ABSTRACTS

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ESTROGEN AND PROGESTERONE RECEPTORS GENE EXPRESSION IN CANINE OOCYTES AND CUMULUS CELLS THROUGHOUT THE ESTROUS CYCLE

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Introduction - The establishment of canine in vitro embryo production protocols is limited by low oocyte meiotic competence following in vitro maturation (IVM) (1). In order to optimize this biotechnology it is necessary to improve the knowledge on the unique physiology of reproduction in this species (2). Even though hormonal supplementation has been widely used during IVM it is not known whether the cumulus-oocyte complexes (COCs) are responsive to such stimulus. In the current study it is hypothesized that estrogen (ERα and ERβ) and progesterone (PR) receptor expression in oocytes and cumulus cells is regulated by the phase of the estrous cycle.

Objectives - To analyze ERα, ERβ and PR gene expression in the canine oocyte and cumulus cells throughout the estrous cycle.

Material and methods - Ovaries from 38 bitches were recovered after ovariohysterectomy and sliced in PBS supplemented with 10% fetal calf serum. Blood samples were collected immediately after surgery for determination of serum estrogen and progesterone concentrations. The phase of the estrous cycle was determined by colpocytology, colposcopy and serum hormonal levels and bitches were grouped within each phase. Oocytes were mechanically denuded by repeated pipetting. Samples were frozen at -80°C until real time PCR. For each phase of the cycle a sample was composed by a pool of 50 oocytes (sample number: proestrus= 3, estrus= 8, diestrus= 5 and anestrus= 5) or a pool of cumulus cells (proestrus= 4, estrus= 7, diestrus= 4 and anestrus= 6). Oocyte and cumulus cells total RNA was isolated using RNeasy® Protect Mini Kit (Qiagen) and reverse transcription was conducted with SuperScript® First-Strand Synthesis for RT-PCR (Invitrogen) as recommended by the manufacturer. Quantification of mRNA abundance was performed using the real-time PCR ABI PRISM 7500 sequence detector system and SybrGreen® as a double stranded DNA-specific fluorescent dye. Transcript relative abundance was determined after normalization using 18S mRNA as an internal reference. Data were subjected to analysis of variance using JMP software.

Results - The mean number of COCs recovered in each phase of the estrous cycle was 114.28 ± 29.67, 135.22 ± 54.22, 99.25 ± 24.47 and 115.5 ± 33.38 for proestrus, estrus, diestrus and anestrus, respectively. There was no effect of phase of the estrous cycle on the relative abundance of ERα, ERβ and PR in oocytes and cumulus cells. Similarly, the proportion of cumulus cells clusters and oocytes expressing ERα, ERβ and PR was not affected by phase. It is possible that this lack of statistical significance in gene expression among phases is due to low sample number per phase. ERα was expressed throughout the cycle in the oocyte (33.33, 25.0, 20.0 and 60.0% for proestrus, estrus, diestrus and anestrus, respectively) and cumulus cells (50.0, 47.14, 25.0 and 66.67% for proestrus, estrus, diestrus and anestrus, respectively). In the oocyte the ERβ was also expressed in all phases of the cycle (33.33, 50.0, 20.0 and 60.0% for proestrus, estrus, diestrus and anestrus, respectively) while in cumulus cells ERβ was only expressed during proestrus (50%) and estrus (14.29%). Even though the exact role of cumulus cells ERβ is not completely understood this study...
suggests only a minor effect during the luteal phase and anestrus. Interestingly, while the oocyte PR was not detected in any moment of the cycle, this receptor was expressed during the proestrus (50%), estrus (42.86%) and anestrus (16.67%) in cumulus cells. The lack of PR indicates that progesterone action on the oocyte is mediated by cumulus cells. Such result is of primordial importance on the development of IVM protocols since the maintenance of cumulus cells seems to be required to ensure progesterone action on oocyte maturation. Moreover, the presence of PR only during proestrus and estrus suggests the role of this hormone on oocyte maturation while PR expression during anestrus points out its role on oocyte priming for the next estrous cycle. The differences in the percentage of positive samples within a phase suggest a dynamic endocrine pattern of gene expression along each estrous cycle phase.

Conclusions - The canine oocyte express ERα and ERβ throughout the estrous cycle. However, there is a lack of PR expression in all these phases. Moreover, in cumulus cells only ERα was expressed along the estrous cycle.

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References