Folliculogenesis, Embryo Parameters, and Post-Transfer Recipient Pregnancy Rate in eFSH-Treated Donor Mares

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Equine follicle-stimulating hormone (eFSH) treatment increases embryo-recovery rate in donor mares. Recipient pregnancy rates per donor cycle were similar between eFSH-treated mares and non-eFSH-treated progesterone- and estradiol-synchronized donor mares. Authors’ address: Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4, Canada; e-mail: tal.raz@usask.ca (Raz). © 2006 AAEP.

1. Introduction

Embryo transfer offers distinct advantages to owners who wish to increase the number of foals born from valuable mares. It is also useful for mares where pregnancy is not desirable, such as in performance mares or in cases where medical issues preclude carrying a foal to term. However, the high cost of equine embryo transfer dictates that only genetically superior animals should be used as donors. Contributing to the expense of embryo transfer are the unique biological features and technical problems that must be overcome in mares. Currently, single-embryo recovery attempts are common in equine-embryo transfer. A 50% embryo-recovery rate per ovulation and a 50–65% pregnancy rate per transferred embryo is expected in commercial embryo-transfer operations.1,2 Economically, there is a need to increase the success rate with embryo transfer.

More efficient and economical embryo transfer relies on the ability to induce multiple ovulations or superovulation in the donor mare, and it also depends on careful and accurate reproductive management of both the donor and recipient mares. Combined treatment with progesterone and estradiol (PE) has been shown to be an effective means of synchronizing estrus and follicle emergence. After PE treatment, human chorionic gonadotropin or deslorelin is usually administered during estrus to align donor- and recipient-mare ovulation. Superovulation potentially increases the efficiency and decreases the cost of embryo transfer by increasing embryo-collection rates.1,3 Superovulation also has been suggested as a critical requirement for other types of assisted reproductive technology in the horse, including oocyte transfer and gamete intrafallopian transfer.1,4 However, the induction of superovulation has been elusive in the mare, and superovulatory treatments are less efficient in mares than in other domestic species.5–8 Equine pituitary extract has been reported to stimulate multiple ovulations in mares but...
was not commercially available. A purified pituitary extract product called equine follicle-stimulating hormone (eFSH) has been investigated and was reported to increase ovulation rate and embryo-recovery rate of donor mares.9–11

There is limited information on the post-transfer recipient pregnancy rate in eFSH-treated cycles compared with control cycles in donor mares. The main objectives of this study were to investigate the effects of eFSH on ovulation rates, embryo recovery, embryo quality, and subsequent pregnancy rates and compare treated mares with control mares.

2. Materials and Methods

Twelve donor mares and thirty-seven recipient mares were used during the physiologic breeding season. Donor mares were treated with PE 17β (150 mg progesterone and 10 mg estradiol, q 24 h, IM) for 8–11 days followed by a PGF2α injection (5 mg, SC or IM) on the last day of PE treatment. Reproductive activity was monitored in two consecutive estrus cycles by daily transrectal palpation and by ultrasonography using a 5-MHz rectal probe to determine the day of ovulation; monitoring continued for 2 days after ovulation. Donor mares with pre- or post-breeding uterine-fluid accumulations were treated daily using saline lavage and antibiotics for ≤3 days post-ovulation or as needed. The first cycle served as a control (control cycle) for the second cycle (eFSH cycle). The eFSH treatment was administered in the second cycle only. Treatments were not randomized.

In the first cycle, mares were administered hCG (2000 IU, IM) after a follicle ≥35 mm was detected. Donor mares were bred 24 h after hCG administration and again every 48 h until ovulation (day 0 = day of first ovulation) using artificial insemination with fresh semen from a stallion of proven fertility. A minimum insemination dose of 100 million morphologically normal and progressively motile spermatozoa extended 1:1 with Kenney extender was used. Eight days after the first ovulation, the donor mares were flushed for embryos.

On the day of embryo flush, mares were administered a PGF2α injection (5 mg, SC or IM), and for the second cycle, eFSH treatment was initiated when a follicle ≥25 mm in diameter was detected. Mares received 12.5 mg eFSH twice daily intramuscularly until one or more follicles ≥35 mm in diameter was present; at that time, hCG (2000 IU, IM) was administered. Mares were inseminated, and embryo-collection attempts were performed just like after the first ovulation.

The AB Technology Vigro Complete Equine Embryo Flush Media (4 l) was used for embryo recovery. Mares were treated with 40 IU oxytocin intramuscularly after 3 l of solution had been recovered. Embryos were identified using a stereomicroscope, enumerated, and scored for quality; as described by McKinnon and Squires,12 poor embryos were given a score of 4, fair a score of 3, good a score of 2, and excellent a score of 1. Embryos were subjectively assessed for age according to their developmental stage (morula/early blastocyst/explanted blastocyst) and size. Vigro Embryo Holding Media was used to rinse and hold the embryos before transfer. Embryos were held at room temperature and transferred within 1 h of recovery. Embryos were aspirated into sterile 0.5 ml straws with 0.25 ml of holding media, loaded into a flexible Universal pipette, and transferred into the uterine body of the recipients using a non-surgical, transcervical approach.

Two to three recipient mares were synchronized for each donor mare. Recipient mares were synchronized using PE or PGF2α to align their ovulations with the donor mares as described previously by Raz et al.11. Recipients that had an ovulation that occurred in a range of 2 days before to 2 days after the donor mare’s ovulation were used. Recipient mares were evaluated for pregnancy status on day 14 post-ovulation using transrectal ultrasonography. Data were evaluated for normality using the Shapiro-Wilk Test and were then analyzed using χ², one-way analysis of variance (ANOVA), or Kruskal-Wallis one-way ANOVA.

3. Results

Twelve donor mares completed the control-cycle treatment, which resulted in 18 ovulations, 6 embryos, and 4 recipient pregnancies. In the eFSH cycle, 12 mares completed the eFSH treatment; however, 2 mares did not ovulate, and therefore, embryo recovery data were available for only 10 mares. Duration of eFSH treatment was 5.3 ± 2.9 days (mean ± SD), range 2–11 days. The results for the eFSH cycle were 28 ovulations, 15 embryos, and 5 recipient pregnancies. Mean number of follicles >30 mm at hCG was significantly higher in the eFSH cycle (mean 3.8 ± 2.0 follicles; range 1–8 follicles) compared with the control cycle (1.6 ± 0.7 follicles, range 1–3 follicles) (p = 0.0003). However, mean number of ovulations per mare was not significantly different between the cycles (Control: mean 1.5 ± 0.5 ovulations, range 1–2 ovulations; eFSH: mean 2.3 ± 1.8 ovulations, range 0–6 ovulations) (p = 0.1429). The ratio of ovulation number to follicles >30 mm was significantly higher in the control cycle (mean 1.0 ± 0.1, range 0.7–1.0) compared with the eFSH cycle (mean 0.7 ± 0.4, range 0–1.0) (p = 0.0249). In the first and second cycles 11 of 12 mares and 10 of 12 mares, respectively, developed post-breeding fluid accumulations and were treated as needed.

Embryo per ovulation rates were not significantly different between the cycles (control: 6/18, 33.3%; eFSH: 15/28, 53.6%; p = 0.2318). Mean embryo number per cycle was significantly higher in the eFSH cycle (mean = 1.3 ± 2.0 embryos per cycle, range = 0–3 embryos per cycle) compared with the control cycle (mean = 0.5 ± 0.7 embryos per cycle, range = 0–2 embryos per cycle, p = 0.0381), and mean embryo number per embryo flush attempt (ex-
cluding the two eFSH mares that failed to ovulate) was significantly higher in the eFSH cycle (mean 1.5 ± 0.9 embryos per flush attempt, range 0–3 embryos per flush attempt) than in the control cycle (mean 0.5 ± 0.7 embryos per flush attempt, range 0–2 embryos per flush attempt) (p = 0.0059). Five pregnancies were established from the eFSH cycles, and four pregnancies were established from the control cycles. Pregnancy rate per donor cycle did not significantly differ between the groups (eFSH control cycles. Pregnancy rate per donor cycle did not significantly higher for embryos recovered from the eFSH cycles. The mean embryo-quality scores in the eFSH cycle were not different compared with the control cycle (eFSH: mean 2.7 ± 0.9, range = 2–4; Control mean 1.8 ± 0.8, range 1–3 grades, p = 0.0620). Mean embryo age at 8 days after the first ovulation was significantly higher for embryos recovered from the control cycle (median; quartiles = 8; 7, 8.5) compared with embryos recovered in the eFSH cycle (median; quartiles = 7; 7, 7.5) (p = 0.0488).

4. Discussion
The treatments were not randomized in this study because of possible carry-over effects of eFSH treatment. PE treatment was chosen for the control cycle, because this estrus synchronization protocol is commonly used in private practice, and it has been shown to provide good estrus and ovulation synchrony in donor and recipient mares. The protocols used in this study likely influenced follicular recruitment. PE treatment resulted in an initial suppression of follicular growth, which was followed by the emergence of a synchronous follicular wave. Duration of PE treatment was chosen to be 8–11 days to stagger the inseminations and to aid in recipient alignment. Early reports on the use of PE did not show differences between 10- and 15-day protocols. In the eFSH cycles, the mares were injected with prostaglandin 8 days after ovulation; however, the eFSH treatment was started based on the attainment of a follicle >25 mm. This meant that the mares started the eFSH treatment in a variable time frame from prostaglandin administration. The rationale for starting eFSH treatment at a follicle size of 25 mm was that treatment is costly and the administration of the hormone should be based on the approximate time of follicular deviation in draft-type mares. The eFSH treatment was administered in this study until the mares had a follicle >35 mm, and then they were given hCG. It is now recommended that treatment with eFSH be discontinued for 30 h before the administration of hCG. This practice is called “coasting” in embryo-transfer protocols. The coasting period has been shown to be beneficial in some bovine embryo-transfer protocols.

In this study, eFSH treatment significantly stimulated follicular development compared with no treatment. Reports of mean ovulation rates in eFSH-treated mares vary from 2.2–3.8 ovulations per cycle. In this study, the mean ovulation rate per mare in eFSH cycles was 2.3 and was within this published range; however, it was not different than control. This was an unexpected finding. The mean ovulation rate in control cycles was 1.5 ovulations per cycle, which was higher than the commonly reported 1.2 ovulations per cycle after hCG administration. The hCG response in this study may be caused by the recruitment of additional follicles, the genetic makeup of the donor mares, the small sample size, or other variables. We also noted that whereas eFSH-treated mares developed more follicles >30 mm, only 70% of those follicles ovulated; however, 100% of the follicles >30 mm in the control cycles ovulated. Failure to ovulate has been reported in a proportion (10–15%) of eFSH-treated mares. In the two eFSH-treated mares in this study that did not ovulate and in some eFSH-treated mares that did ovulate, we observed that follicles continued to grow after the eFSH treatment stopped and/or became static. The cause of the significantly lower ovulation rate per follicle >30 mm in the eFSH cycles has not yet been determined. It is possible that the eFSH treatment may alter the hormonal environment, lower the number of luteinizing hormone receptors, or change the affinity or hormone receptors in large follicles. The effect of eFSH on the hormonal environment and on follicular maturation requires further study.

The eFSH treatment increased donor embryo-recovery rate (eFSH = 1.3 embryos per cycle versus control = 0.5 embryos per cycle) and was within previously published ranges for eFSH-treated mares (1.0–2.6 embryos per cycle). Fifteen embryos were recovered from 12 eFSH cycles, and 6 embryos were recovered from 12 control cycles. Mean embryo age was significantly different between cycles and was lower in eFSH-treated cycles. Because embryo recovery attempts were performed 8 days after the first ovulation was detected and because there were some additional ovulations 24–48 h later in eFSH cycles, we expected to recover <day 8 embryos, which would result in a lower mean embryo age.

Mean embryo-quality score was 1.8 (good to excellent) in the control cycles and 2.7 (fair to good) in the eFSH cycles. The mean embryo-quality scores were not different between the groups, but there was a tendency for lower scores (p = 0.0620) in the eFSH cycles. Lower than anticipated embryo-quality scores have been reported in a previous study in eFSH-treated donor mares, but in that study, there was no control non-eFSH-treated group. It is possible that this is a chance finding, but other possible explanations for the lower embryo-morphology
scores include effects of persistent post-mating inflammation, recent embryo-recovery attempt, or treatment. Embryos with lower morphology grades have impaired embryonic viability. Treatment with FSH for ovarian superstimulation has been shown to impact embryo quality and quantity in other species. A proportion of the embryos from superovulated donor cows are expected to be abnormal; however, high mean embryo-recovery rates make the procedure economical in cattle. In cows, ovarian superstimulation has been proposed to have an effect on oocytes, sperm transport and capacitation, fertilization, oviductal maturation, and embryonic development. These same effects may occur in superstimulated mares. Further studies are required to understand the effect of eFSH on the ovarian, tubal, and uterine environment.

Mean pregnancy per transferred embryo in the control cycles (0.6 ± 0.6) was within the published range. In the eFSH cycles, mean pregnancy per transferred embryo (0.2 ± 0.4) was lower than expected; however, pregnancy per transferred embryo rates were not significantly different between treatments (p = 0.1731). There is limited data on pregnancy rates in recipient mares receiving embryos from eFSH-treated donor mares. We previously reported recipient pregnancy rates per transferred embryo of 27% in prostaglandin eFSH-treated donor mares and 46% in PE eFSH-treated donor mares with an overall per eFSH donor-cycle recipient pregnancy rate of 38%. Hudson et al. reported 65–75% pregnancy rates from vitrified thawed equine embryos selected for good morphology obtained from eFSH-treated mares.

Under our experimental conditions, the recipient pregnancy rates per eFSH-treated donor cycle were not different than control. This was not expected based on the higher follicle numbers at the time of hCG administration and significantly increased embryo-recovery rates in the eFSH-treated mares. The similarity in pregnancy rates may be attributed to the carryover negative effect from the first embryo flush attempt (control cycles) on the results of the second embryo flush attempt (eFSH cycles), the high incidence of post-breeding fluid accumulation, the good response of the control mares to synchronization and hCG, the donor/recipient mare quality factors, the small sample size, or the direct or indirect effect of eFSH on the embryos or reproductive tissues. A future study using a non-treated rest cycle between treatments to eliminate possible negative effects from the first cycle on the second cycle should be performed.

The acceptable post-transfer pregnancy rate obtained using the PE protocol coupled with the reported reliable estrus and ovulation synchrony suggest that this is a good embryo-transfer protocol for use in private practice. More controlled studies are required with larger numbers of eFSH-treated mares to evaluate factors influencing post-transfer recipient pregnancy rates.

Economically, the equine industry needs pharmaceutical agents to help increase the efficiency of embryo transfer where presently the overall success rate in terms of pregnancies per cycle is ~25%. In the development of superovulation protocols, the cost-benefit ratio should be evaluated to determine the effect on reproductive efficiency. Future studies should be directed at fine tuning eFSH treatment protocols to further increase ovulation rates and embryo numbers.

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