen, or a reduced dose of expensive cryopreserved semen. . Other assisted reproductive techniques such as in vitro fertilization have not been very successful in horses. Hysteroscopic or deep horn insemination may have broad application in the future as more producers use cryopreserved semen. They are best performed in mares with good reproductive health. Maiden, barren or well involuted post partum mares in good heat (estrus) may be used. Mares with double ovulations and preovulatory follicles on both ovaries should have semen deposited in each uterine horn near or on the uterine tubal junction because the small volume of the inseminate is unlikely to allow easy movement of the spermatozoa between the uterine horns. Post procedure monitoring is useful in detecting uterine inflammation. The hysteroscopic delivery or deep horn insemination technique may be used for low numbers of spermatozoa in small volumes.



Author	Year	Hysteroscopic insemination dose	Preg rate
Manning et al.	1998	100 mil, body, 10 mil body, 1 mil hysteroscopy, 10 mil normal PM	335 4/12, 17% 2/12, 22% 2/9, 0% 0/11
Vasquez et al.	1998	3.8 million motile PM	30% 3/10
Morris et al.	2000	10.0,5.0,1.0,0.5,0.1,0.001 mil PM in 30 - 50 ul	60% 6/10, 75% 6/8, 64% 16/25, 29% 4/14, 22% 2/11, 10% 1/10
Lindsey et al.	2002	Fresh, Flow sorted fresh 5 mil PM 230 ul and frozen, frozen flow sorted in 230ul	40% 4/10, 38% 6/16 38% 6 16, 13% 2/15
Lindsey et al.	2002	5 mil tot in 100ul, Deep horn non sorted, hysteroscopy non sorted, hysteroscopy sorted	0% 0/10, 50% 5/10, 25%, 5/20
Koene et al	2002	6 mil 0.2 ml,12mil 0.4 ml, 80mil 1.6 mil mot	5.9%, 17.1%, 25.3%
Morris et al.	2003	Frozen 14 mil PM 500ul body, hyster 14 mil PM 500ul, hyster 3.2mil PM 3.0mil 100ul, body 100ul	8/12 67%, 64% 9/14, 47%16/34, 14% 2/14, 8% 1/12

Table 1: References on hysteroscopic insemination in mares

Author	Year	Experiment	Pregnancy Rate
Buchanan et al.,	2000	Body 500 mil, deep horn: 25mil 20cc, 5 mil 1 cc, and 5 mil 0.2 cc	90% 18/20, 57% 12/21, 30% 3/10, 40% 4/10
Woods et al.,	2000	Body 25 million, horn 25 million 2 stallions good vs poor	63% 9/16, 56% 10/18, 29% 4/14, 29% 4/14
Brinsko et al.,	2003	5 mil tot cooled hys vs deep horn	67% 12/18, 56%, 10/18
Reger et al.,	2003	200 mil tot frozen body vs horn at 24, 40 hrs	50% 10/20, 20% 5/20
Nie, et al.,	2003	25 mil tot deep horn GWS, PS, neat, normal stallions	50% 15/30, 43%, 13/30, 33% 10/30

Table 2: References on Deep Horn Insemination

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