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# Low-dose insemination—Why, when and how

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## Abstract

The typical dose for insemination into the uterine body of the mare is  $>300 \times 10^6$  progressively motile spermatozoa (PMS) and an insemination dose of  $>200 \times 10^6$  PMS is recommended for frozen–thawed semen. Low-dose insemination techniques allow for a drastic reduction in the numbers of spermatozoa required to achieve pregnancy. Acceptable pregnancy rates can be achieved with doses ranging from 1 to  $25 \times 10^6$  PMS in volumes ranging from 20 to 1000  $\mu$ L. Two techniques have been described: hysteroscopic insemination and transrectally guided deep horn insemination using a pipette. Similar pregnancy rates can be attained by either method when  $5 \times 10^6$  PMS are used. Hysteroscopic insemination may provide an advantage when the dose is  $1\text{--}3 \times 10^6$  PMS. These techniques have the potential to make more efficient use of frozen–thawed or sex-sorted semen from certain stallions. The use of low-dose insemination to improve fertility of infertile stallions warrants further investigation. © 2005 Elsevier Inc. All rights reserved.

*Keywords:* Mare; Low-dose insemination; Hysteroscopic insemination; Deep horn insemination; Delayed uterine clearance

## 1. Introduction

Low-dose insemination in the mare has been developed to substantially reduce the number of spermatozoa required to achieve pregnancy. With traditional insemination into the uterine body, the recommended dose for fresh semen is typically  $>300 \times 10^6$  progressively motile spermatozoa (PMS) [1–3], and for frozen–thawed semen  $>200 \times 10^6$  PMS is recommended [4]. Results from several studies suggest that the uterotubal junction (UTJ) or caudal isthmus of the oviduct functions as the sperm reservoir in the mare and the

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number of spermatozoa colonizing the UTJ ( $10^2$  to  $10^3$ ) or caudal isthmus ( $10^2$ ) is considerably lower than the number inseminated ( $10^8$  to  $10^9$ ) [5–7]. Based on the inability of lavage of the uterus 4 h post-insemination to reduce pregnancy rates [8,9], the caudal isthmus may serve a more substantial role as the sperm reservoir in the mare. It has been also observed that the UTJ and caudal isthmus may play an important role in selection of sperm that colonize these sites. Populations of spermatozoa that attach to equine oviductal epithelial cells *in vitro* have a higher motility and percentage normal morphology than those of the inseminate [10], and the spermatozoa that colonize the UTJ and ampullary-isthmus junction have a higher percentage of morphologically normal cells (90%) than that of the original ejaculate (65–85%) [6]. This theorized role of the UTJ created interest in exploiting these observations to achieve pregnancy with greatly reduced numbers of cells. Industry pressures to decrease the dose necessary to establish pregnancy include more efficient use of frozen–thawed semen, stallions whose semen freezes poorly, oligospermic stallions, and emerging technologies such as sex-sorted semen [11,12].

Three methods of low-dose insemination resulting in pregnancy have been reported: gamete intrafallopian transfer [13], hysteroscopic insemination [14–16], and deep intrauterine horn insemination using a pipette (hereafter referred to as “deep horn insemination”) [11,17]. Gamete intrafallopian transfer is the surgical deposition of oocytes and spermatozoa directly into the oviduct; sperm doses ranged from  $2\text{--}5 \times 10^5$  [13,18,19]. This paper will focus on non-surgical methods of hysteroscopic and deep horn insemination. Early attempts at developing hysteroscopic insemination described attempting to cannulate the UTJ [14]; however, other studies described deposition of the inseminate onto the UTJ [15,16]. Although it is difficult to compare pregnancy rates across these studies, due to potential differences in fertility of mares, stallions, and expertise of personnel, it appears that deposition onto the UTJ results in equal or higher pregnancy rates than attempts to cannulate the UTJ. Deep horn insemination has also been a successful method of low-dose insemination [11,17,20]. With either method of low-dose insemination, the inseminate dose has been reduced to  $1\text{--}25 \times 10^6$  PMS in volumes ranging from 20 to 1000  $\mu\text{L}$  [11,16,20–22]. Pregnancy rates for deep horn insemination of  $5\text{--}25 \times 10^6$  fresh spermatozoa ranged from 30 to 56% [11,20]. Pregnancy rates for hysteroscopic insemination of  $0.001\text{--}10 \times 10^6$  fresh spermatozoa range from 10 to 75% [14–16,20]. Debate continues over which technique offers higher pregnancy rates, and comparisons between studies is hampered by potential differences in the fertility of the mares, stallions, and expertise of personnel. Direct comparison of these techniques with the same population of mares and stallions showed no difference in pregnancy rates when  $5 \times 10^6$  PMS cooled for 24 h were inseminated [20]. If the insemination dose is decreased to  $1 \times 10^6$ , it has been suggested that pregnancy rates may be higher with hysteroscopic versus deep horn insemination [16]. For a more detailed examination of low-dose insemination in the mare, the reader is directed to previous reviews on this subject [23–25].

## 2. Indications for low-dose insemination

If the inseminate contains  $>50 \times 10^6$  motile cells, then it is unlikely that low-dose techniques will result in improved pregnancy rates compared to uterine body insemination

[26]. However, if the inseminate contains  $<50 \times 10^6$  motile cells, then hysteroscopic or deep horn insemination may improve pregnancy rates compared to uterine body insemination. Several circumstances arise when the available number of cells indicates the use of low-dose techniques. The desire to make more efficient use of frozen–thawed semen has led to the application of these techniques with a reduced dose of frozen–thawed semen. A single fixed-time insemination of  $14 \times 10^6$  motile frozen–thawed cells resulted in similar pregnancy rates, whether semen was deposited in the uterine body or on the UTJ. When the dose was reduced to  $3 \times 10^6$  motile cells, hysteroscopic insemination resulted in pregnancy rates higher than those of body insemination (47% versus 15%) [27]. Sorting of spermatozoa into X- and Y-bearing cells is possible with flow cytometry; however, the sorting rate of 1000 sperm/s limits the practicality of producing an inseminate with  $>50 \times 10^6$  cells [11]. Sex-sorted semen doses are typically in the range of  $5\text{--}25 \times 10^6$  motile cells, and pregnancy rates have ranged from 25 to 60% [11,28,29]. When considering differences in pregnancy rates across studies, it is important to keep in mind the variability in inherent fertility of mares and stallions and the breeding management used by different investigators. Another area of initial intense interest for low-dose insemination was whether these techniques could be used to improve pregnancy rates of oligospermic stallions. Although continued research in this area is warranted, it has been this author's experience and that of others [25] that low-dose insemination does not improve pregnancy rates of oligospermic, teratospermic stallions.

### 3. Low-dose insemination and endometritis

The inflammatory component of low-dose insemination techniques is also the subject of debate. Sieme et al. [26] found a significant interaction between pregnancy rate, method of insemination and reproductive history of the mare. Problem mares (history of pregnancy loss or being barren) had lower pregnancy rates when inseminated hysteroscopically than when inseminated in the uterine body. The reverse was true for normal mares. The incidence of ultrasonically detected fluid post-insemination was not different between mares inseminated by either method, but the potential interaction of the effect of category of mare, method of insemination, and presence of fluid was not examined. In contrast, Morris et al. [23] reported a very low incidence of endometritis (1%) but the method of assessment was not defined. In a study recently completed in our laboratory, the effects of hysteroscopic insemination were compared to uterine body insemination in normal mares to mares with delayed uterine clearance (DUC). Although DUC mares accumulated more fluid 24 and 48 h after insemination than normal mares ( $P < 0.05$ ), there was no difference in the percentage of leukocytes among groups or treatments ( $P > 0.05$ ). There was a strong positive correlation ( $r = 0.98$ ) between the duration of the hysteroscopy and the concentration of leukocytes in normal, but not in DUC mares. Regression analysis of the data determined that if hysteroscopy extended beyond 7 min, endometritis is likely to persist 48 h after the procedure [30]. Some investigators have suggested that deep horn insemination would be more inflammatory than hysteroscopic insemination [23], but to the author's knowledge, the inflammatory response to deep horn insemination in comparison to uterine body or to hysteroscopic insemination has not been critically evaluated.

## 4. Sperm preparation

The range of volumes of commonly used for low-dose insemination is 20–200  $\mu\text{L}$  for fresh or chilled semen, and is 250–500  $\mu\text{L}$  for frozen–thawed semen. Glass wool/ Sephadex (GWS) filtration, Percoll separation (PS), and swim-up techniques have been used to select spermatozoa prior to insemination, with the goal of improving sperm quality. Although it seems logical that application of selection techniques to semen prior to application of low-dose techniques would improve pregnancy outcomes, Nie et al. [31] did not find this to be the case. There was no difference in pregnancy rates of mares inseminated by deep horn insemination with  $25 \times 10^6$  cells selected by GWS, PS, or absolute number (no selection). A tendency ( $P = 0.105$ ) for GWS to improve pregnancy rates was observed. The pregnancy rates of the stallions used in this study were similar to those of previous seasons (same mare population and management considerations). In stallions with good fertility, it is unlikely that selection techniques would produce any increase in per-cycle conception rates. Further studies are needed to determine if selection techniques applied to semen from subfertile stallions prior to low-dose insemination would improve pregnancy rates.

## 5. Hysteroscopic insemination

### 5.1. Endoscope

A 1.6 M video endoscope<sup>1,2</sup> is recommended for hysteroscopic insemination. Cold sterilization with an activated dialdehyde solution<sup>3</sup> is the most common method used to clean video endoscopes between procedures. Extreme care must be taken to thoroughly rinse the exterior and working channels of the endoscope. Sterile saline is preferable to sterile water for this task. Any residual disinfectant or water will be highly spermicidal for the low volumes of semen used with low-dose insemination. Air is then blown through the channels, either by syringe or under pressure, to ensure that all residual fluid is dispelled. It is also preferable to use sterile saline or lactated ringers solution in the reservoir bottle used for flushing during the procedure. Although a standard endoscope can be used for hysteroscopic insemination, the procedure is expedited by the use of a video endoscope. Hysteroscopic insemination requires a team of three operators; it would be extremely difficult for a single person to hysteroscopically inseminate a mare. A video endoscope allows all members of the team to work in concert. With experience, a team of inseminators require little verbal communication; the necessary adjustments by the person holding the scope in the vagina, the person adjusting the direction of the distal end of the endoscope, or the person delivering the semen through the catheter become essentially automatic. This results in short procedure times, which lessens the

<sup>1</sup> EVIS EXERA type 160 Series (Olympus America Inc., Melville, NY, USA; phone: 631 844 5000).

<sup>2</sup> EX-3870K video colonoscopy, EPK-100 video processor (Pentax Precision Instrument Corp., Orangeburg, NY, USA; phone: 800 431 5880).

<sup>3</sup> Cidex<sup>®</sup> (Advanced Sterilization Products, Johnson & Johnson Co., Markham, ON, Canada; phone: 905 946 1611).

degree of inflammation produced by the procedure. If the catheter tip loses contact with the UTJ, inaccurate placement is avoided by immediately discontinuing delivery of the inseminate.

### 5.2. Catheters

Double-lumen<sup>4,5</sup> and single-lumen<sup>6</sup> 200-cm catheters are available for hysteroscopic insemination. The catheter should be at least 20 cm longer than the endoscope. Double-lumen catheters have an inner catheter that is 2 Fr. The single-lumen catheter is 7 Fr, which can present problems with leakage of semen. Double-lumen catheters afford better protection to the semen from any residual fluids present within the channel, and tend to retain the semen within the catheter until it is actively dispelled. If leakage of the semen is encountered, then a three-way stopcock can be attached to the catheter. Double-lumen catheters are considerably more expensive than single-lumen catheters; however, it is possible to clean and sterilize catheters between uses.

### 5.3. Procedure

The procedure is scheduled 24–30 h after administration of either hCG or deslorelin acetate (Ovuplant<sup>®</sup>) to induce ovulation. In most cases, sedation with detomidine (Dormosedan<sup>®</sup>; 6 mg i.v.) alone or in combination with butorphanol (Torbugesic<sup>®</sup>; 4 mg i.v.) facilitates the procedure. With the mare restrained in stocks, the rectum is evacuated of feces, the side of the preovulatory follicle confirmed, and the perineum aseptically prepared. The endoscope is introduced just anterior to the cervix and insufflation of the uterus is begun. Once the openings to the horns can be identified, the airflow may be decreased to minimize mare discomfort and the scope is advanced into the horn ipsilateral to the preovulatory follicle. It is extremely important to maintain the correct orientation of the endoscope. Failure to do so may result in insemination of the contralateral horn. A papilla is the most common morphology of the UTJ, but there is variability in its appearance [14]. Once the UTJ is identified, the catheter with pre-loaded semen is introduced through the biopsy channel of the endoscope until the tip of the outer catheter is just visualized on the monitor. The inner catheter is advanced to touch the UTJ and the semen is deposited. If contact is not maintained between the catheter tip and the UTJ, the semen may fall away and pool in the ventral portion of the horn. Frothing of the semen tends to aid in the inseminate remaining over the surface of the UTJ [16]. After completion of insemination, air within the uterus should be passively or actively removed by suction through the endoscope. A variation of this technique involves rectal guidance of the endoscope to the tip of the horn after vaginal insertion. The mid-portion of the horn is compressed around the endoscope, so that only the proximal tip of the horn ipsilateral to the preovulatory follicle is insufflated [26].

<sup>4</sup> Equine fallopian tube insemination set, V-EFIS-2-200 (Cook Australia, distributed by AB Technology; phone: 800 335 8595, [www.abtechnology.com](http://www.abtechnology.com)).

<sup>5</sup> Large animal insemination set INS200 (Mila Int.; phone: 888 645 2468, [www.milaint.com](http://www.milaint.com)).

<sup>6</sup> Aspiration catheter ASPC2 (Endoscopy Support Services Inc., Brewster, NY, USA; phone: 800 349 3636, [www.endoscopy.com](http://www.endoscopy.com)).

#### 5.4. Deep horn insemination

The procedure is scheduled 24–30 h after administration of either hCG or deslorelin acetate (Ovuplant<sup>®</sup>) to induce ovulation. Initial preparation of the mare is the same as for hysteroscopy, with the exception of sedation. Few mares require sedation for deep horn insemination. With the mare restrained in stocks, feces are evacuated from the rectum and the side of the preovulatory follicle is confirmed. The perineum is prepared and a flexible insemination pipette<sup>7,8</sup> is inserted through the cervix into the uterine body. Manipulation of the uterus per rectum is used to guide the pipette to the tip of the horn. In most cases, the tip of the pipette can be easily palpated in close proximity to the proper ligament of the ovary. The most difficult part of the procedure is advancing the catheter past the base of the horn; there is variability between mares, and even between cycles in the same mare, regarding the ease of catheterization. To straighten out the entrance into the horn, the ipsilateral horn can be extended forward, or the contralateral horn can be pulled caudally. If uncertainty exists regarding the location of the pipette, transrectal ultrasonography can be used to confirm its location. The type of catheter used is personal preference. One catheter<sup>7</sup> has a bulb on the end, which may improve the ease of palpation for some individuals. This catheter<sup>7</sup> can be supplied with an insemination gun that retrieves an empty straw after insemination, simplifying the insemination of multiple straws. It is also available with an inner catheter for use with low volumes of fresh or chilled semen to minimize semen loss. Another catheter<sup>8</sup> is made of a flexible material that permits the creation of a bent tip, which some examiners feel aids in passing the catheter into the base of the horn.

#### Summary

Hysteroscopic and deep horn insemination are techniques that allow for a drastic reduction in the number of spermatozoa necessary to achieve pregnancy. Acceptable pregnancy rates can be achieved with doses ranging from  $1\text{--}25 \times 10^6$  PMS in volumes ranging from 20 to 1000  $\mu\text{L}$ . Pregnancy rates should be the same with either technique unless the dose is less than  $5 \times 10^6$  PMS, in which case hysteroscopic insemination may result in higher pregnancy rates than deep horn insemination.

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<sup>7</sup> Universal Insemination Pipette & Gun, 55 and 65 cm lengths (Minitube of America, Verona, WI, USA; phone: 800 646 4882, [www.minitube.com](http://www.minitube.com)).

<sup>8</sup> IMV flexible equine catheter 007356, 30 (IMV International Corp. Maple Grove, MN, USA; phone: 800 342 5468).

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