

were surgically transferred into recipients' oviducts 2–3 days after detection of ovulation. In Experiment 1, cleavage rates of 1-cell embryos were similar ($p > 0.05$) for treatments and control. However, blastocyst rates were superior for the EG group compared to EG-DMSO ($p < 0.05$). The blastocyst rate of 8-cell embryos was higher ($p < 0.05$) for EG than the EG-DMSO group. In Experiment 2, the pregnancy rate on day 17 after transfer was 63% (five of eight) for equine embryos after ICSI, vitrification and warming.

Keywords: Equine; Embryo; Cryopreservation; Vitrification; ICSI

Table 1

Cleavage and blastocyst rates of bovine embryos vitrified at 1-cell or 8-cell stage

Vitrification method	Embryo stage				
	One cell (% per oocyte)			Eight cell (% per 8-cell embryo)	
	N	Cleavage	Blastocyst	N	Blastocyst
Control	81	86.4 a	25.9 a	81	56.8 c
EG	72	87.5 a	23.6 a	75	42.7 b
EG-DMSO	68	79.4 a	11.8 b	75	14.7 a

Values within columns with different letters (a,b,c) differ ($P < 0.05$).

THE REPEATABILITY OF A CANINE HERPESVIRUS-1 (CHV-1) PCR ASSAY AT ONE COMMERCIAL LABORATORY

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Polymerase chain reaction (PCR) assays for detection of canine herpesvirus-1 (CHV-1) are frequently performed in prebreeding examinations as well as to aid in the diagnosis of causes for pregnancy loss, infertility and neonatal disease. Several laboratories in North America offer PCR assays but virtually nothing is known about the sensitivity, specificity and overall performance of these DNA tests.

We examined the repeatability of a CHV-1 PCR assay offered by a commercial laboratory in Ontario, Canada. The objective of this study was to assess the

agreement of the results of duplicate samples collected from the same animal at the same time.

Convenience sampling of healthy dogs ($n = 30$) of various breeds, ages and fertility histories was performed between July and December 2005. Coded duplicate samples from each dog included EDTA blood, nasal swabs and vaginovestibular swabs (bitches) or preputial swabs (dogs). Laboratory personnel were blinded to the dog identification.

DNA was extracted using a modified DNeasy Tissue Kit (Qiagen Inc., Mississauga ON) protocol. A 267 bp nucleotide sequence of the canine herpesvirus-1 (CHV-1) thymidine kinase (TK) gene was specifically amplified from isolated DNA using a single PCR.

To assess the agreement between duplicate samples, kappa statistics were calculated for nasal swabs (kappa = -0.08), EDTA blood (kappa = 0.19), vestibular swabs (kappa = 0.2) and preputial swabs (kappa = -0.2). Overall agreement among different sampling sites from the same dog was poor (16%). In this convenience sample, which included 15 prepubertal animals, 93.3% of the animals were positive from at least one sampling site.

The PCR assay in this laboratory lacked diagnostic precision. Duplicate samples sent for CHV-1 PCR testing resulted in poor overall agreement. The poor agreement is of concern for the practitioner as well as researcher. Dog breeders need advice on the appropriate collection of samples and interpretation of tests before reasonable control measures can be implemented. Careful validation of the PCR assay, positive and negative control samples verified by virus culture, serology and histology and quality control measures in the laboratory might identify and prevent problems associated with the accuracy and precision of the PCR test for CHV-1.

Keywords: Canine; Canine herpesvirus-1; PCR assay

EFFECTS OF DIFFERENT DOSES OF PGF_{2α} ON THE EQUINE ESTROUS CYCLE

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The natural prostaglandin F-2alpha (PGF_{2α}), dinoprost tromethamine (Lutalyse[®]), is currently the only PGF_{2α} approved by the Food and Drug Administration agency (FDA) for use in horses. It is used at manufacturer

recommended doses of 1 mg/100 lb body weight, or more commonly, at doses of 10 mg/mare. The objective of the present study was to evaluate the effects that single undiluted low doses of the commercially available PGF_{2α} on luteal function and characteristics of the induced estrus and diestrus. We hypothesized that PGF_{2α}-induced estrus would be characterized by ovulation rates and luteal function similar to those associated with control estrous cycles.

Eleven horse mares provided 32 estrous cycles that were randomly assigned to the following treatments: T₁ ($n = 8$ cycles), 2 mL 0.9% saline solution; T₂ ($n = 6$), 10 mg (2 mL) dinoprost tromethamine (DT); T₃ ($n = 11$), 2.5 mg (0.5 mL) DT; T₄ ($n = 7$), 1.25 mg (0.25 mL) DT. Treatments were administered as single doses intramuscularly during mid-diestrus. Ovulations and echotexture of the uterus were determined by palpation per rectum and transrectal ultrasonography. Blood samples were taken at intervals between treatment and subsequent induced estrus, and the plasma samples were stored at -20°C until assayed for progesterone using RIA techniques. The number of days taken to achieve complete luteolysis (defined as a concentration of plasma progesterone being <1.0 ng/ml) was analyzed by one-way ANOVA on ranks and differences between the control group and PGF_{2α} treatment levels were subsequently compared using the Dunn's method (SigmaStat for Windows version 2.03, SPSS Inc., Chicago, IL). Same methods were used to analyze the time taken from ovulation until detection of concentrations of plasma progesterone >3 ng/ml, as an indication of luteal function after ovulation.

All treatments with PGF_{2α} successfully induced luteolysis, as evidenced by analyses of concentrations of plasma progesterone; all mares underwent an estrus period following luteolysis. The number of days (mean $\pm$ S.E.M.) elapsed from treatment to complete luteolysis (concentration of plasma progesterone <1.0 ng/mL) was 4.7 ± 0.2 , 1.6 ± 0.2 , 1.8 ± 0.2 and 2.1 ± 0.3 for treatments T₁, T₂, T₃, and T₄, respectively ($P < 0.05$). Ovulation was detected in every estrus occurring after treatments. Concentrations of plasma progesterone were ≥ 3.0 ng/mL in 29 out of 32 cycles 2 days after detection of ovulation and >5.0 ng/mL in all cycles 3–5 days after ovulation ($P > 0.10$).

Recently, a dose as low as 0.5 mg of PGF_{2α} per mare administered as two injections given 24 h apart have been successfully used to induce luteolysis in horse mares [Irvine, et al. *Equine Vet J* 2002;34:191–4]. In our study, luteolysis was induced with doses as low as 1.25 mg per mare given as a single undiluted injection of Lutalyse®. All PGF_{2α}-induced estrus

in the present study were characterized by normal ovulation and formation of a functional corpus luteum.

Keywords: Horse; Prostaglandin; Estrus cycle; Luteal function

SUPEROVULATION OF MARES: EFFICACY OF A DECREASED eFSH DOSE AND COMPARISON OF OVULATION INDUCING AGENTS

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The use of superovulation in the equine embryo transfer industry is increasing as many breed registries now allow multiple foals to be registered out of the same mare in a single year. The goals of this experiment were to: (1) determine the efficacy of a decreased dose of eFSH in stimulating multiple ovulations and (2) compare the efficacy of hCG and deslorelin in inducing ovulation in eFSH treated mares. Thirty-three normal mares were used for a total of 38 cycles in this study. Mares were examined via transrectal ultrasonography beginning in early April. When a dominant follicle was detected, mares were examined daily to determine the day of ovulation. Once ovulation was confirmed, mares were randomly assigned to one of four treatment groups. Mares in Groups 1 and 2 ($n = 10$ cycles per group) were administered 12.5 mg of eFSH (Bioniche, Inc., Athens, GA) twice daily beginning 5–7 days after ovulation when the majority of follicles were 20–25 mm in diameter. On the second day of treatment, mares were administered 250 μg of cloprostenol intramuscularly. When $\geq 50\%$ of the cohort of developing follicles was ≥ 35 mm, eFSH treatment was discontinued and mares were “coasted” for 36 h and then given either 2500 IU hCG (Group 1) intravenously or 1.5 mg of compounded deslorelin (BET Pharm, LLC, Lexington, KY) intramuscularly (Group 2). Mares in Groups 3 and 4 ($n = 9$ cycles per group) were administered 6.25 mg eFSH twice daily, administered 250 μg of cloprostenol on the second day of treatment, and subsequently given either hCG (Group 3) or deslorelin (Group 4) as described above. All mares were inseminated with one billion progressively motile spermatozoa on the day of hCG or deslorelin administration. A cooled dose of 1 billion progressively motile spermatozoa was inseminated the following day. Mares were subsequently examined daily to detect ovulation. Embryo recovery was attempted 8 days post-ovulation. After the embryo flush, prostaglandins