

Progress Report for Proposed Research Program**Expt 1. Kisspeptin and GPR54 expression in ovine brains, projections to GnRH cells***1a. Distribution and expression of kisspeptin expressing cells.*

We have conducted studies outlining the expression and distribution of kisspeptin cells within the hypothalamus. This was achieved by immunohistochemistry and was performed in ovariectomised (OVX) and OVX + oestrogen ewes. We discovered that the expression of kisspeptin protein is up-regulated in the arcuate nucleus of OVX ewes. Moreover, the effects of oestrogen in down-regulating kisspeptin expression appeared to be greater during the non-breeding (anestrous) season. This data has recently been published in *Endocrinology*.

We have also performed similar experiments in ewes at differing stages of the oestrous cycle. We show up-regulation of kisspeptin protein expression during the late follicular phase of the oestrous cycle concurrent with the onset of the preovulatory LH surge. We are currently preparing this data for publication.

1b. Projections of kisspeptin-producing cells to GnRH cells.

Our data show that a significant percentage of GnRH neurons (approximately 60-100%) receive input from kisspeptin positive terminals. Moreover, this percentage appears to be regulated by breeding season in the ewe. During the breeding season the percentage of GnRH neurons with kisspeptin ‘contacts’ increases significantly. This data has recently been published in *Endocrinology*.

1c. Retrograde labelling from POA and Fos induction by oestrogen.

Retrograde tracing studies are underway and should be completed in 2009.

We show under a condition of oestrogen positive feedback stimulus (leading to a preovulatory LH surge) kisspeptin cells in both the middle and caudal arcuate nucleus become transcriptionally activated. This was shown by Fos induction using dual label immunohistochemistry. This data was presented at the 2008 ESA Meeting and we are currently preparing it for publication.

Expt 2. Effects of kisspeptin on GnRH/LH secretion in relation to breeding season and steroid feedback*2a. Effect of kisspeptin on GnRH/LH secretion.*

Our data show that kisspeptin administration rapidly and robustly stimulates LH secretion in ewes. Interestingly, the degree of response in LH concentrations to kisspeptin varied during different reproductive phases in the ewe. Follow up experiments, using GnRH administering showed that the variable response to kisspeptin was more likely a reflection of the change in the pituitary response to GnRH and not a change in the hypothalamic response to kisspeptin. This data has been submitted for publication.

2b. GPR54 mRNA expression in relation to breeding season and steroid feedback.

This study is currently underway. We have perfected a double label in situ hybridisation technique of determining the level of GPR54 expression directly on GnRH neurons. This technique offers a more histologically discreet and physiological measure than detecting GPR54 expression in the hypothalamic fragments by RT-PCR.

Expt 3. Effects of kisspeptin antagonist on LH release and ovulation*3a. Effect of kisspeptin antagonist on LH secretion*

We have recently shown that intracerebroventricular (ICV) administration of a kisspeptin antagonist reduces the concentration of LH in OVX ewes. This is an important finding in

itself as it demonstrates the critical importance of kisspeptin in maintaining gonadotrophin secretion. These studies are currently submitted for publication.

3b. Effect of kisspeptin antagonist on ovulation

These studies are yet to be conducted and will begin in 2009.

Papers published in 2008 related to this Award

(1) **Smith JT** (2008) Sex steroid control of hypothalamic Kiss1 expression in sheep and rodents: Comparative aspects. *Peptides* In Press.

(2) **Smith JT**, Coolen LM, Kriegsfeld LJ, Sari IP, Jaafarzadehshirazi MR, Maltby M, Bateman K, Goodman RL, Tilbrook AJ, Bentley GE, Clarke IJ, Lehman MN (2008) Variation in kisspeptin and gonadotropin-inhibitory hormone expression and terminal connections to GnRH neurons in the brain: possible medium for seasonal breeding in the sheep. *Endocrinology* 149:1951-1959.

Submitted Papers

(1) **Smith JT**, Saleh S, Clarke IJ () Seasonal and cyclical change in the luteinising hormone response to kisspeptin in the ewe: No effect of kisspeptin on growth hormone, prolactin or cortisol.

(2) Roseweir A, Kauffman A, **Smith JT**, Morgan K, Pielecka-Fortuna J, Pinead R, Tena-Sempere M, Moenter SM, Clarke IJ, Steiner RA, Millar RP () Novel kisspeptin/metastin antagonists as potent inhibitors of gonadotropins.

Conference Abstracts

(1) **Smith JT**, Clarke IJ (2008) Seasonal and cyclical change in the luteinizing hormone response to kisspeptin in the ewe: No effect of kisspeptin on growth hormone, prolactin or cortisol. *Proceedings of the Endocrine Society* 89

(2) Roseweir AK, Kauffman A, **Smith JT**, Morgan K, Pielecka-Fortuna J, Pineda R, Gottsch ML, Tena-Sempere M, Moenter SM, Clarke IJ, Steiner RA, Millar RP (2008) Kisspeptin antagonists. *Proceedings of the Endocrine Society* 89

(3) **Smith JT**, Pereira A, Clarke IJ (2008) Evidence that kisspeptin neurons in the arcuate nucleus are central processors for generating the preovulatory luteinising hormone surge in ewes. *The Endocrine Society of Australia, Annual Scientific Meeting, Melbourne, Australia.*

(4) **Smith JT**. (2008) Sex steroid regulation of Kiss1 expression in the mouse and sheep. *The 1st World Conference on Kisspeptin Signaling in the Brain, Cordoba, Spain.*

(5) Goodman R, Rao A, **Smith JT**, Clarke IJ. (2008) Negative feedback control of Kiss-1 gene expression by estradiol and progesterone in the ewe. *The 1st World Conference on Kisspeptin Signaling in the Brain, Cordoba, Spain.*

(6) Caraty A, **Smith JT**, Clarke IJ. (2008) Kisspeptins and the seasonal control of reproduction in sheep. *The 1st World Conference on Kisspeptin Signaling in the Brain, Cordoba, Spain.*

(7) Roseweir AK, Kauffman A, **Smith JT**, Guerriero K, Morgan K, Pielecka-Fortuna J, Pineda R, Gottsch ML, Tena-Sempere M, Moenter SM, Terasawa, E, Clarke IJ, Steiner RA, Millar RP (2008) Development of kisspeptin antagonists as potent inhibitors of gonadotropins. *The 1st World Conference on Kisspeptin Signaling in the Brain, Cordoba, Spain.*