simulated cycle on the induction of CEH/P using lower numbers of *E. coli* than used previously (1). Ovariectomised greyhound bitches ($n = 14$) were treated with estradiol benzoate and megestrol acetate to induce the simulated estrus and diestrus stages of the reproductive cycle. The bitches were allocated amongst two experiments (Experiment I ($n = 8$); Experiment II ($n = 6$)). *E. coli* strains isolated from a clinical case of pyometra (P3) and from the feces of a clinically healthy dog (F8) were used to inoculate dogs in this study. The P3 strain had five uropathogenic virulence factors (*pap*, *sfa*, *hlyA*, *cnf1* and *fim*) as determined by the polymerase chain reaction. These virulence factors were absent in the F8 strain of *E. coli*. The inocula contained 68–100 colony forming units per mL. One mL of P3 ($n = 5$) or F8 ($n = 3$) inoculum was introduced directly into the uterus on day 10 (d 10) of simulated diestrus (Experiment I). One milliliter of P3 ($n = 3$) or F8 ($n = 3$) inoculum was introduced directly into the uterus on simulated estrus day 4 (d 4) (Experiment II). The bitches were observed daily for general health, and patterns of food and water intake. After inoculation, blood samples and vaginal smears were collected every 2 days and ultrasound examination of the reproductive tract was performed every 3 days. Necropsies were performed 4 days (Experiment II ($n = 2$)) and 14 days (Experiment I ($n = 8$); Experiment II ($n = 4$)) after the inoculation. CEH/P was induced in four of five bitches inoculated with P3 and two of three bitches inoculated with F8 during simulated diestrus. Although the disease produced by P3 *E. coli* showed early and severe clinical signs of infection, the disease was indistinguishable from the F8 infected uteri in histopathological studies. CEH/P did not develop in bitches inoculated with either of the strains of *E. coli* during simulated estrus. The results demonstrated that, in this model, the stage of simulated estrous cycle and not the presence of UVFs was the important determinant for the induction of CEH/P.

Keywords: Dog; *Escherichia coli*; Cystic endometrial hyperplasia; Pyometra; Uropathogenic virulence factors

USE OF BOVINE EMBRYOS TO ESTABLISH METHODS FOR VITRIFICATION OF EARLY EQUINE EMBRYOS

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Assisted reproduction has been fundamental for conservation of valuable equine genetics. The objective of this study was to develop an efficient cryopreservation protocol for early equine embryos (two to eight cells) after ICSI. Bovine embryos were used as a model to investigate procedures for equine embryos. In Experiment 1, bovine embryos were produced by IVF in four replicates by standard procedures using semen from two bulls. The experimental design used two embryological stages and two vitrification methods. Embryos were vitrified as 1-cell embryos (potential zygotes 20 h post-IVF, $n = 221$) and 8-cell embryos (2.5 days after IVF, $n = 231$) using two open-pulled straw (OPS) vitrification techniques. In Method 1 (EG-DMSO) [Mol Reprod Dev 51:53–8], embryos were exposed to 7.5% EG and 7.5% DMSO in HCDM1 for 3 min. Embryos were then transferred into a 20-μl drop of 16.5% EG, 16.5% DMSO and 0.5 M galactose in HCDM1 for 30 s, loaded in an OPS with 1 μl of medium by capillary action, and plunged into liquid nitrogen. In Method 2 (EG) [Fertil Steril 77:422–3], embryos were exposed to 10% EG in HCDM1 for 5 min, moved into a 20-μl drop of 40% EG and 0.6 M galactose in HCDM1 for 30 s, and loaded and plunged as in Method 1. For Methods 1 and 2, removal of cryoprotectants after warming took place in three steps: 1, 0.5, and 0.25 M galactose in HCDM1 for 3 min each at 38 °C. One-cell embryos were cultured in CDM-1 for 2.5 days and in CDM-2 for 4.5 days. Eight-cell embryos were cultured in CDM-2 for 4.5 days after warming. Cleavage rates were evaluated for 1-cell embryos, and blastocyst rates were determined for 1- and 8-cell embryos. Data (Table 1) were analyzed by ANOVA with replicates in the model, and means were compared using Fisher's protected LSD. In Experiment 2, equine oocytes were obtained from preovulatory follicles by ultrasound-guided, transvaginal aspirations. Sperm were injected into oocytes, which then were cultured in DMEM/F12. Two- to eight-cell embryos ($n = 8$) were vitrified and warmed as described above (Method 2 in Experiment 1) but with H-DMEM/F12 as base media. Warmed embryos ($n = 8$)

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 $PGF_{2\alpha}$ ON THE EQUINE ESTROUS CYCLE

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