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Abstract

The reproductive management of mares inseminated with frozen-thawed semen can be similar to that for mares inseminated with cooled semen. Examination of the mare with ultrasonography once a day and insemination of frozen-thawed semen 24 and 40 h after human chorionic gonadotropin (hCG) injection provided similar fertility to those examined three or four times a day and inseminated within 6 h post-ovulation. Furthermore, by inseminating the mare with 400 x 10^6 sperm twice in a cycle, the number of frozen-thawed sperm used to obtain pregnancy was identical to that of mares inseminated once post-ovulation. Deep uterine insemination into the uterine horn by a rectally guided approach did not improve pregnancy rates over those of mares inseminated into the uterine body.

1. Introduction

Expanded use of frozen semen in the equine industry is dependent on simplified breeding strategies. Mares bred with frozen semen are often examined four to six times per day and inseminated immediately before or within 6 h post-ovulation. This is based on the premise that frozen-thawed spermatozoa do not survive as long in the mare's reproductive tract as fresh or cooled semen. Furthermore, this strategy is partly because stallion owners provide only limited doses of frozen semen for each mare. If multiple doses of frozen semen were available each cycle, mares could be examined only once per day with ultrasonography and inseminated once or twice in a cycle. There are two criticisms of this strategy: (1) more semen per cycle would be used and (2) the mare would develop greater post-breeding endometritis if inseminated twice in a cycle with frozen-thawed spermatozoa. Decreasing the dose per insemination or depositing the semen at the tip of the uterine horn may allow one to decrease the number of sperm used to breed mares with frozen semen. Workers in France [1,2] demonstrated that fertility was improved when mares were inseminated more than once in a cycle with frozen semen. Recently, Metcalf [3] reported that insemination of mares twice in a cycle with frozen semen did not impair fertility and did not increase the incidence of post-breeding endometritis.

This report contains results of a controlled study (Colorado State University) and results of a clinical field trial (Italy).

2. Materials and Methods

Controlled Study

The objective of the first experiment was to determine embryo recovery rates of mares inseminated with either 800 x 10^6 total frozen-thawed sperm 6 h post-ovulation or 400 x 10^6 total frozen-thawed sperm at 24 and 40 h after administration of human chorionic gonadotropin (hCG). Forty light-horse mares between 3 and 15 yr of age were synchronized with altrenogest [a] for 10 days, followed by prostaglandin on day 10. On returning to estrus, once a mare had acquired a follicle > 35 mm and uterine edema was observed, hCG [b] (2500 IU) was administered intravenously at 8:00 p.m. Mares were randomly assigned to one of two groups. Group 1 was scanned at 6:00 a.m., 12:00 p.m., and 6:00 p.m. until ovulation was detected, at which time they were inseminated with 800 x 10^6 frozen-thawed spermatozoa. Two mares ovulated between 6:00 p.m. and 6:00 a.m. and were excluded from the data. Mares in group 2 were scanned once daily with ultrasonography and inseminated 24 and 48 h after administration of hCG with 400 x 10^6 frozen-thawed spermatozoa. Frozen semen for this trial was obtained from three stallions. Each stallion was represented equally in both groups, and his semen was used to inseminate 12 - 14 mares. Ejaculates were frozen in a lactose-ethylenediamine tetra-acetic (EDTA) extender at a concentration of 400 x 10^6 sperm/ml in 0.5 ml. Within 5 min of thawing semen, mares were inseminated with the appropriate dose into the body of the uterus.
Between 7 and 9 days after ovulation, embryo flushes were performed on all mares, and embryo recovery was recorded. The objective of the second experiment was to determine if insemination into the uterine horn produced higher pregnancy rates than insemination into the uterine body. Forty mares were randomly assigned to one of two groups: body versus horn insemination. As mares developed a follicle > 35 mm and uterine edema, hCG was administered at 8:00 p.m. Mares that had received hCG more than twice in a breeding season were administered deslorelin acetate [c] at 10:00 p.m. At 24 and 40 h after administration of hCG, or 30 and 46 h after deslorelin acetate administration, mares were inseminated with 200 x 10^6 frozen-thawed spermatozoa into either the uterine body (group 1) or uterine horn (group 2). A dose of 200 x 10^6 frozen-thawed spermatozoa was selected to allow insemination of only one straw and to determine if insemination of a low number of frozen-thawed sperm into the tip of the uterine horn could improve pregnancy rates over insemination into the uterine body. The 0.5-ml frozen-thawed straw was loaded into a universal pipette catheter [d] that was seated in a universal pipette [e]. In group 1, semen was deposited just inside the cervix in the body of the uterus. In group 2, semen was deposited at the tip of the horn ipsilateral to the preovulatory follicle or ovulation. The inseminator palpated the horn and ovary transrectally and guided the pipette to the tip of the horn. At 7 - 9 days post-ovulation, mares were flushed for embryos, and embryo recovery rates were recorded.

**Clinical Field Trial**

Forty-eight Warmblood, cycling mares to be inseminated with commercial frozen semen were used. Mares were < 16 yr of age. Seven Warmblood stallions were selected for their freezability and frozen semen fertility. Only doses containing at least 250 x 10^6 progressively motile spermatozoa and having a minimum of 30% progressive motility were used to inseminate mares. Mare were inseminated according to one of two insemination protocols. Group 1 mares (n = 18) were examined with ultrasound 12 h after hCG injection and then every 4 h until ovulation was detected. A single insemination was then performed within 6 h post-ovulation. Group 2 mares (n = 30) were examined with ultrasound daily and inseminated at 24 and 40 h after hCG injection. The second insemination was always performed, even if ovulation was detected at 40 h post-hCG. Both groups of mares were scanned with ultrasound 24 h after the last insemination to identify post-insemination fluid accumulations. Pregnancies were detected at 14 days post-ovulation and again at days 30 and 50 of gestation.

**3. Results**

**Controlled Study**

In experiment 1, embryo recovery rates were not different when mares were inseminated using timed insemination (24 and 40 h after administration of hCG) versus insemination after ovulation (11 of 20, 55% versus 12 of 20, 60%, respectively). In experiment 2, embryo recovery rates were lower for mares inseminated in the ipsilateral horn versus into the uterine body (4 of 20, 20% versus 10 of 20, 50%, respectively).

**Clinical Field Trial**

Pregnancy rates were not different for mares inseminated once within 6 h post-ovulation (83.3%) versus those inseminated 24 and 40 h after hCG administration (86.6%). These pregnancy rates are quite high for frozen semen and probably reflect the selection of very fertile stallions. Accumulation of uterine fluid post-insemination was not different (P > 0.05) between the two groups of mares. Uterine fluid was observed in 11.7% of the cycles in mares inseminated once versus 9.5% of the time for mares inseminated twice during the cycle. Furthermore, there was no incidence of embryonic loss between 15 and 30 days for either group of mares in this study.

**4. Discussion**

One of the major deterrents to the use of frozen semen is the increased mare management required for proper timing of insemination in relation to ovulation. However, the results of both the controlled study and the clinical study demonstrated that daily examinations with ultrasonography were sufficient when mares were inseminated at a fixed time after hCG administration. Although post-ovulatory insemination resulted in similar fertility, these mares had to be examined three to four times per day to time the insemination within 6 h post-ovulation. Woods et al., [4] have demonstrated that delaying insemination beyond 8 h after ovulation resulted in a depression of fertility with fresh semen. Vidament et al., [1] in a large field trial in France, demonstrated that more than one insemination per cycle with frozen-thawed spermatozoa increased pregnancy rates. The basis as to why timed insemination 24 and 40 h after hCG administration provides acceptable fertility is based on having sperm available in the female reproductive tract covering a period of time from 18 to 52 h after hCG administration. Assuming that a mare ovulates any time during this interval, sperm in one of the two inseminates should be viable and be available for fertilization.

One argument against insemination of mares more than once in each cycle is the increased use of semen. However, the controlled study demonstrated that halving the dose and inseminating mares twice during the cycle resulted in similar fertility compared with mares inseminated with a full dose of semen within 6 h after ovulation. It has been suggested that
insemination twice during the cycle with frozen-thawed semen may result in a higher incidence of post-breeding endometritis. However, the incidence of mares having uterine fluid 24 h after insemination in the controlled study was similar between the two groups. Furthermore, in the clinical trial, the incidence of post-breeding endometritis was also similar between the two groups; less than 10% of the cycles resulted in fluid accumulation 24 h after insemination. Metcalf et al., [3] had previously demonstrated that insemination with frozen-thawed semen twice during the cycle did not increase the incidence of post-breeding endometritis. Possibly, one of the reasons for the low incidence of post-breeding endometritis in this study was selection of mares for the study. In the controlled study, mares were between 3 and 15 yr of age, and in the clinical study, all mares were less than 16 yr of age. It is likely that if older mares are inseminated twice in a cycle with frozen-thawed spermatozoa, the incidence of post-breeding endometritis may be greater.

Once semen is deposited into the body of the uterus, the spermatozoa move relatively quickly to the uterotubal junction or the distal isthmus of the oviduct. Of the millions of sperm placed in the uterus, only a few thousand spermatozoa can be found in the oviduct [5]. There are several studies that demonstrate that pregnancies can be obtained with low numbers of spermatozoa if the sperm are placed onto the uterotubal junction [6-8]. The second controlled study was designed to determine if deep uterine insemination would improve pregnancy rates when a fourth of the normal dose of frozen-thawed spermatozoa was inseminated. Unexpectedly, in this study, pregnancy rates were higher if mares were inseminated into the uterine body versus the uterine horn. It may be that the inexperienced technician did not properly position the catheter onto the uterotubal junction or perhaps the added insult of inseminating the mare twice (24 and 40 h after hCG) may have caused a more severe uterine reaction that decreased fertility.

Recently in our laboratory, Lindsey et al., [9] reported a higher pregnancy rate with videoendoscopic insemination of 5 x 10^7 spermatozoa versus insemination of the same sperm number using deep uterine insemination. Rigby et al., [8] reported similar pregnancy rates for videoendoscopic insemination versus deep uterine, rectally guided insemination. Further studies with larger numbers of mares are needed to truly evaluate the advantage of insemination to the uterotubal junction using a rectally guided approach. The difference may also have been because of chance caused by the low number of mares in these studies. However, recently, a lower pregnancy rate was reported [6] if problem mares were inseminated on the uterotubal junction (UTJ) compared with those inseminated in the uterine body.

Footnotes

[e] Minitube pipette, Minitube of America, Inc., Verona, WI 53593-0187.
[f] Sieme et al., Celle, ND Germany, (Personal Communication) 2003.

References


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