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The localization of kisspeptin and kisspeptin receptor in the canine ovary during different stages of the reproductive cycle

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Kisspeptin is a neuropeptide involved in the hypothalamic regulation of reproduction in many species. Our group mapped the distribution and nature of interaction of kisspeptin and gonadotropin releasing hormone immunoreactive neurons in the canine hypothalamus.1 Recent studies have revealed kisspeptin within the ovaries of rats, Siberian hamsters and humans, indicating a local role in reproduction.2 However, the role of kisspeptin in the ovary is poorly understood in the bitch. This study investigated the presence and location of kisspeptin protein (kiss1) and kisspeptin receptors (kiss1-R) in the canine ovary during different stages of the reproductive cycle (pre-pubertal, anestrus and cycling) by means of immunohistochemical staining. Ovaries from 24 bitches of mixed breed of age ranging from 10 weeks to 10 years, presented at local veterinary clinics for routine ovariohysterectomy, were collected and grouped based on reproductive stage (pre-pubertal, anestrus, proestrus, estrus and diestrus). The ovaries were then fixed in 4% paraformaldehyde for 5 days, followed by 20% and 30% sucrose in 0.1M phosphate buffered saline respectively for 3 days each, and then sectioned on a cryostat (40µm). Kiss1 and kiss1-R immunoreactive cells were identified using the immunoperoxidase method with rabbit primary antibodies and Nickel 3, 3’-Diaminobenzidine as the chromogen. Labelled cells were examined using an Olympus CH30 microscope and images captured using a Nikon Eclipse Ti microscope with a Nikon Digital sight DS-U3 camera. The presence or absence of immunoreactive Kiss1 and Kiss1-R was recorded without any quantification of the levels of expression within cells. Being an observational study, no statistical analysis was performed. Immunoreactive kiss1 was found in the oocytes during all stages of the estrous cycle, in the granulosa cells during all stages except anestrus, and in the corpus luteum (CL) during diestrus. Kiss1 was absent in the ovaries of pre-pubescent bitches. Immunoreactive kiss1-R was consistently found in the oocytes, primordial follicles, the granulosa cells and CL in cycling bitches. The finding of kiss1-R in the granulosa cells is suggestive that kisspeptin and progesterone may be linked as this pattern of staining is seen in animals that show preovulatory luteinization of follicles during estrus, and in birds where progesterone is produced by the granulosa cells.3 Kiss1-R was also observed in the ovaries of pre-pubescent and anestrous bitches. These findings are suggestive of a possible role of kisspeptin in oocyte proliferation, development and maturation of granulosa cells, and progesterone production. This study provides a starting point for the establishment of a canine model for kisspeptin regulation within the ovary. Future quantitative investigations into the amount of kiss1 and kiss1-R present in the bitch’s ovaries at different stages of the estrous cycle could explain more about any fluctuations in the concentration of kisspeptin across the estrous cycle.

1 Scott CJ, Buchholz V, Norman ST. The distribution of kisspeptin and Gonadotrophin – Releasing Hormone (GnRH) neurons in the hypothalamus of the dog. Proc 2nd world conference on kisspeptin signalling in the brain – Tokyo Japan 2012