

Abstracts

Opening Session

IMPROVED OVULATION AND EMBRYO RECOVERY RATES IN MARES TREATED WITH PORCINE FSH

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High cost and limited availability still prevent the widespread use of equine pituitary extract (eFSH) for the induction of superovulations in mares. Furthermore, it has been associated with overstimulation and disappointing ovulation and embryo recovery rates. Porcine FSH (pFSH) is an affordable product that moderately increased the number of ovulatory follicles in mares. The aims of this study were (a) to determine an effective dose of pFSH required to increase ovulation rate and (b) establish a practical multiple ovulation protocol for improved embryo recovery rates.

Two thoroughbreds and three warmblood mares were each treated with two dose regimens of pFSH during consecutive cycles. Each mare underwent each treatment protocol at least twice. Untreated, spontaneous cycles of the same mares served as controls. On Day 6 post-ovulation each mare was started on twice daily IM injections of either 10 or 25 mg of pFSH (Folltropin-V; Bioniche, Bellville, ON, CA) and was treated until follicles reached an average diameter of 35 mm (including all follicles >25 mm). At this time, mares received 1500 IU of hCG IV and were inseminated with one billion progressively motile

spermatozoa from a fertile stallion. Treatment with pFSH was discontinued and daily ultrasonographic examinations were continued until all previously recorded follicles had either ovulated or become atretic. Embryo recovery flushes were performed on Day 7 post-last ovulation and each mare was given a single IM injection of PGF2 α (5 mg Dinoprost; Lutalyse, Upjohn, Kalamazoo, MI, USA).

The mean number of ovulations and recovered embryos per cycle were calculated for each mare and treatment regimen. *T*-tests for paired variables were used to compare numbers of ovulations and recovered embryos between treatment regimens. The mean duration of pFSH treatment was 6.72 (± 1.32) days per cycle. Mares had more ovulations during treated cycles than during control cycles ($P < 0.005$). Dose of pFSH had no effect on the number of ovulations per cycle, but fewer embryos were recovered from mares that received the lower dose of pFSH ($P < 0.05$).

Table 1

Mean numbers of ovulations and recovered embryos ($\pm$ S.D.) in five mares during spontaneous estrous cycles and after treatment with either 10 or 25 mg pFSH

Group	No. of cycles	Ovulations/cycle	Embryos/cycle
Control	33	1.12 (0.12) a	Not assessed
25 mg pFSH	15	2.28 (0.30) b	1.32 (0.61) c
10 mg pFSH	10	2.2 (0.57) b	0.7 (0.57) d

Values with different letters (a,b) are different ($P < 0.005$). Values with different (c,d) are different ($P < 0.05$).

We conclude that porcine FSH offers an affordable, readily available means of significantly increasing the ovulation rate and embryo yield in embryo donor mares. The lower embryo recovery rate after treatment of

mares with the lower dose of pFSH requires further investigation.

Keywords: Mare; Multiple ovulations; Porcine FSH; Embryo recovery

EFFECT OF POLYVINYL ALCOHOL (PVA) IN VITRIFICATION OF EQUINE EMBRYOS

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It has been demonstrated that equine embryos can be vitrified, warmed, and transferred, achieving comparable pregnancy rates to fresh or cooled embryos. At the present time, bio-security issues limit practitioners and owners from importing or exporting embryos vitrified in a fetal calf serum media. Therefore, a demand exists for an alternative solution which would allow for importation and exportation of vitrified embryos. The goal of this pilot study was to compare pregnancy rates between embryos vitrified in fetal calf serum and those vitrified in polyvinyl alcohol.

Light horse mares were chosen based on the quality of their reproductive tract. Mares were bred to a stallion with proven fertility. Ovulation was induced in 13 mares by administration of 2500 IU of human chorionic gonadotropin (hCG) (Chorulon[®], Intervet, Holland) or a GnRH analog once a preovulatory follicle >35 mm, associated with good uterine edema, was detected. A non-surgical embryo flush procedure, using a commercial media (EmCare[®], ICP bio, New Zealand), was performed on day 6.5 post-ovulation. The embryos were evaluated using the IETS embryo grading method. Late morula or early blastocyst stage embryos with a grade of 1–2 and <300 µm in diameter were randomly assigned to one of two treatment groups. Embryos in both groups were washed four times in a commercial holding medium (EmCare[®], ICP bio, New Zealand). Group 1 ($n = 7$) embryos were vitrified in media containing fetal calf serum. Embryos in Group 2 ($n = 6$) were vitrified in media containing polyvinyl alcohol. Vitrified embryos were warmed after randomly selecting them from a liquid nitrogen tank. A direct non-surgical transfer, into an available recipient that ovulated approximately 48 h after the donor mare, was performed once the fluid inside the straw was allowed to equilibrate.

A 71% (five of seven) pregnancy rate was established in the group containing fetal calf serum. An 83% (five of six) pregnancy rate was established in the group containing polyvinyl alcohol. Pregnancies were visualized, per rectum, using ultrasonography beginning 5

days post-transfer. Pregnancies were evaluated until a heart beat was detected. Results of this pilot study may indicate late morula and early blastocyst equine embryos (<300 µm) can be collected and vitrified in media containing polyvinyl alcohol without losing embryo viability. This optional media has the added benefit of qualifying for importation and/or exportation. Future projects, using larger numbers of embryos, are needed to validate this present pilot study.

Keywords: Equine; Embryo transfer; Fetal calf serum; Polyvinyl alcohol; Vitrification

FERTILITY AND ULTRASTRUCTURAL ANALYSIS OF FROZEN–THAWED DOG SPERMATOZOA WITH DIFFERENT CONCENTRATIONS OF EQUEX STM PASTE

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Two experiments (EXP) were designed to investigate the effect of different concentrations of Equex STM paste (EQ) on the ultrastructural changes and fertility of frozen–thawed dog spermatozoa extended with or without EQ. EXP 1 studied the ultrastructure of frozen–thawed dog spermatozoa diluted in Tris egg yolk (TEY) extender containing 0, 1.5 (E15) and 2.5% of EQ (E25) and 5.0% glycerol. Semen was collected from four fertile German Shepard dogs. The sperm rich fraction was divided into four samples. One sample was prepared immediately after collection for transmission electron microscopy (TEM). The three remaining samples were diluted in each of the different extenders in a two step dilution before equilibration to a final sperm concentration of $100 \times 10^6 \text{ ml}^{-1}$, were packed in 0.5 ml straws and frozen in liquid nitrogen. For TEM, the samples were examined in a JEM 1200 EX II 60–80 kV TEM. In each specimen, 100 spermatozoa were evaluated. Sperm characteristics on fresh semen (motility [MOT, % motile], velocity [VEL, 0–5], vital stain [VS, % alive], plasma membrane integrity [HOS, % intact], acrosome morphology [ACR, % intact]), post-thawing index ([frozen–thawed sperm characteristic/fresh sperm characteristic] $\times 100$) and % intact spermatozoa (TEM) were analyzed by analysis of variance with the GLM procedure. EXP 2 studied whether the addition of 1.5% of EQ to frozen–thawed dog semen improved the fertility after uterine insemination. Fourteenth mixed breed bitches aged between 1 and 4 years old, weighing between 5 and 25 kg were divided into