

The Effect of Postinsemination Endometritis on Fertility of Frozen Stallion Semen

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In normal mares subjected to heterospermic insemination, postbreeding endometritis does not appear to be detrimental to the fertility of a second dose of frozen semen that is artificially inseminated within 6–10 hours of the first insemination. Author's Address: Honahlee, PC, 14005 SW Tooze Road, Sherwood, Oregon 97140. © 2000 AAEP.

Introduction

In mares, a physiological endometritis ensues following the deposition of semen into the uterus.¹ This response is thought to be due to the antigenic nature of spermatozoa, specifically the activation of complement, and may be modulated by the presence of seminal plasma component of the semen.² The removal of seminal plasma during cryopreservation of spermatozoa may account for the longer duration of the inflammatory reaction that often accompanies insemination with frozen-thawed semen.³ This reaction, following insemination with fresh semen, appears to be greatest at 12 hr after insemination, gradually subsiding in 24–36 hr.³ In vitro studies indicate that sperm motility is most significantly depressed at 12 hr following insemination as well.⁴

Some of the highest reported pregnancy rates using frozen semen have been the result of a breeding protocol where mares are bred both pre- and post-ovulation with 6–10 hr between inseminations.⁵ Since the cost of a single breeding dose of frozen semen is often quite expensive, researchers have questioned if the second dose, inseminated in such a proximity to the first dose, is truly necessary for acceptable pregnancy rates, or the sperm are simply

phagocytized by the incipient endometritis, never to reach the oviduct.

The present experiment, using heterologous spermatozoa, was designed to determine if the fertility of frozen-thawed semen is lost when inseminated within 4–10 hr of a previous dose of frozen-thawed semen.

Materials and Methods

Mare candidates for this study were selected based on age, history, and a normal prebreeding examination. Mares in estrus (n = 11) were examined daily via transrectal palpation and ultrasound examination^b until a dominant softening follicle that measured ≥ 30 mm and prominent endometrial folds were detected. At this time, HCG^c (1500–5000 IU IM) was administered. Mares were examined more frequently, and at 34–40 hr following HCG administration, they were inseminated with the frozen-thawed semen from one stallion (Stallion A) just prior to ovulation. Ovulation was documented by the presence of a *corpus hemorrhagicum* on ultrasound evaluation and the mares were inseminated with semen from another stallion (Stallion B) 6–10 hr after the first insemination. Pregnancy was confirmed by serial ultrasound examinations.

NOTES

Frozen-thawed semen from 8 stallions, with each insemination dose containing approximately 300×10^6 progressively motile spermatozoa extended to a volume ranging from 1.5–5 ml, was used in this study. The frozen-thawed semen from each stallion had a per-cycle fertility rate $\geq 30\%$. Although the stallion selection was made by the mare owner, the semen was randomly assigned to be either the pre- or postovulatory dose. One mare served for two consecutive years and Stallions A and B were reversed during the second year.

Parentage of the foals was determined by DNA analysis of mane and tail hair follicle samples.

Results

Pregnancy was detected in 9/11 mares bred (82% pregnancy rate). Eight mares conceived on the first cycle and the remaining mare was bred through 3 cycles before a 14-day-old pregnancy was detected (72% first heat conception rate). One of these mares experienced a double ovulation and appeared to have twins at 12 days and only a singleton pregnancy at 20 days postovulation. All pregnant mares ($n = 9$) delivered a single live foal.

Stallion A (preovulatory insemination) proved to be the sire of 4 foals and Stallion B (postovulatory insemination) proved to be the sire of 5 foals. The mare that was used for two consecutive years and had the order of stallions reversed produced both foals from postovulatory insemination (Stallion B). There was no significant difference between pregnancy rates of pre- or postovulatory inseminations (Stallion A or Stallion B).

Discussion

It appears that postbreeding endometritis, resulting from insemination with frozen semen, is not associated with infertility of subsequent doses of frozen-thawed semen, even when the subsequent insemination is performed during the time of peak polymorphonuclear leukocyte (PMN) activity and sperm motility derangement. This study suggests that spermatozoa, extended to very small volumes of frozen-thawed semen and introduced into a mare's uterus that is subjected to the physiological inflammation concomitant with exposure to spermatozoa from the first insemination, can actually enter the oviduct and fertilize ova. This phenomenon lends support to the concept of selective PMN phagocytosis of abnormal, infertile spermatozoa that may be concomitant with postbreeding endometritis.

Heterospermy has been used in the food animal industry for many years to evaluate male fertility and enhance pregnancy rate and/or litter size.⁶ It is interesting to note that in the bovine industry, the fertility of an insemination dose consisting of multiple sires will rise to the level of the most fertile sire.^d In fact, a heterospermic dose of semen may demonstrate higher fertility than any of the sires

individually.^c This synergistic effect may be related to the period of time before ovulation during which a female is artificially inseminated; there may be "competition" among sperm from different sires for oviductal binding or more likely, capacitation rates may vary between spermatozoa from different sires and a heterospermic insemination dose may maximize pregnancy rates by ensuring the availability of capacitated sperm for fertilization regardless of the interval to ovulation.

Of the 9 pregnant mares in this study, 8 conceived on the first cycle (88%); this first cycle pregnancy rate is higher than those reported in other studies using frozen semen,¹⁰ albeit the results are from a very limited sample size. Still, the results may suggest that heterologous sperm may indeed enhance fertility in the horse as well.

Finally, the results in the present study suggest that a second dose of frozen-thawed semen inseminated postovulation is not a waste of the veterinarian's time and the client's money. Furthermore, the timing of insemination with respect to ovulation remains controversial; this study supports researchers who claim that high pregnancy rates can be achieved with frozen-thawed semen when the mare is inseminated both immediately prior to and following ovulation, rather than a single breeding dose pre- or postovulation.

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References and Footnotes

1. Kottilainen T, Huhtinen M, Katila T. Sperm-induced leukocytosis in the equine uterus. *Theriogenology* 1994;41: 629–636.
2. Troedsson MHT, Lliu IKM, Ing M, et al. Mechanism of sperm-induced endometritis in the mare. *Biol Reprod* 1995; 52(Suppl 1):307.
3. Katila T. Onset and duration of uterine inflammatory response after insemination with fresh semen. *Biol Reprod* 1:515–517.
4. Troedsson MHT, Alghamdi A, Laschwitich T, et al. Sperm motility is altered in uterine secretions in mares with postbreeding endometritis, in *Proceedings*. 44th Annu Conv Am Assoc Equine Practnr 1998;66–67.
5. Metcalf L. Maximizing reproductive efficiency in private practice: the management of mares and the use of cryopreserved semen, in *Proceedings*. Soc Theriogenol 1995;155–159.
6. Vanberboom RJ, McCauley TC, Tappan R, et al. Bovine reproductive biotechnology. In: Youngquist RS, ed. *Current therapy in large animal theriogenology*. WB Saunders Philadelphia 1997:457–472.
7. Loomis PR. Artificial insemination of horses: where is it going?, in *Proceedings*. Soc Theriogenol 1999;325–336.

^aAloka Diagnostic Ultrasound, 10 Fairfield Blvd., Wallingford, CT 06492.

^bSteris Laboratories, Inc., Phoenix, AZ 85043.

^cTroedsson MHT. Submitted for publication.

^dSaacke RG. Submitted for publication.

^eSaacke RG. Personal communication.

Endometritis - inflammation of the uterine mucosa of polymicrobial etiology. Endometritis during labor (chorioamnionitis) is a polymicrobial infection of membranes and amniotic fluid. Acute endometritis and endomyometritis do not belong to the frequently occurring diseases requiring the doctor to provide emergency care. [1], [2], [3], [4], [5], [6], [7], [8]. Epidemiology. You can imagine that such a mechanism of therapeutic effect of drugs that reduce the uterus is effective in endometritis. With myometrites, the contractility of the uterus is difficult to correct, and in the case of involvement in the inflammatory process of uterine veins, the appointment of strongly fast, but short-acting uterotonic agents can promote the spread of thrombi. The evaluation of stallion semen in aspects of fertility control and its use for artificial insemination. J. Reprod. Fert. 2002. Post-thaw spermatozoa plasma membrane integrity and motility of stallion semen frozen with different cryoprotectants. Theriogenology 58(2-4):245-249. Hintz, H.F. 1993. The effects of seminal plasma on sperm during storage . 23 2.6. As sperm longevity and stallion fertility vary markedly between individuals, it is of interest for the equine industry to investigate the factors producing these effects. Stallion SP influences the function and survival of ejaculated sperm during in vitro storage and in the female genital tract, and some of the stallion variation could perhaps be explained by inherent differences in the composition of SP. The addition of K to semen extenders seems to improve motility of stallion and human sperm, but the effect depends on the proportion of SP retained in the sample during storage (Padilla and Foote 1991, Karow et al.