PROGRAMMING FOR PUBERTAL DEVELOPMENT BY BREASTFEEDING, HEIGHT, ADIPOSITY AND ADIPOSITY REBOUND

Alina German[1], Jay Belsky[2], Michael Shmoish[3], Renate Houts[4], Ze'ev Hochberg[5]

1 Pediatric endocrinology, Clalit HMO, Haifa, Israel
2 Human Development, University of California, USA
3 Bioinformatics Knowledge Unit, Technion, Haifa, Israel
4 Psychology and Neuroscience, Duke University, USA
5 Pediatric Endocrinology, Rambam Medical Center, Haifa, Israel

Context: Hereditary, environmental, and stochastic factors determine the onset of puberty in a unique environment. In an animal experiment we previously showed that lactation duration programs for growth, adiposity and developmental tempo.

Hypotheses: Early life event – breastfeeding, and body composition program both boys and girls for the pubertal onset as a reproductive strategy.

Methods: We analyzed longitudinal data from 1991-2006 of the NICHD and Human Development Study of Early Child Care and Youth Development (SECCYD) considering the onset of puberty (Tanner stage P2, B2 or G2 and menarche age [M]) as a function of maternal M, breast feeding, adiposity rebound age (AR), height SDS and BMI during childhood (age 36 mo) and juvenility (age 84 mo).

Results: In 659 girls, the AR occurred at age 62.4±14.7mo (M±SD), breast development stage-2 (B2) at 9.8±0.7y, pubic hair stage-2 (P2) - 10.3±0.7y, and M - 12.3±1.2y. In breastfed, the age of AR correlated positively (+ve) with B2 (p=0.001) and M (p=0.01). B2 correlated +ve with maternal M (p=0.00016), AR (p=0.001), and –ve with childhood (p=8.06E-0.8) and juvenile height (p=4.99E-0.9) and juvenile BMI (p=6.92E-0.8). M, but not B2, was later in breast fed, (p=0.0015), +ve correlated with AR (p=0.008), and –ve with juvenile height (p=0.0006) and BMI (p=1.64E-0.5), and childhood BMI (p=0.016). In 706 boys, AR occurred at age 65.0±15.4mo, genital development stage-2 (G2) at 10.3±0.5 y and P2 at 11.4±0.7y. Breast feeding duration didn't correlate with pubertal onset. P2 (p=0.008) but not G2 correlated +ve with AR. G2 correlated +ve with maternal M (p=0.006) and –ve with childhood (p=4.3E-5) and juvenile height (p=0.0003) but not with BMI.

Conclusions: Breastfeeding programs girls but not boys for slower maturational tempo, eventually by providing secure attachment. AR sets the ground for pubarche in boys only, and for puberty in girls only. Mothers' puberty has strong influence on boys' and girls' puberty. Childhood and less so juvenile height program the onset of puberty in both sexes and M, whereas adiposity influences puberty in girls only.

A NOVEL HOMOZYGOUS MISSENSE MUTATION IN STEROIDOGENIC FACTOR 1 CAUSE COMPLETE 46,XY SEX REVERSAL AND ASPLENIA

Ehud Banne[1], Yotam Kaufman[2], Ariella Weinberg-Shukron[2], Abdulsalam Abulibdeh[1], Pinchas Renbaum[2], Efrat Levy-Lahad[2], **David Zangen[1]**

1 Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Jerusalem, Israel

2 Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel

Background: Steroidogenic Factor 1 (SF-1) is important for steroidogenic and nonsteroidogenic tissue differentiation. Most reported SF-1 mutaions are heterozygous and result in mild to severe Disorders of Sex Development (DSD). We describe a unique clinical presentation of a novel homozygous SF-1 mutation.

Patient andMethods: Following routine ultrasonographic studies for abdominal pain revealling asplenia and the absence of a uterus in a 13.5 prepubertal usually healthy girl; karyotype was found to be 46XY. Further imaging,adrenal function testicular histology studies and molecular sequencing of the StAR and SF-1 genes were performed.

Results: CT studies confirmed asplenia, absence of uterus and ovaries, with gonads (testis) present in the inguinal regions. Peripheral blood smear revealed Howell Jolly bodies and pokilocytosis characterizing asplenia. Electrolytes, aldosterone as well as basal and ACTH stimulated cortisol, and 17–OH-progesterone were normal. Testicular histopathology revealed absence of germinal cells, no preneoplastic markers and very few Leidig cells. A homozygous G to A mutation in a cross species conserved region in exon 4 of the SF1 gene was identified, resulting in a non tolerable (SIFT program) R103Q substitution at the 103 position.

Conclusions: This novel homozygous SF-1 mutation is the first XY-DSD case worldwide associated with human asplenia, mimicking the asplenia in SF-1 knockout mice. The specific absence of testicular germinal precursors and Leidig cells indicates that SF-1 is specifically required for their differentiation but not for the presence and secretory function of Sertoli cells . The variety of phenotypes in SF-1 heterozygous mutations singles out this “clean” homozygous presentation illustrating the crucial role of the F1 box domain of SF-1 in severe DSD and asplenia. Asplenic and alternatively DSD patients should be screened for the reciprocal childhood asymptomatic condition.

ADAPTIVE PROGRAMMING FOR LIFE HISTORY TEMPO BY LACTAION DURATION IN RATS

Yonatan Crispel[1], Oren Katz[1], Ze'ev Hochberg[1]

1 Rappaport Faculty of Medicine and Research Institute, Technion Israel Institute of technology, Haifa, Israel

Hereditary, environmental, and stochastic factors determine developmental tempo in a unique environment, but their relative contribution to the phenotypic outcome is only partly known. *The subject of this study was the plasticity of maturation and developmental tempo in rat, the role of weaning from lactation and their trans-generation inheritance.* METHODS: Sprague-Dawley pups (generation F1), which usually wean on d21, were weaned by transfer to foster non-lactating mothers on d16, d21 or d26. On d60 females (F) and males (M) were mated within the weaning groups and F2 and F3 generation pups were investigated for d10 body composition by NMR, adult BMI, development and puberty. RESULTS: Early weaned F1 rats (d16) matured faster, with vaginal opening (VO) at d35±1 as compared to d37±1 for d26 weaned ($p < 0.001$). d16 weaned had their estrous (E) on d40±1 as compared to d42.5±1.5 for d26 weaned ($p<0.001$). Testicular growth of d16 F1M started on d35±1, as compared to d36.2±0.4 in d26 F1M ($p<0.0001$). BMI of d26-late weaned rats grew greater - by d60 and d90 they were 0.22±0.02 ($p<0.05$) and 0.25±0.01 g/cm2 as compared to 0.21±0.02 and 0.22±0.01 g/cm2, respectively, in d16 early weaning M ($p<0.05$). Overweight in late-weaned was associated with glucose intolerance (by OGTT) – peak glucose 258±40 vs 155±15 mg%, $p<0.0001$), and insulin resistance (by ITT) – nadir glucose 62±15 vs. 42±6, $p<0.01$, as well as reduced motor activity as measured in an open field ($p<0.05$) . Generation F2 d10 pups had greater fat deposit if their parents d16-weaned on as compared to offspring of parents d26-weaned (7±2 vs 2±1%, $p<0.0001$), whereas their lean body mass was smaller (73±2 vs. 82±3%, $p<0.05$). Offspring of late weaned d26 group (F2) postponed their infantile maturation: pups of d26 parents development fur at d10.4±1.6 (no difference between M and F) vs. d8.7±0.7 in F2 d16 ($p<0.05$). Ear detachment was also later on d13.1±0.6 in d26 F2 pups vs. d10.7±0.8 in d16 F2 pups ($p<0.05$). Eye opening occurred on d16.4±0.5 in d26 F2 vs. d15.7±0.5 in d16 F2M ($p<0.05$). VO, but not E, was earlier in F2 and F3 than F1 generations in d16 animals, but did not change in d21 and d26 groups. Testicular growth started earlier in F2 and F3 (d32.9±0.3) than F1 generations (35±1, $p<0.0001$) in d16 animals. Conclusions: Life history tempo adaptively shifts to slower when infancy, as defined by lactation, prolongs, and faster when infancy is shorter. The former is associated with characteristic modern life indolence: lesser motor activity, overweight, glucose intolerance and insulin resistance. Shifts in VO and testicular growth, but not E, increase from F1 to F3 generations.

DENDRITIC CELLS ARE INDISPENSABLE FOR OVULATION

Adva Cohen-Fredarow[1], Ari Tadmor[1], Tal Raz[1], Michl Neemam[1], Gil Mor[2], Nava Dekel[1]

1 Biological Regulation, Weizmann, Rehovot, Israel
2 Obstetrics and Gynecology, Yale University School of Medicine, New Haven, USA

<u>Introduction:</u> The analogy between ovulation and an inflammatory response has been suggested since the early eighties. This idea took into account the fact that during ovulation follicles become hyperemic, produce prostaglandins and synthesize a hyaluronan-rich extracellular matrix. Moreover, the preovulatory LH surge stimulates the expression of inflammatory-associated genes, increases vascular permeability and is followed by invasion of immune cells into the ovarian tissue.

<u>Aims:</u> We hypothesized that the immune cells, specifically dentritic cells (DCs), play a role in ovulation and corpus luteum formation. Our study was designed in order to test this hypothesis.

<u>Methods:</u> We used either wild type (WT) or transgenic mice, in which the expression of a DCs marker is conjugated to YFP, allowing their microscopic detection. Another transgenic mouse model used in this study enabled conditional depletion of DCs. All mice were subjected to the commonly used protocol of superovulation-induction by gonadotropins. The abundance of DCs was quantified by flow cytometry and changes in the vascular system were monitored by stereoscopic imaging.

<u>Results:</u> We found that DCs are recruited to the ovary in response to the ovulatory stimulus provided by human chorionic gonadotropin (hCG). Along this line, depletion of DCs blocked gonadotropin-induced ovulation, prevented the hCG-induced elevation of systemic progesterone and interfered with hCG-stimulated lymphangiogenesis. In addition, in the absence of DCS the profile of hCG-induced gene expression was altered

<u>Conclusion:</u> Our results provide, for the first time, strong evidence for the involvement of the ovarian DCs in controlling the ovulatory response further specifying their role in this process.

GNRH REGULATES TRANSCRIPTION OF THE GONADOTROPIN SUBUNIT GENES THROUGH ITS EFFECTS ON CHROMATIN

Andrea Wijeweera[1], Philippa Melamed[1]

1 Biology, Technion, Haifa, Israel

The synthesis and release of the luteinizing hormone (LH) and follicle stimulating hormone (FSH) are regulated by the gonadotropin releasing hormone (GnRH). GnRH regulates transcription of these genes at the level of chromatin, through displacing histone deacetylases, allowing subsequent histone acetylation. We hypothesize that transcriptional activation of the subunit genes by GnRH involves the induction of a sequence of histone modifications, including monoubiquitination of histone H2B at lysine K120 (H2BK120ub) and trimethylation of histone H3 at lysine 4 (H3K4me3), both of which were previously shown to be implicated in yeast and mammalian transcriptional regulation.

In the absence of GnRH, the murine gonadotrope αT3-1 cells express the α-subunit gene (αGSU). Upon GnRH stimulation, the expression of both β-subunit genes is induced and a further increase in αGSU expression is observed. In the basal state, ChIP studies indicate that both the LHβ and FSHβ promoters contain twice the amount of histones H2B and H3 as compared to the αGSU promoter. Additionally, while the higher levels of H2BK120ub are found on the LHβ promoter, the αGSU promoter was observed to have a five fold increase in levels of H3K4me3 as compared to the β-subunit gene promoters. Levels of total RNAPII and Ser5 phosphorylated RNAPII were also found to be significantly higher on the αGSU promoter than on the β-subunit gene promoters. In the presence of GnRH, ChIP studies normalized against levels of total H3 present at the promoters, demonstrated an increase in H3K4me3 on all promoters. ChIP studies normalized against levels of total H2B demonstrated that GnRH also increases H2BK120ub levels on all three subunit gene promoters.

These results indicate that, in the basal state, the αGSU promoter, unlike both the LHβ and FSHβ promoters, adopts a more “open” chromatin conformation that is already marked with modifications associated with actively transcribed genes. Stimulation with GnRH results in a further increase in levels of both H2BK120ub and H3K4me3 on all promoters, suggesting that GnRH regulates gonadotropin subunit gene transcription through the induction of various histone modifications.

ANTI-ANGIOGENIC EFFECTS OF THROMBOSPONDIN-1 IN OVARIAN GRANULOSA AND ENDOTHELIAL CELLS

Svetlana Farberov[1], Rina Meidan

1 Department of Animal Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

The thrombospondins (TSPs) comprise a family of extracellular proteins that participate in cell-to-cell and cell-to-matrix communication. Thrombospondin - 1 (TSP-1) is a multi-modular protein, that can exert its anti-angiogenic activity through multiple mechanisms involving different active sequences: binding and sequestration of FGF_2, activation of latent transforming growth factor (TGF-ß1) and/or other effects mediated by TSP-1 binding to its CD36 receptor. The relevance of TSP-1 to ovarian physiology was demonstrated in our recent studies in which we showed that this protein was specifically induced in the corpus luteum that undergoes luteolysis and was also dramatically induced in vitro by prostaglandin F2α in both granulosa cells (GC) and luteal endothelial cells (LEC). However, the effects of TSP-1 on luteal cells were not yet well defined. To that end we first examined the effects of TSP-1 in both GC and LEC under basal or exogenous FGF_2. FGF_2 dose-dependently enhanced cell migration and proliferation, whereas TSP-1 inhibited FGF_2 actions in both cell types. Furthermore, treatment with TSP-1 promoted endothelial cell apoptosis, evident by activation of caspase-3 and fragmentation of the nuclei. We next studied the effects of TSP-1 silencing by using siRNA constructs. These constructs effectively reduced TSP-1 gene and protein by 70% and 85%, respectively. While TSP-1 was silenced, FGF_2 mRNA levels were elevated in both LEC and GC, as well as CD36 in GC. In contrast, TGF-ß1 expression in LEC was reduced. Interestingly, in contrast to cells transfected with scrambled siRNA sequences, silenced LEC did not respond to added FGF_2 with cell proliferation, suggesting elevated endogenous bioavailable FGF_2.

These observations suggest that: 1) TSP-1 negatively regulate survival and viability of luteal cell types. 2) TSP-1 inhibits both the expression and the activity of FGF_2. 3) Changes in expression pattern of genes in TSP-1 silenced cells may further shed light on its actions.

DNA METHYLATION IN THE REGULATION OF GONADOTROPIN GENE EXPRESSION

Yahav Yosefzon[1], Philippa Melamed[1]
1 Biology, Technion, Haifa, Israel

Gonadotropin-releasing hormone (GnRH) induces expression of luteinizing hormone (LH) and follicle stimulating hormone (FSH). The genes encoding these hormones are quiescent soon after birth and their expression is re-initiated at puberty in response to GnRH. Thus GnRH is able to overcome repression of these genes, and we have shown that it reverses histone deacetylase-mediated repression and induces histone modifications at the LHβ and FSHβ promoters. However we hypothesized that GnRH activation of these genes involves additional modifications of the chromatin, including perhaps DNA demethylation. To test this, we have examined DNA methylation at the LHβ and FSHβ gene promoters and how it is affected by GnRH in immature αT3-1 gonadotropes that do not express the β-subunit genes, and in mature LβT2 cells that do express them. Using methylated DNA immunoprecipitation (MeDIP) and bisulfite sequencing, we show that both LHβ and FSHβ promoters are methylated in αT3-1 cells, with more methylated CpGs on the LHβ promoter than on the FSHβ promoter. Treatment with 5-Aza-dC, an inhibitor of DNA methyltransferases, increased LHβ but not FSHβ expression, while GnRH treatment shifted the levels of methylation at some of the CpGs on the LHβ gene. Moreover in the LβT2 gonadotropes, there is less methylation at the LHβ gene than in αT3-1 cells, while at the FSHβ gene it is no different. Thus LHβ methylation status appears related to the levels of gene expression and it changes with development and/or GnRH-treatment. Although mechanisms of DNA demethylation are still not understood, functional demethylation may be conferred by conversion of the methylcytosine to a hydroxymethylcytosine by Tet enzymes. Some of the Tet enzymes are developmentally down-regulated and we found Tet1 and Tet2 at higher levels in the immature than the mature gonadotropes, and also saw that GnRH regulates their expression. Moreover, Tet1 knockdown increased expression of the LHβ gene. We are currently testing the effects of the Tet enzymes and GnRH on hydroxymethylation of this gene.