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Kisspeptin and its receptor are expressed in canine trophoblast cells

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In humans, placental kisspeptin contributes to the regulation of implantation. During a previous study, we detected the kisspeptin and kissR gene in the canine placenta. The aim of this study was to assess the protein expression in uterine and placental cells. Tissues were collected from bitches after ovariohysterectomy and assigned to groups: pre-implantation, day 10-12, n=9; post-implantation, day 18-25, n=13; mid-gestation, day 30-40, n=7; early metestrus, day 10-12, n=3. Immunohistochemistry was performed by using the streptavidin-biotin peroxidase complex technique for detection of kisspeptin (kiss), kisspeptin receptor (kissR), pan-Cytokeratin and Vimentin. Serial sections were prepared and incubated with each of the primary antibodies (kisspeptin10, Rabbit PAb, Merck Millipore; Kiss1R/GPR54, Rabbit PAb, Novus Biologicals). Sections were then incubated with biotin-conjugated second step antibody (Zymed Laboratories, Inc.; San Fransisco, CA, USA) and thereafter with streptavidin-peroxidase complex (Zymed Laboratories). Labelling was ‘visualized’ with amino ethyl carbazole (AEC substrate kit; Invitrogen, Camarillo, Canada). Sections were counterstained with Gill’s haematoxylin. Negative controls were incubated with normal serum instead of primary antibody. The immunoreactivity was evaluated semi-quantitatively at x200 magnification over an area of 0.050 mm² and the percentage of positive stained cells assessed. Labelling intensity was quantified using a four-point scale: 0=no labelling; 1=weak; 2=moderate; 3=intense labelling. An immuno-reactivity score (IRS) was obtained by multiplying the labelling intensity score (0-3) with the percentage of immunolabelled cell types investigated. The IRS ranged from 0 to 300. The statistical significance was analysed by Wilcoxon test using PASW-Statistic 18 (SPSS, IBM). Homogeneity of groups were determined by the Shapiro-Wilk test. The confidence interval was determined as P<0.05. All values are expressed as mean ± SE. The kiss and kissR signals varied among groups and individuals. Labeling for kiss in the vascular endothelial cells and tunica media of larger vessels were present in the pre-implantation period only. KissR staining of vessels was recorded in all gestational periods including non-pregnant uteri with highest IRS score in the post-implantation period. In general, luminal (surface) epithelial cells were negative for both kiss and kissR with the exception of kiss in the controls. Staining for kiss was more intense in the superficial uterine glands of the non-pregnant than the pregnant uterus; expression diminished following implantation. KissR staining was negative in the superficial uterine glands in all groups. Deep uterine glands stained negative or weak for both kiss and kissR. IRS for kiss was more pronounced in the non-pregnant uterus (IRS:26) compared to all gestational periods investigated. In myocytes, kiss expression was significantly higher in mid-gestation than in the pre-implantation period (in the deep glandular layer P<0.05; sponge zone P<0.01). For kissR, the highest IRS scores were seen in mid-gestation (IRS:70) and post-implantation (IRS:56). In the placental labyrinth, fetal trophoblast cells, predominantly syncyto-trophoblasts, stained weak to moderately for kiss in the post-implantation (IRS:22) and mid-gestation (IRS:29) periods. A similar localization and staining intensity was observed in the post-implantation period for kissR (IRS:24). Although labeling intensity of the

Abstract truncated at 1 page maximum length