ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

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Leptin and leptin receptor gene expressions in the canine corpus luteum during diestrus, pregnancy and after aglepristone induced luteolysis

Balogh, O1; Kowalewski, MP2; Reichler, IM1

1Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, Zurich, CH 8057 and
2Institute of Veterinary Anatomy, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, Zurich, CH 8057

obalogh@vetcinics.uzh.ch

INTRODUCTION AND OBJECTIVES: Leptin is a primarily adipocyte-derived hormone and its serum levels parallel the amount of fat reserves. It plays a role in controlling reproductive processes centrally at the hypothalamo-pituitary level, and directly in reproductive tissues e.g. the ovary, where leptin is expressed together with its receptors (lepRs) suggesting an autocrine/paracrine effect (1). In the cyclic bovine corpus luteum (CL), leptin and lepRs increase significantly by the mid-luteal phase and are low again in the regressing CL. During pregnancy, expression levels are comparable to that of mid- and late-CL and do not change during gestation (2). Leptin seems to influence luteal steroidogenesis either directly or indirectly by modulating the actions of metabolic hormones e.g. GH, insulin, IGF-I. These effects may differ between species, phase of the CL, in vitro culture conditions and hormone dosages used. There is ample data on the relationship of leptin and adiposity in the dog, but no sufficient information is available on its involvement in canine reproduction. Plasma leptin levels were not different between male and female dogs irrespective of their body condition score (3). Another study (4), however, found higher levels in females than in males that also varied by cycle stage in the bitches showing a significant increase in estrus compared to proestrus and diestrus. The inconsistency between the two reports may be due, at least in part, to differences in methodology and in the animal population. Leptin was detected by immunohistochemistry in the non-pregnant canine ovary with abundance in luteinized granulosa cells and in the CL however, the phase of the CL was not specified in that study. Luteal lepRs were not found either (5). The reproductive physiology of the dog is different from that of many domestic animals and from humans, as the gestational and cyclic luteal phases are of comparable length and the CL is the sole source of progesterone (P4) during pregnancy (6). Therefore, regulatory processes and the role of players involved in the formation, maintenance and regression of the CL may be different from other species. Our goal was to characterize changes in luteal leptin and lepR gene expressions in cyclic diestrous and pregnant dogs, and in pregnant bitches treated at mid-gestation with the antiprogestagen aglepristone.

MATERIALS AND METHODS: Groups (n=5-6) of healthy bitches (2-8 years, different breeds) were spayed on day 5, 15, 25, 35, 45, 65 post ovulation (p.o.) during the non-pregnant diestrus or on pregnancy day 8-12 (pre-implantation; n=5), 18-25 (post-implantation; n=5), 35-45 (mid-gestation; n=5) and at prepartal luteolysis (n=3). Additionally, mid-gestation dogs were treated with aglepristone (10mg/kg BW s.c. 24 h apart) and ovariohysterectomized 24 h (n=5) and 72 h (n=5) after the second treatment. Pregnancy on day 8-12 was confirmed by detecting embryos in uterine flushes. CL were collected and preserved for RNA extraction. Total RNA was isolated, DNase treated and reverse transcribed. Semiquantitative real-time (TaqMan) PCR was performed to quantify the amount of canine leptin and lepR mRNA. One-way ANOVA with Tukey’s multiple comparison was used to test for differences between groups. Level of significance was set at P<0.05. Statistical calculations were carried out with IBM® SPSS® Statistics Version 19 program package.

RESULTS: In the non-pregnant diestrus, leptin expression in the CL was significantly higher on day 15 and 35 than on day 5 p.o., and significantly lower on day 25, 45 and 65 compared to day 35 p.o.. LepR mRNA levels were lower on day 35 than on day 65 (P<0.05). There was a significant increase in luteal leptin expression from the pre-implantation to post-implantation period of pregnancy. There were no significant changes observed afterwards. LepR expression did not change from pre-implantation to mid-gestation and increased significantly at prepartal luteolysis. In the aglepristone-treated pregnant group, CL leptin mRNA was not different from mid-gestation to 24 h post-treatment and decreased significantly from 24 to 72 h after aglepristone administration.

CONCLUSION: Both leptin and lepR are expressed in the canine CL and their expression levels change significantly during the luteal lifespan. Leptin expression is increased in the first half of pregnancy and non-pregnant diestrus corresponding with highest P4 levels, suggesting a possible interaction between locally produced leptin and luteal steroidogenesis. LepR expression seemed to be inversely related to leptin mRNA levels after day 25 p.o. of diestrus and, therefore, may differently modulate luteal availability of leptin in the first and second half of the CL phase. These fluctuations were not detected during gestation except for a significant upregulation at prepartal luteolysis. This may be due to biological differences between pregnant and cyclic diestrous bitches, and/or less frequent tissue sampling. Luteal leptin expression is significantly downregulated by aglepristone without affecting lepR expression, although its mechanism of action is not known. Further studies are needed to elucidate the role of leptin and its receptor in CL function in the bitch.