ABSTRACTS

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Progesterone (PR), estrogen (α and β) and oxytocin (OTR) genes expression in the oviduct and uterus of pregnant and nonpregnant bitches

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OBJECTIVES AND METHODS: The aim of the present study was to assess hormone receptor genes expressed (PR, OTR, ER-α and ER- β) in the oviduct and uterus during canine pregnancy. For this purpose, 19 pregnant bitches and 5 non-pregnant bitches were ovariohysterectomized (OSH). The pregnant bitches were inseminated, castrated in programmed days and allotted to four groups according to the gestational ages: (Group A: 05 females with eight days of gestation, Group B: 05 females with 12 days of gestation, Group C: 05 females with 21 of gestation, Group D: 04 females with 60 days of gestation). The non-pregnant females were ovariohysterectomized 12 days after the onset of the LH preovulatory surge (Group E). After the OSH, oviduct and uterine tissue samples were obtained and frozen in cryotubes (- 80°C) These samples were extracted using TRIzol Reagent (Life Technologies, Carlsbad, CA, USA), according to the manufacturer’s protocol. Total RNA amounts were determined using a NanoDrop Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Total RNA (2 μg) was treated with Amplification Grade Deoxyribonuclease I (Life Technologies Corporation, Carlsbad, CA, USA). The RNA structural integrity was assessed by capillary electrophoresis by using a bioanalyzer (2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA) calculating the RNA Integrity Number (RIN). The cDNA was synthesized using High Capacity cDNA archive kit (Life Technologies, Carlsbad, CA, USA), according to the manufacturer’s protocols. The resulting cDNA samples were aliquoted and stored at -20°C. After these steps, the target and reference gene expression levels were detected by reverse transcription quantitative real-time polymerase chain Reaction (RT-qPCR) using an ABI 7300 Real Time PCR System (Life Technologies Corporation, Carlsbad, CA, USA) and Power SYBR Green PCR Master Mix 2x (Life Technologies Corporation, Carlsbad, CA, USA), with thw purpose to investigate the genes: progesterone receptor (PR), oxytocin receptor (OTR), alpha- estrogen receptor (ER-α) and beta-estrogen receptor (ER- β). The expression stability of reference genes glyceraldehydes-3-phosphate dehydrogenase (GAPDH), 18S and Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was assessed through geNorm VBA applet for Microsoft Excel (1). The reactions were performed in duplicate. Data were statistically analyzed and expressed as mean ± SD. Differences were considered significant with a p value of < 0.05.

RESULTS: The mRNA PR expression in the oviduct not changed at all pregnancy stages (Group A, B, C and D) and nonpregnant bitches (Group E), P = 0.2849. The mRNA PR expression in the uterus on the early stages of pregnancy (Group A: 1.818 ± 0.3028) and cyclic diestrus (Group E: 2.103 ± 0.1720) were higher than the others (Group B: 1.121 ± 0.1493, Group C: 1.0538 ± 0.285 and Group D: 06.962 ± 0.0466) indicating a variation on the mRNA PR expression during pregnancy. This condition is similar to what occurs in sheep (2). Concerning the mRNA ERβ expression in the oviduct was a significant reduction with the pregnancy advance (P = 0.0111), while in control females (Group E: 0.2919 ± 0.05105), the mRNA Erf expression was significantly higher than females in early (Group A: 0.0714 ± 0.02788) and final pregnancy (Group D: 0.0120 ± 0.0020) and similar to group B (0.2142 ± 0.09591 ) and group C (0.1343 ± 0.05064). The mRNA ERβ expression in the uterus was higher in Group E (5.231 ± 1.243) and Group B (5.261 ± 0.3164) when compared to group D or late gestation (0.008667 ± 0.0008819) and Group A or early gestation (3.204 ± 0.2430), but similar in Group C (3.408 ± 0.8875). The mRNA ER α expression was significantly higher in the oviduct with pregnancy advance (P = 0.0040). We have seen that the final stage of gestation (Group D: 0.9430 ± 0.1096) occurs higher expression of mRNA ER α in the uterus, compared to the other phases (P = 0.0005). Probably, the increase in serum progesterone causes the decrease the mRNA Erβ expression and increased the mRNA ER α expression in the oviduct and uterine tissue. The opposite was observed by Hatoya et al, (2003) in studies with hypothalamus, pituitary and ovary during the canine estrous cycle. The mRNA OTR expression in the oviduct was lower than the uterus in the group E (P = 0.0273) and final pregnancy or group D (P = 0.0147). The expression of this receptor in oviduct and uterus were higher in the final stages of gestation, groups C and D (P = 0.0300 and P = <0.0001, respectively) when compared to other phases. In pregnant sheep, the decrease in serum progesterone concentration causes an increase estrogen receptor (ER) and this consequently causes an increase in oxytocin receptors (OTR) (4).

CONCLUSION: The serum progesterone concentration probably exerts a direct control on the mRNA PR and ER (α and β) expression and indirectly on mRNA OTR expression in the bitch oviduct and uterus.


(4) Spencer TE, Bazer FW. Conceptus signals for establishment and maintenance of pregnancy. Reproductive Biology and Endocrinology 2004; 2:1-15