

## **Immunohistochemical evidence for kisspeptin signaling in equine gonadotrophs**

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Kisspeptins (Kp) are a family of amidated peptides known to initiate and maintain reproductive function in all mammalian species studied to date. Our laboratory and others have provided immunohistochemical evidence for a kisspeptin Gonadotropin Releasing Hormone (GnRH) neuronal mechanism in the equine hypothalamus and have demonstrated that IV administration of equine kisspeptin decapeptide (eKp 10) elicits a rise in peripheral luteinizing hormone (LH) concentrations in mares. We hypothesized that Kp has a direct effect on equine gonadotrophs to elicit LH synthesis and secretion. Both GnRH receptor and Kiss1r are  $G\alpha_{q/11}$  coupled receptors and a rise in intracellular calcium concentration is necessary for LH secretion. By quantifying the rise in intracellular calcium after exposure of equine primary pituitary cells to GnRH and/or eKp 10, our laboratory identified 3 distinct populations of equine pituitary cells: 1 population responded to both eKp 10 and GnRH, a second population responded to only eKp 10, and a third population responded only to GnRH. Although these findings supported a pituitary mechanism for eKp 10 at the level of the gonadotroph, pre-treatment of diestrus mares with GnRH antagonist (antide 1.0 mg IV) eliminated any measurable change in peripheral LH after 1.0 mg eKp 10 IV. The body of evidence for kisspeptin regulation of pituitary cell function is growing. Objective was to establish immunohistochemical evidence for Kp signaling at the level of the equine pituitary gland. To confirm Kiss1r expression in the equine pituitary and to determine if gonadotrophs express Kiss1r, coimmunofluorescence studies against Kiss1r (AKR 001, Alomone Labs, Israel) and the LH subunit  $\beta$  (518B7, courtesy of JF Roser) were performed on frozen diestrus mare hemipituitaries ( $n = 8$ ). Images of immunolabeled tissues were captured using scanning confocal microscopy and analyzed via direct cell counting of at least 4 fields per tissue per mare, using DAPI to identify individual cells. Based on cell counts, on average 26.7% of all cells within the equine pituitary expressed Kiss1r and 21.17% of all pituitary cells were positive for LH $\beta$ . Additionally, in congruency with previous data, 3 populations were identified including Kiss1r only positive cells (18.2%), LH $\beta$  only positive cells (13.3%) and cells positive for both Kiss1r and LH $\beta$  (8.4%). Data provided evidence that cells of the equine pituitary, including gonadotropes, were primed to directly respond to kisspeptin signaling and that further exploration to understand the role of kisspeptin in pituitary function is needed.

**Keywords:** Equine, kisspeptin, pituitary, immunohistochemistry

