before (time 0), 24, and 48 hours after cooling. In the second experiment, 2 estrous cycles of 15 mares were used for fertility assessment. Mares were examined every other day by transrectal ultrasonography (SonoScape A6®, China). Once a preovulatory follicle was detected (i.e. ≥ 35 mm in the presence of endometrial edema > 1, 0 absent and 3 max), ovulation was induced with 250 µg of histrelin acetate. At induction, semen from 1 jack was collected (n = 28), extended in either SKM or SKM-CLC, and cooled for 24 hours. Mares were randomly and equally assigned in a crossover for breeding with either extender 24 hours after induction of ovulation. Thereafter, mares were examined daily to detect intrauterine fluid accumulation and ovulation. Mares received oxytocin (20 units) to prevent intrauterine fluid accumulation. Pregnancy diagnosis was carried out on day 15 day after ovulation and mares received dinoprost (5 mg) intramuscularly to induce estrus. Data were analyzed with GraphPad Pris 8.0.1. (GraphPad, San Diego, CA). Semen parameters were analyzed with a Mixed model and Tukey's as posthoc. Pregnancy diagnosis was assessed with Fisher's Exact test. Significance was set at $p \leq 0.05$. There were no differences ($p > 0.05$) in TM, PM, RAP, PMI, and MMP for semen extended in either extender at time 0. There was a reduction in TM, PM, RAP, PMI, and MMP over time across groups; however, semen extended with SKM-CLC had superior ($p < 0.05$) TM, PM, RAP, PMI, and MMP than semen extended in SKM at 24 and 48 hours postcooling. Mares bred with semen extended in SKM had lower ($p < 0.05$) conception rate (13%, 2/15 cycles) than mares bred with SKM-CLC (47%, 7/15 cycles). Incorporating CLC to SKM extender improved semen parameters and fertility of cooled donkey semen

**Keywords:** Donkey, semen cryopreservation, extenders, chilling, cholesterol

## Luteinizing hormone receptor activation stimulates endothelial adhesion of neoplastic canine T-lymphocytes

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Previous research from our laboratory has demonstrated that luteinizing hormone receptors (LHR) are expressed in neoplastic lymphocytes in canine lymph nodes and that activation of the LHR with human chorionic gonadotropin (hCG) increases LHR gene expression and cell proliferation in isolated neoplastic T-lymphocytes. The objective of the current study was to determine if hCG activation of LHR in neoplastic T-lymphocytes increase their adhesion to an endothelial cell monolayer. The hypothesis was that increasing hCG concentration induces a dose-dependent increase in neoplastic T-lymphocyte adhesion. Canine aortic endothelial cells (#Cn304-05, Cell Applications, Inc.) were cultured to form a monolayer. Endothelial cells were activated with tumor necrosis factor-alpha for 12 hours. Immortalized T-cell lines isolated from 3 dogs (CLC, EMA, CLK) with multi-centric lymphoma were cultured for 72 hours with increasing concentrations of hCG (from 4 - 4,000 IU/ml). Neoplastic T-lymphocytes were then fluorescently labeled (CytoSelect LeukoTracker, Cell Biolabs, Inc.) and added to the endothelial monolayer. After a 2-hour incubation, non-adherent cells were removed by washing. Images of adherent cells were digitally captured (#QIC-F-M-12-C, QImaging) at 400 x magnification using fluorescent microscopy (#DM4000B, Leica Microsystems). Adherent cells were then quantified on a fluorescence plate reader (Synergy 2, Biotek) using 50% gain. Four replicates of each T-cell line were used for each assay and the assays were repeated 3 times. Results (mean ± SEM) were expressed as a fold of baseline and compared between different hCG concentrations using a one-way analysis of variance (GraphPad Prism). Significance was defined as $p < 0.05$. Activation of LHR in neoplastic lymphocytes increased cell adhesion in a dose-dependent manner in all 3 cell lines (CLC: $p = 0.030$; EMA: $p = 0.016$; CLK: $p = 0.004$). Increases in hCG concentrations stimulated more neoplastic T-lymphocyte adhesion (Figure). This is the first study to demonstrate that activation of LHR in neoplastic canine lymphocytes increases endothelial cell adhesion. These results could explain why gonadectomized dogs with elevated circulating LH concentrations develop lymphoma at higher rates than intact dogs.

**Keywords:** Cancer, dog, human chorionic gonadotropin, lymphoma

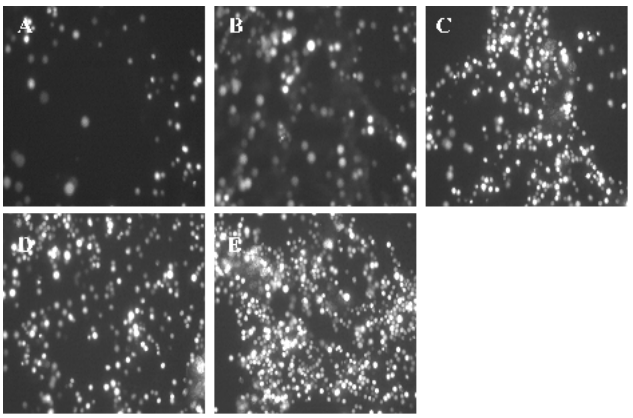


**Figure.** Neoplastic T-lymphocyte adhesion A: hCG 0 IU/ml; B: hCG 4 IU/ml; C: hCG 40 IU/ml; D: hCG 400 IU/ml; E: hCG 4000 IU/ml.