Final Report for Proposed Research Program 2008-2009

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 and published 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.

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 been published.

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

Initial retrograde (and anterograde) tracing studies are underway. We now have evidence to suggest kisspeptin fibres in the median eminence originate from the arcuate nucleus.
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 has now been published.

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 administrating 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. To confirm this we have now directly measured the GnRH (in portal blood) response to kisspeptin. Unlike LH, the GnRH response does not appear to vary during different reproductive phases in the ewe. This data is now in preparation for publication.

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

This study has been completed. We have perfected a double label in situ hybridisation technique of determining the level of GPR54 expression directly on GnRH neurons. We are now quantifying the data.

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 now published.

3b. Effect of kisspeptin antagonist on ovulation
This study has been completed. Preliminary analysis of the data suggests that treatment with a kisspeptin antagonist prevents (or severely dampens) the preovulatory LH surge. Importantly, our experimental designed utilised an estrogen (injection) induced surge model. Because of this, we eliminated the possibility of the antagonist preventing the rise in estrogen responsible for the surge and show the antagonist blocks the surge itself. These data are currently under analysis and will be presented at the ICN meeting in 2010 (France). It is predicted the data will be published in 2010.

Papers published in relation to this Award
1) Backholer K, **Smith JT**, Clarke IJ (2009) Melanocortins may stimulate reproduction by activating orexin neurons in the dorsomedial hypothalamus and kisspeptin neurons in the preoptic area of the ewe. Endocrinology In Press. Accepted 12th September 2009
2) Smith JT, Li Q, Pereira A, Clarke IJ (2009) Kisspeptin neurons in the ovine arcuate nucleus and preoptic area are involved in the preovulatory luteinizing hormone surge. Endocrinology In Press. Accepted 12th September 2009
3) Smith JT, Saleh S, Clarke IJ (2009) Seasonal and cyclical change in the luteinizing hormone response to kisspeptin in the ewe. Neuroendocrinology 90:283-291.
4) Roseweir A, Kauffman A, **Smith JT**, Guerriero K, Morgan K, Pielecka-Fortuna J, Pinead R, Gottsch MG, Tena-Sempere M, Moenter SM, Teresawa E, Clarke IJ, Steiner RA, Millar RP (2009) Discovery of potent kisspeptin antagonists delineate physiological mechanisms of gonadotropin regulation. Journal of Neuroscience 29:3920-3929.
5) Smith JT (2009) Sex steroid control of hypothalamic Kiss1 expression in sheep and rodents: Comparative aspects. Peptides 30:94-102.
6) 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 and Clarke IJ (2009) Seasonal breeding as a neuroendocrine model for puberty in sheep. Molecular and Cellular Endocrinology
2) Backholer K, **Smith JT**, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ (2009) Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology

Conference Abstracts
1) Smith JT, Shahab M, Pau KYF, Clarke IJ (2009) Kisspeptin neurons in the arcuate nucleus and preoptic area are central regulators for the preovulatory luteinizing hormone surge in the sheep and monkey. Proceedings of the Society for Neuroscience
2) Barrell GK, Ridgway MJ, Wellby M, **Smith JT**, Clarke IJ (2009) Effect of kisspeptin on reproductive function in seasonally acyclic red deer hinds. The Endocrine Society of Australia and New Zealand Society of Endocrinology, Annual Scientific Meeting, Adelaide, Australia.
3) Smith JT, Periera A, Shahab M, Pau KYF, Clarke IJ (2009) Hypothalamic expression of the kisspeptin gene (Kiss1) and the RFamide-related peptide (RFRP) gene during the

menstrual cycle of a non-human primate. The Endocrine Society of Australia, Annual Scientific Meeting, Adelaide, Australia.
4) Puspita Sari I, **Smith JT**, Clarke IJ (2009) Reduced RF-amide related peptide (RFRP) gene expression in the follicular phase of the ewe estrous cycle permits increased LH secretion from gonadotropes. The Endocrine Society of Australia, Annual Scientific Meeting, Adelaide, Australia.
5) Jacobi JS, **Smith JT**, Clarke IJ (2009) Evidence for a rapid effect of estradiol on Kisspeptin Cells in the Ewe. The Endocrine Society of Australia, Annual Scientific Meeting, Adelaide, Australia.
6) 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) The Development of Kisspeptin Antagonists for Therapeutic Interventions. 56th Annual Meeting of the Society-for-Gynecologic-Investigation. Glasgow, Scotland.
7) 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
8) 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
9) 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.
10) 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.
11) 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.
12) 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.
13) 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.