Development of Hysteroscopic Insemination of the Uterine Tube in the Mare

Stephen T. Manning, DVM; Pamela A. Bowman, AHT; Lorrie M. Fraser, DVM; and Claire E. Card, DVM, PhD

The hysteroscopic insemination of the uterine tube (HIT) is a technique that may be used in mares to achieve pregnancies at a dramatically lower sperm cell dosage than is required by using traditional techniques. Authors’ address: Dept. of Herd Medicine and Theriogenology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada. Dr. Manning’s current address is W. A. Burwash, Equine Services Ltd., Box 2 Site 29, RR 12, Calgary, Alberta T3E 6W3, Canada. © 1998 AAEP.

1. Introduction
The hysteroscopic insemination of the uterine tube (HIT) has been used in humans to reduce the number of sperm cells required for conception.1–3 The technique of hysteroscopy has been used to examine visually the endometrium of subfertile or infertile mares for evidence of inflammation, fibrosis, or atrophy, and to visualize palpable or ultrasonographically visible abnormalities such as endometrial or lymphatic cysts, tumors, intramural hematomas, adhesions, and congenital anomalies.4 To our knowledge, the use of HIT has not been reported in the horse. We investigated the potential for the use of this technique in the mare.

2. Materials and Methods
Preliminary investigations included the examination of equine reproductive tracts (N = 100) obtained from a slaughter plant. The uterine ostium of the uterine tube was identified, and a series of needles (18–25 g) or catheters (<5 French) were inserted into the uterine tube to determine the diameter, which was found to be less than 2 French. The average volume required to fill a uterine tube was 0.3 ml.

Complete tracts (n = 86) were classified as estrus, diestrus, transitional, or anestrus based on ovarian structures. The external appearance of the ostium (opening) was imaged by using an Olympus stereomicroscope a connected to a data-acquisition–data-analysis imaging program. b The mean diameter and area of the ostium were determined. A variability in the external morphology but not in the diameter (analysis of variance; p = 0.25) or area (p = 0.39) of the uterine tube was observed, but it was unrelated to physiologic status (chi square; p = 0.56).

There were five main morphologic categories: papilla (36%), complex (32%), diffuse (17%), double ridge (11%), and other (4%). There was a within-mare variability in morphologic type. In the second experiment, 12 mixed-breed light horse mares were selected for a breeding trial based on the following: age (<8 years of age), good endometrial biopsy scores (I or IIA), healthy reproductive tracts, and no history of reproductive problems. The mares were teased
daily and examined frequently by palpation and ultrasound while in estrus, i.e., from May to October, 1997. Estrous mares with endometrial edema and follicles > 35 mm were inseminated. Mares with firm preovulatory follicles were treated with 2000 IU IM of human chorionic gonadotrophin at insemination. Pregnancy rates were determined at 14 days postovulation by using ultrasonography. One stallion was used for all of the inseminations. The stallion has a satisfactory BSE evaluation and had sired a number of foals prior to use in the study. A complete semen evaluation was performed on all ejaculates. Semen was extended in a milk-based extender. The four treatments consisted of an insemination once with (1) 100 million fresh extended progressively motile, morphologically normal (PMMN) sperm cells, (2) 10 million PMMN fresh extended sperm cells, (3) HIT with 10 million PMMN sperm cells, and (4) HIT with 1 million PMMN sperm cells. Insemination volumes were 100 million PMMN, <12 ml; 10 million PMMN, 5.2 ml; 10 million HIT, 0.25 ml; and 1 million HIT, <0.16 ml. Sperm cells were collected, extended, and centrifuged at 300 × g for 10 min for the 10 million HIT. For HIT, mares were tranquilized (detomidine 0.01 mg/kg and butorphanol 0.1 mg/kg IV) and were administered clenbuterol (200–300 µg IV). A videoendoscope was manually placed into the uterus, and the cervix was held closed around it. With continuous insufflation, the endoscope was advanced into the horn ipsilaterally to the preovulatory follicle; the uterine ostium of the uterine tube was visualized; cannulation was attempted with a custom made, sheathed (8 French), Teflon-coated catheter (3.5 French); and extended semen was deposited. The time to perform the procedure varied from 5 to 20 min.

3. Results

Pregnancy rates were as follows: control, 100 million PMMN, 4/12 (33%); control, 10 million PMMN, 2/12 (17%); HIT, 10 million PMMN, 0/11 (0%); and HIT, 1 million PMMN, 2/9 (22%). In 20 HIT cycles, two mares had transient intraluminal fluid accumulation after HIT; none of the control artificial insemination mares accumulated fluid.

4. Discussion

HIT is technically challenging, and a further refinement in equipment will be required to increase the ease and repeatability of the procedure. These results show that HIT is a technique that may be used to achieve pregnancies in mares at a much reduced sperm cell dose than is possible by using traditional techniques. The lack of pregnancies in the 10 million HIT group may be explained by the larger insemination volume that may have caused passage of the semen out of the uterine tube into the abdomen, by an inflammation of the tubal epithelium from the larger PMMN sperm cell dose, or by an experimental error in which semen was not accurately or completely deposited into the uterine tube. In the 1 million HIT group in our study, three of the nine mares inseminated failed to ovulate within 3 days of insemination. Hence, if the data are reported as pregnancies obtained from mares ovulating within 72 h of insemination, the pregnancy rate in this group would be 2/6 (33%). The HIT procedure may have applications in the future in assisting and improving the reproductive capability of valuable or subfertile stallions, as well as in maximizing the use of cryopreserved semen. Hysteroscopic tubal cannulation could potentially be used for other assisted reproductive procedures in the mare, such as embryo transfer and gamete intrafallopian tubal transfer.

References and Footnotes


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