Mitochondria-to-nucleus cross-talk: upregulation of mitochondrial protease transcription by 'protein stress' in steroid making cells

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Introduction: Being a highly metabolic organelle, mitochondria are subjected to various stresses such as ROS and unfolded protein accumulation. While undergoing functional differentiation, the mitochondria in steroidogenic cells are exposed to stress impacts exceedingly more than these organelles in any other cell type. That is due acquisition to the key proteins essential for steroid synthesis from cholesterol, i.e., the mitochondrial inner membrane monooxygenase CYP11A1, and STAR protein that translocates cholesterol substrate to CYP11A1. Upon completion, STAR is imported and floods the mitochondrial matrix with its inactive form. We suggest that the latter event imposes a 'protein stress' effect with potential organellar damage, to which the mitochondria operate a defense machinery of chaperone/proteases complexes acting to neutralize the 'ticking bomb' and rapidly degrade STAR. We addressed the question whether mitochondrial 'protein stress' can generate signaling to the nucleus and generate an upregulation of the organelle protease gene transcription (YES!).

Patients/ Methods: co-immunoprecipitations (coIP), in situ protein degradation assays by metabolic labeling, protein knock-down by siRNA, qRT-PCR and promoter analysis assays. The experimental models included hormone administered rat models and various cell lines.

Results: (a) STAR can physically interact with the membrane-bound m-AAA mitochondrial chaperone/protease complex (AFG3L2/SPG7), (b) Once reaching the mitochondrial matrix, a rapid STAR degradation is launched and proceeds consecutively by at least three proteases: the matrix LON protease, continuing with the inner membrane homo or hetero-oligomeric form of the AFG3L2/paraplegin protease/chaperone complexes, and a third yet unknown protease that concludes the elimination mission, (c) Simulation of mitochondrial 'protein-stress' caused by over-expression of STAR resulting in upregulation of Afgel2, Spg7 and Lon mRNAs levels and promoter activities. Consistent with this observation we show that hormonal induction of STAR in rats results in upregulation of the above proteases mRNAs levels in the ovary.

Conclusions: Our studies unravel the mechanism by which the mitochondria in steroidogenic cells respond to organelle stress. We named this effect as protein overload, or 'protein stress'. The findings suggest that in-house mitochondrial proteases serve as 'gate-keepers' of these organelles and prevent damage by a rapid removal of the threatening proteins. Proper activities of the proteases we describe herein are vital, as previously shown in the fatal neurodegenerative disorders hereditary spastic paraplegia and spinocerebellar ataxia type 28. We show that elevated mitochondrial 'protein stress' can signal upregulation of protease gene transcription in the nucleus.

Effect of sperm ligands on forward motility, hyperactivation and acrosome reaction, via ERK-mediated cascade

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Introduction: Mammalian sperm are activated by sperm ligands but the nature of the stimulating ligands is still not known. The signaling involved in ligand-stimulated spermatozoa is also not yet clarified and the main question in sperm biology is the identification of these sperm ligands and their mechanism of action. Mitogen activated protein kinases (MAPKs) are key regulatory enzymes in signal transduction. MAPKs are known to be involved in proliferation, differentiation, cell cycle control, apoptosis and transformation. We have recently characterized human sperm MAPKs, and implicated ERK and p38 in forward and hyperactivated motility and acrosome reaction (AR).However, the nature of the sperm ligands that activate ERK and p38 are not yet known. We have decided to concentrate our initial efforts on two potential sperm ligands, namely EGF and TGF-β3. We decided to compare their ability to activate ERK, and in parallel to stimulate forward and hyperactivated motility and acrosome reaction. Furthermore, we intend to find out if the activation of forward and hyperactivated motility and acrosome reaction by the above sperm ligands is mediated by ERK.

Patients/ Methods: Sperm samples from healthy donors were obtained from Sheba Medical Centre Sperm Bank, Tel-Hashomer Hospital.

Results: EGF and TGF-β3 activated ERK within 5 minutes. The effect was persistent and still detectable in sperm after capacitation. Secondly, we explored the effect of these ligands on human spermatozoa forward and hyperactivated motility. Incubation of normal spermatozoa with EGF, increased forward motility within the first 5 minutes, and hyperactivation after capacitation. Incubation with TGF-β3 increased both forward and hyperactivated motility within the first 5 min. Later, we examined whether the EGF-induced forward motility is mediated via ERK-dependent mechanism, by adding a selective inhibitor. Indeed, pre-incubation with the inhibitor reduced the percentage of motile sperm and abolished the effect of EGF on forward motility. As a final point, we investigated the effect of both EGF and TGF-β3 on AR and whether this effect is mediated by ERK. The preliminary results have shown that both ligands induced AR.

Conclusions: EGF and TGF-β3 stimulate sperm motility and AR via an ERK-mediated cascade.

Molecular pathways leading to GnRH-induced cell proliferation and death in gonadotropes

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Introduction: The gonadotropes are a population of cells in the pituitary that play a pivotal role in the mammalian reproductive system. When exposed to gonadotropin-releasing hormone (GnRH), these cells undergo several intracellular modifications leading to production and secretion of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). GnRH is also involved in the gonadotrope development and we have already reported that it leads to proliferation of immature, partially differentiated gonadotropes. However, this response is no longer seen in mature, fully differentiated gonadotropes, in which GnRH decreases cell numbers instead. Several MAPK cascades are activated by GnRH in the gonadotropes. However, the mechanisms downstream to the MAPK cascades that are responsible for mediating the GnRH-induced cell proliferation and death are not yet understood. Moreover, it is not clear at which point GnRH stops inducing proliferation and which event is responsible for this switch. We hypothesize that the Bcl-2 family proteins, Bax and Hrk, as well as prohibitin are at least partially responsible for mediating the effects of GnRH on proliferation or cell death of the gonadotropes. Here, we show that GnRH increases the levels of cleaved PARP in mature gonadotropes, while leading to their slight decrease in immature gonadotropes. Additionally, our data shows that GnRH leads to an increase in the levels of Bax and Hrk in mature gonadotropes, while decreasing the levels of Bax in immature gonadotropes, and that GnRH does not change the levels of Bcl-2 in either cell type. In addition to Bax, Hrk and Bcl-2, we have also found an effect of GnRH on prohibitin, a protein that may also be involved in preventing cell proliferation. We have previously reported that the protein levels of prohibitin are higher in the nuclei of mature gonadotropes, when compared to immature gonadotropes. Here, we show that GnRH increase the prohibitin mRNA levels in mature gonadotropes. We have also found that the prohibitin levels in the nuclear fraction decrease following GnRH treatment, indicating export of the protein from the nucleus. Our data indicates that this export is ERK1/2-dependent.

Conclusions: Together, our findings suggest that the different effects of GnRH on mature and immature gonadotropes with regard to proliferation and apoptosis may be at least partially caused by the different effects of the hormone on Bax, Hrk and prohibitin.

Sphingosine kinase is not upregulated by GnRH or its analogues

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Introduction: The mechanisms whereby GnRH-a decreases ovarian failure in young women exposed to gonadotoxic chemotherapy are unknown. One of the suggested possible mechanisms is the gonadal up regulation of Sphingosine-1-Phosphate [S1P], an antiapoptotic molecule. To examine such a possible mechanism we have evaluated the activity of Sphingosine Kinase [SK] which is the physiological enzyme generating S1P.

Patients/ Methods: Human granulosa cells were retrieved by follicular aspiration for IVF, after informed consent. The granulosa cell cultures [GCC] were established after separation of the GC's on Percoll and cultured in M199 with FCS and antibiotics. After 2-3 days and preincubation in serum free medium, the GC's were incubated with native GnRH, GnRH-agonist, GnRH-antagonist, dimethyl-sphingosine (DMS, an SK inhibitor), and control medium. After 24 hours, the cells were trypsinized, lysed, and the SK activity was determined by conversion of added Sphingosine to SK followed by TLC separation with radioactive P32, by phosphoimaging. ATP- P32 was added for labeling SK, according to the method of Sara Spiegel [Richmond, VA, USA].

Results: There was no significant change in SK activity following incubation with either GnRH or its analogues. Neither the agonist, nor the antagonist or native GnRH affected SK activity. Our results are validated by the fact that DMS inhibited SK activity

Conclusions: Neither GnRH, nor its analogues increase the activity of SK in GCC, in vitro. However, S1P might be still possibly involved in the protective mechanism of GnRH-a against chemotherapy associated gonadotoxicity through an inhibitory effect on S1P-phosphatase.

The roles of MAPK in human sperm functions

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Introduction: Human spermatozoa are the final product of a complex differentiation process that takes place in the testes. These cells swim through the female reproductive tract to reach the fertilization site and transfer the male DNA into the egg. During their course they encounter various signals such as hormones, that make them capacitated, and only then they are capable to fertilize the egg. MAPKs are crucial signaling proteins that convey signals of multiple stimulations, such as proliferation, differentiation, motility and stress into cells. The signal is conveyed by protein phosphorylation. Spermatozoa are known as transcriptionally quiet cells, and therefore phosphorylations are considered to be major means of signaling. We investigated the PKC/MAPK pathway in human sperm flagellar motility, hyperactivated motility and acrosome reaction.

Patients/ Methods: Ejaculates were obtained by masturbation from healthy donors after 3 days of sexual abstinence. After liquefaction, semen was layered on top of a percoll gradient (40/80), centrifuged and spermatozoa were separated, washed and diluted into Ham's F-10 that was added with 4 mg/ml BSA. The cells were then incubated in 37C at 5% CO2. Stimulants were added for the indicated times.

Results: ERK1/2 and p38 are localized to the sperm mid-piece and tail. In response to PMA, a known stimulator of PKC, we observed a marked increase in ERK phosphorylation that could be abolished by specific PKC and MEK1/2 inhibitors. PMA also stimulated flagellar and hyperactivated motility, as well as the acrosome reaction. The PMA-induced increase in flagellar and hyperactivated motility could be abolished by the MEK1/2 inhibitors. However, p38 inhibitors could increase flagellar and hyperactivated motility. The PMA-induced acrosome reaction could be markedly decreased by both MEK1/2 inhibitors and p38 inhibitors.

Conclusions: We could demonstrate that ERK1/2 are activated downstream to PKC in human sperm, and stimulate sperm flagellar motility, hyperactivation and acrosome reaction. P38 has an inhibitory role in sperm hyperactivation and flagellar motility, and a stimulatory role in the PMA-induced acrosome reaction.

The role of FYN kinase in the release from metaphase in Mammalian oocytes

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Introduction: Meiosis in mammalian oocytes starts during embryonic life and arrests for the first time before birth, at prophase of the first meiotic division. The second meiotic arrest occurs after spindle formation at metaphase of the second meiotic division in selected oocytes designated for ovulation. The fertilizing spermatozoon induces the release from MII arrest only after deactivation of the oocyte's spindle assembly checkpoint (SAC). Src family kinases (SFKs) are nine non-receptor protein tyrosine kinases that regulate many key cellular functions. Fyn is an SFK expressed in many cell types, including oocytes, that regulates many cellula. Our aim was to study the involvement of Fyn in the organization of the meiotic spindle and in the exit from metaphase during meiosis in rat and mouse oocytes, as a model for mammalian oocytes.r functions.

Patients/ Methods: Endogenous Fyn kinase was inhibited either by exposing the oocytes to SFKs inhibitors (SU6656 or PP2) or by microinjecting them with dominant negative form of Fyn (DN-Fyn) complementary RNA (cRNA) together with Histone-H2-RFP and β-Tubulin-GFP cRNAs. Fyn localization, spindle structure and chromosomes segregation were assessed by live cell confocal microscopy or immunostaining.

Results: Exposure of oocytes at the metaphase of either first or second meiotic divisions to SFKs inhibitors resulted in disruption and condensation of the spindle structure, reduction of the spindle size, misalignment of the chromosomes and appearance of microtubule (MTs) filaments throughout the cytoplasm in a time and dose dependent manner. Fyn co-localized with the spindle MTs under the inhibitory effect of SU6656 and even after recovery from the drug. Microinjection of DN-Fyn cRNA into the oocytes or exposing them to SU6656 or PP2 inhibited the exit from meiotic and mitotic metaphases. After washing the inhibitor, the spindle recovered and the oocytes gained the ability to exit from metaphase.

Conclusions: Altogether, it is suggested that Fyn plays an essential role in signaling events that involve the SAC pathway and hence in regulating the exit from metaphase during meiosis.

Deciphering the luteal transcriptome: Insights into possible mechanisms regulating corpus luteum regression

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Introduction: Prostaglandin F2α (PGF2α) is the principal luteolytic hormone however, despite its widespread use, the mechanisms by which it induces luteolysis are not well defined. In the mature corpus luteum (CL) PGF2α initiates a series of events culminating in its demise. However, during early luteal phase the gland is refractory to exogenous PGF2α. This lack of responsiveness cannot be attributed to a deficiency of PGF2α receptors and in fact various cell responses were observed. We hypothesized that functional genomics and individual luteal cell isolation will be instrumental in elucidating these mechanisms

Patients/ Methods: Microarray and bioinformatics analyses were used to screen genes differentially expressed between day (D)4 and D11 CLs (PGF2α refractory and responsive, respectively) collected 4h and 24h after PGF2α administration. The mRNA and protein expression of selected genes were validate by qPCR and western blots, respectively. Cell localisation of selected genes was studied by: i) endothelial (EC) and steroidogenic cells enriched from CL, using BS-1 coated magnetic beads and ii) small and large–like luteal cells obtained after in vitro luteinization as well as cultured luteal EC

Results: Microarray studies revealed robust differences in luteal gene expression on d4 versus d11 of the luteal phase. Over 500 transcripts differentially expressed in D4 vs D11 were identified. Seventy six genes unique to the early luteal stage were identified as genes involved in cell cycle. There were 164 genes whose expression increased on d11 only, most of them are involved in apoptoic cell death and immune response. We then selected 11 genes affecting major cellular pathways that were differentially expressed in these two developmental stages. Quite a few of these genes are known to be involved in angiogenesis: FGF2, PTX3, thrombospondins (THBS 1/2) and their cell adhesion receptor –CD36 were expressed in the steroidogenic and EC compartments of the CL alike. However while FGF2, a proangiogenic factor, was markedly elevated by PGF2α on D4 CL, THBS1 and 2, which inhibit angiogenesis, were induced only in the mature gland undergoing luteolysis. Interestingly, PTX3 known to bind and inhibit FGF2 action was inversely expressed with this growth factor. Selectins, E and P adhesion molecules, were restricted to luteal EC. Surprisingly, a similar pattern was observed for galanin (GAL), a neuropeptide found in brain. Its localization to luteal EC or any EC type is revealed here for the first time. Neuregulin1 (NRG1) was more abundant in the steroidogenic luteal cells and specifically in the luteinized theca cells. As with GAL, NRG-1 expression was stimulated by PGF2α only on D11 (PGF2α-responsive) but not D4 CL (PGF2α-refractory)

Conclusions: These studies are beginning to decipher the long sought after mechanisms involved in PGF2α- induced luteolysis

Elucidation of mechanisms of the reciprocal cross-talk between Gonadotropin-Releasing Hormone (GnRH) and Prostaglandin receptors.

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Introduction: We have recently described a novel GnRH receptor signaling pathway mediated by the prostaglandins PGF2α and PGI2, which acts through an autocrine/paracrine modality to limit autoregulation of the GnRH receptor and inhibit LH, but not FSH release. Here we further explore the cross-talk between GnRH and the PGs receptors.

Results: GnRH stimulates arachidonic acid (AA) release from LβT2 gonadotrope cells via the Ca2+-independent phospholipase A2 (iPLA2) and not via the more common Ca2+-dependent cytosolic phospholipase A2α (cPLA2α). LβT2 cells express various PLA2 family members such as sPLA2IIE, sPLA2V, iPLA2-A, iPLA2-γ, cPLA2β and cPLA2γ, but surprisingly not the common cPLA2α. AA release was followed by a marked induction of COX-1 and COX-2 by GnRH, via the PKC/c-Src/PI3K/MAPK pathway. COX-2 transcription by GnRH is mediated by the two NFkB sites and the C/EBP site within its promoter. Indeed, GnRH stimulates p65/RelA phosphorylation (22-fold) in LβT2 cells and the two NFkB sites apparently act as a composite response element. Although GnRH stimulates cAMP formation in LβT2 cells, we found no role for cAMP acting via the CRE site in the COX-2 promoter. PGF2α, PGI2 or PGE2 had no effect on basal- or GnRH-stimulated ERK, JNK and p38MAPK activation and cellular Ca2+ elevation. Although, PGF2α, PGI2 and PGE2 reduced GnRH-stimulated cAMP formation, we could not correlate it to the inhibition of GnRH receptor expression, which is exerted only by PGF2α and PGI2.

Conclusions: The inhibition by PGF2α and PGI2 of the autoregulation of GnRH receptor expression is most likely mediated via inhibition of GnRH-stimulated phosphoinositide turnover and not by inhibition of Ca2+ elevation and MAPK activation.

Sibling affinity and environmental influences on the infancy-childhood transition age population-based sample of Israeli infants

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Introduction: The transition from infancy to childhood (ICT) at age 7-13 months is marked by a growth spurt, when GH-IGF-1 axis activity sets in. The ICT age correlates negatively with, and determines 50% of the final adult height. Delayed ICT results in adult short stature. Predictive adaptation to low or high energy availability modifies the timing of transition to adjust adult size to energy resources. Hypothesis: The ICT age is affected by the household environment and is governed by genetic factors.

Patients/ Methods: The study examined growth pattern in 239 boys and 261 girls from well-baby clinics, including 48 pairs of monozygotic (MZ), 58 dizygotic (DZ) twins and 288 siblings (SB), who were measured repeatedly for body weight and length over the first two years of life. Age at ICT was estimated using the Karlberg's ICP model without correcting for gestational age. The followings potential predictors of the ICT timing were evaluated: birth weight and length, maternal body weight and height at infants' birth, Apgar score, parental education and occupation, and season of year at infant's birth.

Results: Significant sex difference was found for the ICT age(11.0±1.9 mo, boys vs 10.3±2.0 mo, girls, $p<0.0001$), and for body length at 24m (mean F -1.0SDS, M -1.2 SDS, $p=0.02$). The ICT age showed significant sib-sib correlations, with highest within-pair correlation in MZ twins, $r=0.88$, vs. $r = 0.74$ in DZ and $r=0.439$ in SB (all at $p<0.0001$). SB correlation was significantly ($p<0.01$) lower than either DZ or MZ correlation. However, MZ and DZ correlations did not differ significantly ($p>0.05$).

Conclusions: 1.Significant intra-pair sibling correlations clearly suggest the existence of considerable familial effect in ICT age variation. It has a common environment component shared by siblings, and in particular twins, who share intrauterine and familial environment at a given time. 2. The contribution of genetic factors remains to be clarified.

Pelvic ultrasound in girls with precocious puberty is a useful adjunct in diagnosis and therapy monitoring

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Introduction: Gonadotropin-releasing hormone analogs (GnRHa) are known to be efficacious in the treatment of central precocious puberty (CPP). However, there are no clear-cut criteria for initiation of therapy or the means for monitoring suppression during treatment. We have previously shown that pelvic ultrasound can be used to differentiate CPP from premature thelarche (PT). The contribution of pelvic ultrasonogrphy to the decision on treatment initiation and to treatment monitoring has not been extensively studied. Aim: To prospectively assess the use of pelvic ultrasound in documenting progression of precocious puberty before GnRHa therapy and in monitoring suppression during therapy.

Patients/ Methods: Girls referred because of appearance of breast buds before age 8 years were recruited consecutively. All underwent general and endocrine evaluation. The diagnosis of CPP (n=25) was based on the clinical judgment of an experienced clinician after 6 months of follow up. Transabdominal pelvic ultrasound was performed with a 5-MHz real-time sector scanner on referral, 3 and 6 months later, and every 6 months thereafter.

Results: Before treatment a significant increase in height –SDS (p=0.003), uterine volume (p<0.01), fundus diameter (p<0.05) and endometrial thickness (p<0.001) was observed after 3 months of follow-up in girls with CPP but not in controls (girls with PT). Three months after beginning therapy there was a significant decrease in uterine length (mean 4.2±0.6 vs 3.8±0.7 cm , p=0.01), uterine volume (4.4±2.2 vs 3.0±1.0 ml , p<0.003) and ovarian volume (3.2±2.3 vs 1.9±1.0, p<0.01) but no significant change in height-SDS or bone age to chronological age ratio. No further changes in either height-SDS or ultrasound measurements were documented during 2 years of treatment.

Conclusions: The increase in uterine measurements indicating progression of puberty may be used as an adjunct when considering GnRHa treatment. The significant decrease in both uterine and ovarian measurements as soon as 3 months after therapy initiation suggest that ultrasound may be an early useful means for monitoring suppression.

The protective effect of GnRH-a against chemotherapy associated ovarian failure in stem cell transplantation [SCT]

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Introduction: GnRH-a has been shown to decrease ovarian failure in young women exposed to gonadotoxic chemotherapy. Recently, we have reported on a young woman, similarly treated, who has successfuly delivered twice after two bone marrow transplantations, against all odds.

Patients/ Methods: To examine whether GnRH-a may minimize the risk of premature ovarian failure [POF], in young women undergoing gonadotoxic chemotherapy conditioning for stem cell transplantation [SCT], the ovarian function of 98 young women, age 15-40, who have undergone SCT for various indications, were retrospectively evaluated. Of the 85 evaluable patients, 50 were treated with GnRH-a during the aggressive conditioning chemotherapy before SCT, whereas 35 did not. POF vs cyclic ovarian function[COF] was defined by regular cycles, hormonal profile, ultrasound, and/or pregnancy.

Results: Patients treated with GnRH-a resumed COF in 34% [17/50] compared to 11% [4/35] in women who did not receive GnRH-a, $P<0.05$. Lymphoma patients benefitted significantly from the GnRH-a cotreatment, $P=0.023$, whereas the others did not. 71.4% of the Hodgkin Llymphoma [HL] and 50% of the non-HL patients who received GnRH-a had COF after SCT vs 2/11 controls [no GnRH-a].

Conclusions: Administration of GnRH-a before and during gonadotoxic chemotherapy may minimize POF in lymphoma patients undergoing SCT, but not in leukemias and other indications. Due to the retrospective nature of this study, larger, prospective and RCT are awaited.

Correlations between pre- and postnatal measurements of penile and clitoral size

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Introduction: Ultrasound examination, usually at mid-gestation, is routinely used in standard clinical obstetric practice for the prenatal detection of various syndromes, fetal growth status, determination of fetal sex and detection of anatomic malformations, including external genitalia anomalies. A recently established reference range for prenatal penile length in relation to gestation age has led to frequent incorporation of micropenis into the prenatal diagnostic profile. Findings outside the normal range often cause parental anxiety, lead to further evaluation and sometimes to pregnancy termination. Comparisons of pre- and postnatal penile and clitoral size are lacking. Our objective was to correlate pre- and postnatal measurement of penile width and length and clitoral height and length.

Patients/ Methods: Fetal penile width and length and clitoral hieght and length in singleton pregnancies of randomly selected pregnant woman, were measured twice by high-resolution ultrasonography. Postnatal measurements were carried out twice during the first postnatal week. Correlation between pre- and postnatal measurements were calculated by the Pearson correlation test.

Results: Paired pre- and postnatal measurements were performed in 45 males and 47 females. The correlations between measurements of fetal penile and clitoral length and width/height and week of gestation were highly significant ($p \leq 0.001$). Correlations between fetal and postnatal penile length and width were not significant. There was significant correlation between fetal clitoral length and height and postnatal clitoral length ($r=0.34$, $p=0.019$, $r=0.36$, $p=0.012$, respectively).

Conclusions: The lack of correlations between pre- and postnatal penile measurements and the significant correlation between fetal and postnatal clitoral length suggests that while prenatal findings in females might be reliable indicators of postnatal measurements, this is not the case in males. This uncertainty in fetal penile measurements mandates exercising caution in parent counseling.

46, XX infant (SRY-Negative) with bilateral ovotestis, is there a causing gene?

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Introduction: Ovotesticular Disorder of Sexual Development (OT-DSD) (true hermaphroditism) is a rare disorder of sexual differentiation characterized by ambiguous external genitalia and gonads having both ovarian and testicular elements. Differentiation of testicular tissue in 46, XX individuals occurs either in XX males (mostly expressing the SRY gene), or in individuals with OT-DSD usually SRY negative. Although they are sporadic cases, there are some reports on familial recurrence. We report a rare case of scrotal hypospadias, and bilateral ovotesticular DSD with its unique clinical and molecular genetic analysis.

Patients/ Methods: Serum hormonal levels, HCG stimulation test, karyotype and gonadal biopsy were performed followed by PCR studies of DNA from peripheral leukocytes and gonadal tissues investigating the presence of the SRY gene. SNP microarray for homozygosity mapping is being performed.

Results: Karyotype was 46, XX. HCG test showed significant rise in testosterone levels. Pathology revealed the unique finding of bilateral similar ovotestis. PCR in peripheral leukocytes was SRY negative, while in gonadal tissues SRY expression was observed in the ovotesticular (testicular) tissue. SNP homozygosity mapping currently performed has early indication for a linkage which may direct to candidate genetic studies.

Conclusions: The bilateral existence of both ovarian and testicular tissues in the same gonad is rare. The presence of SRY in the ovotestis while negative in serum is unique even in OT-DSD. The far consanguinity in this case enables linkage (SNP) studies that may provide evidence that bilateral XX OT-DSD is caused by genes which are crucial in gender determining stages of sex development.