

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

two groups ($n = 7$) and were inseminated with semen frozen in TEY extender containing 0% (CON) or 1.5% of EQ (TRT). One hundred million progressively motile spermatozoa were used for each uterine insemination using Norwegian catheter 2–5 days after ovulation. The pregnancy rate and number of fetuses were analyzed by using the CATMODE and GLM procedure. In EXP 1, the post-thawing indices were higher in semen diluted with TEY + EQ compared to semen diluted with TEY extender alone (MOT 0.8 versus 0.4 ± 0.02 ; VEL 0.9 versus 0.8 ± 0.03 ; VS 0.7 versus 0.6 ± 0.02 ; HOS 0.8 versus 0.6 ± 0.02 ; ACR 0.8 versus 0.6 ± 0.02 ; $P < 0.01$). Furthermore, there were no significant differences in all sperm parameter between E15 and E25 with the exception of MOT (0.73 versus 0.79 ± 0.02 ; $P < 0.01$). Semen diluted with TEY extender alone had a lower percent of intact spermatozoa at TEM compared to semen diluted with TEY + EQ (0.5 versus 0.7 ± 0.05 ; $P < 0.04$). Semen diluted with TEY + EQ 2.5% had fewer percent of intact spermatozoa at TEM compared to semen diluted with TEY + EQ 1.5% (0.6 versus 0.8 ± 0.05 ; $P < 0.03$). In EXP 2, the pregnancy rate and the litter size were numerically higher but not significantly different in the TRT group compared to the CON group (71% [5/7] versus 43% [3/7], $P < 0.29$; 2.1 versus 1.1 ± 0.6 , $P < 0.29$; respectively). In conclusion, the addition of 1.5% or 2.5% of EQ to the TEY extender improved post-thawing indices of dog spermatozoa. Although in most sperm parameters studied there were no differences between 1.5% and 2.5% EQ, the ultra structural study of frozen–thawed sperm with TEM showed a decrease in the percentage of intact spermatozoa with TEY + 2.5% EQ compared to 1.5%. There was a trend in improving pregnancy rates with the use of TEY + 1.5% EQ compared with TEY.

Keywords: Semen; Canine; Frozen; Ultrastructure; Spermatozoa

EFFECT OF THE GnRH ANTAGONIST, ACYLINE, ON CANINE TESTICULAR PARAMETERS

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GnRH antagonists competitively block GnRH receptors sites at the pituitary gland, exerting an immediate inhibitory effect on the gonadal axis. Acyline, is a third generation GnRH antagonist, that has been found to be safer and more effective at suppression and maintenance of suppression of gonadotrophins than

earlier compounds. The aim of this study was to test the effect of acyline on some testicular parameters in male dogs. Secondary, acyline safety was also assessed.

Six reproductively normal, 2–6 years old, mixed and pure bred (Beagle, German shepherd, Bull Mastiff) dogs were followed up weekly for three periods (PRE, POST1 and POST2) of 4 weeks each. At the end of the first period, they were administered acyline (NICHHD, NIH, USA) $330 \mu\text{g/kg}$ SC. Follow up included general physical examination, scrotal diameter, testicular consistency, libido and erection at semen collection and semen volume, concentration, motility and morphology. Before treatment and then on days 15, 30 and 60 after treatment blood samples were taken for hemogram and biochemical serum determinations. Quantitative data were analyzed by least-squares ANOVA using the General Linear Models Procedure (PROC GLM, SAS[®]), and categorical data analysis by PROC CATMOD, SAS[®]. The mathematical model included the main effect of period. Orthogonal contrasts were also used to test differences among periods. The level of significance set at $P 0.05$.

Individual responses to treatment varied markedly among animals. Testicular consistency and scrotal diameter slightly, but not significantly, decreased in POST1 in all the animals. Libido and erection was unaltered throughout the study, with the exception of three dogs in which they were absent during 2 weeks of POST1. Semen volume, total concentration and motility were significantly lower in POST than in PRE ($P < 0.05$). There was a clear impairment of these parameters, reaching nadir values ($\leq 0.2 \text{ cm}^3$, 0.5×10^6 and 30%, respectively) around week 2 of POST1, and then slowly improving to the end of the study, when three animals regained PRE values. Spermatozoa morphological abnormalities significantly increased during POST2 ($P < 0.05$), they were mainly represented by ≥ 40 proximal droplets and head abnormalities in some dogs. No animal presented hematologic, serum biochemical, local nor systemic side effects related with the treatments throughout the study periods.

These results probably reflect the 2-week gonadotrophin and testosterone suppression to castrate levels that have been described for this drug in other species. It is concluded that the GnRH antagonist, acyline severely and reversibly deteriorated semen quality without side effects in these treated dogs. If this deterioration is enough to provoke infertility remains to be determined. Further, pharmacokinetic, endocrine and clinical studies are still necessary before GnRH antagonists could