Tuesday April 20

Session Co-Chairs: Aleisha Moore and Allan Herbison

5:40 pm Maria Phylactou (Imperial College London)

5:50 pm Inmaculada Velasco Aguayo (IMIBIC)

6:00 pm Eliana Aerts (West Virginia University)

6:10 pm Silvia Leon (Harvard Medical School/Brigham & Women's Hospital)

6:20 pm Margaret Mohr (UCLA**)**

6:30 pm Nimisha Nandankar (Rutgers University)

6:40 pm Teresa Chou (University of California, San Diego)

6:50 pm Thatiane Ramalho (UFMG)

(all times EDT [New York time])

PERFORMANCE OF PLASMA KISSPEPTIN AS A BIOMARKER FOR MISCARRIAGE IMPROVES WITH GESTATION DURING THE FIRST TRIMESTER

Maria Phylactou[a], Ali Abbara[a], Maya Al-Memar[b], Christopher Kyriacou[b], Pei Chia Eng[a], Rans Nadir[a], Chioma Izzi-Engbeaya[a], Sophie A. Clarke[a], Edouard G. Mills[a], Elisabeth Daniels[a], Lechun Huo[a], Ewa Pacuszka[a], Lisa Yang[a], Bijal Patel[a], Tricia Tan[a], Paul Bech[a], Alexander N Comninos[a], Hanine Fourie[b], Tom W Kelsey[c], Tom Bourne[b,d], Waljit S Dhillo[a]

a. Section of Endocrinology and Investigative Medicine, Imperial College London, Hammersmith Hospital, London, UK.
b. Tommy's National Centre for Miscarriage Research, Queen Charlotte's and Chelsea Hospital, Imperial College London, London, UK.
c. School of Computer Science, University of St Andrews, St Andrews, UK.
d. KU Leuven, Department of Development and Regeneration, Leuven, Belgium.

Context: Miscarriage is the commonest pregnancy complication, but there are currently no biomarkers in clinical use to predict pregnancy loss. Kisspeptin is highly expressed in placental syncytiotrophoblasts and has emerged as a putative regulator of placentation. Preliminary data suggest that circulating kisspeptin levels are reduced in women with miscarriage, but its discriminatory performance at different gestations during the first trimester has not been assessed.
Objective: Compare the performance of kisspeptin and beta human chorionic gonadotropin (βhCG), both alone and in combination, as biomarkers for miscarriage throughout the first trimester.
Methods: Women with confirmed intrauterine pregnancy underwent serial ultrasound scans and blood sampling every 1-2 weeks during the first trimester. The ability of plasma kisspeptin and βhCG levels to distinguish healthy asymptomatic pregnancies (n=265; 557 samples) from those with miscarriage (n=95; 173 samples) was assessed.
Results: Gestation-adjusted circulating kisspeptin and βhCG levels were lower, by 79% and 70% respectively, in samples from women with miscarriage than healthy pregnancies (P <0.0001). The area under the ROC curve for identifying miscarriage during the first trimester was: kisspeptin 0.874 (95%CI 0.844-0.904), βhCG 0.859 (95%CI 0.820-0.899), and for the sum of both markers 0.916 (95%CI 0.886-0.946). The performance of kisspeptin to identify miscarriage improved with increasing first trimester gestational week, whereas that of βhCG worsened. The odds of miscarriage decreased by 35% for every 100 pmol/L increase in plasma kisspeptin during the first trimester (95% CI 32% - 38%) (P<0.0001). Gestation-adjusted kisspeptin and βhCG levels were even lower in samples taken with closer proximity to the day of miscarriage. A decision tree model incorporating kisspeptin, βhCG and gestational age had 83-87% accuracy for the prediction of miscarriage.
Conclusion: Plasma kisspeptin is a promising biomarker for miscarriage and provides additional predictive value to βhCG alone, especially at later first trimester gestations.

DISSECTING THE ROLES OF KNDY-DERIVED KISSPEPTINS IN THE CONTROL OF REPRODUCTION AND METABOLISM USING A NOVEL *TAC2*-SPECIFIC KISS1 KO (TAKKO) MOUSE LINE

Inmaculada Velasco[1-3], Delphine Franssen[1,4], Francisco Ruiz-Pino[1,5], Encarnación Torres[1-3], Elvira Rodríguez[1-3], Miguel Ruiz-Cruz[1-3], Silvia León[1-3], Diego Luque-Cordoba[1], Feliciano Priego-Capote[1], Krisztina Kukoricza[6], Fuping Zhang[6], Suvi Ruohonen[6], Matti Poutanen[6], María Jesús Vázquez[1-3], Manuel Tena-Sempere[1-3,5]

[1]Instituto Maimónides de Investigación Biomédica de Cordoba (IMIBIC)
[2]Department of Cell Biology, Physiology and Immunology, University of Córdoba
[3]Hospital Universitario Reina Sofía, Cordoba, Spain
[4]GIGA-Neurosciences Unit. University of Liège, Liège, Belgium
[5]CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Spain
[6]Institute of Biomedicine, University of Turku, Finland.

Kiss1 neurons are master regulators of puberty and fertility. In addition, kisspeptins have been recently proposed to participate in the control of other bodily functions, including weight homeostasis and metabolism. A prominent population of Kiss1 neurons is located in the arcuate nucleus (ARC), where a proportion of Kiss1 neurons co-express the neuro-peptides, Neurokinin B (NKB; encoded by *Tac2*) and Dynorphin (Dyn), named KNDy neurons. However, NKB-only and Kiss1-only neurons are also found in the ARC. Yet, the relative contribution of the different subsets of Kiss1 neurons to the control of reproductive function and metabolism remains unexplored. We report here the generation and characterization of a novel mouse line with conditional ablation of *Kiss1* in *Tac2*-expressing neurons, namely the TaKKO mouse, to dissect the precise roles of KNDy-born kisspeptins in vivo.

TaKKO mice of both sexes displayed a substantial decrease of ARC *Kiss1* expression, whose suppression was greater in females and already detectable at the infantile-pubertal period. Yet, *Tac2* mRNA levels remain unaltered. Despite the drop of ARC *Kiss1*/kisspeptin levels, puberty onset occurred at normal timing in both sexes. However, in females, gonadal weights were markedly decreased in young adult (2-mo-old) TaKKO mice, which displayed also reduced basal LH levels, disturbed LH pulsatility and perturbed sex steroid levels. In good agreement, ovarian histology and fertility tests denoted a suppression of reproductive capacity already at this early age in females, while testicular histology and fertility of TaKKO males were totally conserved. Premature ovulatory failure was confirmed in 12-mo-old TaKKO females. Additionally, metabolic analyses pointed out that ablation of *Kiss*1 in KNDy neurons leads to changes in body composition and energy expenditure, together with reduced locomotor activity in both sexes. Altogether, our data indicate that KNDy-born kisspeptins are dispensable/compensable for puberty but required for maintenance of female reproductive function and adult metabolic homeostasis.

EFFECT OF ABLATING NK3R-EXPRESSING NEURONS WITHIN THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS ON PUBERTY ONSET, THE LH SURGE, AND RESPONSE TO INTRAVENOUS SENKTIDE IN SHEEP.

Eliana Aerts[1], Max Griesgraber[1], Elizabeth Bowdridge[1], Steve Hardy[1], Robert Goodman[1,2], Casey C Nestor[3], Stan Hileman[1,2]

[1]Dept. of Physiology and Pharmacology and [2]Dept. of Neuroscience, West Virginia University, Morgantown, WV 26508
[3]Dept. of Animal Science, North Carolina State University, Raleigh, NC 27695

Genetic disruption of the neurokinin B (NKB) receptor, NK3R, delays or prevents puberty in humans, but not mice. As there is no evidence for this in sheep, we tested the hypothesis that arcuate (ARC) cells expressing NK3R are important for ovine puberty by using intra-ARC injections of an NK3-saporin conjugate (NK3-sap) to ablate cells expressing NK3R, including those containing kisspeptin. Prepubertal, ovary-intact ewe lambs (7 months of age) received one of three treatments: ARC NK3-sap injection (n=6), ARC Blank-sap injection (control; n=3), or sham surgery (control; n=2). Blood sampling began 14 days post-surgery (day 0) and elevated plasma progesterone concentrations were used as an indicator of puberty onset. Control ewes began cycling 29.8 $\pm$ 4.8 days after start of sample collection while cycles were absent to the end of the collection period (77 days) in all but one NK3-sap injected ewe. After day 77, ewes were subjected to an estrogen-induced LH surge protocol. All ewes exhibited an LH surge with no difference in time to LH surge peak, but there was a tendency ($p=0.08$) for lower LH surge amplitudes in NK3-sap ewes (133.6 $\pm$ 24.2 ng/ml) than in controls (208.2 $\pm$ 48.6 ng/ml). Finally, all ewes were injected i.v. with senktide (500 µg), an NK3R-agonist, and saline (vehicle; 3 ml). In NK3-sap injected ewes, LH concentrations following senktide treatment were significantly higher ($p<0.01$) than after saline injection, while they also tended to be higher ($p=0.08$) than saline in controls. Tissue is currently being assessed to identify neural populations ablated by NK3-sap injection, but these are very likely to include ARC kisspeptin neurons. Taken together, we suggest that ARC NK3R-expressing neurons (including those containing kisspeptin) are essential for puberty onset in sheep, but are less so for the LH surge and are not necessary for the response to i.v. senktide.

SEX SPECIFIC PUBERTAL AND METABOLIC REGULATION OF KISS1 NEURONS VIA NHLH2

Silvia Leon[1], Rajae Talbi[1], Elizabeth A. McCarthy[1], Chrysanthi Fergani[1], Kaitlin Ferrari[1], Lydie Naule[1], Ji Hae Choi[1], Rona S. Carroll[1], Ursula B. Kaiser[1], Alejandro Lomniczi[2], Victor M. Navarro[1].

[1]Department of Medicine, Division of Endocrinology, Diabetes and Hypertension, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA
[2]Division of Neuroscience, Oregon National Primate Research Center, Beaverton, Oregon, USA

Hypothalamic Kiss1 neurons control gonadotropin-releasing hormone (GnRH) release through the secretion of kisspeptin. Kiss1 neurons serve as a nodal center that conveys essential regulatory cues for the attainment and maintenance of reproductive function. Despite this critical role, the mechanisms that control kisspeptin synthesis and release remain largely unknown. Using Drop-Seq data from the arcuate nucleus of adult mice and *in situ* hybridization, we identified Nhlh2 as a transcription factor (TF) of the basic helix-loop-helix family enriched in Kiss1 neurons. JASPAR/Ciiider analysis revealed a binding site for Nhlh2 in the *Kiss1* and *Tac2* genes, but not in *Pdyn*. *In vitro* luciferase assays evidenced a robust enhancer action of Nhlh2 on human *KISS1* and *TAC3* promoters. The recruitment of Nhlh2 to the *KISS1* and *TAC3* promoters was further confirmed through chromatin immunoprecipitation. *In vivo* ablation of *Nhlh2* from Kiss1 neurons using *Kiss1*-cre:*Nhlh2*$^{fl/fl}$ (Kiss1^{Nhlh2KO}) mice induced a male-specific delay in puberty onset and subfertility, in line with a decrease in arcuate *Kiss1* expression. Females retained normal reproductive function albeit with irregular estrous cycles. Further analysis of male Kiss1^{Nhlh2KO} mice revealed higher susceptibility to metabolic challenges in the release of luteinizing hormone (LH) and impaired response to leptin. Overall, Nhlh2 in Kiss1 neurons contributes to the metabolic regulation of kisspeptin and NKB synthesis and release, with implications for the timing of puberty onset and regulation of fertility in male mice.

PROGESTERONE RECEPTORS IN RP3V KISSPEPTIN NEURONS ARE SUFFICIENT FOR ESTROGEN POSITIVE FEEDBACK OF THE LH SURGE

Margaret A. Mohr[1], Lourdes A. Esparza[2], Paige Steffen[2], Paul E. Micevych[1]*, and Alexander S. Kauffman[2]*

[1]Dept. of Neurobiology, University of California, Los Angeles, Los Angeles, CA
[2]Dept. of OBGYN and Reproductive Sciences, University of California, San Diego, La Jolla, CA
*co-senior authors

Estradiol (E2)-induced positive feedback culminates in the preovulatory surge of luteinizing hormone (LH), a critical event in reproduction. In rodents, estrogen positive feedback is thought to be regulated by kisspeptin neurons of the hypothalamic rostral periventricular nucleus (RP3V; also termed the AVPV). RP3V kisspeptin neurons co-express estrogen and progesterone receptors (PGR) and are activated during the LH surge. While E2's effects on kisspeptin neurons have been well-studied, progesterone's effects are less understood. We previously showed that peripheral E2 increases hypothalamic neuroprogesterone (neuroP) synthesis, which is required for the LH surge. Using an *in vitro* model of RP3V neurons, we also previously demonstrated that astrocyte-derived neuroP signals through nuclear and membrane PGRs and augments kisspeptin expression and release. *In vivo*, transgenic mice lacking PGR exclusively in kisspeptin cells (termed KissPRKOs) have diminished fecundity and do not generate normal LH surges, indicating that PGR signaling in kisspeptin cells is required for proper positive feedback. However, since PGR was knocked out from all kisspeptin cells, that study was unable to determine exactly which specific kisspeptin population in the brain mediates the necessary action of PGR on the LH surge. Here, we used Cre-targeted AAVs to re-introduce PGR selectively into RP3V kisspeptin neurons of adult KissPRKO females, and tested whether this rescues the occurrence and magnitude of the LH surge. We found that targeted upregulation of PGR in kisspeptin neurons exclusively in the RP3V is sufficient to restore proper E2-induced LH surges in KissPRKO females. This indicates that the RP3V kisspeptin population is a key target of the necessary progesterone action for LH surge generation, whereas PGR in other kisspeptin populations is not required for this event. These findings further highlight the critical importance of progesterone signaling, along with E2 signaling, in the positive feedback induction of LH surges and ovulation.

MICE WITH TARGETED ARCUATE KISSPEPTIN DELETION EXHIBIT DISRUPTED LH PULSATILITY AND IMPAIRED FERTILITY

Nimisha Nandankar[1], Ariel L. Negrón[1], Andrew Wolfe[2], Jon E. Levine[3], and Sally Radovick[1]

[1]Dept of Pediatrics, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ
[2]Division of Physiological and Pathological Sciences, National Institutes of Health, Bethesda, Maryland, USA
[3]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA

Hypothalamic kisspeptin is synthesized in two discrete nuclei – the anteroventral periventricular (AVPV) and the arcuate (ARC). AVPV *Kiss1* is necessary for the female pre-ovulatory luteinizing hormone (LH) surge and mediates estrogen-induced positive feedback regulation of GnRH and LH. In contrast, ARC *Kiss1* neurons are major regulators of pulsatile release of GnRH and LH, and mediate estrogen-induced negative feedback regulation of GnRH and LH. Previous studies have not fully separated the distinct roles of *Kiss1* in the AVPV versus ARC regarding downstream signaling for the maturation and maintenance of the reproductive axis. Consequentially, we generated a Prodynorphin-Cre/Kiss$^{fl/fl}$ knockout (KO) mouse model targeting ARC *Kiss1* to uncover the differential roles of ARC and AVPV *Kiss1*. After confirming ARC specific *Kiss1* loss through immunohistochemistry and qRT-PCR, we conducted a characterization of our KO model and found that females have persistent diestrus. We confirmed our hypothesis that conditional deletion of *Kiss1* in KNDy neurons would disrupt or ablate episodic GnRH/LH pulsatile release, where serial tail-tip blood collection demonstrated that KO mice of both sexes had decreased pulse frequency. To examine the end-organ effect of the loss of ARC kisspeptin, we examined gametogenesis, gonad morphology, and fertility. Hematoxylin and eosin (H&E) staining of serial-sectioned whole ovaries demonstrated that KO females had a complete absence of corpora lutea and arrested folliculogenesis, suggesting anovulation. 75% of the examined testes of KO males had a striking decrease in mature sperm in the seminiferous tubules. Furthermore, KO mice of both sexes exhibited a significant hypogonadal phenotype in the diminished size and weight of their gonads. In a controlled, continuous mating paradigm with proven WT mates, KO females were completely infertile, whereas KO males exhibited subfertility in accordance with their variable spermatogenesis phenotype. We conclude that ARC kisspeptin is necessary for estrous cyclicity, GnRH/LH pulsatility, gametogenesis, and ultimately fertility.

THE EXPRESSION OF THE HOMEODOMAIN TRANSCRIPTION FACTOR SIX3 WITHIN KISSPEPTIN NEURONS IS NECESSARY FOR FEMALE FECUNDITY IN MICE

Teresa Chou, Shanna N. Lavalle, Jacqueline Hernandez, Nay Chi P. Naing, Karen J. Tonsfeldt, and Pamela L. Mellon

Department of Obsterics, Gynecology, and Reproductive Sciences, Center for Reproductive Science and Medicine, University of California, San Diego, La Jolla, CA, USA

Kisspeptin neurons serve as critical regulators of reproduction, mediating puberty onset, ovulation, and pulsatile GnRH/luteinizing hormone (LH) secretion. Homeodomain transcription factors, such as SIX3, have been implicated in fertility, although the role of these proteins in regulating kisspeptin neuron activity is unclear. In mice, the loss of a single *Six3* allele results in reproductive impairments in both male and female mice. Prior studies found that deleting SIX3 specifically from GnRH neurons did not recapitulate the same phenotypes produced from a global heterozygous knockout of the *Six3* allele and imposed no reproductive abnormalities, suggesting that SIX3 populations outside of GnRH neurons are contributing to reproduction. We hypothesized that SIX3 in kisspeptin neurons is required for maximal fertility. We demonstrated for the first time that *Six3* is expressed in kisspeptin neurons in adult mice. We then used Kiss-Cre and Six3-Flox mouse lines to selectively delete *Six3* from kisspeptin neurons *in vivo*. In female mice, we found that the loss of SIX3 from kisspeptin neurons increased estrous cycle length and reduced female fecundity, but resulted in no changes to LH levels during diestrus or in the ability to induce an LH surge. On the morning of an induced LH surge, when LH levels are expected to remain low, LH levels were significantly increased in mice lacking SIX3 from kisspeptin neurons. Additionally, on the morning of the LH surge, *Kiss1* gene expression in anteroventral periventricular (AVPV) kisspeptin neurons was unchanged by the loss of SIX3, but *cFos* intensity was increased. Finally, we investigated transcriptional regulation *in vitro*, and found that SIX3 represses the human *KISS1* promoter but does not regulate the mouse *Kiss1* promoter.

KISSPEPTIN PREVENTS BONE LOSS CAUSED BY LACK OF TESTOSTERONE IN RATS

Thatiane V. Ramalho; Patrícia C. Henriques, Daniela O. Gusmão; Mariana S. Santos Soraia Macari; and Raphael E. Szawka

Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Deficiency of sex steroid hormones is related to bone loss in both in men and women. Besides controlling gonadal axis, kisspeptin may have direct effects on bone remodeling. In this study, we evaluated the effect of kisspeptin-10 (Kp10) on bone resorption caused by lack of testosterone. Adult rats were either orchiectomized (ORX) or subjected to sham surgery (Sham). Twenty one days after surgery, ORX rats were treated daily with Kp10, its antagonist kisspeptin-234 (Kp234), testosterone, or vehicle. Body weight, oxygen consumption, adiposity, femur length, and hormonal parameters, were measured. Femur samples were analyzed by computed micro-computed tomography, histomorphometry, and quantitative polymerase chain reaction. Treatment with Kp10 caused no changes in the metabolic parameters investigated compared to Sham or ORX rats. Prostate and seminal vesicle weight as well as the concentrations of luteinizing hormone, testosterone, estradiol, and prolactin in ORX rats treated with Kp10 were similar to those of ORX rats. ORX rats displayed trabecular bone loss in the femur, whereas Kp10 prevented this outcome similarly to testosterone. Moreover, femoral cortical bone was increased by Kp10 compared with ORX rats treated with Kp10+Kp234 or testosterone. Histological analyses demonstrated increased osteoblast number and fewer osteoclasts in ORX rats treated with Kp10 or testosterone than in ORX rats. Kp234 blocked all these bone effects of Kp10. In addition, orchiectomy increased bone expression of *Rankl*, *Opg* and *Runx2*, whereas Kp10 treatment restored their levels. However, the effects on gene expression were not blocked by Kp234. Our findings provide evidence of a selective effect of kisspeptin on bone homeostasis, which can protect against bone loss induced by the decline in sex steroids. This action is independent of the kisspeptin effects on the gonadal axis and seems to involve direct modulation of osteoblasts and osteoclast.