Alternate Methods for Artificial Insemination

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Much of the information on how many sperm are needed for insemination of mares in order to achieve maximum fertility were determined in studies conducted at Colorado State University in the 1970's. Since that time, new information has been published regarding sperm transport and sperm storage within the female reproductive tract. Within a matter of a few hours, sperm are transported from the site of insemination (uterine body) to the oviduct. Although 0.5 to 1 billion sperm are generally inseminated, only a few thousand actually enter the oviduct and attach to the oviductal lining. Those sperm that do enter the oviduct are selected by the uterus as the most morphologically normal and motile. Based on this information, it seemed reasonable that, if one needs to use low numbers of sperm for insemination, placing the sperm at the end of the uterine horn onto the opening of the oviduct (uterotubal junction [UTJ]) would be advantageous.

Recently, low-dose AI has been used for various situations, such as: stallions that are popular and overbooked, limited semen is available because of the stallion's age, frozen semen is in limited supply, stallions with poor quality semen which requires centrifugation through a gradient prior to AI, and mares that have severe post-breeding endometritis.

Methods for low-dose AI include sperm injection (one sperm, one egg); surgical oviductal insemination, called gamete intrafallopian tube transfer (GIFT) requiring only 100-200 thousand sperm; deep-horn AI (50 to 100 million sperm); and videoendoscopic AI (5 to 100 million sperm). This article will focus on low-dose AI using deep-horn insemination or videoendoscopic AI.

Much of the earlier work done on low-dose AI was through the use of a videoendoscope. Morris et al. (2000) inseminated mares hysteroscopically with 10, 5, 1, 0.5, 0.1 or 0.001 million fresh motile sperm. Pregnancy rates per cycle of >60% were achieved in mares inseminated with 10, 5 or 1 million sperm. Lindsey et al. (2002) utilized hysteroscopic AI as a means of inseminating sex-sorted sperm. Five million sex-sorted or non-sorted sperm were deposited onto the uterotubal junction. Ten of 30 mares became pregnant after insemination of 5 million (1/100th the normal dose) sperm.

Hysteroscopic AI also has been used as a means of inseminating low numbers of frozen/thawed sperm. In a clinical setting (Matthews, see SBS Winter 2001-2002 Newsletter article "Technology To The Rescue"), mares were inseminated hysteroscopically with 100 million sperm twice in the cycle resulting in a 56% pregnancy rate/cycle. Petersen et al. (2002) inseminated mares with either 50 million sperm at 24-hr intervals or 500 million fresh and 500 million cooled sperm at 24-hr intervals. Embryo recovery was similar for mares bred with 50 million sperm hysteroscopically (7/11, 64%) versus those inseminated with 500 million sperm (4/11, 37%). In a similar study (Alvarenga and Leao, 2002), 24 mares were assigned to the following groups: 1) 10 million sperm selected by Percoll and inseminated hysteroscopically onto the UTJ; 2) hysteroscopic insemination of 10 million non-selected sperm onto the UTJ; and 3) 400 million sperm inseminated into the uterine body. All mares were inseminated 0 to 8 hr after ovulation. There was no difference in pregnancy rates among groups. However, when data from the two hysteroscopic groups were combined, pregnancy rates were greater than those of controls. Based on these studies, acceptable pregnancy rates can be obtained from insemination of low numbers of frozen/thawed sperm by using a videoendoscope.

Alternative methods of inseminating low numbers of sperm have been sought since the videoendoscope is expensive and time consuming. Lindsey et al. (2005) compared pregnancy rates after deposition of 20 million sex-sorted sperm onto the UTJ with either a flexible videoendoscope or by rectally guided, deep-uterine insemination. Sperm were stored at either 5ºC or 15ºC for 18 hr prior to sorting. Pregnancy rates were higher when sperm were stored at 15ºC and inseminated hysteroscopically (72%) as compared to sperm stored at 5ºC and inseminated using the rectally guided technique (38%). There was a tendency for fewer mares to become pregnant following rectally guided insemination (38%) compared to hysteroscopic insemination (55%) when sperm were stored at 5ºC, sorted and then inseminated. In contrast, Brinsko et al. (2003) reported no difference in pregnancy rates for mares inseminated with 5 million sperm using a long pipette directed to the UTJ (10/18, 56%) compared to those inseminated hysteroscopically onto the UTJ (12/18, 67%). In another study (Nie et al., 2003), mares were inseminated deep in the uterine horn with 25 million sperm selected by glass wool/Sephadex filtration, Percoll, or no separation. Sperm selection method did not affect pregnancy rate and overall 38 of 90 (42%) became pregnant.

Recently, a study in Germany (Sieme et al., 2004) examined the effect of different AI techniques and sperm dose on fertility of 187 normal mares and 85 mares with abnormal reproductive history. The factors evaluated were AI technique (body; rectally guided, deep-horn; hysteroscopic AI onto the UTJ), storage method (fresh, frozen), AI volume (0.5, 2, 12 ml), and sperm dose (50 or 300 million fresh sperm, 100 or 800 million frozen/thawed sperm). Mares were inseminated once per cycle 24 hr after administration of hCG (fresh semen) or 30 hr after hCG for insemination with frozen/thawed sperm. In normal mares, hysteroscopic AI with fresh semen gave better pregnancy rates than body AI, whereas in problem mares, hysteroscopic AI resulted in lower pregnancy rate than insemination into the uterine body. Reger et al. (2003) also reported higher pregnancy rates for body AI than rectally guided, deep-horn AI.

These studies leave many questions as to which method of AI should be chosen. The factors that need to be considered are type of mare (young or old, normal or abnormal), type of semen (fresh, cooled, frozen), volume of insemination and cost of the procedure. Theoretically, deep-horn insemination of a low sperm dose in a small volume should decrease post-breeding endometritis. However, this has not been proven and, in at least one study, pregnancy rates were decreased with AI onto the UTJ.

For proper insemination onto the UTJ, the volume should be less than 0.5 ml. Volumes greater than 0.5 ml quickly drain from the UTJ and sperm numbers at the UTJ are diminished. Probably the most important factors in selecting the method of AI are the number and quality of sperm. Hysteroscopic AI seems to be justified when sperm numbers are very low (5 to 25 million) and when sperm are damaged (poor quality frozen/thawed or sex-sorted). It is likely that when sperm numbers are relatively high (50 to 100 million), then rectally guided, deep-horn AI may provide the same fertility as hysteroscopic AI. With both techniques, the skill of the technician may also affect the pregnancy results.

Is there any benefit to deep horn or hysteroscopic AI when normal doses of frozen-thawed semen are used? Hysteroscopic AI when normal doses of frozen-thawed sperm are used is not justified. Deep-horn insemination of a normal dose of frozen-thawed sperm is also probably not justified, but the time and effort needed for deep-horn AI is minimal compared to hysteroscopic AI, and thus this technique is commonly being used.

It is obvious that more studies with larger mare numbers are needed in which methods of insemination are evaluated.

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