Reproductive Endocrinology 2011

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The biology and endocrinology of reproduction is a wide field and lively research area attracting considerable attention as the implications concern our everyday life. More than 1,000 papers published in the last year were found under the search terms used of which 26 were selected. This chapter hosts a selection of papers dealing with many important aspects of reproductive endocrinology including the function of new and old genes, puberty, gametogenesis, rescue of fertility, lifestyle and other environmental factors in reproduction, prenatal origin of reproductive disorders and many others. In other chapters of this book there are also several additional publications dealing with subjects related with reproduction. For sure there were other excellent papers published within the field during the past year, some which we might have missed in our search and yet others which were not possible to include due to space limitation. The aim has been to present a mix of experimental and clinical publications advancing our knowledge of the reproductive endocrinology field. The present selection of papers obviously represents our own bias but we hope you find them interesting to read and helpful for your activity in clinical and experimental pediatric endocrinology.

Reviews

Reproduction in females: the role of the early life environment

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Hum Reprod Update 2011;17:210–27.

Background: There is compelling evidence that long-term health and physiological function are modified by events that occur early in life and involve interactions between the genome and the developmental environment. That reproductive function may similarly be influenced by early life events has been established in selected human populations.

Methods: This is a literature study highlighting early life influences on reproduction with a particular focus on nutritional impacts, providing a brief overview with reference to some key studies in both the human and animal literature.

Results: The impact of early life events on reproductive health and disease risk is poorly understood but it is thought to be clear that nutrition, during the developmental lifespan, plays an important role.

Conclusion: Improved insight into the underlying mechanisms is likely to have significant implications for our current understanding of reproductive disorders, and therefore for the health and reproductive potential of future generations.

That already the prenatal life is of great importance for long-term health was implicated by Barker [1] in 1990, and the 'prenatal origin of adult disease theory' was also reviewed in *EMBO Reports* [2] last year. The review by Sloboda et al. shows that a good maternal health is important also for long-term effects on the reproductive potential. Thus, proper maternity care during pregnancy is not only important in a short-term perspective for the mother and fetus, but also for reproductive functions in generations to come.

Maternal alcohol consumption during pregnancy and semen quality in the male offspring: two decades of follow-up

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Hum Reprod 2010;25:2340–5.

Background: Concurrent alcohol exposure has been associated with reduced fecundity. The present study investigates the association between maternal alcohol consumption during pregnancy, semen quality and levels of reproductive hormones in young, adult men.

Methods: From a Danish pregnancy cohort established in 1984–1987, 347 sons were selected for a follow-up study conducted in 2005–2006. Semen and blood samples were analyzed for conventional semen characteristics and reproductive hormones, respectively, and results were related to prospectively self-reported information on maternal alcohol consumption during pregnancy.

Results: The sperm concentration decreased with increasing prenatal alcohol exposure. The adjusted mean sperm concentration among sons of mothers drinking ≥ 4.5 drinks per week during pregnancy was approximately 32% lower compared with men exposed to <1.0 drink per week. The semen volume and the total sperm count were also associated with prenatal alcohol exposure; sons prenatally exposed to 1.0–1.5 drinks per week had the highest values. No associations were found for sperm motility, sperm morphology or any of the reproductive hormones, including testosterone.

Conclusion: These results indicate that prenatal exposure to alcohol may have a persisting adverse effect on Sertoli cells, and thereby sperm concentration.

Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index

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Hum Reprod 2011;26:1000–11.

Background: Prenatal exposure to tobacco smoking is a risk factor for reduced semen quality. The aim of the present study was to investigate possible associations between prenatal smoking and reproductive hormones, pubertal development or adult BMI while controlling for the effects of current smoking in young adulthood.

Methods: A cross-sectional study (1996–2006) including 3,486 Danish men (median age 19 years) participating in a semen-quality study. Data were obtained from questionnaires, physical examinations, semen analyses and assessments of reproductive hormones. The main outcome measures were markers of pubertal onset, BMI, reproductive hormones and semen variables.

Results: Maternal smoking during pregnancy was associated with earlier onset of puberty, lower final adult height, higher BMI, smaller testicles, lower total sperm counts, reduced spermatogenesis-related hormones and higher calculated free testosterone. If not exposed prenatally, men's own smoking was associated with increased total testosterone but unchanged free-T. For smokers who had been exposed prenatally, total testosterone was increased but free-T was reduced due to higher levels of sex hormone-binding globulin.

Conclusion: Prenatal exposure to tobacco may lead to faster pubertal development possibly caused by a higher free-T, and to higher adult BMI and impairment of testicular function. These findings may not be clinically relevant for the individual but are of public health importance, and add to the knowledge of effects of tobacco smoking.

Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat

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Hum Reprod 2011;26:235–44.

Background: More than half of pregnant women in the Western world report intake of mild analgesics, and some of these drugs have been associated with anti-androgenic effects in animal experiments. Many of the anti-androgenic compounds are, like the mild analgesics, potent inhibitors of prostaglandin synthesis.

Methods: In a prospective birth cohort study, 2,297 Danish and Finnish pregnant women completed a questionnaire and 491 of the Danish mothers participated in a telephone interview. The testicular position of newborns was assessed by trained pediatricians. In rats, the impact of mild analgesics on anogenital distance after intrauterine exposure was examined, together with the effect on ex vivo gestational day 14.5 testes.

Results: The use of mild analgesics (particularly during second trimester) was dose-dependently associated with congenital cryptorchidism in the Danish birth cohort. The association was not found in the Finnish birth cohort. Intrauterine exposure of rats to paracetamol led to a reduction in the anogenital distance and mild analgesics therefore reduced testosterone production in ex vivo fetal rat testes.

Conclusion: There was an association between the timing and the duration of mild analgesic use during pregnancy and the risk of cryptorchidism. Our results suggest that intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders.

... but

No secular trend over the last decade in sperm counts among Swedish men from the general population

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Hum Reprod 2011;26:1012–6.

Background: Based on historical data, a decline in sperm counts during the years 1940–1990 has been suggested and etiologically linked to a concomitant increase in the incidence of testicular cancer. The present study, focusing on possible changes in sperm parameters among young Swedish men during the past 10 years, was specifically designed in order to answer the question of whether there is a continuing decline in sperm counts.

Methods: During the period 2008–2010, 295 young (17–20 years; median 18) men born and raised in Sweden were recruited at the age they were supposed to undergo medical examination prior to military service. Their semen parameters were compared with those of a similar cohort of men ($n = 216$) recruited in the year 2000–2001.

Results: No significant changes in sperm concentration, semen volume or total sperm counts were found. The proportion of progressively motile spermatozoa also remained unchanged.

Conclusion: Between the years 2000 and 2010, no evidence of time-related deterioration of semen parameters was found among young Swedish men from the general population. This finding does not exclude that such a decrease may have taken place before year 2000.

A decreasing sperm count among men has been a concern for many years, and in these three Danish and Danish/Finnish studies the prenatal exposure of smoke, alcohol or mild analgesics all show a negative effect on sperm count. In addition, in utero exposure of smoking also affects timing of puberty. The decrease in sperm count has been suggested to be due to new hazards in the environment, such as exposure to endocrine disruptors, and/or changes in lifestyle, like fast food, tobacco, alcohol, etc. As a positive contrast to these reports, a newly published study on young Swedish men

could not find a decrease in sperm count among the general population during the last 10 years. This could implicate that the decreasing sperm count could be leveling out, which would be a potential break of a negative trend, or support the notion that there are yet unexplained regional differences in these observations.

Mechanism of the year **Progesterone-induced Ca^{2+} influx in human sperm**

The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm

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Nature 2011;471:382–6.

Background: In the oviduct, cumulus cells that surround the oocyte release progesterone. In human sperm, progesterone stimulates a Ca^{2+} increase by a non-genomic mechanism. The Ca^{2+} signal has been proposed to control chemotaxis, hyperactivation and acrosomal exocytosis of sperm. However, the underlying signaling mechanisms remain an enigma.

Methods: cAMP levels, changes in $[\text{Ca}^{2+}]_i$ and pH_i were measured in sperm, obtained from healthy volunteers. A patch-clamp technique was used in whole-cell configuration.

Results: Both progesterone and alkaline pH stimulated a rapid Ca^{2+} influx with almost no latency, incompatible with a signaling pathway involving metabotropic receptors and second messengers. The Ca^{2+} signals evoked by alkaline pH and progesterone are inhibited by the Ca(v) channel blockers NNC 55-0396 and mibefradil. Patch-clamp recordings from sperm reveal an alkaline-activated current carried by mono- and divalent ions that exhibits all the hallmarks of sperm-specific CatSper Ca^{2+} channels. Progesterone substantially enhances the CatSper current. The alkaline- and progesterone-activated CatSper current is inhibited by both drugs.

Conclusion: Progesterone activates the sperm-specific, pH-sensitive CatSper Ca^{2+} channel. These results resolve a long-standing controversy over the non-genomic progesterone signaling. In human sperm, either the CatSper channel itself or an associated protein serves as the non-genomic progesterone receptor. The identification of CatSper channel blockers will greatly facilitate the study of Ca^{2+} signaling in sperm and help to define further the physiological role of progesterone and CatSper.

Progesterone activates the principal Ca^{2+} channel of human sperm

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Nature 2011;471:387–91.

Background: The steroid hormone progesterone, released by cumulus cells surrounding the egg, is a potent stimulator of human spermatozoa. It attracts spermatozoa towards the egg and helps them penetrate the egg's protective vestments. Progesterone induces Ca^{2+} influx into spermatozoa and triggers multiple Ca^{2+} -dependent physiological responses essential for successful fertilization, such as sperm hyperactivation, acrosome reaction and chemotaxis towards the egg. As an ovarian hormone, progesterone acts by regulating gene expression through a well-characterized progesterone nuclear receptor. However, the effect of progesterone upon transcriptionally silent spermatozoa remains unexplained and is believed to be mediated by a specialized, non-genomic membrane progesterone receptor. The identity of this non-genomic progesterone receptor and the mechanism by which it causes Ca^{2+} entry remain fundamentally unresolved questions in human reproduction.

Methods: The mechanisms of the non-genomic action of progesterone on human spermatozoa were elucidated by identifying the Ca^{2+} channel activated by progesterone. By applying the patch-clamp

technique to mature human spermatozoa, nanomolar concentrations of progesterone were found to dramatically potentiate CatSper, a pH-dependent Ca^{2+} channel of the sperm flagellum.

Results: Human CatSper is synergistically activated by elevation of intracellular pH and extracellular progesterone. Interestingly, human CatSper can be further potentiated by prostaglandins, but apparently through a binding site other than that of progesterone. Since the experimental conditions did not support second messenger signaling, CatSper or a directly associated protein serves as the elusive non-genomic progesterone receptor of sperm.

Conclusion: Given that the CatSper-associated progesterone receptor is sperm-specific and structurally different from the genomic progesterone receptor, it represents a promising target for the development of a new class of non-hormonal contraceptives.

The ovarian hormone progesterone classically binds to a nuclear receptor initiating gene transcription. How could the transcriptionally inactive human spermatozoon be stimulated in preparation for fertilization? The question has long been an enigma for reproductive biologists. The two articles above, published in the same issue of *Nature*, provide an unexpected answer: progesterone activates a sperm-specific calcium ion (Ca^{2+}) channel called CatSper [3]. Progesterone rapidly activates intracellular signaling in human sperm, regulating key aspects of their physiology. The ion channel, CatSper, unique to the sperm tail, seems to transmit the signal of the steroid hormone progesterone [3]. The CatSper-associated receptor is indicated as a potential target for a new class of contraceptives, which raise hopes for future development along this line to finally achieve an effective oral contraceptive for men.

Genes: an old gene

Widespread presence of human BOULE homologs among animals and conservation of their ancient reproductive function

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PLoS Genet 2010;6:e1001022.

Background: Sex-specific traits that lead to the production of dimorphic gametes, sperm in males and eggs in females, are fundamental for sexual reproduction. The sex-biased genes that underlie these sex-specific traits are under strong selective pressure, and as a result of adaptive evolution they often become divergent. Indeed out of hundreds of male or female fertility genes identified in diverse organisms, only a very small number of them are implicated specifically in reproduction in more than one lineage.

Methods: DNA sequences (from known BOULE homologs) from various species were retrieved from the literature and the Genbank database. In the absence of EST or cDNA information, a representative species from the same phylum whose genome had been completely sequenced was used. The identified homologs were verified by BLASTing against the human protein database, which should identify human Boule as the top hit sequence with highest similarity. A phylogenetic analysis was done. Mice with the BOULE gene knocked out were generated.

Results: BOULE homologs were present in the genomes of representative species of each of the major lineages of metazoans and exhibit reproductive-specific expression in all species examined, with a preponderance of male-biased expression. Loss of mouse Boule prevents sperm production, resulting in a global arrest of spermatogenesis in remarkable similarity to that of *Drosophila* Boule mutants.

Conclusion: There is conservation of a reproductive protein throughout *Eumetazoa*, with a predominant testis-biased expression in diverse bilaterian species, and conservation of a male gametogenic requirement in mice. This shows an ancient gametogenesis requirement for Boule among *Bilateria* and supports a model of a common origin of spermatogenesis.

BOULE turns out to be an ancient gene. As highlighted in *Nature* (<http://www.nature.com/nature/journal/v466/n7305/pdf/466417f.pdf>) the BOULE gene seems to have been conserved for at least 600

million years. Not surprisingly it also seems that the generation of sperm has a common origin among animals. The gene was mainly expressed in the testis and its deletion, in a mouse model, resulted in male infertility due to the lack of mature spermatozoa. This indicates a common origin for male gametogenesis among metazoans and reveals high conservation of this reproduction-specific protein among bilaterian animals.

SRY function still an enigma

Identification of SOX3 as an XX male sex reversal gene in mice and humans

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J Clin Invest 2011;121:328–41.

Background: Sex in mammals is genetically determined and is defined at the cellular level by the sex chromosomes. The Y chromosome-linked gene sex-determining region Y (SRY) is believed to be the master initiator of male sex determination in almost all mammals, functioning to upregulate expression of its direct target SOX9. Data suggest that SRY evolved from SOX3, although there is no direct functional evidence to support this hypothesis. Indeed, loss-of-function mutations in SOX3 do not affect sex determination in mice or humans.

Methods: To further investigate Sox3 function in vivo, the authors generated transgenic mice overexpressing Sox3 and examined the obtained phenotypes.

Results: In one of the transgenic lines, Sox3 was ectopically expressed in the bipotential gonad and resulting in frequent complete XX male sex reversal. Further analysis indicated that Sox3 induced testis differentiation in this particular line of mice by upregulating expression of Sox9 via a similar mechanism to Sry. Strengthening the clinical implications of these findings the authors also identified genomic rearrangements within the SOX3 regulatory region in 3 patients with XX male sex reversal.

Conclusion: The reported data suggest that SOX3 and SRY are functionally interchangeable in sex determination and support the notion that SRY evolved from SOX3 via a regulatory mutation that led to its de novo expression in the early gonad.

For two decades SRY has been known as the male sex-determining factor in somatic sex differentiation, but its precise function still remains elusive. The present view is that SRY acts as a master switch controlling the expression of a set of downstream genes with defined functions in the maturation process, SOX9 being the most well studied. The present paper adds SOX3 to the list of genes involved in male gonadal differentiation and reveals it constitutes yet another target gene to explore in DSD cases with XX male sex reversal.

Common variants in DGKK are strongly associated with risk of hypospadias

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Nat Genet 2011;43:48–50.

Background: Hypospadias is a common congenital malformation of the male external genitalia but the causes are largely unknown, although failure of prenatal androgen action is thought to play a major role.

Methods: The authors performed a genome-wide association study using pooled DNA from 436 individual cases with hypospadias and 494 controls of European descent and selected the highest ranked SNPs

for individual genotyping in the discovery sample, an additional Dutch sample of 133 cases and their parents, and a Swedish series of 266 cases and 402 controls.

Results: Individual genotyping of two SNPs in DGKK, encoding diacylglycerol kinase κ , produced compelling evidence for association with hypospadias in the discovery sample (allele-specific odds ratio (OR) = 2.5, $p = 2.5 \times 10^{-11}$ and OR = 2.3, $p = 2.9 \times 10^{-11}$, respectively) and in the Dutch and Swedish replication samples. Expression studies showed expression of DGKK in preputial tissue of cases and controls, which was lower in carriers of the risk allele of rs1934179 ($p = 0.047$).

Conclusion: It is proposed that DGKK is a major risk gene for hypospadias.

Hypospadias is a relatively common malformation in boys associated with a need for intervention that is not trivial and often negatively influencing the quality of life of those affected. Although undermasculinization due to failure of androgen action is a frequently proposed cause, convincing proof of such association is rare in the individual case. This may be due to a transient androgen failure at sensitive time window(s) of the prenatal development that is no longer detectable at birth, but other causes should not be neglected. This report shows that there seems to be a strong link of DGKK to hypospadias opening up for more research to find novel causes of this common malformation in boys. However, the role of this particular gene in the pathogenesis of hypospadias, if any, remains to be determined.

New paradigms and focus on puberty **Impact of diet on pubertal timing**

Relation of isoflavones and fiber intake in childhood to the timing of puberty

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Am J Clin Nutr 2010;92:556–64.

Background: It has been suggested that phytoestrogens and dietary fiber can affect pubertal timing. Whether intake of isoflavone, the major class of dietary phytoestrogens, and fiber in healthy white children before their pubertal growth spurt (age at take-off (ATO)) was associated with puberty timing was examined.

Methods: Multivariate regression analyses were performed in 227 participants with 3-day weighed dietary records and information on potential confounders at baseline. In a subsample ($n = 111$), urinary isoflavones were determined in 24-hour urine samples by gas chromatography-mass spectrometry analysis. Pubertal timing was examined by using ATO and chronologic ages at pubertal stage 2 for breast development (B2) or gonadal development, peak height velocity, and menarche or voice break.

Results: Girls whose diet was in the highest dietary isoflavone tertile experienced Tanner stage 2 for breast development and reached peak height velocity (approximately 0.7 and 0.6 years respectively), later than girls whose diet was in the lowest isoflavone tertile. In boys, dietary isoflavones were not associated with pubertal markers.

Conclusion: Girls, but not boys, with higher prepubertal isoflavone intake appear to enter puberty at a later age. Fiber intake in this population sample was not relevant for pubertal timing.

Epidemiologic studies have implicated that both a later age at peak height velocity and menarche are associated with a reduced risk of breast cancer. Thus, the considerable delay of pubertal timing associated with higher dietary isoflavones may contribute to the prevention of breast cancer in later life. The beneficial effects on breast cancer risk were particularly highlighted in relation to consumption of a diet rich in phytoestrogen before puberty [4] or during adolescence [4, 5]. Phytoestrogens could be implicated in puberty timing, which is recognized as an established risk factor for a number of hormone-related cancers besides breast cancer. A recent cross-sectional study in 9-year-old healthy girls proposed that a higher phytoestrogen intake was associated with later breast development [6]. Cheng et al. confirm that a high intake of isoflavones postponed puberty

onset in girls, but were without effect in boys. This finding could have an impact on future nutritional recommendations during childhood and adolescence. The data also represents a confounder in the ongoing discussion of an earlier start of puberty in many countries. The biological mechanism involved remains to be determined, but one suggestion may be that phytoestrogens could be sensed by a feedback mechanism at the hypothalamic pituitary level without exerting detectable agonist action in the periphery.

Follow-up on a Yearbook 2008 paper
Don't skip breakfast if you want a son (pp. 84–85)

Female gender preselection by maternal diet in combination with timing of sexual intercourse – a prospective study

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Reprod Biomed Online 2010;21:794–802.

Background: Natural sex selection methods have been applied for several decades, but their use and effectiveness are still a matter of debate.

Methods: The present study assessed the efficacy of a maternal diet low in sodium and high in calcium, in combination with timing of intercourse well before ovulation as a method to improve the chances of conceiving a girl.

Results: 150 couples entered the study, compliance with diet was assessed through mineral analyses of blood, and timing of intercourse relative to ovulation was determined by ovulation tests. Based on mineral blood values and timing of intercourse of 28 participants, a prediction rule for conceiving a girl was constructed and was tested prospectively for validity on a subsequent group of 50 women. In this latter group, 21 women satisfied the criteria of the prediction rule and 16 gave birth to a daughter.

Conclusion: The combination of maternal diet with timing of intercourse is capable of increasing the probability of conceiving a girl ($p = 0.005$). The observed percentage of female babies for all 32 women satisfying the prediction rule was 81% (95% CI 68–95%).

A paper by Alexenko et al. [7] in 2007 presented in the *ESPE Yearbook 2008* showed that a in murine model a high caloric diet favored male offspring. In agreement with this, Mathews et al. [8] in 2008 showed similar results in a study on humans, where mothers with a high caloric intake prior to conception had a higher proportion of boys. Here the authors present that a low-sodium, high-calcium diet in combination with timing of intercourse will favor female offspring. Thus, by adjusting the food on the dinner plate it is possible to predict the sex of the children! The biological mechanisms involved are obscure.

Development of steroid signaling pathways during primordial follicle formation in the human fetal ovary

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J Clin Endocrinol Metab 2011;96:1754–62.

Background: Ovarian primordial follicle formation is critical for subsequent human female fertility. It is likely that steroid, and especially estrogen, signaling is required for this process. The aim of the present study was to identify and characterize key members of the steroid-signaling pathway expressed in the second trimester human fetal ovary.

Methods: This is an observational study of the female fetus, quantifying and localizing steroid-signaling pathway members. Ovaries were collected from 43 morphologically normal human female fetuses from women undergoing elective termination of second trimester pregnancies. mRNA transcript levels, immunolocalized key steroidogenic enzymes and steroid hormone receptors were measured.

Results: Levels of mRNA encoding the steroidogenic apparatus and steroid receptors increased across the second trimester. CYP19A1 transcript increased 4.7-fold during this period with intense immunostaining for CYP19A detected in pre-granulosa cells around primordial follicles and somatic cells around oocyte nests. ESR2 was localized primarily to germ cells, but the androgen receptor was exclusively expressed in somatic cells. CYP17A1 and HSD3B2 were also localized to oocytes, whereas CYP11A1 was detected in oocytes and some pre-granulosa cells.

Conclusion: The human fetal ovary expresses the machinery to produce and detect multiple steroid signaling pathways, including estrogenic signaling, with the oocyte acting as a key component.

The number of primordial follicles is the reproductive reserve in adult women, and thus determines the reproductive status. This study shows that the steroid signaling pathways are active already in the early fetal ovary. The number of germ cells in the ovary peaks already in fetal month 6, from there on the amount decline until menopause, where they are finished completely. From the second trimester the primordial follicles are being formed, and studies have shown that the PI3K pathway is important for the maintenance of the primordial follicles in rodents [for a review, see 9]. The present study shows novel data on local steroid hormone signaling during this key period of human primordial follicle formation. Thus, the fetal ovary contains the machinery to produce and detect steroid hormones, especially estrogen. Therefore, it is likely that estrogens play an important role in intraovarian signaling and primordial follicle formation in the human ovary.

For masculinization

Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty

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Int J Androl 2011 (E-pub ahead of print).

Background: Incomplete prenatal masculinization of boys is relatively common resulting in hypospadias and/or cryptorchidism. Experimental animal studies show that measurement of anogenital distance (AGD) and/or penis length may provide lifelong 'read-outs' of androgen exposure during the masculin-

ization programming window in prenatal development. However, variation in postnatal androgen exposure during minipuberty and later may complicate interpretation of such measurements. This is important to clarify if such measurements are to be applied to humans. The present aim was to evaluate effects of prenatal and/or postnatal manipulation of androgen production/action on growth of AGD and the penis in rats.

Methods: Pregnant rats were treated daily before and after birth with either vehicle, di(*n*-butyl)phthalate (DBP) or flutamide in prenatal + postnatal treatment combinations. DBP impairs androgen production whereas flutamide impairs androgen action. The male offspring were killed on postnatal day 8 (prepuberty), 25 (early puberty) or 90 (adulthood) when AGD was measured, the penis dissected out and its weight and length measured; plasma testosterone and ventral prostate weight were measured at day 90 to assess endogenous androgen exposure.

Results: In controls, penis length, girth and AGD increased from day 8 to day 90. Significant inhibition of penis growth and final length and girth was induced by treatments that inhibited postnatal androgen action. Conversely, growth and ultimate (adult) AGD was inhibited by prenatal inhibition of androgen production whereas postnatal androgen inhibition had negligible effect. Nevertheless, AGD and penis length were highly correlated at every age. However, altered endogenous androgen exposure may confound interpretation of changes in adults exposed prenatally/postnatally to DBP/flutamide.

Conclusion: The authors concluded that the AGD provides a lifelong guide to prenatal androgen exposure whereas penis size reflects both pre- and postnatal androgen exposure.

Most pediatric endocrinologists are faced with cases of newborn boys with a small phallus (micropenis) and anxious questions from the parents whether the condition will be restored by nature or require/responds to treatment. This report addresses this issue in an experimental animal model. The relevance of the findings in humans remains to be determined which is important for our understanding of the pathogenesis behind and possibilities of treatment of undermasculinization disorders in boys. Biomarkers of androgenization in neonatal boys are also highly wanted to serve as phenotypic markers of adverse actions of endocrine disruptors and other influences but it is too early to say if AGD is robust enough to serve such a purpose.

Important for clinical practice and research

Counseling of a couple faced with a prenatal diagnosis of Klinefelter syndrome

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Acta Paediatr 2011;100:917–22.

Background: Klinefelter syndrome (KS) is a common genetic disorder that is often diagnosed late in life or not at all. When a prenatal diagnosis of KS is made, a couple is faced with an unfamiliar and unexpected diagnosis in most cases. The aim of this article was to give clues to prenatal counseling in this situation.

Methods: The authors reviewed the evidence about the phenotype of KS according to the circumstances of diagnosis and its use in counseling couples faced with a prenatal diagnosis of this common condition.

Results: The information provided to couples facing a prenatal diagnosis of KS should ideally be based on longitudinal studies of unselected individuals, including those diagnosed prenatally. Indeed, there are several reasons to think that the phenotype of individuals diagnosed prenatally is globally less severe than in those diagnosed postnatally. Based on these studies, the evidence to be explained to couples to help them make an informed decision about the pregnancy was concluded to be the following: except for rather tall height, generally normal appearance throughout life; increased risk of learning disabilities; spontaneous puberty, reduced testicular size, usual need for testosterone supplementation

from adolescence onward; increased risk of gynecomastia; sexual orientation similar to the general male population; infertility, but with the possibility of having biological offspring with assisted reproductive techniques.

Conclusion: Cohort studies including individuals with KS diagnosed prenatally are not yet available but wanted to provide an evidence base for the counseling.

The evidence base for prenatal counseling in Klinefelter syndrome is weak since most cases are discovered in adult life or not at all. The 'typical' clinical picture presented by textbooks or web sources often consulted by parents is not representative of the average case, but more of the severe end of the clinical spectrum. This paper is food for thought and useful as a reference for the pediatric endocrinologist asked to counsel the pregnant couple in the case of a prenatal diagnosis of Klinefelter syndrome and other similar conditions. Requests for such counseling is increasing due to increased availability of prenatal diagnostics and an older age of both mothers and fathers when expecting their children.

AMH reference values from birth to adulthood

Serum levels of anti-müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients

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Changes in anti-müllerian hormone throughout the lifespan: a population-based study of 1,027 healthy males from birth (cord blood) to the age of 69 years

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Background: Anti-müllerian hormone (AMH) is a testis-specific marker in newborns. In adult women, AMH is related to the ovarian follicle pool. Little is known about AMH in girls. The objective of the study in females was to provide a reference range for AMH in girls and adolescents and to evaluate AMH as a marker of ovarian function. The aim of the study in males was to describe the ontogeny of AMH secretion in healthy individuals.

Methods: AMH levels were investigated in males and females through life. AMH was measured in 926 healthy females as longitudinal values during infancy as well as in 172 Turner syndrome (TS) patients according to age, karyotype and ovarian function. In males a population-based study of healthy volunteers was performed. Participants included 1,027 healthy males from birth to 69 years. A subgroup was followed up longitudinally through the infantile minipuberty and another group through puberty. Serum AMH was determined by a sensitive immunoassay.

Results: In females AMH was undetectable in 54% of cord blood samples and increased in all (37 of 37) infants from birth to 3 months. From 8 to 25 years, AMH levels were stable, with the lower level of the reference range clearly above the detection limit. AMH levels were associated with TS-karyotype groups as well as with ovarian function. As a screening test of premature ovarian failure in TS, the sensitivity and specificity of AMH <8 pmol/l was 96 and 86%, respectively.

In males serum AMH was above the detection limit in all samples with a marked variation according to age and pubertal status. The median AMH level in cord blood was 148 pmol/l and increased signifi-

cantly to the highest observed levels at 3 months. AMH declined at 12 months and remained at a relatively stable level throughout childhood until puberty, when AMH declined progressively with adults exhibiting 3–4% of infant levels.

Conclusion: In females AMH seems to be a promising marker of ovarian function in healthy girls and TS patients. In males the authors found detectable AMH serum levels at all ages, with the highest measured levels during infancy. At the time of puberty, AMH concentrations declined and remained relatively stable throughout adulthood. The potential physiological role of AMH and clinical applicability of AMH measurements remain to be determined. The presented data may serve as normative reference values from birth to adulthood in both males and females.

AMH has long been known as a Sertoli cell marker and an important factor behind the regression of the anlagen of female internal genitalia in the male fetus during prenatal sex differentiation. However, its role in peri- and postnatal life has been less well recognized. These two studies from the same research group present reference values of AMH in boys and girls from birth to adulthood and in Turner syndrome patients. The clinical usefulness of these data is yet to be fully explored but the full potential of AMH measurements most certainly requires more time to develop.

Do pediatric chief residents recognize details of prepubertal male genital anatomy?

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Clin Pediatr 2010;49:756–9.

Background: This study evaluated how well pediatric chief residents could label anatomic structures, recognize circumcision, and discern abnormal anatomy on three photographs of male prepubertal genitalia. Aspects of pediatric training in sexual abuse and clinical practice issues regarding routine genital examination of a male patient were also explored.

Methods: The authors asked respondents to identify anatomic structures, recognize circumcision, and assign a Tanner stage to prepubertal male genitalia and to recognize an abnormal finding.

Results: Only 7% of chief residents were able to correctly identify basic structures on the photo of a circumcised prepubertal male. Only 22% correctly recognized the abnormal example as hypospadias. Basic recognition of anatomic structures and circumcision did not achieve 100% accuracy, while an abnormal condition was missed by the majority of respondents.

Conclusion: These data suggest a need to address education about the male genital examination in greater detail during pediatric residency training.

Can we trust the records of a male genital examination in patient files from pediatric specialist care? The answer from this study is NO, which points to a problem to use retrospective patients' records as a major source of information in clinical research in this area. Another question is if this conclusion is valid also for other examinations than genital anatomy, such as Tanner staging in general. It is well known that determination of testicular volume by palpation, using an orchidometer as a reference, is not a very robust measure when comparing different examiners.

Concomitant mutations in the P450 oxidoreductase and androgen receptor genes presenting with 46,XY disordered sex development and androgenization at adrenarche

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Pubertal presentation in seven patients with congenital adrenal hyperplasia due to P450 oxidoreductase deficiency

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J Clin Endocrinol Metab 2011;96:E453–62.

Background: Undervirilization in males, i.e. 46,XY disordered sex development (46,XY DSD), is commonly caused by either lack of androgen action due to mutant androgen receptor (AR) or deficient androgen synthesis. P450 oxidoreductase (POR) is a crucial electron donor to all microsomal P450 cytochrome (CYP) enzymes including 17 α -hydroxylase (CYP17A1), 21-hydroxylase (CYP21A2) and P450 aromatase. Mutant POR causes congenital adrenal hyperplasia with combined glucocorticoid and sex steroid deficiency. P450 oxidoreductase deficiency (ORD) commonly presents neonatally, with disordered sex development in both sexes, skeletal malformations, and glucocorticoid deficiency. The authors conducted two studies on ORD patients. The objective of the first study was to analyze the clinical and biochemical phenotype in a 46,XY individual carrying concomitant POR and AR mutations and to dissect their impact on phenotypic expression. The aim of the second study was to describe the clinical and biochemical characteristics of ORD during puberty.

Methods: In the first study the authors characterized the clinical and biochemical phenotype, genetic identification, and functional analysis of POR missense mutation by yeast microsomal coexpression assays for CYP17A1, CYP21A2 and CYP19A1 activities. In the second study the clinical, biochemical, and genetic assessment of 7 ORD patients (5 females, 2 males) presenting during puberty was conducted.

Results: The patient in the first study presented neonatally with 46,XY DSD and was diagnosed as partial androgen insensitivity syndrome carrying a disease causing AR mutation (p.Q798E). She was raised as a girl and gonadectomized at the age of 4 years. At 9 years, progressive clitoral enlargement prompted reassessment. Urinary steroid analysis was indicative of POR deficiency, but surprisingly androgen production was normal. Genetic analysis identified compound heterozygous POR mutations (p.601fsX12/p.Y607C). In vitro analysis confirmed p.Y607C as a pathogenic mutation with differential inhibition of steroidogenic CYP enzymes. The second study revealed that the predominant findings in females were incomplete pubertal development (four of five) and large ovarian cysts (five of five) prone to spontaneous rupture. Pubertal development in the 2 boys was more mildly affected, with some spontaneous progression. Urinary steroid profiling revealed combined CYP17A1 and CYP21A2 deficiencies indicative of ORD in all patients; all but 1 failed an appropriate cortisol response to ACTH indicative of adrenal insufficiency. Diagnosis of ORD was confirmed by direct sequencing, demonstrating disease-causing POR mutations.

Conclusion: For the first case it was concluded that both mutant AR and POR were likely to contribute to the neonatal presentation with 46,XY DSD. Virilization at the time of adrenarche appears to suggest an age-dependent, diminishing disruptive effect of both mutant proteins. This case further highlights the importance to assess both gonadal and adrenal function in patients with 46,XY DSD.

Disordered puberty can be the first sign leading to a diagnosis of ORD. Appropriate testosterone production during puberty in affected boys but manifest primary hypogonadism in girls with ORD may indicate that testicular steroidogenesis is less dependent on POR than adrenal and ovarian steroidogen-

esis. Ovarian cysts in pubertal girls may be driven not only by high gonadotropins but possibly also by lack of meiosis-activating steroids caused by mutant POR.

P450 oxidoreductase is a relatively new player behind the clinical spectrum of disorders affecting pre- and postnatal sexual differentiation and function. These two studies from the same group give better insights into the role of P450 oxidoreductase in DSD and puberty and present some phenotypic characteristics of affected patients. The described findings may be helpful to better guide the molecular workup and delineate the etiology of unresolved cases of DSD and reproductive dysfunction later in life.

Too fast for genes – rapid response of the reproductive system to environmental change

Plasticity of the reproductive axis caused by social status change in an African cichlid fish. I. Pituitary gonadotropins

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Plasticity of the reproductive axis caused by social status change in an African cichlid fish. II. Testicular gene expression and spermatogenesis

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Background: Social position in a dominance hierarchy is often tightly coupled with fertility. Consequently, an animal that can recognize and rapidly take advantage of an opportunity to rise in rank will have a reproductive advantage. Reproduction in all vertebrates is controlled by the brain-pituitary-gonad (BPG) axis. In certain strains of fish, GnRH neurons at the apex of this axis are under social control. However, little is known about how quickly social information is transformed into functional reproductive change, or about how socially controlled changes in GnRH neurons influence downstream actions of the BPG axis including the cellular and molecular composition of the testes to regulate the reproductive capacity.

Methods: The authors created an opportunity for reproductively suppressed males to ascend in status and then measured how quickly the perception of this opportunity caused changes in mRNA and protein levels of the pituitary gonadotropins and the testis.

Results: Gonadotropin transcripts rose rapidly in the pituitary after suppressed males perceived an opportunity to ascend. In contrast, mRNA levels of GnRH receptor-1 remained unchanged during social transition but were higher in stable dominant compared with subordinate males. In the circulation, levels of both gonadotropins were also quickly elevated. For the testis, the results showed rapid upregulation of mRNA levels of FSH receptor and several steroid receptor subtypes during social ascent. In contrast, LH receptor was not elevated until 72 h after ascent, but this increase was coincident with elevated circulating androgens and early stages of spermatogenesis, suggesting a role in steroidogenesis. The spermatogenic potential of the testes, as measured by cellular composition, was also elevated before the overall increase in testes size.

Conclusion: The results showed that the pituitary is stimulated extremely rapidly after perception of social opportunity. The findings in subordinate males suggest that the BPG axis and spermatogenesis are maintained at a subthreshold level in anticipation of the chance to gain a territory and become reproductively active.

For survival of the species it seems logical that power and social status are favored when aiming for reproductive success of the fittest. However, the biological mechanisms involved and the adaptability

of the system to allow rapid changes in social structures to take effect are obscure. These two papers, taken together, demonstrate that the brain-pituitary-gonadal axis in a lower species (fish) is stimulated extremely quickly after perception of a social opportunity to rise in rank. The purpose is presumably to allow suppressed males to rapidly achieve higher reproductive success in a dynamic social environment dependent on high reproductive capacity. Obviously, the observed changes are too fast to allow genetic selection and demonstrate the immediate impact of a rapid social climbing on the reproductive behavior. It is up to the reader to translate these interesting observations to a human context.

Impact of the close environment

Brothers delay menarche and the onset of sexual activity in their sisters

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Background: The higher costs of sons compared with daughters extends to a negative effect of brothers on the lifetime reproductive success of their siblings in subsistence and preindustrial societies. In societies with fewer resource constraints, one might expect that these effects would be limited or non-existent.

Methods: This study investigates the costs of brothers and sisters in a contemporary Western society of adult Australians.

Results: Girls with elder brothers had a delayed age at menarche. Younger brothers were associated with delayed onset of sexual activity in sisters, but not in brothers. Neither younger nor elder brothers influenced fitness parameters (number of pregnancies, number of children, age at first pregnancy or age at first birth) in siblings of either sex.

Conclusion: This study provides evidence that brothers negatively affect their sisters' onset of reproductive maturity and sexual activity; however, this delay is not associated with a fitness cost in contemporary Australia. The authors suggest this is due to the long period of independence prior to childbearing.

This is a study from the social sciences indicating that the 'pubertal clock' can be directly influenced by environmental cues operating in the close family context. This is of interest as it explores a human context and constitutes another indication that reproductive fitness is favored at many levels of complexity. Apparently, older but not younger brothers influenced age of menarche of their sisters. The mechanisms involved in the pathway from the selective environmental signaling within the family to the described biological processes remain obscure.

Social consequences of early onset of puberty

Outcomes of early pubertal timing in young women: a prospective population-based study

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Background: Early pubertal timing in girls is associated with psychosocial problems throughout adolescence, but it is unclear whether these problems persist into young adulthood. This is an important issue for the pediatric endocrinologist in the decision process to treat or not in cases of precocious puberty.

Methods: The authors analyzed outcomes in adolescence and young adulthood in girls in a longitudinal study. The data for this study were from a prospective population-based study ($n = 1,420$), which initially recruited children at ages 9, 11 and 13, and followed them into young adulthood. Pubertal timing was defined on the basis of self-reported Tanner stage and age at menarche. Outcome measures included functioning related to crime, substance use, school/peer problems, family relationships, sexual behavior, and mental health in adolescence (ages 13–16) as well as crime, substance use, education/socioeconomic status, sexual behavior, and mental health in young adulthood (ages 19 and 21).

Results: In adolescence, early-maturing girls displayed higher levels of self-reported criminality, substance use problems, social isolation, early sexual behavior, and psychiatric problems. By young adulthood, most of these differences had attenuated. Functioning for early maturers improved in some areas; in others, on-time and late maturers had caught up with their early-maturing peers. Nevertheless, early-maturing girls, particularly those with a history of adolescent conduct disorder, were more likely to be depressed in young adulthood compared to their counterparts. Early maturers were also more likely to have had many sexual partners.

Conclusion: The effects of early pubertal timing on adolescent psychosocial problems were wide ranging but diminished by young adulthood for all but a small group.

There is an ongoing discussion on whether or not there is a recent trend of an earlier start of puberty in the general population, at least in certain countries. As a consequence, in the USA the normative reference age for precocious puberty has been lowered for certain ethnic groups. In addition to looking for possible causes behind this novel trend, other important questions are if there is need to worry about any medical or social consequences and if intervention should be considered for an increasing number of subjects. The present report adds food to this discussion and the decision by the pediatric endocrinologist to treat early puberty or not. The results support previous findings presented by Johansson and Ritzén [10] in a long-term follow-up study discussing the social and societal consequences of an earlier onset of puberty in the general population.

New hope Regenerative medicine to produce sperm in vitro

In vitro production of functional sperm in cultured neonatal mouse testes

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Nature 2011;471:504–7.

Background: Spermatogenesis is one of the most complex and longest processes of sequential cell proliferation and differentiation in the body, taking more than a month from spermatogonial stem cells, through meiosis, to sperm formation. The whole process, therefore, has never been reproduced in vitro in mammals, nor in any other species with a very few exceptions in some particular types of fish.

Methods: The authors employed an organ culture technique to show that neonatal mouse testes which contain only gonocytes or primitive spermatogonia as germ cells can produce spermatids and sperm in vitro with serum-free culture media.

Results: Spermatogenesis was maintained over 2 months in tissue fragments positioned at the gas-liquid interphase. The obtained spermatids and sperm resulted in healthy and reproductively competent offspring through microinsemination. In addition, neonatal testis tissues were cryopreserved and, after thawing, showed complete spermatogenesis in vitro.

Conclusion: The methodology may be applicable through further refinements to a variety of mammalian species, which will serve as a platform for future clinical application as well as mechanistic understanding of spermatogenesis.

Complete meiosis from human-induced pluripotent stem cells

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Background: Gamete failure-derived infertility affects millions of people worldwide; for many patients gamete donation by unrelated donors is the only available treatment. Embryonic stem cells can differentiate in vitro to germ-like cells, but they are genetically unrelated to the patient.

Methods: The authors used an in vitro protocol aiming to achieve complete differentiation of human-induced pluripotent stem (hiPS) cells to post-meiotic cells.

Results: The authors succeeded for the first time to produce complete meiosis from hiPS cells. Unlike previous reports using human embryonic stem cells, post-meiotic cells arose without the overexpression of germline-related transcription factors. Moreover, the authors consistently obtained haploid cells from hiPS cells of different origin (keratinocytes and cord blood), produced with a different number of transcription factors, and of both genetic sexes, suggesting the independence from the epigenetic memory of the reprogrammed somatic cells.

Conclusion: This work using a relatively simple protocol brings us closer to the production of personalized human gametes in vitro.

Research in regenerative medicine is rapidly moving and attracts much attention as reflected by publications in highly ranked journals. The two cited papers hold promises that we are soon facing the possibility to produce functional human male haploid germ cells in vitro. This success was achieved by employing relatively simple and non-costly organ culture methodology and stem cell technology. Such set up and readily available hiPS cells are expected to be every man's tool in clinical centers worldwide. In the context of the pediatric endocrinologist, these techniques add to the potentials for fertility preservation in prepubertal boys receiving gonadotoxic treatment for childhood cancer.

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