Reproductive Biology Satellite Abstracts

RB1: Pubertal disorders: A window into the neuroendocrine control of reproductive development and fertility

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The hypothalamic-pituitary-gonadal (HPG) axis controls puberty and reproduction and is tightly regulated by a complex network of excitatory and inhibitory factors. Delayed or absent activation of the HPG axis results in delayed puberty or hypogonadotropic hypogonadism, whereas early activation results in central precocious puberty (CPP). In recent years, many genes have been identified in this complex network, providing insight into the regulation of GnRH secretion. These advances were heralded by the discovery of the kisspeptin system as a critical component for the activation of GnRH secretion, and followed by the discovery of the tachykinin, neurokinin B, and its role in puberty initiation, in turn, through regulation of kisspeptin secretion. More recently, we identified loss-of-function mutations in the *MKRN3* gene, encoding makorin ring finger protein 3, as an important cause of CPP. MKRN3 is an imprinted gene on chromosome 15q11.2 in the Prader-Willi Syndrome critical region, with expression only from the paternally inherited allele. MKRN3 is expressed at high levels in the mouse hypothalamus prepubertally and decreases prior to puberty onset, suggesting that it acts as a 'brake' or inhibitor of GnRH secretion. MKRN3 is the first factor to be identified that likely has an inhibitory role on puberty in humans. The discovery of this new genetic link to early puberty will help to diagnose the cause of precocious puberty or to identify children at risk for developing precocious puberty, and the elucidation of the mechanisms by which MKRN3 regulates GnRH secretion will bring new insights into reproductive physiology.

RB2: In vitro maturation of oocytes: culture systems tailored for oocytes from growing antral follicles

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Oocyte in vitro maturation (IVM) is a reproductive technology that makes use of oocytes from patients or animals that have received minimal or no gonadotrophin stimulation. Whilst this brings many advantages, this typically means oocytes are collected from small – medium sized antral follicles, and these oocytes are still in the process of acquiring the capacity to support subsequent embryo development. For example, an essential signalling network required for natural oocyte maturation and ovulation, the EGF signalling cascade, is grossly underdeveloped in these oocytes. A broad objective of my work is to restore in vitro, as far as possible, the natural processes that occur during oocyte maturation in vivo. For example, inducing EGF signalling during IVM leads to notable improvements in subsequent oocyte quality as assessed by blastocyst yield post-IVM. This research has implications for the efficacy of human and veterinary clinical IVM and for fertility preservation for cancer patients.

RB3: Dynamin 2 is essential for mammalian gametogenesis

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The dynamin family of proteins play important regulatory roles in membrane remodelling and endocytosis, especially within brain and neuronal tissues. In the context of reproduction, dynamin 1 (DNM1) and dynamin 2 (DNM2) have recently been shown to act as key mediators of sperm acrosome formation and function. However, little is known about the roles that these proteins play in the developing testicular germ cells. In this study, we employed a DNM2 germ cell-specific knockout model to investigate the role of DNM2 in gametogenesis. We demonstrate that ablation of DNM2 in early spermatogenesis results in germ cell arrest during prophase I of meiosis, subsequent loss of all post-meiotic germ cells and concomitant sterility. These effects become exacerbated with age, and ultimately result in the demise of the spermatogonial stem cells and a Sertoli cell only phenotype. $DNM2^{-/-}$ females were also infertile and failed to form follicles beyond the preantral stage. Dynamin was also essential for the final stages of oocyte maturation with meiotic completion or MII spindle maintenance disrupted in a dynamin inhibitor-specific manner. Our findings identify an important role for Dynamin 2 during male and female gametogenesis from the earliest stages of gametogenesis through to maturation of the mature spermatozoa and oocyte.

RB4: The follicular microenvironment during oocyte maturation

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The attainment of developmental competency of an oocyte occurs gradually in the maturing follicle. Investigating the follicular microenvironment during this maturation process will identify the critical pathways involved and determine the optimal biochemical composition in which the bovine oocyte matures. The overall objective was to characterise the key molecular and biochemical pathways as well as the composition of the microenvironment provided by the follicular fluid, of growing and preovulatory follicles in the NZ dairy cow. This information was then utilised to develop physiologically-relevant *in vitro* maturation media to test whether cumulus cell-oocyte complexes matured in a microenvironment similar to that in which they were extracted from, improved embryonic outcome following *in vitro* fertilisation.

With the exception of three amino acids (alanine, glycine and hydroxyproline) in the cow, overall concentrations of amino acids (N=19) were markedly lower in follicular fluid of mature (preovulatory), compared to growing, follicles. Moreover, the majority of amino acids measured in follicular fluid of growing and preovulatory follicles were approximately 100% and 65% of that in plasma, and significantly lower than that in commercial IVM media. From this information, two physiological bi-phasic media were developed that reflected either the median values (good) or the mean lower tenth percentile values (poor) of amino acids in follicular fluid of growing (early) or preovulatory (late) follicles.

The blastocyst rate of COCs matured in poor and good media were lower and similar, respectively compared to that in commercial media. Overall, the expression levels of genes identified as being critical to follicular maturation in COCs matured in physiologically-relevant media were similar to that of *in vivo* matured COCs, but lower than that of COCs matured in commercial IVM media.

In summary, this study highlights the importance of understanding the microenvironment of a maturing follicle. Species-specific information of the follicular microenvironment may lead to the refinement of *in vitro* maturation media and improvements in the efficiency of current artificial reproductive technologies.

RB5: Splicing up your sex life; Why men are failing to produce in the bedroom

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Male infertility is a very common condition, with reports suggesting that one in 15-20 men of reproductive age are affected. Understanding why or how men produce defective sperm is a question that has remained elusive. We have used a combination of proteomic and genomic screens to identify both those proteins and genes responsible for building defective sperm in men. Interestingly, about 40 proteins are commonly dis-regulated within infertile sperm. Significantly, we have found that many of these proteins show atypical alternate splicing patterns. One such mechanism that explains this was the observation that several alternate-splicing regulators were up-regulated within defective sperm. As such, in proof of concept, we replicated this condition using transgenic flys. Our data show that sperm overexpression RNA-splicing regulators showed typical patterns of “male-factor” infertility, including (i) decreased amounts of sperm production, (ii) head morphology defects and (iii) poor sperm motility.

RB6: Regulation of follicular growth and ovulation rate: insights from sheep

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Growth, maturation and ovulation of the ovarian follicle is a key determinant of fertility in mammals. Furthermore, the number of follicles ovulating at each reproductive cycle (i.e. ovulation rate) sets the upper limit for litter size in species that may ovulate multiple follicles at each reproductive cycle. Sheep are an excellent model for understanding the regulation of follicular development given that they have considerable plasticity in ovulation rate, with ovulation rates for natural, unstimulated cycles ranging from 1 to over 10 depending on the genetics and environment (particularly nutrition) of the ewe. Classically, the role of hormones produced by the hypothalamus and pituitary have been investigated when trying to understand control of ovarian follicular development and ovulation rate. However, it is becoming ever clearer that factors produced and acting within the ovary itself are key regulators of ovarian follicular development and ovulation rate. Cocaine- and amphetamine-regulated transcript (CART) has recently been postulated to be such a factor. Instead of a positive role supporting the development of the follicle, CART is thought to actively suppress follicle development, targeting follicles for atresia. We have recently shown that CART is differently expressed in ewes with differing ovulation rates and this presentation will focus on understanding the role of CART, as well as other factors, in regulating follicle development in sheep.

RB7: Dissecting leptin actions on the central pathways controlling puberty

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In mammals, adequate circulating concentrations of the hormone leptin, produced by adipose tissue, are required for puberty onset. Deficient leptin signaling attributable to diet-induced leptin resistance is associated with infertility in humans and rodents, and treatments for human infertility show a decreased success rate with increasing body mass index. Leptin does not act directly on the GnRH neurons that control reproduction, but their critical fertility-permitting actions are confined to brain neurons. So far, no one population of neurons has been shown to be absolutely critical for metabolic control of fertility. Interestingly however, mice with leptin receptor knockout targeted to neuronal populations that produce the neurotransmitter GABA exhibit delayed puberty and subfertility in both sexes. It is likely that leptin signals via a heterogenous and redundant network of GABAergic neurons to critically regulate GnRH neurons. Recently, we showed that the transmission of metabolic information to the hypothalamo-pituitary-gonadal axis is mediated by at least in part leptin receptors on AgRP neurons. These results provide new insights into the mechanisms that cause infertility attributable to malnourishment.

RB8: Overexpression of AMH inhibits secondary ovarian follicle development in vivo

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Anti-Müllerian hormone (AMH) regulates ovarian function by inhibiting the activation of primordial follicles. There is also evidence that AMH affects developing follicles *in vitro* but evidence of similar actions *in vivo* remains elusive. In a transgenic mouse line (Thy1.2-AMH^{Tg}) generated in our lab, over-expression of AMH causes an infertility phenotype in the females. The oocytes of these females can be fertilised and implant in the uterus but most embryos are resorbed prior to parturition. Preimplantation embryos from Thy1.2-AMH^{Tg} mice show increased rates of developmental abnormalities compared to wild type females but recombinant AMH has no effect on embryo development *in vitro*. In the ovary primordial follicle activation rates were slightly reduced in Thy1.2-AMH^{Tg} mice when compared to wild type mice but the numbers of preantral follicles were greatly reduced in Thy1.2-AMH^{Tg} mice. More research is needed to determine if altered oocyte development in the Thy1.2-AMH^{Tg} mice is the cause of the subsequent miscarriage.

RB9: PCOS - The ignored syndrome!

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It is more than 80 years since polycystic ovary syndrome (PCOS) was first described but patients still do not know what causes it. There is both a genetic predisposition and a fetal origin, cause unknown, of developing PCOS. Only treatments to alleviate the symptoms of PCOS, not cure or prevent it, are available. The name PCOS is does not even correctly describe the ovaries and they are also not necessary for a diagnosis of PCOS. Women with PCOS not only experience infertility (menstrual irregularity, anovulatory infertility, miscarriage) and symptoms of excess androgen (hirsutism, acne, central adiposity), but also they have substantially increased risk of becoming obese, insulin resistant and of developing non-alcoholic fatty liver disease, dyslipidaemia, depression and type 2 diabetes. It can be estimated that up to 35% of all diabetes in women of reproductive age is related to PCOS. Given that alone one would expect research into PCOS to be a priority. Alas that is certainly not the case.

RB10: Does androgen excess have an etiological role in polycystic ovary syndrome (PCOS)?

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Polycystic ovary syndrome (PCOS) is a complex disorder characterised by reduced fertility, due to ovulatory disturbance and reproductive hormone dysregulation involving luteinising hormone (LH) hypersecretion and hyperandrogenism. Women with PCOS are also predisposed to metabolic disturbances such as obesity, insulin resistance, and dyslipidemia, with an increased risk of cardiovascular disease and type 2 diabetes. The origins of PCOS remain unknown, hence mechanism-based treatments are not feasible and current management is suboptimal as it relies on the treatment of symptoms. Hyperandrogenism is the most consistent PCOS characteristic, however it is unclear if androgen excess, is a cause or a consequence of PCOS. As androgens mediate their actions via the androgen receptor (AR), we combined a hyperandrogenised PCOS mouse model with global and cell-specific AR resistant (ARKO) mice to uncover the sites of androgen action that mediate the development of the PCOS phenotype. Analysis of these mouse models implies that extra-ovarian and not intra-ovarian AR actions are key sites of androgen action in generating the PCOS phenotype.

Polycystic ovary syndrome (PCOS): Is it all in our heads?

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Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder and the leading cause of anovulatory infertility. Characterised by hyperandrogenism, menstrual dysfunction and polycystic ovaries, PCOS is commonly considered an ovarian disease. However, impaired steroid hormone negative feedback to the hypothalamic-pituitary axis is suggestive of impairments at the very top of this axis, making the brain a prime candidate in both the ontogeny and pathology of PCOS. Our work, in a prenatally androgenized (PNA) mouse model of the syndrome, has identified changes within the gonadotropin-releasing hormone (GnRH) neuronal network that controls pituitary gonadotropin secretion and ultimately fertility. Specifically, anatomical studies have identified that GnRH neurons receive greater synaptic input from GABAergic neurons originating within the arcuate nucleus (ARN) of the hypothalamus. ARN GABA neurons are largely steroid hormone sensitive but are less sensitive to progesterone in PNA animals, suggesting that these circuit changes may reflect and mediate the neuroendocrine pathology of PCOS. More recently we have investigated the functional impact of selective ARN GABA neuron activation with Cre-dependent optogenetics. Specific activation of ARN GABA neurons leads to a dramatic and long lasting increase in lutienizing hormone (LH) secretion, suggesting that ARN GABA neurons may be responsible for driving the hyperactive GnRH/LH system and associated downstream consequences of PCOS. In addition, although increased GABA innervation to GnRH neurons in PNA mice is present prior to the pubertal onset of PCOS-like features, androgen receptor blockade can restore normal GABA innervation to GnRH neurons and some aspects of reproductive function. These findings provide compelling support for the key role of the brain and specific brain circuits in the development and pathophysiology of androgen induced forms of PCOS.

RB12: Non-invasive testing of embryo quality during IVF

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During IVF cycles multiple embryos are produced, therefore selecting the best quality embryo for transfer is critical to improving success rates and decreasing the time to pregnancy. Morphological evaluation at both the cleavage and blastocyst stage of development remains the primary method of embryo selection currently. However blastocyst grading is subjective, with significant levels of intra- and inter-user variability. In addition, blastocyst quality is rarely reflective of the embryo genetics; poor quality blastocysts are often euploid and, in many cases, high quality blastocysts are aneuploid. Direct genetic assessment of embryos through cell biopsy (PGS) is a valuable tool for embryo selection and may improve success rates by selecting only euploid embryos, however it is an invasive procedure. Therefore, identifying non-invasive strategies of embryo selection remains an important challenge of reproductive medicine. Evidence from our laboratory and others indicates that embryos release cell free DNA into their surrounding culture media. This DNA may represent a non-invasive source of genetic material on which to perform preimplantation genetic testing. However key issues need to be addressed, including whether this cell-free DNA is purely of embryonic origin, and whether there is consistency in results given the small quantity and the degraded nature of this DNA. Should non-invasive testing be reliable it will reduce the risk to the embryo, and may increase access for patients by offering a cost-reduction compared to conventional screening.

RB13: Profiling the placental epigenome in pre-eclampsia to predict pregnancy outcomes

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Pre-eclampsia is a dangerous and common placental condition that can lead to premature labour, seizures and death of mother and infant. Unfortunately, diagnosis is difficult as the clinical symptoms do not present until after mid-gestation. The only treatment option is early-induced delivery of the placenta and baby, despite fetal immaturity. Recent evidence suggests that epigenetics plays a critical role in the onset and severity of pre-eclampsia, and our extensive genome-wide DNA methylation sequencing of placental tissue from pre-eclamptic and normal pregnancies shows significant differences in methylation. We are currently determining whether our panel of candidate epigenetic biomarkers for pre-eclampsia can be identified in the circulating cell-free placental DNA that comprises approximately 10% of a pregnant women's blood plasma. We aim to identify a DNA methylation signature of pre-eclampsia in maternal plasma that can be used clinically to predict women who are at risk of developing this threatening condition of pregnancy.

RB14: Central adaptations to pregnancy

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Many changes occur in the pregnant female to prepare her for the physiological demands of motherhood. As well as the more obvious changes to the uterus and mammary glands, the brain is also undergoing a number of modifications that will result in marked changes to her body functions. The control of hormone secretion changes, such that the pattern of secretion is unique to the pregnant or lactating state. Her appetite increases, and she gains fat, in anticipation of the huge energy demands of lactation. Her reproductive cycle is stopped, focusing energy on the present pregnancy. She also becomes less responsive to stressors in her environment, and no longer mounts a fever in response to infection. These changes protect the baby from being exposed to factors that might harm development. Remarkably, there is increased neurogenesis in the maternal brain during pregnancy, contributing to changes in mood and behavior that accompany childbirth.

Prolactin is the hormone responsible for lactation, but also exert a broad range of actions in the body. We have identified widespread prolactin receptor expression throughout the hypothalamus, and have been investigating the hypothesis that prolactin actions might coordinate the multiple adaptive changes that occur in the maternal brain during pregnancy and lactation. We have developed a mouse line allowing conditional deletion of prolactin receptors from specific populations of hypothalamic neurons, and have now characterized a number of functions of prolactin in the brain. Most notably, we have demonstrated that prolactin receptor expression in the medial preoptic area is critical to the normal expression of maternal behavior. The data support the hypothesis that prolactin (and its pregnancy-specific homologue placental lactogen) provide a key afferent signal to the brain conveying information about the novel physiological states of pregnancy and lactation.

RB15: Should New Zealand support human embryo research?

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In September 2013 I started to plan (with my PhD student) a clinical trial of day 3 versus day 5 embryo transfer as part of an IVF cycle. And we immediately ran into what has become an insurmountable road block. Current clinical practice is to transfer embryos on day 3 or day 5 and the decision is based on view that day 5 transfer is superior. So we have seen a change in practice from predominantly day 3 transfer to day 5 transfer. The reason for this change it that it appears that day 5 is superior as most studies report on pregnancy rate per embryo transfer from a single transfer. As fewer embryos survive to day 5 then the denominator is smaller and makes it seem to many observers that indeed the pregnancy rate is better. In clinical studies this is known as selection bias. A clinical trial overcomes this limitation. Women would be randomised to receive a single embryo on day 3 or day 5. Remaining embryos are frozen. If no pregnancy occurs then the frozen embryos are transferred in subsequent cycles one by one. The cumulative live birth rate is then measured from both fresh and frozen embryos. The denominator is women who start the cycle, not women who get an embryo transferred.

However, this research has not been able to progress as the Health and Disability Ethics committee informed us that as this research involved the 'use of embryos" that this proposed research was not permitted. Discussions with the ECART chair resulted in two legal opinions which supported the view that the research could not progress. As the legal opinions were protected (and not able to be released) we then requested the Obudsmans office to intervene. Once we received the full legal opinions we have now reached a stalemate. The Associate Minister of Health has declined to meet to discuss embryo research. I have been advised by ECART to do an observational study which I have declined to do as it is not an appropriate study design.

This paper will explore the New Zealand policies with regard to embryo research and how we might make steps towards a new set of guidelines.

RB16: The ethical and biological implications of human oocyte freezing

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Oocyte vitrification, a technique that has rapidly become a standard of care in little more than a decade, offers women the opportunity to preserve fertility when faced with fertility-threatening disease or age-related limits to natural conception. While there are reports that frozen oocytes perform as well as fresh oocytes, commercial egg banks report a 10% rate of poor outcomes with oocytes from young donors preselected based on good performance in a fresh cycle. The reason for this variation from fresh to frozen is unknown; thus the variable nature of outcomes using frozen oocytes has significant implications on patients electing fertility preservation. The application of this still relatively unproven technology presents unique ethical dilemmas, particularly as it is promoted commercially for elective egg freezing. In addition to the societal implications of oocyte freezing, the physiology underling poor outcomes presents researchers with unique challenges. While oocyte vitrification yields consistently high survival and fertilization rates, normal and timely completion of the first mitotic division or progression to embryonic genome activation can be altered, resulting in embryos that lack the competence to develop to the blastocyst stage. Vitrification activates oocytes briefly with an increase of intracellular calcium before the activation cascade is arrested through suspended animation in liquid nitrogen, only to be restarted with the injection of sperm after warming. The impact of process variation, both in terms of an individual egg's responsiveness to activation and in the timing of activation before and after freezing, are poorly understood. Clinical examples of aberrant development following oocyte vitrification will be presented along with potential molecular mechanisms.

RB17: What are the paracrine contributions of mesenchymal stem/stromal cells to placental vascularisation?

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Background: The placenta's ability to exchange nutrients/gases between mum and baby is dependent on the establishment of an extensively branched network of blood vessels within it. However, this network is inadequate in pregnancies complicated by intrauterine growth restriction (IUGR). Mesenchymal stem/stromal cells (MSCs) from many tissues have pro-angiogenic properties that primarily result from their secretion of paracrine factors. In the placenta, MSCs reside in a perivascular niche where they likely influence angiogenesis. However, differences in the paracrine contributions of placental MSCs (pMSCs) throughout gestation, or in pathological pregnancies, have not been examined. Therefore, we aimed to determine how paracrine factors secreted by pMSCs influence normal and abnormal placental angiogenesis.

Methods: MSCs were isolated from first-trimester (n=5), term (n=5) or IUGR (n=4) placentae, phenotyped by multi-colour flow cytometry, and used to condition serum-free media (pMSC-CM). Differences in pMSC secretomes were determined by Quantibody Cytokine Arrays. The effect of pooled pMSC-CM on angiogenesis was determined over 16 hours using endothelial tube formation assays in conjunction with time-lapse microscopy.

Results and Conclusions: First-trimester and term pMSCs had different secretomes and completely different sets of pro-angiogenic cytokines were secreted by term pMSCs and first-trimester pMSCs. In angiogenesis tube formation assays, endothelial cells treated with first-trimester pMSC-CM formed shorter tubes with fewer branching points than those treated with term pMSC-CM (n=5, p=<0.001). These data suggest that pMSC contribute to placental vascularisation differently throughout gestation, which may correspond to different stages of vessel formation and expansion.

A significant inhibition of tube length and branching points was also observed in wells treated with IUGR pMSC-CM in comparison to term pMSC-CM (n=5, p=<0.001), suggesting that dysregulation of paracrine factors produced by IUGR pMSCs may contribute to inadequate vascular development in these placentae. Future work aims to identify specific cytokines responsible for the anti-angiogenic effects of IUGR pMSC-CM.

RB18: Minor changes to gene expression during mouse embryonic gonadal development, have long-term consequences for reproductive function in the adult

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The distinction between sexes is one of the most obvious examples of morphological dimorphism in the animal kingdom. Differences between the reproductive organs stem from a fate decision made during early gonadal development. While there has been extensive research into the differentiation of the ovaries and testes, there is much to learn about the urogenital ridge, a bipotential tissue that makes this all possible. The LIM-homeobox gene, Lhx9 is a transcription factor critical for the formation of the urogenital ridge.

To investigate the gene networks that underlie bipotential gonad formation, we used chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq). We identified ~5000 putative target genes of Lhx9, including some with known roles in gonad development, confirming Lhx9 is crucial for this process. RT-qPCR confirmed altered expression of some of these target genes in Lhx9+/- gonads. Gene ontology analyses showed that Lhx9 target genes are involved in biological processes such as angiogenesis, cell growth/proliferation and cell signaling.

Additionally, we found that Lhx9+/- adult mice, while initially fertile, develop gonadal abnormalities as they age. Analysis of the role of Lhx9 in oocyte quality and fertility is being undertaken using heterozyogous mice. Gene expression of several cell-type markers will be measured in both later stage embryonic gonad and adult gonads to assess changes to gene expression.

Having an understanding of the genetic pathways that underpin the formation and growth of the urogenital ridge are vital to understanding how abnormalities arise, this is particularly relevant in the context of disorders of sex development and infertility.

RB19: Fertility awareness

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Female fertility declines with age. One of the biggest factors in increasing infertility rates is couples, and especially women, leaving it too late to have their families. Ovarian aging exhibits no obvious signs, therefore, women who delay pregnancy later in life may be faced with unexpected fertility issues. The ability to accurately predict a woman's reproductive lifespan is becoming of increasing importance, however current techniques, lack long-term accuracy and predictability. Raising awareness of declining fertility is an important topic that needs to be addressed, but first, we need to be aware of current knowledge of fertility decline. University students are the group of people most likely to postpone parenthood, yet several international studies have shown that they overestimate their fertility. We designed a questionnaire where University students were asked to answer questions related to their awareness of fertility decline in spontaneous and IVF pregnancies, and methods they considered would prolong their reproductive lifespan. Our study has shown that New Zealand University students overestimated the rates of pregnancy for both spontaneous natural and IVF pregnancies. Students are mainly aware of the availability of Assisted Reproductive Technologies, however overestimate their effectiveness. Few students mentioned non-medical or well-being initiatives as measures to prolong parenthood. It is important University students are aware of the rate of fertility decline in women, as although Assisted Reproductive Technologies can be effective at times, they are not a guaranteed solution to an aging woman's fertility. New Zealand University students, like other cohorts, demonstrated an overestimation regarding the chances of a female's pregnancy and predicted the fertility decline to occur much later than it does in reality.

RB20: Female fertility preservation

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Improvements in cytotoxic therapies over the last two decades have resulted in increased long term survival rates from various types of cancer. For young women with malignant disease, the option to potentially preserve their fertility by cryopreserving ovarian tissue before commencing cytotoxic treatment is now a realistic option with over 80 babies born from the technology. The successful preservation of functional follicles had been initially established following xenografting of human frozen ovarian cortex. More recently, unequivocal evidence of successful cryopreservation of primordial follicles has been demonstrated by live birth via IVF following autografting to a heterotopic site in a woman who had previously undergone bilateral oophorectomy. However, for patients with leukaemia there is a high risk of reintroduction of disease with grafting and, therefore, other technologies such as in vitro follicle growth and in vitro maturation of oocytes from small antral follicles hold the best prospect for future fertility potential in these patients.

RB21: Resveratrol: Potential role in treatment of PCOS

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Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder among women in reproductive age. It is associated with hyperandrogenism, anovulation, and metabolic dysfunction including insulin resistance and low-grade systemic inflammation. While the etiology of PCOS is still poorly understood, extensive evidence points to hyperandrogenism as the key aspect of this condition. Our *in vitro* studies identified resveratrol, a natural polyphenol, as a potentially promising novel treatment of ovarian hyperandrogenism. Exposure of rat theca-interstitial cells to resveratrol resulted in a potent concentration-dependent inhibition of cell growth *via* a reduction of DNA synthesis and stimulation executioner caspases leading to apoptosis. We also found that resveratrol reduces androgen production by theca-interstitial cells by inhibition of Cyp17a1 mRNA expression. Recently, we completed a double-blind, placebo-controlled trial evaluated the effects of resveratrol on women with PCOS. Resveratrol treatment led to a significant decrease of total testosterone. In parallel, resveratrol induced a comparable decrease of DHEAS, a decrease of fasting insulin level and an increase of the Insulin Sensitivity Index. We propose that resveratrol improves ovarian and adrenal hyperandrogenism, at least in part, by improving insulin sensitivity and reducing insulin level.

RB22: Towards 'natural AI': generating allogeneic sperm in the testis of genetically sterilised sheep

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Mammalian male germline transmission depends on the RNA-binding gene *NANOS2* and *NANOS2* homozygous knockout males (-/-) are sterile. We aim to produce chimeric 'absolute transmitter' rams whose own germline has been replaced with that of an elite cloned donor embryo. This will be achieved by genetically disabling spermatogenesis and complementing the vacant sperm niche with germline-competent embryonic donors. For proof-of-concept, *NANOS2*$^{-/-}$ nuclear transfer (NT) host embryos have been aggregated with wild-type NT donor embryos carrying a red fluorescent protein (RFP) reporter. Once RFP cells have colonised the empty testes, all progeny sired by the transmitter rams will possess the donor haplotype. Successful developmental compensation would provide an alternative to artificial insemination (AI) in extensive farming systems, accelerating genetic gain through rapidly disseminating high-value sperm by 'natural AI'.

We employed CRISPR-Cas9 to disrupt *NANOS2* in male ovine fetal fibroblasts (OFFs). A single-stranded homology-directed repair (HDR) template was designed to mediate a small insertion, introducing a stop codon and *Taq1* restriction site. Using Taqman hydrolysis probes to quantify mutant and wild-type variants by droplet digital PCR, up to 15% HDR events were observed. Following single cell seeding, 45 clonal strains were isolated, comprising 11% homozygous mutant (5/45), 4% heterozygous (2/45), 62% wild-type (28/45), and 22% NHEJ *NANOS2* mutants. DNA sequencing and *Taq1* digest confirmed biallelic editing for 4/5 *NANOS2*$^{-/-}$ clones.

For embryo complementation, wild-type OFFs were modified by using the *Sleeping Beauty* transposase to insert *RFP* controlled by the constitutive *CAGGS* promoter. Cell clones with uniformly high *RFP*-expression were isolated and used for NT. *NANOS2*$^{-/-}$ NT blastocyst development was significantly lower than for isogenic *RFP* donors (9/203 = 4% vs 25/206 = 12%, respectively, $P<0.01$). Following transfer of 9 *NANOS2*$^{-/-}$, 14 *RFP* and 1 *NANOS2*$^{-/-}$*RFP* complemented blastocysts into recipient ewes, we obtained 0%, 21% and 0% pregnancies, respectively, which are currently ongoing.

RB23: Evolutionary comparisons of cytochrome P450 aromatase: structural insight into activity

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Estrogen is an essential mammalian hormone with major roles in reproduction, menopause, osteoporosis and hormone-dependent cancers. It is therefore critical to understand the molecular mechanism of estrogen synthesis. The biosynthesis of estrogens from androgens involves multiple hydroxylations of androgens. This is catalyzed by cytochrome P450 aromatase (P450arom) bound to the endoplasmic reticulum. Despite its importance, the X-ray crystal structure of P450arom was only obtained in 2009 [1] and its molecular mechanism still remains very poorly understood.

We have used a combination of biological, chemical and biophysical approaches to discover the molecular basis that is critical for the regulation of P450arom activity. We directly compared the human and porcine gonadal P450arom, as porcine gonadal P450arom has very low catalytic efficiency, with a ten-fold higher affinity (K_m) for a substrate (androstenedione) and ten-fold reduction in turn over (V_{max}) than the human P450arom [2]. We recombinantly expressed these proteins and compared their interactions with lipid membrane and also with the electron donor protein cytochrome P450 oxidoreductase [3]. We identified that human and not the porcine gonadal isoform acts as a homo-dimer. Using *in silico* analysis of the X-ray crystal structure we identified the site of homo-dimerisation in human P450arom and showed that these critical amino acids were substantially different in the porcine gonadal aromatase, rendering it capable of acting only as a monomer [4]. We conclude that the lower affinity and higher activity with reduced release of intermediate metabolites by the human isoform is as a consequence of its ability to form homo-dimers. However, there is more to understanding how these dimers function. Here we present how these proteins form functional structure that regulate the activity of aromatase and oestrogen biosynthesis.

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2. Corbin, C. J.; Mapes, S. M.; Lee, Y. M.; Conley, A. J., *Structural and functional differences among purified recombinant mammalian aromatases: glycosylation, N-terminal sequence and kinetic analysis of human, bovine and the porcine placental and gonadal isozymes*, Mol. Cell. Endocrinol. (2003) 206, 147.
3. Praporski, S., Ng, S., Nguyen, A., Corbin, C.J., Mechler, A., Zheng, J., Conley, A.J., Martin, L.L., *Organization of Enzymes Involved in Sex Steroid Synthesis: Protein-protein interactions in lipid layers*, J. Biol. Chem. (2009), 284(48) 33224-33232.
4. Martin L.L., Holien J.K., Mizrachi D., Corbin C.J., Conley A.J., Parker M.W., Rodgers R.J., *Evolutionary comparisons predict that dimerization of cytochrome P450 aromatase increases enzymatic activity and efficiency*, J Steroid Biochem. Mol. Biol. (2015) 154, 294-301.

RB24: The role of arcuate nucleus kisspeptin neurons in the generation of luteinising hormone pulses

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Kisspeptin signalling through its receptor Kiss1r is essential for reproductive function. Despite the dogma that the kisspeptin-expressing (KP) neurons in the arcuate nucleus (ARN) are an essential component of the gonadotropin-releasing hormone (GnRH) neuron pulse generator, the field has lacked the tools to definitely examine this hypothesis. Combining a sequential blood collection procedure with transgenic mice and the inhibitory optogenetic tools archaerhodopsin (ArchT) and halorhodopsin (halo) has allowed us to remotely and reversibly control the activity of the ARN kisspeptin neurons and probe their role in the generation of GnRH and luteinising hormone (LH) pulses. Adeno-associated viral vectors (AAVs) were injected bilaterally into the ARN of KP-cre mice to specifically and exclusively target the expression of ArchT or halo to the ARN KP neurons. During the sequential blood sampling procedure, the ARN KP neurons were illuminated with 532nm laser light via an indwelling bilateral fiberoptic cannula for 30min. Illumination with 532nm light, and not 473nm light, resulted in an inhibition in LH secretion for at least the duration of illumination in KP-cre mice expressing either ArchT or halo in ARN KP neurons. Illumination of the ARN KP neurons of wildtype mice injected with the AAVs did not alter LH secretion. Taking advantage of the strong rebound excitation of ARN^{KISS} neurons following inhibition with halorhodopsin, we found that re-setting the activity of the ARN KP neurons resulted in a re-setting of pulsatile LH secretion. These data indicate that the ARN KP neurons are critical for pulsatile secretion of GnRH and LH and likely represent the so-called “GnRH pulse generator”.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
RB25	Activation of Arcuate Neuropeptide Y Neurons Alters Luteinising Hormone Secretion in Mice	Eulalia Coutinho	Department of Physiology, Otago School of Biomedical Sciences; Centre for Neuroendocrinology, University of Otago, Dunedin, NZ
RB26	Potential mechanisms underlying the relationship between gestational nutrition and post-natal fertility	Peter Raymond Smith	AgResearch Invermay, Mosgiel, NZ; Department of Anatomy, University of Otago, Dunedin, NZ
RB27	Mitochondrial DNA content in oocytes from transgenic AMH over-expressing mice	Savana Woodcock	Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, NZ
RB28	Changes in prolactin responsiveness of glutamate and nNos neurons in the female mouse brain during lactation	Rosie Brown	Department of Anatomy and Centre for Neuro-endocrinology, School of Biomedical Sciences, University of Otago, Dunedin; Maurice Wilkins Centre for Molecular Biodiscovery, NZ
RB29	Deciphering the role of arcuate GABA neurons in fertility regulation with chemogenetic tools *in vivo*	Elodie Desroziers	Department of Physiology, Otago School of Biomedical Sciences; Centre for Neuroendocrinology, University of Otago, Dunedin, NZ
RB30	Deletion of protein tyrosine phosphatase 1B from forebrain neurons does not prevent the onset of diet-induced infertility in female mice	Caroline Ancel	Centre for Neuroendocrinology and Department of Anatomy, University of Otago School of Medical Sciences, Dunedin, NZ
RB31	Investigating the role of microglia in	Sarah Holland	Department of

	polycystic ovary syndrome (PCOS)		Physiology, Otago School of Biomedical Sciences; Centre for Neuroendocrinology, University of Otago, Dunedin, NZ
RB32	TRPV regulation of magnocellular neurosecretory cell activity in lactation	Alexander Seymour	Centre for Neuroendocrinology, Brain Health Research Centre and Department of Physiology, University of Otago, Dunedin, NZ
RB33	Optogenetic activation of arcuate nucleus GABA neurons in vivo elicits luteinizing hormone secretion in mice	Mauro Silva	Centre for Neuroendocrinology, Department of Physiology, University of Otago, Dunedin, NZ
RB34	The role of activin C in follicular development	Karen Reader	Department of Anatomy, University of Otago, Dunedin, NZ
RB35	Paraventricular nucleus kisspeptin fibres arise from kisspeptin cell bodies located in the periventricular nucleus in the mouse	Rachael Augustine	Brain Health Research Centre, Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, NZ
RB36	The role of RFRP neurons in murine puberty onset and anxiety	India L. Sawyer	Department of Anatomy and Centre for Neuroendocrinology, University of Otago School of Biomedical Sciences, Dunedin, NZ
RB37	Prolactin effects on kisspeptin fibre expression in the paraventricular and supraoptic nucleus of the mouse	Shalini Kumar	Brain Health Research Centre, Centre for Neuroendocrinology and Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin, NZ
RB38	Regulation of the maternal hypothalamic-pituitary-adrenal axis	Papillon Gustafson	Centre for Neuroendocrinology,

	by prolactin		University of Otago, Dunedin, NZ
RB39	Finding CLARITY: Visualising the Gonadotrophin-Releasing Hormone neuron	Mel Prescott	Department of Physiology, Otago School of Biomedical Sciences; Centre for Neuroendocrinology, University of Otago, Dunedin, NZ
RB40	TRPV1 Expression in the Supraoptic Nucleus of Pregnant Rats	Emily Brown	Brain Health Research Centre, Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, NZ
RB41	Using AMH as a predictor of fertility in sheep	Laurel Quirke	AgResearch, Invermay Agricultural Centre, Mosgiel, NZ
RB42	Using DREADDs to elucidate the role of AgRP neurons in the control of reproduction	George Connolly	Department of Anatomy and Centre for Neuroendocrinology, University of Otago School of Biomedical Sciences, Dunedin, NZ

RB25: Activation of Arcuate Neuropeptide Y Neurons Alters Luteinising Hormone Secretion in Mice

Coutinho, E.A.[1,2], Desroziers, E.[1,2], Prescott, M.[1,2], Campbell, R.E.[1,2]

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It is well known that the gonadotropin-releasing hormone (GnRH) neurons regulate the pulsatile secretion of luteinising hormone (LH) and follicle stimulating hormone (FSH) by the pituitary. However, the components of the GnRH neural circuit and how this circuit is altered in pathological conditions like polycystic ovary syndrome (PCOS) remain unclear. It is crucial to identify the afferent neurons that play an important role to sense and integrate humoral signals like hormones and relay this information to GnRH neurons. Recent work from our lab in a mouse model of PCOS suggests that GABAergic neurons in the arcuate nucleus of the hypothalamus (ARN) may serve as important afferent inputs to the GnRH neurons. Our finding that 1/3rd of these ARN GABA neurons co-express neuropeptide Y (NPY), has led us to investigate this subpopulation. NPY has long been implicated in the regulation of fertility, though the specific role of ARN NPY neurons in vivo in regulating GnRH neuron activity and LH release is still unknown. In the present study, we used the stimulatory Designer Receptors Exclusively Activated by Designer Drugs (DREADD) 'hM3Dq' to activate ARN NPY neurons and then measured the output of GnRH neurons in conscious mice. As expected, we found that activation of NPY neurons by injection of clozapine-N-oxide (CNO), a specific DREADD ligand, increased food intake in mice with unilateral or bilateral expression of hM3Dq-mCherry. As a readout of GnRH neuron activity, we measured LH using ELISA from serial blood samples collected every 6 minutes before and after injection of saline or CNO. Interestingly, in ovariectomised (OVX) mice, injection of CNO decreased baseline LH secretion compared to saline and slowed the pulsatility of LH secretion, which suggests a slowing of GnRH pulse generation. This study demonstrates an important role for ARN NPY neurons in the afferent regulation of GnRH neurons.

RB26: Potential mechanisms underlying the relationship between gestational nutrition and post-natal fertility in Sheep

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Restricted gestational nutrition has been shown to impact the fertility of female offspring. The mechanisms underlying these impacts have yet to be established, however alterations to fetal germ cell development are thought to be important.

Ewes were placed on restricted or maintenance nutrition for the first 55 days of gestation, then given access to pasture ad libitum. In contrast to previous studies, this resulted in increased indicators of fertility in female offspring, and increased germ cell numbers in fetal ovaries at day 75 of gestation.

RNAseq analysis of day 55 and 75 fetal ovaries showed 69 genes differentially expressed between nutritional groups at day 55, and 145 genes differentially expressed at day 75. Affected canonical pathways included a number related to nitric oxide production and Ca^{2+} transport (day 55 & 75) and vitamin C metabolism and REDOX reactions (day 75 only), while gene ontology terms were predominantly related to ion transport and protease inhibitors.

It is hypothesized from this data that increased nitric oxide produced in response to restricted nutrition may increase DNA damage in germ cells which would lead to the negative effects on fertility in female offspring. However, the negative effects of nitric oxide may have been negated by the change in redox reaction pathways following the introduction of an ad lib diet, promoting positive effects of nitric oxide e.g. angiogenesis, cell proliferation.

The impact of decreased expression of protease inhibitors is less clear. Proteases play key roles in both germ cell autophagy/cell survival (proteases produced by the ATG genes) and apoptosis (caspases). Thus the impact of protease inhibitors on germ cells may be substantial.

This study has identified some potential mechanisms underlying the relationship between gestational nutrition and post-natal fertility. However, more research is required to determine the extent to which these mechanisms underlie this relationship.

RB27: Mitochondrial DNA content in oocytes from transgenic AMH over-expressing mice

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Female mice overexpressing Anti Mullerian Hormone have problems relating to early embryonic development, including blastocyst degeneration and increased miscarriage compared to wildtypes, however, the cause for these problems is unknown. We hypothesise that poor oocyte quality may be responsible for this phenotype. The mature oocyte contains the store of mitochondria needed for subsequent early embryonic development. Therefore, mitochondrial number below a certain threshold indicates a low-quality oocyte.

This project aims to investigate mtDNA content as an indicator of mitochondrial number in oocytes of AMH^{Tg} female mice compared to wildtype female mice to determine a possible reason for their infertility.

Oocytes were harvested and mitochondrial DNA was extracted by 10 rounds of freeze-thaw lysis. qPCR was then performed on AMH^{Tg} oocytes and wildtype oocytes to quantify mtDNA content between the two groups.

There was no significant difference in mtDNA copy numbers between AMH^{Tg} and wildtype oocytes ($P=0.218$). Therefore, the expansion of the mitochondrial population in developing oocytes does not appear to be defective in AMH^{Tg} mice. The developmental defects in AMH^{Tg} female dams are overt hence it will be surprising if the oocytes exhibit normal indications of quality. Future research will investigate other indicators of poor oocyte quality to determine the cause of the sub-fertility phenotype in AMH^{Tg} mice.

RB28: Changes in prolactin responsiveness of glutamate and nNos neurons in the female mouse brain during lactation

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The anterior pituitary hormone, prolactin, acts through the prolactin receptor in the maternal brain to induce numerous critical adaptations during pregnancy and lactation. Although we have identified extensive expression of prolactin receptor throughout the mouse forebrain, the identity (and therefore function) of many of these prolactin-responsive neurons remains unknown. As large populations of glutamatergic and nNos-expressing cells have previously been described in many of these regions, we aimed to identify whether prolactin acts on glutamatergic and nNos neurons in the virgin female mouse brain. Secondly, we aimed to investigate whether there are changes in prolactin signaling in these cells during lactation, when prolactin levels are chronically elevated.

Using immunohistochemical labeling of prolactin-induced phosphorylated signal transducer and activator of transcription 5 (pSTAT5) as a marker of activated prolactin receptors, we identified populations of prolactin-responsive glutamatergic neurons in the rostral preoptic area, medial preoptic area and ventromedial hypothalamus. Furthermore, many of these prolactin-responsive glutamatergic neurons also expressed nNos. By conditionally deleting prolactin receptors (*Prlr*$^{lox/lox}$) exclusively from glutamatergic neurons, using a mouse with cre recombinase expression restricted to cells that express the vesicular glutamate 2 transporter (VGlut-Cre), we demonstrated that the ventromedial hypothalamic prolactin-responsive neurons consists of a homogenous population of cells that all co-express glutamate and nNos. All pSTAT5 labelling was lost in this nucleus in glutamatergic cell-specific prolactin receptor knockout mice. In contrast, the preoptic area consists of heterogeneous populations of prolactin-responsive cells with subpopulations of cells expressing glutamate, nNos, both glutamate and nNos or neither cellular marker. In response to endogenously-elevated levels of prolactin during lactation, many of these populations showed significantly increased levels of prolactin-induced pSTAT5 labelling. These data provide the first description of populations of glutamatergic and nNos neurons that respond to prolactin, with changes in responses during lactation.

RB29: Deciphering the role of arcuate GABA neurons in fertility regulation with chemogenetic tools in vivo

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Polycystic ovary syndrome (PCOS) is the most common infertility disorder in women worldwide. In a mouse model reflecting the clinical phenotype of hyperandrogenism, impaired steroid hormone feedback and infertility, we identified an alteration in the gonadotropin-releasing hormone (GnRH) neuronal network. Specifically, GnRH neurons receive greater synaptic input from GABA neurons (GABA-N) residing in the arcuate nucleus (ARN)1. To address the functional relevance of ARN GABA-N in the regulation of fertility, we are using the chemogenetic tool: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Using a Cre/lox approach, we expressed the designer receptor hM3Dq specifically in ARN GABA-N via stereotaxic injection into the ARN of vesicular GABA transporter (VGAT-Cre) mice. The delivery of the designer drug (CNO) to activate hM3Dq was coupled with serial tail-tip blood sampling to detect luteinizing hormone (LH) secretion, a readout for GnRH secretion, in gonadally intact male (n=12) and female (n=9) mice or ovariectomised female (n=4) mice. Using this approach, we have been able to accurately target hM3Dq to ARN GABA-N, elicit food intake, and trigger cFos expression specifically in ARN GABA-N after peripheral injection of 1.5mg/kg of CNO. To date, there is no evidence that the activation of ARN GABA-N by an acute peripheral injection of CNO elicits LH secretion. This result is in contrast with recent unpublished data from our lab showing a clear LH release driven by the activation of ARN GABA-N with optogenetic stimulation (Mauro Silva, unpublished results), suggesting that the acute activation of ARN GABA-N via DREADDs is not sufficient to trigger GnRH/LH release. Therefore, we are currently investigating whether chronic ARN GABA-N activation achieved through CNO delivery in the water over a 2 week period will impact estrous cyclicity and the LH pulse profile.

1. Aleisha M. Moore and Rebecca E. Campbell, The neuroendocrine genesis of polycystic ovary syndrome : a role for arcuate nucleus GABA neurons. Journal of Steroid Biochemistry and Molecular Biology. 2015

RB30: Deletion of protein tyrosine phosphatase 1B from forebrain neurons does not prevent the onset of diet-induced infertility in female mice

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Obesity impacts a number of hormonal functions, including reproduction. Leptin, an adipocyte-secreted hormone, acts on the hypothalamus to inhibit food intake and increase energy expenditure and most obese individuals develop hyperleptinemia and leptin resistance. Leptin signalling is regulated by multiple mechanisms, including tyrosine phosphorylation. Protein tyrosine phosphatase 1B (PTP1B) is an important negative regulator of leptin signalling through the dephosphorylation of JAK2. PTP1B is a ubiquitously-expressed enzyme and shows enriched expression in areas of high leptin receptor expression in the brain, including in the hypothalamus. Consistent with its role as a negative regulator of leptin signaling, PTP1B knockout in mice results in lean and leptin hypersensitive animals, but the effects on high calorie diet (HCD)-induced infertility have yet to be established. In this study, we used neuron-specific PTP1B knockout mice, expressed on a DBA/2J background strain prone to diet-induced infertility, to elucidate whether PTP1B mediates the effects of HCD on the onset of female infertility. HCD-fed PTP1B knockout mice were somewhat protected from diet-induced obesity (82% of HCD-fed control body weight after 11 weeks on the diet, but 142% of normal chow-fed controls; both $P<0.05$), but showed no improvement in glucose homeostasis compared to their HCD-fed wild-type littermates. Examination of the average number of litters, age at which the dams delivered their last litter, and circulating LH levels revealed no improvement in diet-induced infertility in HCD-fed PTP1B knockout mice, compared to HCD-fed controls. This study indicates that PTP1B knockout in female mice does not prevent the onset of diet-induced infertility, in spite of moderately preventing diet-induced obesity. In contrast, we recently showed that deletion of another leptin signalling inhibitor, SOCS3, rescued mice from diet-induced infertility for about 1.5 months. Therefore, while it is likely that multiple factors contribute to diet-induced infertility, PTP1B does not appear to be a major player.

RB31: Investigating the role of microglia in polycystic ovary syndrome (PCOS)

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Polycystic Ovary Syndrome (PCOS) is the leading cause of anovulatory infertility worldwide. Although typically thought of as an ovarian disorder, there is now clear evidence that the brain is involved. Changes in neuronal inputs to rostral preoptic area (rPOA) Gonadotrophin Releasing Hormone (GnRH) neurons have been identified in a prenatally androgenized (PNA) mouse model of the syndrome [1]. These changes to GnRH inputs are dependent upon androgen signalling and likely contribute to the downstream ovarian pathology. The mechanisms responsible for this modified neuronal circuitry within PCOS remain unclear. Microglia respond to inflammation and produce an extensive repertoire of neuroactive molecules that regulate synaptogenesis and axon migration [2]. We hypothesized that microglia may play a role in the modified brain wiring of PCOS. Microglial coverage and morphology were analyzed in different hypothalamic nuclei in PNA and control female mice (treated prenatally with dihydrotestosterone or a vehicle during gestational days 16-18) and treated with either flutamide (an androgen receptor blocker) or a VEH oil solution from postnatal day 40-60 (n=6-7/groups). Nickel-DAB immunohistochemistry with the Iba-1 primary antibody was used to stain for microglia and the microglial coverage and morphological state was analyzed with Image J software. Our initial results showed no treatment effect on microglial coverage within the rPOA or Arcuate Nucleus (ARN). However, our preliminary results of microglia morphology (representing activational state) within the ARN showed the number of microglia cells in the “surveying/resting” state (i.e. with thin ramified processes) is reduced in PNA mice following androgen receptor blockade. Taken together, our preliminary results suggest no change microglial coverage, but rather a reduction in the number of “surveying” microglia within the ARN following flutamide treatment within PNA mice. These preliminary results suggest that the neuronal plasticity in PCOS-like mice associated with androgen receptor blockade may involve changes in microglia activational state.

1. Moore AM, Prescott M, Marshall CJ, Yip SH & Campbell RE. (2015). *Enhancement of a robust arcuate GABAergic input to gonadotropin-releasing hormone neurons in a model of polycystic ovarian syndrome*. Proceedings of the National Academy of Sciences 112, 596-601.

2. Béchade C, Cantaut-Belarif Y & Bessis A. (2013). *Microglial control of neuronal activity*. Frontiers in Cellular Neuroscience 7.

RB32: TRPV regulation of magnocellular neurosecretory cell activity in lactation

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Vasopressin and oxytocin are hormones that are produced by magnocellular neurosecretory cells in the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus. A major role of vasopressin is to control body water balance. Under normal physiological conditions, vasopressin regulates body fluid balance via transient receptor potential vanilloid (TRPV) channels. During lactation, a reduction in the threshold for vasopressin secretion reduces osmolality, leading to retention of body water to aid lactation. However, the mechanisms that lead to altered vasopressin secretion are unclear.

We made electrophysiological recordings from SON magnocellular neurosecretory cells *in vivo* in anaesthetised female virgin and lactating rats. Oxytocin and vasopressin neurons were distinguished by excitation following intravenous cholecystokinin, which only occurs in oxytocin neurons. The TRPV antagonist ruthenium red was perfused through the SON via a microdialysis probe for 1 h. Ruthenium red reduced the firing rate of some vasopressin neurons, and had no effect on others, but overall caused a reduction in the firing rate of the population in both virgin rats ($n = 11$) and lactating rats ($n = 8$; main effect of reproductive status, $P = 0.81$, main effect of time, $P < 0.001$, two-way repeated measures ANOVA), despite osmolality being ~ 10 mosmol kg^{-1} lower in lactating rats compared to virgin rats ($P < 0.01$, Student's *t*-test). Ruthenium had no overall effect on oxytocin neuron activity in virgin rats ($n = 7$; one-way repeated measures ANOVA, $P = 0.51$).

These results suggest that TRPV channels on vasopressin neurons are activated at lower osmolality during lactation, which might lower the threshold for vasopressin secretion. Therefore, increased TRPV channel activation in vasopressin neurons might cause increased water retention in pregnancy and lactation.

RB33: Optogenetic activation of arcuate nucleus GABA neurons *in vivo* elicits luteinizing hormone secretion in mice

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Gamma-amino butyric acid (GABA) neurons are considered key regulators within the gonadotropin-release hormone (GnRH) neuronal network controlling gonadotropin secretion and fertility. GABA neurons located in the arcuate nucleus (ARN) project directly to GnRH neurons. ARN GABA neuronal inputs on GnRH neurons are elevated in prenatally androgenized female mice that exhibit increased luteinizing hormone (LH) levels. Although typically an inhibitory neurotransmitter in the brain, GABA can excite GnRH neurons through activation of GABA type A receptor ($GABA_AR$). Therefore, we hypothesized that optogenetic activation of ARN GABA neurons triggers GnRH/LH secretion in mice. Transgenic mice expressing Cre recombinase in Vesicular GABA transporter (VGAT)-producing neurons were used to deliver channelrhodopsin expression exclusively in ARN GABA neurons. Diestrus female (N=6) and male (N=6) $VGAT^{+/-}$::TdTomato mice were bilaterally injected in the ARN with a cre-dependent viral vector, which express channelrhodopsin ChETA variant (AAV2/9-EF1α-DIO-*ChETA*-eYFP; 3.15 x 10^{13} GC/Ml). A control group (N=6) was composed of female mice $VGAT^{+/-}$::TdTomato mice injected with a control vector AAV2/9-eYFP (AAV2/9-EF1α-DIO-eYFP; 6.69 x 10^{13} GC/Ml) and C57BL6 mice injected with AAV2/9-ChETA-eYFP. Animals were implanted with a 100-µm-diameter optic fiber in the rostral preoptic area (rPOA) under isoflurane anaesthesia. Light pulses (473 nm) were delivered at 2 and 20 Hz (5 ms) over a period of 10 min and tail-tip blood samples (3 □L) were collected every 6 min. LH levels were measured by ELISA method. Optogenetic activation of ARN GABA neuron terminals in the rPOA induced a robust increase in LH following 20 Hz-stimulation but not a 2 Hz-stimulation in both females and males. LH levels remained higher than baseline values for over 30 minutes. The highest amplitude of LH release happened at 14, 17 and 23 minutes, after light was turned on. Control animals did not show any response to blue light stimulation. Our data provides the first evidence that a specific population of GABA neurons residing in the ARN are functionally relevant in GnRH neuron activation of LH secretion, suggesting an important role in the regulation of fertility in both sexes.

RB34: The role of activin C in follicular development

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Activins and inhibins play important roles in the development, growth and function of ovarian follicles. Activin C consists of a homo-dimer of the activin-βC subunit. It is expressed in the oocytes, granulosa cells, thecal cells and surface epithelium of mouse and human ovaries, and in the granulosa-like cells of adult granulosa cell tumours. However, the function of activin C in the ovary is currently unknown. Activin-βC can form a hetero-dimer with the activin-βA subunit and can bind to the activin A receptor, ACVR2[1,2]. Mice lacking inhibin-α develop granulosa cell tumours in their ovaries which secrete activin A, and the growth of these tumours has been shown to be reduced by increased activin C expression[3,4].

The aim of this study was to identify whether overexpression of activin C modulates early follicular development in mice with normal levels of inhibin-α and inhibin-α null mice. Stereology techniques were used to quantify oocyte and follicular diameters, and the percentage of different follicular types in ovaries from wild-type mice and those under-expressing inhibin-α, and/or over-expressing activin C, at 5-6 weeks of age. Overexpression of activin C did not appear to alter follicular development when compared to wild-type mice but it did modulate the development of abnormal early stage follicles in inhibin-α null mice. These results provide further evidence of a role for activin C in both normal and cancerous ovaries.

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RB35: Paraventricular nucleus kisspeptin fibres arise from kisspeptin cell bodies located in the periventricular nucleus in the mouse

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The hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei contain magnocellular neurons that synthesize oxytocin, which is released into the circulation from the posterior pituitary gland in response to action potential firing. Oxytocin promotes uterine contractions during parturition and milk let-down during lactation. Central kisspeptin administration increases oxytocin neuron activity in anaesthetized late-pregnant rats (days 18 – 21 of gestation) but not in non-pregnant rats. We have unpublished immunohistochemistry data from mice that show increased kisspeptin projections to the PVN and SON in late pregnancy. Kisspeptin fibres were seen to wrap around the cell bodies and the apical dendrites of oxytocin neurons. To identify the origin of these kisspeptin fibres, we injected a retrograde tracer into the PVN of pregnant mice. The fluorescent retrobeads are taken up by axon terminals in the PVN and retrogradely transported along the axon to the cell body. Four days after PVN injection, mice were perfused with 4% paraformaldehyde and brains were processed for immunohistochemistry to identify kisspeptin cell bodies and co-localization of green fluorescent retrobeads. The periventricular nucleus of the hypothalamus showed co-expression of green retrobeads in kisspeptin cell bodies. Other kisspeptin neuron populations in the anteroventral periventricular nucleus and arcuate populations did not contain retrobeads. Taken together, these results show increased kisspeptin fibre innervation in the PVN and SON at the end of pregnancy in the mouse and that these fibres arise from kisspeptin cell bodies located in the periventricular nucleus. As yet the functional significance of the increase in kisspeptin fibres during pregnancy is unknown but it appears likely to increase the excitability of oxytocin neurons for birth.

RB36: The role of RFRP neurons in murine puberty onset and anxiety

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RF-amide related-peptide (RFRP) neurons are thought to modulate reproductive function and stress responses[1]. Using transgenic mice which have stimulatory and inhibitory designer-receptors exclusively activated by designer-drugs (DREADDs) selectively expressed in RFRP neurons via a Cre-loxP system, we aim to explore the reproductive and behavioural effects of RFRP neurons non-invasively in vivo and to elucidate whether changes to RFRP neuronal tone can modulate puberty onset. To validate the efficacy of this model, we first investigated corticosterone concentrations from whole blood before and after stimulation of RFRP neurons using the synthetic DREADD ligand clozapine-n-oxide (CNO), or their inhibition with CNO during restraint-stress. RFRP neuronal stimulation was sufficient to elevate corticosterone levels from 36.1±7.9 and 46.1±5.9 ng/ml to 79.3±15.6 and 81.4±11.1 ng/ml, in females and males respectively ($p=0.036$ and $p=0.007$), whereas CNO was without effect in controls not expressing DREADDs ($p>0.05$). Inhibition of RFRP neuronal activity was insufficient to significantly abolish the corticosterone response to restraint-stress (restrained controls: 160.3±28.3 and 180.8±29.6 ng/ml vs restrained inhibitory DREADD-expressing mice: 201.4±31.0 and 113.4±25.0 ng/ml, for females and males respectively; $p=0.38$ and $p=0.08$). Current experiments are examining the role of RFRP neurons in reproductive function. In postnatal-day 26 mice, RFRP neurons will be chronically stimulated or inhibited (5 days of CNO delivered in drinking water) until the onset of puberty. We hypothesise RFRP stimulation will delay puberty onset in females, while it may accelerate puberty onset in males, since RFRP-3 administration modulates gonadotropin release in a sexually dimorphic manner (inhibiting the LH surge in female mice and stimulating LH secretion in male mice[2]). Tests assessing anxiety-like and depressive-like behaviours will determine the effects of transient changes in RFRP tone on stress responses. Elucidating the role of murine RFRP neurons is an important step towards understanding their role and therapeutic potential in human infertility and mental illness.

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RB37: Prolactin effects on kisspeptin fibre expression in the paraventricular and supraoptic nucleus of the mouse

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In New Zealand, approximately 7.4% of births are premature, which increases the risk of long-term health problems in the offspring. Labour starts with uterine contraction and so appropriate activation of the uterus during delivery ensures the normal timing of birth. The stimulus for uterine contraction is the posterior pituitary hormone, oxytocin. We have shown that kisspeptin excites the neurons that secrete oxytocin only in late pregnancy and that the kisspeptin fibre density around oxytocin neurons increases during pregnancy. Prolactin is another hormone that is proposed to signal pregnancy to the maternal brain. Both kisspeptin neurons and oxytocin neurons express prolactin receptors. Hence, we aim to determine whether prolonged subcutaneous (SC) prolactin infusion increases kisspeptin fibre density in the hypothalamic paraventricular and supraoptic nuclei (where the cell bodies of oxytocin neurons are located) in virgin wild-type mice. Immunohistochemistry (IHC) will be carried out for kisspeptin and oxytocin to determine whether kisspeptin fibres innervate the paraventricular and supraoptic nuclei after prolonged infusion (through SC mini-osmotic pumps for seven days) of ovine prolactin or vehicle. IHC for the canonical intracellular messenger activated by prolactin receptor, phosphorylated signal transducer and activator of transcription factor 5 (pSTAT5), will also be carried out to determine whether pSTAT5 is present in key cell populations in the brain that respond to prolactin and have prolactin receptors, as a positive control. This study will help us to understand whether prolonged activation of prolactin receptors, as occurs in pregnant mice, can increase kisspeptin fibre density around oxytocin neurons in virgin mice.

RB38: Regulation of the maternal hypothalamic-pituitary-adrenal axis by prolactin

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During pregnancy and lactation, the activity of the hypothalamic-pituitary-adrenal (HPA) axis is suppressed. The hormone prolactin may play a role in mediating this suppression as concentrations are elevated during pregnancy and lactation and prolactin has known anti-stress actions[1]. This study investigated this proposal using a mouse model. *In situ* hybridisation revealed a decrease in the number of *Crh* mRNA-expressing neurons in the hypothalamic paraventricular nucleus (PVN) of pregnant (day 18; 55.6 ± 9.0 cells per section) and lactating (day 7; 97.4 ± 4.9) mice in comparison to diestrous controls (186.8 ± 18.7; $p < 0.01$, Tukey-Kramer test; n = 6-7). Removal of the pups (24 h), and thus suckling-induced prolactin secretion, restored CRH neuron number (180.1 ± 19.7; n = 7).

To investigate the role of prolactin during pregnancy in suppressing *Crh* mRNA expression, a transgenic mouse model (*$Prlr^{lox/lox}$/CamK-Cre)* was used to conditionally delete prolactin receptors (PRLRs) from forebrain neurons[2]. Widespread reductions in phosphorylated signal transducer and activator of transcription 5 (pSTAT5) expression (marker of PRLR activation), measured by immunohistochemistry, confirmed the success of this approach, although pSTAT5 expression remained in the PVN. *Crh* mRNA expression was then characterised using *in situ* hybridisation in virgin and pregnant (day 16-18) *$Prlr^{lox/lox}$/CamK-Cre* mice and *Cre*-negative controls (n = 3-5). *Crh* mRNA-expressing cell number was significantly reduced in pregnant animals ($F(1,14) = 82.37$, $p < 0.0001$, two-way ANOVA), however, widespread deletion of PRLRs in the brain did not reverse this effect ($F(1, 14) = 0.1980$, $p = 0.6632$). These data indicate that prolactin signalling is not necessary for the suppression of the CRH neurons during pregnancy. However, the persistence of pSTAT5 in the PVN suggests PRLR deletion may have been incomplete in this nucleus. Thus, it remains possible that prolactin targets neurons in the PVN to mediate its anti-stress actions.

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RB39: Finding CLARITY: Visualising the Gonadotrophin-Releasing Hormone neuron

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The Gonadotrophin-Releasing Hormone (GnRH) neuron is the final downstream neuron of the neuronal network controlling reproductive function. The GnRH neurons are dispersed throughout the rostral forebrain in a diffuse distribution, possessing remarkably long dendritic processes. This makes targeting and visualising the neurons for anatomical studies using classical approaches particularly challenging. Previous studies suggest long dendrites of GnRH neurons project millimetres through the rostral forebrain towards the median eminence (ME), and that some dendrites then transition into axons that ramify throughout the ME. However it has not been possible to visualise the entire dendritic projection of individual neurons located in the rostral preoptic area (rPOA). This study used advances in viral-mediated transduction tools combined with optical clearing to assess GnRH neuron morphology in its entirety. Female GnRH-cre mice (n=4) were injected with cre-dependent AAV channel rhodopsin (ChR) linked to mCherry, into the rPOA, to drive the expression of ChR-mCherry, which becomes docked in the membrane exclusively in GnRH neurons. Thick (1.5 mm) brain slices were cleared using the CLARITY method, immunostained for mCherry, and imaged using a Nikon 1A confocal with long-working distance objective. mCherry expression was restricted to GnRH neurons in the rPOA and thin fibre processes. Dendritic projections were indentified extending all the way to the ME and subsequently branching. Morphological features of the GnRH neuron, such as spines, could also be visualised along the length of the dendrite. Combining the viral delivery of conditionally expressed fluorophores into a select population of GnRH neurons, coupled with optical clearing is currently a promising approach to image the entire GnRH neuron *in situ* without risk of tissue loss. These findings support the presence of a dendrite-axon process in rPOA GnRH neurons, and raise additional questions about the functional role of this unique structure in the regulation of fertility.

RB40: TRPV1 expression in the supraoptic nucleus of pregnant rats

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Pregnant females have increased water retention to sustain placental blood supply for the developing child. Vasopressin is a neuropeptide hormone that promotes water retention and is secreted from the posterior pituitary gland by magnocellular neurons in the hypothalamic supraoptic and paraventricular nuclei. Secretion of vasopressin is activated by mechanosensitive TRPV channels that are present on the membrane of magnocellular neurons. TRPV (1, 2 and 4) channels are activated by membrane shrinkage due to high plasma osmolality (hyperosmolality). In pregnancy, water retention causes hypo-osmolality but vasopressin levels are similar to, or higher than, those in non-pregnant females. Therefore, the osmotic threshold for vasopressin secretion is lowered in pregnancy. However, the mechanism that reduces the threshold is unknown. Here, we tested the hypothesis that increased TRPV expression by vasopressin neurons decreases the osmotic threshold for vasopressin secretion in pregnancy. Blood samples and brains were collected from six female virgin and six late-pregnant (gestation day 21) Sprague-Dawley rats. Plasma osmolality was 305.6 ± 2.1 mosmol/kg in virgin rats and 293.4 ± 2.9 mosmol/kg in late-pregnant rats ($P < 0.0001$, unpaired t-test), confirming reduced osmolality during pregnancy. Brains were sectioned through the supraoptic nucleus of the hypothalamus and double-label immunohistochemistry was performed for vasopressin and TRPV1. There was no difference in the percentage of double-labelled cells in the supraoptic nuclei of virgin (7.4 ± 1.5%) and pregnant rats (10.7 ± 2.8%, $P = 0.3115$, unpaired t-test). Therefore, a change in the numbers of TRPV1-expressing vasopressin neurons does not lower the osmotic threshold for vasopressin secretion in pregnant rats.

RB41: Using AMH as a predictor of fertility in sheep

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The ability of a farmer to be able to detect and identify highly fertile ewes at a young age could provide the farmer with information on which to base management decisions (particularly around replacement stock) and therefore establish solutions to optimise profitability. Previous studies have demonstrated a relationship between plasma anti-Mullerian Hormone (AMH) concentration and fertility in cattle, however little is known about whether this relationship exists in sheep.

Ewe lambs born in 2013 (259) had a blood sample taken as a hogget (approximately 6 months of age, 2014) and as a 2-tooth (approximately 18 months of age, 2015). For each ewe, key measurements were recorded at birth, puberty, mating, gestation, and lambing. AMH concentrations were measured using the AnshLabs Ovine AMH ELISA kit (AL-155).

The main findings of this study are: (1) AMH levels increase with age, however the variation between individuals decreases with age; (2) AMH2014 levels and birth weight were significant predictors of whether a hogget would have a high number of antral follicles ($P=0.004$); and (3) there was evidence ($P=0.002$) that AMH2014 concentration and mate weight 2015 were related to NLB in 2015. Ewes with increased AMH concentrations were slightly more apt ($P<0.08$) to be pregnant than ewes with low AMH concentrations as both a hogget and a 2-tooth.

The objective of this study was to establish whether the reproductive potential of a sheep can be predicted by AMH measurement. We have shown that there is a relationship between AMH and a number of measures of fertility, however the data is very variable and AMH concentrations explain only a small fraction of this variation. Future work would aim to ensure AMH measurements are as precise as possible and identify alternate mechanisms that drive sheep fertility.

RB42: Using DREADDs to elucidate the role of AgRP neurons in the control of reproduction

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Agouti-related peptide (AgRP) neurons are thought to inhibit the activity of hypothalamic gonadotrophin-releasing hormone (GnRH) neurons which control fertility, but the nature of this role remains unclear. The activity of these neurons is modulated by metabolic hormones such as leptin, insulin and ghrelin. Because of this, these neurons act as an integrative system to convey metabolic cues to the reproductive system. Through using new 'Designer Receptors Exclusively Activated by Designer Drugs' (DREADDs) technology, we can now selectively activate and silence these AgRP neurons non-invasively *in vivo* using the synthetic ligand CNO to observe the effect of AgRP neuron activity in reproduction. To validate new mouse lines expressing excitatory (hM3Dq) or silencing (hM4Di) DREADDs under the control of Cre recombinase specifically in AgRP neurons, we chronically treated these mice with CNO administered through the drinking water (0.025 mg/ml). A pronounced body weight gain of 13.7% in response to AgRP neuronal excitation and loss of 11.5% in response to AgRP neuronal silencing was observed, which is consistent with the known orexigenic effects of these neurons. Using these validated mice, we are now evaluating reproductive activity in response to the activation or silencing of AgRP neurons. We hypothesize that increasing the tone of AgRP neurons will inhibit reproductive function, while decreasing the tone will have the opposite effect. Puberty onset, estrous cycles, mating success and LH concentrations will be evaluated in AgRP neuron excited, inhibited and non-DREADD expressing control mice (n=10/group). These experiments are currently underway. If AgRP neurons are inhibitory to reproduction, we would expect to see later puberty onset and time to mating, as well as a decreased LH concentration in the AgRP stimulated mice. AgRP silenced animals may show earlier puberty and increased reproductive function. These will reveal for the first time how AgRP neurons manipulate the HPG axis.