

CANINE OVARIAN TUMOR CELL LINES: A MODEL TO STUDY PROGESTERONE AND PROSTAGLANDIN BIOSYNTHESIS IN THE CANINE OVARY

Angelika E. Stock ^{1,2}, Lucie Paradis ³ and Jean Sirois ¹

Centre de Recherche en Reproduction Animale, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe¹, Hôpital Vétérinaire Rive-Sud, Brossard ² and Clinique Vétérinaire Lucie Paradis, Sillery ³, Province of Québec, Canada

Compared to other species, there is little information about the regulation of steroid and prostaglandin production in the canine ovary. Our goal was to establish an in vitro system to study ovarian steroidogenesis and prostaglandin production in the bitch. When a 4-year old Golden Retriever bitch was presented to our clinic with the history of good general health, but infertility and a prolonged estrous period, explorative laparotomy revealed an abnormally large ovary (0.7 x 1 x 2 inches) on the left side. Ovariohysterectomy was performed. Macroscopically, the enlarged ovary was of blue color on the surface and showed a variety of solid and hemorrhagic-necrotic areas when cut in half. Based on the histological examination a sex-cord stromal tumor of benign nature was diagnosed. Due to the cornification of the vaginal epithelium typical for a bitch in estrus and a progesterone level of 1.5 ng/ml, the tumor had been hormonally active, which prompted us to culture cells from this tumor in vitro. The tissue was minced and digested in medium containing collagenase, hyaluronidase and bovine serum albumin. Cells were washed several times and then plated in 24 well culture plates (100 000 cells/well) in DMEM-Ham F12 Medium (1:1) supplemented with insulin, hydrocortisone, transferrin, non-essential amino acids and 10 % fetal bovine serum. Half of the plated cells were cultured in the presence of 17-beta-estradiol (E2, 500 ng/ml). Medium was changed twice a week. After about 2 months in culture, small clones of tumor cells emerged and a total of 56 clones were isolated, 51 from cells that had been cultured in the presence and 5 clones from cells that had been cultured in the absence of E2. Clones were separated to establish different cell lines, subcultured and frozen. Preliminary testing of 8 cell lines demonstrated that they produce progesterone (P4) and prostaglandin E2 (PGE2). Stimulation with forskolin (10 µM) caused a 2.3- to 12-fold increase in P4 production depending on the cell line, but had no marked effect on PGE2 production. However, phorbol 12-myristate 13-acetate (100 nM) caused up to a 4 to 12 -fold increase in PGE2 depending on the cell line and in some of the 8 cell lines, treatment with 100 I.E. hCG doubled PGE2 secretion compared to controls. The establishment of canine ovarian cell lines is novel and may provide a valuable tool to study mechanisms of progesterone and prostaglandin production in the canine ovary.

KEYWORDS- canine ovary, ovarian tumor, steroidogenesis, prostaglandins