

CONCENTRATION OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I), LEPTIN, GROWTH HORMONE (GH), ESTRADIOL AND PROGESTERONE (P₄) IN FOLLICULAR CYSTS OF LACTATING DAIRY CATTLE

H. Ortegon¹, C. Tracey¹, J.S. Walton², J. Petrik³, W.H. Johnson¹

¹Department of Population Medicine, University of Guelph, Ontario, Canada N1G 2W1; ²Department of Animal Sciences, University of Guelph, Ontario, Canada N1G 2W1; ³Department of Biomedical Sciences, University of Guelph, Ontario, Canada N1G 2W1

It is believed that nutritional, physiological and pathological factors influence formation of follicular cysts in post-partum dairy cows. However, it is suspected that local factors within the follicular cyst environment have also a contribution. The role of GH, IGF-I in follicular cell function and induction of estradiol and LH receptors, respectively, as well as P₄ is reported for normal cows, but the peripheral or local role of these substances in follicular cysts remains unclear. Leptin appears to be the link between nutrition and reproduction, but is not documented in cows with follicular cysts (FC). The aims of the present study were to identify circulating and follicular protein concentrations of GH, IGF-I, estradiol, P₄ and leptin in a group of cows with follicular cysts (cases), compared to a group of normal cows (CTL). Twenty-eight postpartum (35–60 DIM) lactating Holstein cows were identified as having naturally occurring follicular cysts and were compared to equal number of controls under the same conditions. Ultrasound, palpation and P₄ concentrations were used to select cows with large follicles (>25 mm) persisting more than 7 days in absence of a CL, with low (<1.0 ng/mL) P₄ concentrations. Ultrasound-guided transvaginal follicular aspiration (Aloka SSD-500 with a ASU-66 curvilinear array) was performed to collect follicular fluid from cases and CTL. Blood samples were collected at aspiration for hormonal determination by radioimmunoassay. Data was analyzed using the SAS program (SAS Institute, Cary, NC, USA) for differences of least squares means and correlation analysis. Concentration of IGF-I was higher ($P = 0.001$) in FC as compared to FF of CTL (mean $\pm$ S.E.M.) (106.69 ± 6.34 ng/mL versus 79.74 ± 6.05 ng/mL, respectively); GH concentration was similar in FC and FF of CTL cows (1.76 ± 0.29 ng/mL versus 1.23 ± 0.20 ng/mL, respectively). Estradiol was lower ($P = 0.01$) in FC as compared to FF of CTL (326.96 ± 107.03 ng/mL ver-

sus 557.35 ± 85.47 ng/mL); while leptin FF concentration was similar between groups (FC versus CTL) (6.96 ± 0.7 ng/mL versus 6.72 ± 0.6 ng/mL, respectively). However, serum leptin was higher ($P = 0.0001$) in cases than CTL. Intrafollicular P₄ levels were higher ($P = 0.001$) in FC compared to CTL (5646 ± 2987.7 ng/mL versus 384.28 ± 81.9 ng/mL, respectively). However, when the volume of the cyst was controlled as a confounder, only a trend ($P = 0.19$) was determined. The significant differences between SER and FF compartments suggest independence between both. Higher follicular IGF-I concentration indicates active FC IGF-I production, which did not result ($r = 0.06$) in higher intrafollicular estradiol production, but was correlated ($r = 0.53$; $P = 0.05$) with P₄. This indicates an influence of IGF-I in production of P₄ in FC. Lower estradiol concentration may not be enough to induce and LH surge an ovulation in cystic cows playing a critical role in development of FC. Leptin and GH appear not to be involved in follicular cyst formation, as per the similar levels compared to normal cows and the lack of correlation with other follicular substances. It is possible that other intrafollicular factors, such as P₄, which is present in high amount in FF of cystic cows, may be involved in lowering estradiol production by affecting specific steroidogenic enzymes and proteases necessary for estradiol synthesis, providing the first step in follicular cyst formation.

Keywords: Cows; Ovulatory follicles; Serum; Follicular fluid; Follicular cysts

THE INFLUENCE OF STAGE OF SIMULATED ESTROUS CYCLE AND THE PRESENCE OF UROPATHOGENIC VIRULENCE FACTORS ON THE OCCURRENCE OF *E. COLI* INDUCED CYSTIC ENDOMETRIAL HYPERPLASIA/PYOMETRA COMPLEX IN THE BITCH

N. Arora, G.F. Browning, P.J. Wright

Department of Veterinary Science, The University of Melbourne, 250 Princes Hwy, Werribee 3030, Vic., Australia

Cystic endometrial hyperplasia/pyometra complex (CEH/P) can be induced in ovariectomised bitches during simulated diestrus by intra-uterine inoculation of *Escherichia coli* with five uropathogenic virulence factors (UVFs; Arora, et al. A model for cystic endometrial hyperplasia/pyometra in the bitch Theriogenology, in press). The aim of this study was to determine the influence of the presence of UVFs and the stage of

simulated cycle on the induction of CEH/P using lower numbers of *E. coli* than used previously (1). Ovariectomized 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

L.F. Campos-Chillon, T.K. Suh, G.E. Seidel Jr., E.M. Carnevale

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523, USA

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- μ L drop of 16.5% EG, 16.5% DMSO and 0.5 M galactose in HCDM1 for 30 s, loaded in an OPS with 1 μ L 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- μ L 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 ($n = 8$)