

## Methods used to detect pituitary luteinizing hormone (LH) response to kisspeptin and gonadotropin-releasing hormone (GnRH) challenge

### Methods

#### *Pituitary LH response to Kisspeptin and GnRH Challenge*

Kisspeptin and GnRH challenges were carried out following 2.5 years of treatment in the luteal phase and early follicular phase of the cycle, respectively. Animals were bled every other day beginning at Day 8 of the cycle to check for progesterone rise, and the kisspeptin administration was carried out between Day 17 and 21 of the cycle following confirmation of serum progesterone greater than 1.0 ng/ml. Average serum progesterone levels at the time of kisspeptin administration was  $4.44 \pm 0.39$  ng/ml. GnRH administration was typically performed between Day 0 and 5 of the early follicular phase in the following cycle. On the day of the GnRH challenge, the average serum progesterone level was  $0.11 \pm 0.02$  ng/ml and the average estradiol serum level was  $38.63 \pm 2.93$  ng/ml. Animals were initially sedated with ketamine:xylazine mixture (8–15 mg/kg) or telazol (3–5 mg/kg) followed by intubation and sedation with isoflurane at 1–2% in 100% oxygen at a flow rate of 1–2 l/min. Dual femoral catheters were placed. Blood was collected at -15, 0, 15, 30, 45, 60, 75, 90 and 105 min with 0 min corresponding to the time of either kisspeptin or GnRH administration. Kisspeptin-10 (Phoenix Pharmaceuticals, Catalogue # 0-48-56) was administered at 8 µg/kg (Plant *et al.*, 2006). GnRH (National Hormone and Peptide Program) was administered at 250 ng/kg (McGee *et al.*, 2012). LH values were plotted and area under the curve (AUC) from zero

was calculated in Prism GraphPad. The change in LH from 0 to 15 min was also calculated.

### Results

Following 2.5 years of treatment kisspeptin was administered during the luteal phase to assess the function of the hypothalamic portion of the hypothalamic pituitary gonadal axis. Kisspeptin administration (8 µg/kg intravenously) resulted in an increase in LH levels (Supplementary Fig. 2A, left); however, there were no differences between the four treatment groups in either the total LH AUC or the change in LH 15 min following kisspeptin administration (Supplementary Fig. 2A, middle and right). During the early follicular phase of the next cycle GnRH was administered to probe the pituitary portion of the hypothalamic pituitary gonadal axis. GnRH administration (250 ng/kg intravenously) resulted in a mild increase in LH release (Supplementary Fig. 2B, left). Once again, there were no group differences in either LH AUC or the 15-min change in LH following GnRH administration (Supplementary Fig. 2B, middle and right).

### References

- Plant, Ramaswamy, DiPietro. Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the Juvenile Monkey (*Macaca mulatta*) elicits a sustained train of gonadotropin-releasing hormone discharges. *Endocrinology* 2006;147:1007–1013.
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