ABSTRACTS

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ImmunoLocalization of progesterone receptors in the canine oviduct around ovulation

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OBJECTIVE AND METHODS: In the bitch, oocyte maturation, sperm storage and capacitation, fertilization and early embryo development all take place within the oviducts under high circulating progesterone (P4) levels (1). In order to investigate the potential effects of P4 on the canine oviduct, the cellular distribution of P4 receptors (PR) was studied in the ampulla, isthmus and utero-tubal junction (UTJ) during anoestrus and around ovulation in Beagle bitches. Eighteen Beagle bitches from our experimental kennel were used in this study. The experimental protocol was approved by the ethical committee of the Alfort National Veterinary School. Ovariec-tomies were performed at 6 stages (n=3 bitches per stage) of the estrous cycle based on vaginal smears, plasma P4 concentration and ovarian ultrasonography: anoestrus, before the LH peak (Pre-LH), after the LH peak and before ovulation (Pre-Ov), one day (Day 1), four days (Day 4) and seven days (Day 7) after ovulation. Following ovariec-tomy, the oviducts were trimmed free of surrounding tissues, divided into 3 regions i.e. ampulla, isthmus and UTJ, snap frozen in liquid nitrogen and stored at -80°C until use.

For western-blot analysis, a canine ovary collected prior to the LH peak was homogenized in a lysis buffer for protein extraction. Proteins (50 µg) were migrated on a SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were incubated with a monoclonal mouse anti-human PR antibody (MA1-410; Affinity BioReagents) diluted at 2 µg/ml. Membranes were washed, incubated with an anti-mouse HRP secondary antibody (Jackson ImmunoResearch), washed again then incubated with enhanced chemiluminescence reagent detection solution (Super Signal West Pico, Pierce). Finally, a film was exposed to the membranes to visualize protein expression.

For immunohistochemistry, oviduct sections were embedded in OCT and serially sectioned (7 µm) using a cryostat. Sections were fixed for 10 min in acetone at 4°C. Non-specific protein binding was inhibited by incubation with porcine serum (10% dilution in PBS) for 30 min. Sections were incubated with the anti-PR antibody described above and diluted at 1 µg/ml, then washed and incubated with an anti-mouse biotinylated secondary antibody (LSAB kit, Dako). Negative controls were obtained by replacing the primary antibody by mouse IgG at the same dilution. Sections were then treated with 3% H2O2 and incubated with a streptavidin-horseradish peroxidase complex (LSAB kit). The signal was detected using a solution of diaminobenzidine (DAB). Finally, sections were counterstained with alcian blue and mounted for examination under light microscopy.

RESULTS: Western-blot analysis confirmed that both PR-A and PR-B isoforms of the nuclear P4 receptor were specifically detected in the canine ovary as ≈90 and ≈110 kDa proteins, respectively. Examination of PR localisation in the canine oviduct revealed intense nuclear staining in the luminal epithelium, stromal cells and muscular layer in the ampulla, isthmus and UTJ. No staining of the cytoplasm was observed in any cell group at any stage. No differences in the localisation pattern were observed between ampulla, isthmus and UTJ. The staining intensity in the luminal epithelium was high from Pre-LH peak to Day 1 then decreased at Day 4 and Day 7 in the three oviduct regions. In the stromal and muscular layers, the staining intensity and the proportion of stained nuclei were high at Pre-LH peak and Pre-Ov stages then decreased from Day 1 to Day 7 in the three oviduct regions. During anoestrus, the staining for PR in the three sections was very weak in the luminal epithelium and absent in other oviduct layers. Immunostaining was absent in all negative controls.

CONCLUSION: This is the first report of PR localization in the isthmus and UTJ in the canine oviduct. PR localization in the ampulla recorded in this study is in keeping with the results of a previous immuno-study conducted in Beagle bitches (2). A decrease in PR immunostaining and in P4 binding site concentrations was previously reported from proestrus to early metestrus in the canine oviduct (2,3), in accordance with the present study. Furthermore, these results are consistent with previous data from our laboratory that showed a significant decrease in PR mRNA levels from Pre-LH peak to Day 7 in the ampulla, isthmus and UTJ (4). The differential expression of PR in various compartments of the canine oviduct suggests that P4 is indeed an important regulator of tubal functions around ovulation in the bitch.


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