Effect on fertility of uterine lavage performed immediately prior to insemination in mares

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EQUINE

Objective—To determine the effect on fertility of large-volume uterine lavage with lactated Ringer's solution (LRS) performed immediately prior to insemination in mares.

Design—Prospective randomized controlled study.

Animals—20 mares.

Procedure—Control mares (n = 10) were inseminated with 1 billion (estimated before cooling) progressively motile spermatozoa that had been cooled in a passive cooling unit for 24 hours. Mares (n = 10) in the treatment group were inseminated with 1 billion progressively motile spermatozoa (cooled as described for control mares) immediately after uterine lavage with 4 L of sterile LRS.

Results—There were no significant differences in pregnancy rates or size of the embryonic vesicle on days 12, 13, and 14 after ovulation between control and treated mares.

Conclusions and Clinical Relevance—Results indicate that uterine lavage with LRS can be performed immediately prior to insemination without adversely affecting fertility in mares. This is clinically important, because insemination may be necessary when a mare has inflammation-associated fluid (detectable ultrasonographically) in the uterus; removal of the fluid is desirable, because it adversely affects spermatozoal motility and fertility. This situation typically arises when mares require rebreeding after they have developed persistent mating-induced endometritis or are inseminated multiple times in a 24-hour period (during the period of physiologic mating-induced inflammation), which is a common practice when using cooled or frozen-thawed semen. (*J Am Vet Med Assoc* 2003;222:1108–1110)

It is known that a physiologic endometritis occurs after breeding in all mares as a result of deposition of semen in the uterine lumen,[1,2] and that spermatozoa and other components of the inseminate induce the inflammatory response.[3] Reproductively healthy mares spontaneously eliminate this mating-induced uterine contamination and inflammation between 24 and 48 hours after breeding,[1] whereas mares susceptible to endometritis fail to do so[4] and develop persistent mating-induced endometritis. Mares that develop persistent mating-induced endometritis accumulate fluid within the uterine lumen because of impaired (primarily delayed) physical clearance of inflammatory material.[4-6] Factors that may contribute to this delayed clearance in susceptible mares include low myometrial activity,[6] inadequate lymphatic drainage from the uterus,[7] and abnormal reproductive conformation (such as a pendulous uterus located ventral to the pelvic brim).[8]

Intrauterine fluid that accumulates during persistent mating-induced endometritis may adversely affect fertility by impairing motility and viability of spermatozoa subsequently inseminated while the uterus is inflamed[9,10] or by inducing embryonic loss if endometritis persists through day 5 after ovulation, at which time the embryo enters the uterine lumen from the oviduct,[11] and the corpus luteum becomes sensitive to **prostaglandin $F_{2\alpha}$** (**$PGF_{2\alpha}$**).[12,13] Therefore, routine treatment for persistent mating-induced endometritis is directed at enhancing clearance of accumulated fluid from the uterus and includes the use of ecbolic agents such as oxytocin[14] and $PGF_{2\alpha}$,[15] which may be used alone or in combination with large-volume uterine lavage.[16] Although uterine lavage can be performed as soon as 4 hours after insemination without adversely affecting fertility in mares,[17,18] the effect of performing uterine lavage immediately prior to insemination has not been reported. Lavage of the uterus prior to insemination may be required when rebreeding becomes necessary in a mare that has developed persistent mating-induced endometritis or when multiple inseminations are performed in a 24-hour period, which is a common practice when using cooled or frozen-thawed semen. However, it is plausible that uterine lavage performed immediately prior to insemination could adversely affect fertility by removing physiologic secretions that are beneficial or necessary for spermatozoal viability and fertility (eg, capacitation)[19] or by leaving residual lavage fluid in the uterine lumen that may alter intrauterine pH or osmolarity or cause excessive dilution of the spermatozoa.[20] The purpose of this study was to determine whether large-volume uterine lavage performed immediately prior to insemination adversely affects fertility in mares.

Materials and Methods

Our study was conducted during April and May of 2002. Twenty mixed-breed mares, 3 to 12 years of age, weighing 300 to 500 kg (660 to 1,100 lb) were included in the study. The reproductive tracts of mares were examined 4 times weekly via transrectal palpation and ultrasonography. Mares with an ovarian follicle ≥ 30 mm in diameter were examined daily until ovulation was detected. Ovulation was defined as disappearance of ovarian follicles > 35 mm in diameter between 2 successive daily examinations, and was confirmed by subsequent identification of the corpus luteum via transrectal ultrasonography. The day during which ovulation was detected was defined as day 0.

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Mares with a follicle ≥ 35 mm in diameter were treated with **human chorionic gonadotropin**[a] (**hCG**; 5 U/kg [2.3 U/lb], IV) and were randomly assigned to the treatment or control group. Ten control mares were inseminated with 1 billion (estimated before cooling) progressively motile spermatozoa that had been cooled in a passive cooling unit for 24 hours. Ten mares in the treatment group were inseminated with 1 billion progressively motile spermatozoa (cooled as described for control mares) immediately after uterine lavage with 4 L of sterile **lactated Ringer's solution** (**LRS**). Mean number of progressively motile spermatozoa in each insemination dose prior to cooling was 1.08×10^9 (range, 1.02 to 1.19×10^9 spermatozoa). Semen from 1 fertile stallion was collected with an artificial vagina. After collection, the semen was evaluated for gel-free volume, concentration, and progressive motility of spermatozoa using standard procedures. Each insemination dose was extended with warm (37°C [98.6°F]) skimmed milk glucose extender[b] to a final concentration of 25 to 50 million spermatozoa/mL and placed into a cooled semen transporter[c] until it was used for insemination (approx 24 hours). Insemination of all mares was performed 24 hours after administration of hCG. Each mare was inseminated only once.

Immediately prior to insemination of treated mares, uterine lavage was performed with 4 L of warm (37°C) sterile LRS.[d] To administer the lavage, each mare was restrained in stocks; the tail was wrapped, and the perineal area was cleaned with soap[e] and then rinsed with water and dried. A sterile 80-cm balloon-tipped catheter[f] was placed through the cervix, and the balloon was inflated with a volume of approximately 80 cm^3 of air. One liter of warm LRS was infused into the uterine lumen and removed via gravity flow; this was performed 4 times. At the completion of the lavage procedure, the uterus was examined via transrectal ultrasonography to determine whether lavage fluid remained in the uterine lumen. When residual lavage fluid was identified in the uterus, the height of the pooled residual fluid was measured and scored using a modification of the method described by Adams et al.[13] An intrauterine fluid score was assigned on the basis of the maximum height of the pooled fluid: ≤ 10 mm, score = 1; 11 to 19 mm, score = 2; 20 to 39 mm, score = 3; and ≥ 40 mm, score = 4. After assignment of a score, no action was taken to remove the fluid from the uterus. Immediately after the lavage procedure, the perineal area was washed, rinsed with water, and dried; insemination was performed via a standard technique.

Pregnancy status was determined by means of transrectal ultrasonography once daily on days 12, 13, and 14 after ovulation. Diagnosis of pregnancy in a mare required identification of an embryonic vesicle that changed location within the uterine lumen or increased in diameter during the interval between 2 consecutive daily examinations. Statistical analyses were performed with computer software.[g] Pregnancy rates between the 2 groups were compared with the Fisher exact test; the mean diameter of the embryonic vesicle on days 12, 13, and 14 in control and treated mares was compared using a randomized-block ANOVA. Values of $P < 0.05$ were considered significant.

Results

Nine mares in each group ovulated within 48 hours after administration of hCG (ie, within 24 hours following insemination), and 1 mare in each group ovulated within 72 hours after hCG treatment. There were no significant differences in pregnancy rate or size of the embryonic vesicle on days 12, 13, and 14 between control and treated mares (**Table 1**). One mare in each group synchronously ovulated 2 follicles; both of those mares became pregnant with twins. Six of 10 treated mares had fluid (detected ultrasonographically) remaining in the uterine lumen immediately after the lavage was completed, with fluid scores of 1 (n = 3), 2 (2), and 3 (1). Five of the 6 mares with residual lavage fluid in the uterus became pregnant; the non-pregnant mare had a fluid score of 1.

Table 1—Pregnancy rate and diameter of the embryonic vesicle (mean ± SD) on days 12, 13, and 14 after ovulation (day 0) in 10 control mares inseminated with 1 billion (estimated before cooling) progressively motile spermatozoa that had been cooled in a passive cooling unit for 24 hours and 10 mares inseminated with 1 billion (similarly cooled) progressively motile spermatozoa immediately after uterine lavage with 4 L of lactated Ringer's solution

	Mares	
End point	**Control**	**Treated**
Pregnancy rate	9/10	7/10
Diameter of embryonic vesicle (mm)*		
Day 12	8.6 ± 2.1	8.7 ± 2.8
Day 13	12.2 ± 3.1	12.6 ± 3.0
Day 14	16.2 ± 3.4	17.3 ± 4.1

*One mare in each group had twin embryos from synchronous double ovulations; data for both embryos in each mare were included in the analysis.

Discussion

These results indicated that uterine lavage with LRS performed immediately prior to insemination did not adversely affect fertility in mares, because there were no differences in pregnancy rates or diameter of the embryonic vesicle on days 12, 13, and 14 after ovulation between control and treated mares. This finding is clinically important, because insemination of a mare that has inflammation-associated fluid (detectable ultrasonographically) in the uterus may be necessary, yet it is known that such fluid adversely affects equine spermatozoal motility[9,10] and fertility.[9] This situation typically arises when mares require rebreeding after they have developed persistent mating-induced endometritis or when mares are inseminated multiple times within a 24-hour period (during the period of physiologic mating-induced inflammation), which is a common practice when using cooled or frozen-thawed semen.

It is plausible that uterine lavage performed immediately prior to insemination could adversely affect fertility through mechanisms previously outlined. Our data, however, indicate that none of these potential adverse effects occurred after uterine lavage was performed as described. Residual lavage fluid was observed in the uteri of 6 of 10 mares at the completion of the lavage procedure, yet 5 of those mares became pregnant, which suggests that residual lavage fluid did not impair spermatozoal viability or fertility. The mare with residual lavage fluid that did not become pregnant was 1 of 3 mares that had a fluid score of 1, whereas 3 other mares with higher fluid scores (2 or 3) all became pregnant; therefore, there was no clear evidence of a relationship between the amount of residual lavage fluid and pregnancy outcome.

It is important to note that LRS was used for the lavage procedure in our study. Although other fluids (such as physiologic saline [0.9% NaCl] solution) may give similar results, their use immediately prior to insemination of mares cannot be advocated until they are tested in a similar manner. There are considerable differences in pH and osmolarity among proprietary saline and electrolyte solutions that could affect their suitability for use

immediately prior to insemination. For example, the LRS solution used in this study had a pH of 6.5 (range, 6.0 to 7.5) and osmolarity of 273 mOsmol/L, whereas physiologic saline solution from the same manufacturer[d] has a pH of 5.0 (range, 4.0 to 7.0) and osmolarity of 308 mOsmol/L. Semen extenders that are compatible with maintenance of spermatozoal motility and fertilizing capacity have a pH of 6.5 to 7.5 and osmolarity of 300 to 400 mOsmol/L.[21] It seems likely that similar characteristics would be desirable in a lavage solution used immediately prior to insemination, because lavage fluid may remain in the uterine lumen at the time of insemination. Although the osmolarity of the LRS used in our study was lower than the value suggested for semen extenders, there was no evidence that this characteristic adversely affected spermatozoal viability and fertility.

In our study, it is also important to note that LRS was not supplemented with antimicrobials or antiseptic agents such as povidone-iodine, which could have a deleterious effect on spermatozoal viability or fertility depending on properties of the specific agent or concentration used. The primary benefit of the use of uterine lavage to treat persistent mating-induced endometritis is the physical clearance of accumulated fluid from the uterine lumen; therefore, the need for antimicrobials or antiseptic agents in the lavage fluid is questionable. When performing a lavage immediately prior to insemination, the addition of an antiseptic agent such as a povidone-iodine is clearly contraindicated because of its deleterious effect on spermatozoal motility, even at extremely low concentrations.[22]

The need to perform uterine lavage immediately prior to insemination is predicated on evidence that fluid that accumulates in response to inflammation adversely affects spermatozoal motility and fertility; however, it appears that lavage may only be necessary when the amount of fluid in the uterus is detectable ultrasonographically, because mares can be inseminated during the period of physiologic mating-induced endometritis without adversely affecting fertility.[23] This indicates the viability and fertility of the spermatozoa inseminated into the inflamed uterine environment were not adversely affected; however, none of the mares in that study[23] had amounts of fluid in the uterus that were detectable ultrasonographically at the time of insemination.[h] Therefore, performing uterine lavage in mares immediately prior to insemination is warranted when fluid, resulting from inflammation, is detectable ultrasonographically (echogenic appearance) in the uterus at the time insemination is planned. Our data indicate that uterine lavage with LRS can be performed immediately prior to insemination without adversely affecting fertility and is, therefore, an appropriate treatment strategy in such circumstances.

[a]Chorulon, Intervet Inc, Millsboro, Del.
[b]EZ-Mixin CST, Animal Reproduction Systems, Chino, Calif.
[c]Equitainer, Hamilton Research Inc, South Hamilton, Mass.
[d]Baxter Healthcare Corp, Deerfield, Ill.
[e]Ivory, Procter & Gamble, Cincinnati, Ohio.
[f]No. VEUF80, Bivona Inc, Gary, Ind.
[g]SAS version 8.01, SAS Institute Inc, Cary, NC.
[h]Metcalf E, Honahlee, PC, Sherwood, Ore: Personal communication, 2001.

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