Reproductive Endocrinology

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Reproductive endocrinology is a lively and interesting research area, with implications for the entire human life cycle. Major achievements have been made in this field during the past year. A broad search of relevant papers published during this period gave more than 1,000 hits which were screened and sorted to various topics for final selection. The selected 24 papers have advanced the field and cover different areas of reproductive endocrinology, including basic science, translational and clinical publications. It is obvious that not everything can be covered within the frame of this chapter. The research area hosts multiple topics and you will find that also other chapters of this *Yearbook* deal with subjects linked to reproduction. We hope you find the present selection interesting and worthwhile reading, and that you will make use of it in your daily professional activities.

New mechanism

On the association between cryptorchidism and testis cancer

Germ cell development in the postnatal testis: the key to prevent malignancy in cryptorchidism?

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Front Endocrinol (Lausanne) 2012;3:176

Background: To permit normal postnatal germ cell development, the mammalian testis undergoes a complex, multistaged process of descent to the scrotum. Failure of any part of this process leads to congenital cryptorchidism, wherein the malpositioned testis finds itself at the wrong temperature after birth, which leads to secondary germ cell loss and later infertility and risk of cancer.

Methods and Results: In this review the authors present recent studies showing that neonatal gonocytes transform into the putative spermatogenic stem cells between 3 and 9 months, and this initial postnatal step is deranged in cryptorchid testes. In addition, it is thought the abnormally high temperature may also impair apoptosis of remaining gonocytes, allowing some to persist to become the possible source of carcinoma in situ and malignancy after puberty.

Conclusions: The biology of postnatal germ cell development is of intense interest, as it is likely to be the key to the optimal timing for orchidopexy to prevent infertility and malignancy.

Cryptorchidism and testicular germ cell tumors: comprehensive meta-analysis reveals that association between these conditions diminished over time and is modified by clinical characteristics

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Background: The risk of testicular germ cell tumors (TGCT) is consistently associated with a history of cryptorchidism (CO) in epidemiologic studies. Factors modifying the association may provide insights regarding etiology of TGCT and suggest a basis for individualized care of CO.

Methods: To identify modifiers of the CO-TGCT association, the authors conducted a comprehensive, quantitative evaluation of epidemiologic data. Human studies cited in PubMed or ISI Web of Science

indices through December 2011 and selected unpublished epidemiologic data were reviewed to identify 35 articles and one unpublished dataset with high-quality data on the CO-TGCT association. Association data were extracted as point and 95% confidence interval estimates of odds ratio (OR) or standardized incidence ratio (SIR), or as tabulated data. Values were recorded for each study population, and for subgroups defined by features of study design, CO and TGCT. Extracted data were used to estimate summary risk ratios (sRR) and evaluate heterogeneity of the CO-TGCT association between subgroups.

Results: The overall meta-analysis showed that a history of CO is associated with a fourfold increased TGCT risk (RR = 4.1; 95% CI 3.6–4.7). Subgroup analyses identified five determinants of stronger association: bilateral CO, unilateral CO ipsilateral to TGCT, delayed CO treatment, TGCT diagnosed before 1970, and seminoma histology.

Conclusions: Modifying factors may provide insight into TGCT etiology and suggest improved approaches to managing CO. Based on available data, CO patients and their parents or caregivers should be made aware of elevated TGCT risk following orchidopexy, regardless of age at repair, unilateral vs. bilateral non-descent, or position of undescended testes.

These two studies deal with several aspects of the pathophysiology and consequences of cryptorchidism with focus of its association with germ cell cancer. Cryptorchidism is the most common congenital malformation in boys and is a concern of all pediatricians as the consequences of treatment failure are infertility and a substantially increased risk of testicular cancer. The pathogenesis of germ cell cancer in relation to cryptorchidism has been extensively discussed as has the most optimal age for treatment by orchidopexy. Very early surgical correction has been advocated recently which is supported by the paper of Hutson et al. The exact numbers of the increased cancer risk have also been a subject of debate and whether it is affected or not by treatment. These two review papers are recommended to pediatric endocrinologists who want to remain updated on these ongoing discussions.

New mechanisms Sex determination

Sex reversal in C57BL/6J XY mice caused by increased expression of ovarian genes and insufficient activation of the testis-determining pathway

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PLoS Genet 2012;8:e1002569

Background: Sex reversal can occur in XY humans with only a single functional WT1 or SF1 allele or a duplication of the chromosome region containing WNT4, but XY mice with only a single functional Wt1, Sf1, or Wnt4 allele, or mice that overexpress Wnt4 from a transgene, reportedly are not sex-reversed. Because genetic background plays a critical role in testis differentiation, particularly in C57BL/6J (B6) mice, the hypothesis that Wt1, Sf1, and Wnt4 are dosage-sensitive in B6 XY mice was tested.

Methods and Results: A reduced Wt1 or Sf1 dosage in B6 XY(B6) mice was found to impair testis differentiation, but no ovarian tissue developed. When a Y(AKR) chromosome replaced the Y(B6) chromosome, these otherwise genetically identical B6 XY mice developed ovarian tissue. In contrast, reduced Wnt4 dosage increased the amount of testicular tissue present in Sf1^{+/-} B6 XY(AKR), Wt1^{+/-} B6 XY(AKR), B6 XY(POS), and B6 XY(AKR) fetuses. Therefore the authors propose that Wt1(B6) and Sf1(B6) are hypomorphic alleles of testis-determining pathway genes and that Wnt4(B6) is a hypermorphic allele of an ovary-determining pathway gene. The latter hypothesis is supported by the finding that expression of Wnt4 and four other genes in the ovary-determining pathway are elevated in normal B6 XX E12.5 ovaries. B6 mice seem to be sensitive, at least in part, to XY sex reversal because they carry Wt1(B6) and/or Sf1(B6) alleles that compromise testis differentiation and a Wnt4(B6) allele that promotes ovary differentiation and thereby antagonizes testis differentiation. Addition of a 'weak' Sry

allele, such as the one on the Y(POS) chromosome, to the sensitized B6 background results in an inappropriate development of ovarian tissue.

Conclusions: Wt1, Sf1, and Wnt4 are dosage-sensitive in mice, this dosage sensitivity is genetic background-dependent, and the mouse strains described in the article are good models for the investigation of human dosage-sensitive XY sex reversal.

A genetic basis for a postmeiotic X versus Y chromosome intragenomic conflict in the mouse

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Background: Intragenomic conflicts arise when a genetic element favors its own transmission to the detriment of others. Conflicts over sex chromosome transmission are expected to have influenced genome structure, gene regulation, and speciation. In the mouse, the existence of an intragenomic conflict between X- and Y-linked multicopy genes has long been suggested but never demonstrated. The Y-encoded multicopy gene Sly has been shown to have a predominant role in the epigenetic repression of postmeiotic sex chromatin (PMSC) and, as such, represses X and Y genes, among which are its X-linked homologs Slx and Slxl1.

Methods and Results: Mice that are deficient for both Sly and Slx/Slxl1 showed that Slx/Slxl1 has an opposite role to that of Sly, in that it stimulates XY gene expression in spermatids. Slx/Slxl1 deficiency rescues the sperm differentiation defects and near sterility caused by Sly deficiency and vice versa. Slx/Slxl1 deficiency also causes a sex ratio distortion towards the production of male offspring that is corrected by Sly deficiency. All in all, the data show that Slx/Slxl1 and Sly have antagonistic effects during sperm differentiation and are involved in a postmeiotic intragenomic conflict that causes segregation distortion and male sterility. This is undoubtedly what drove the massive gene amplification on the mouse X and Y chromosomes. It may also be at the basis of cases of F1 male hybrid sterility where the balance between Slx/Slxl1 and Sly copy number, and therefore expression, is disrupted.

Conclusions: This study demonstrates a competition between X- and Y-related genes that occurs in mammals. It also provides a biological basis for the concept that intragenomic conflict is an important evolutionary force which impacts on gene expression, genome structure, and speciation.

PRC1 coordinates timing of sexual differentiation of female primordial germ cells

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Background: In mammals, sex differentiation of primordial germ cells (PGCs) is determined by extrinsic cues from the environment. In mouse female PGCs, expression is stimulated by the retinoic acid gene 8 (Stra8) and meiosis is induced in response to retinoic acid provided from the mesonephroi. Given the widespread role of retinoic acid signaling during development, the molecular mechanisms that enable PGCs to express Stra8 and enter meiosis in a timely manner are unknown.

Methods and Results: Gene dosage-dependent roles in PGC development for Ring1 and Rnf2, two central components of the Polycomb repressive complex 1 (PRC1), are identified. Both paralogues are essential for PGC development between days 10.5 and 11.5 of gestation. Rnf2 is subsequently required in female PGCs to maintain high levels of Oct4 and Nanog expression, and to prevent premature induction of meiotic gene expression and entry into meiotic prophase. Chemical inhibition of retinoic acid signaling partially suppresses precocious Oct4 downregulation and Stra8 activation in Rnf2-deficient female PGCs. Chromatin immunoprecipitation analyses show that Stra8 is a direct target of PRC1 and PRC2 in PGCs.

Conclusions: These data demonstrate the importance of PRC1 gene dosage in PGC development and in coordinating the timing of sex differentiation of female PGCs by antagonizing extrinsic retinoic acid signaling.

These three articles deal with gene dosage and competing genes in gonadal development. The first study shows important findings of how a missing allele, or the dosage of it, can lead to total sex reversal. XY humans with just a single functional WT1 or SF1 allele have an XX phenotype while XY mice with a similar genotype are not sex reversed. Correa and colleagues now report dosage-sensitive effects with these genes in a strain of mouse (C57BL/6J) known to be sensitive to disruptions in factors that affect testis differentiation. The C57BL/6J mice heterozygous for WT1 and SF1 exhibited impaired testis differentiation. Indeed, when combined with a weak Sry allele, the gonads were developed into an ovarian like tissue. This system could be a valuable tool to understand the dosage-sensitive sex reversal in humans. The study was highlighted in *Biology of Reproduction* [1]. The second paper presents a competition between genes in sex determination. In this battle between two genes on the X chromosome (Slx/Slxl1) and one at the Y (Sly), Cocque et al. show that the winner will be passed on to the next generation in a higher frequency than the anticipated 1:1 ratio. The paper by Cocque et al. was also highlighted in *Biology of Reproduction* [2]. In the third paper, a gene that coordinates the timing of sexual differentiation in female primordial germ cells is studied. Here, again, a dosage-dependent mechanism in development is shown. In female PGCs, retinoic acid plays an important role in timing of sex differentiation. The retinoic acid gene 8 is shown to be a direct target of PRC1 in these cells and its dosage is important for PGC development and coordination of sex differentiation.

These three articles all show examples on how genes are competing, and how their respective dosage/level influence expression, passage to the next generation and sex determination, both in vivo (mice) and in vitro (primordial germ cells). This implicates a multigenic origin of at least some of these disorders in humans.

New genes

Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination

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Background: The combination of ataxia and hypogonadism was first described more than a century ago, but its genetic basis has remained elusive.

Methods: The authors performed whole-exome sequencing in a patient with ataxia and hypogonadotropic hypogonadism, followed by targeted sequencing of candidate genes in similarly affected patients. Neurologic and reproductive endocrine phenotypes were characterized in detail. The effects of sequence variants and the presence of an epistatic interaction were tested in a zebrafish model.

Results: Digenic homozygous mutations in RNF216 and OTUD4, which encode a ubiquitin E3 ligase and a deubiquitinase, respectively, were found in three affected siblings in a consanguineous family. Additional screening identified compound heterozygous truncating mutations in RNF216 in an unrelated patient and single heterozygous deleterious mutations in 4 other patients. Knockdown of *rnf216* or *otud4* in zebrafish embryos induced defects in the eye, optic tectum, and cerebellum; combinatorial suppression of both genes exacerbated these phenotypes, which were rescued by non-mutant, but not

mutant, human RNF216 or OTUD4 messenger RNA. All patients had progressive ataxia and dementia. Neuronal loss was observed in cerebellar pathways and the hippocampus; surviving hippocampal neurons contained ubiquitin-immunoreactive intranuclear inclusions. Defects were detected at the hypothalamic and pituitary levels of the reproductive endocrine axis.

Conclusions: The syndrome of hypogonadotropic hypogonadism, ataxia, and dementia can be caused by inactivating mutations in RNF216 or by the combination of mutations in RNF216 and OTUD4. These findings link disordered ubiquitination to neurodegeneration and reproductive dysfunction and highlight the power of whole-exome sequencing in combination with functional studies to unveil genetic interactions that cause disease.

This is the first paper to link a new mechanism (defective ubiquitination) to reproductive dysfunction and neurodegeneration. This multi-author paper is also a beautiful example and role model of how collaborative efforts by basic science and clinical researchers employing cutting-edge technology can create a complete story, starting from a single well-phenotyped patient. The work has advanced our knowledge by adding another identifiable cause of hypogonadotropic hypogonadism that may appear in the borderland of pediatric endocrinology and neuropediatrics.

Novel functions of an old factor

INSL3 as a biomarker of Leydig cell functionality

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Background: Insulin-like factor 3 (INSL3) is a small peptide hormone made and secreted uniquely by mature Leydig cells in the testes of all mammals. Importantly, this expression and secretion appears to be constitutive and therefore reflects the differentiation status and number of the Leydig cells present, differing thereby from testosterone which is acutely and homeostatically regulated by the hormones of the HPG axis.

Methods and Results: The authors reviewed the role of INSL3 as a marker of Leydig cell differentiation and function. Measurement of INSL3 either as mRNA in the testis, or as secreted peptide circulating in the blood, provides an excellent assessment of Leydig cell differentiation, for example during fetal development, puberty or in aging, or following exposure to endocrine disrupting agents. Whereas INSL3 is proving increasingly useful as a biomarker for testis status, less is known about its functions, particularly in the adult male.

Conclusions: Current evidence points to autocrine, paracrine and endocrine roles of INSL3, acting through the G-protein-coupled receptor called RXFP2, although more research is required to characterize these functions in detail.

The Leydig cell peptide INSL3 is well known for its role in testicular descent in male fetuses although defective INSL3 or its receptor RXFP2 is a rare cause of cryptorchidism. A novel role of INSL3 is emerging linking it to Leydig cell number and status in the postnatal testis. However, the exact function of INSL3 in postnatal testicular biology and dysfunction is yet to be determined. The present review paper is an update of the current status of INSL3 and its receptor with some focus on its potential usefulness as a testicular marker in pathological conditions and postnatal testicular development.

Testis tissue explantation cures spermatogenic failure in c-Kit ligand mutant mice

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Background: Male infertility is most commonly caused by spermatogenic defects or insufficiencies, the majority of which are as yet incurable. Recently, the authors succeeded in cultivating mouse testicular tissues for producing fertile sperm from spermatogonial stem cells.

Methods and Results: In this study the authors showed that one of the most severe types of spermatogenic defect mutant can be treated by the culture method without any genetic manipulations. The *Sl/Sl(d)* mouse was used as a model of such male infertility. The testis of the *Sl/Sl(d)* mouse has only primitive spermatogonia as germ cells, lacking any sign of spermatogenesis owing to mutations of the c-kit ligand (KITL) gene that cause the loss of membrane-bound type KITL from the surface of Sertoli cells. To compensate for the deficit, the authors cultured testis tissues of *Sl/Sl(d)* mice with a medium containing recombinant KITL and found that it induced the differentiation of spermatogonia up to the end of meiosis. They further discovered that colony-stimulating factor-1 (CSF-1) enhances the effect of KITL and promoted spermatogenesis up to the production of sperm. Microinsemination of haploid cells resulted in delivery of healthy offspring.

Conclusions: This study demonstrated that spermatogenic impairments can be treated in vitro with the supplementation of certain factors or substances that are insufficient in the original testes.

Male infertility caused by genetic defects in germ or somatic cells in the testis is regarded as an incurable disorder. In the present study the authors used a cell culture method to revive fertility in male mice that failed to make sperm due to a genetic defect (mutations in Kit ligand (KITL), stem cell factor, and steel factor). To correct the spermatogenic deficit in *Sl/Sl(d)* mice, the authors employed a cell culture system with spermatogonial stem cells present in testicular tissue from *Sl/Sl(d)* mice and added recombinant KITL. This procedure induced differentiation of spermatogonia up to the end of meiosis. To reach final differentiation colony-stimulating factor 1 (CSF-1), was added which is known to function together with KITL in other cell systems. CSF-1 enhanced the effect of KITL and prompted spermatogenesis, resulting in production of sperm. These sperm were functional and gave rise to apparently healthy offspring. This in vitro culture model may be a useful tool not only for studying the mechanisms of spermatogenesis, including the exact role of CSF-1, but potentially also to provide a new opportunity to treat certain types of male infertility in humans. This article is highlighted in *Biology of Reproduction* [3].

The ontogeny of cKIT⁺ human primordial germ cells proves to be a resource for human germ line reprogramming, imprint erasure and in vitro differentiation

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Background: The generation of research-quality, clinically relevant cell types in vitro from human pluripotent stem cells requires a detailed understanding of the equivalent human cell types.

Methods: In this study, 134 human embryonic and fetal samples were analyzed, from 6 to 20 developmental weeks, to identify the stages at which cKIT⁺ primordial germ cells (PGCs), the precursors of gametes, undergo whole-genome epigenetic reprogramming with global depletion of 5mC, H3K27me3 and H2A.Z, and the time at which imprint erasure is initiated and 5hmC is present.

Results: Using five alternative in vitro differentiation strategies combined with single-cell microfluidic analysis and a bona fide human cKIT⁺ PGC signature, the authors show the stage of cKIT⁺ PGC formation in the first 16 days of differentiation.

Conclusion: The study creates a resource of human germ line ontogeny that is essential for future studies aimed at in vitro differentiation and unveiling the mechanisms necessary to pass human DNA from one generation to the next.

Human germ line development begins with the formation of PGCs that express the tyrosine kinase receptor cKIT. Little is known about the developmental progression (ontogeny) of cKIT⁺ PGCs. On the basis of the mouse model, it is clear that PGCs must undergo whole-genome epigenetic reprogramming to remove cytosine methylation from imprinted genes and restore totipotency. Gkoutela et al. have performed a time identifying study of gene expression in more than a hundred human embryonic and fetal samples from developmental weeks 6 to 20, and determined the stage when cKIT⁺ PGCs undergo whole-genome epigenetic reprogramming. Given the fundamental role of epigenetic reprogramming in the germ line, it is essential to characterize reprogramming in human PGCs because mouse and human genomes are separated and diverse strategies may have evolved to execute it. The results show that reprogramming in the human cKIT⁺ lineage follows a protracted series of events that are similar but not identical to that of the mouse. Once the major molecular landmarks of human PGC reprogramming are known, this information will be critical to assessing PGC differentiation and reprogramming in vitro. It also helps identifying problems that must be overcome to generate a functional germ line from human pluripotent stem cells. This study was highlighted in *Biology of Reproduction* [4].

Restoring fertility in sterile childhood cancer survivors by autotransplanting spermatogonial stem cells: are we there yet?

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Background: Current cancer treatment regimens do not only target tumor cells, but can also have devastating effects on the spermatogonial stem cell pool, resulting in a lack of functional gametes and hence sterility. In adult men, fertility can be preserved prior to cancer treatment by cryopreservation of ejaculated or surgically retrieved spermatozoa, but this is not an option for prepubertal boys since spermatogenesis does not commence until puberty.

Methods and Results: Cryopreservation of a testicular biopsy taken before initiation of cancer treatment, followed by in vitro propagation of spermatogonial stem cells and subsequent autotransplantation of these stem cells after cancer treatment, has been suggested as a way to preserve and restore fertility in childhood cancer survivors. This strategy, known as spermatogonial stem cell transplantation, has been successful in mice and other model systems, but has not yet been applied in humans. Although recent progress has brought clinical application of spermatogonial stem cell autotransplantation in closer range, there are still a number of important issues to address.

Conclusions: In this paper, the authors describe the state of the art of spermatogonial stem cell transplantation and outline the hurdles that need to be overcome before clinical implementation.

Late effects of cancer treatment in childhood is now an established clinical focus area in most pediatric endocrinology centers, since endocrine problems are among the most common problems encountered in this expanding group of new survivors in pediatrics. Future reproductive health and particularly fertility issues is a major concern in many of these children. Postpubertal patients with potential to donate semen that can be cryopreserved for later use are better off in this respect but for prepubertal boys there is yet no evidence-based methodology to protect their future fertility. The paper by Struijk and co-workers deals with the emerging technology to collect testicular tissue by biopsy for collection of spermatogonial stem cells prior to gonadotoxic cancer treatment. These cells are then expanded by in vitro propagation and later used for autotransplantation to rescue fertility. This technology has been proven to be functional in animal models but is not yet established in humans. The paper presents the technology and the problems to be overcome before clinical implementation.

Female reproductive health after childhood, adolescent, and young adult cancers: guidelines for the assessment and management of female reproductive complications

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Background: As more young female patients with cancer survive their primary disease, concerns about reproductive health related to primary therapy gain relevance. Cancer therapy can often affect reproductive organs, leading to impaired pubertal development, hormonal regulation, fertility, and sexual function, affecting quality of life.

Methods: The Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancer (COG-LTFU Guidelines) are evidence-based recommendations for screening and management of late effects of therapeutic exposures. The guidelines are updated every 2 years by a multidisciplinary panel based on current literature review and expert consensus.

Results: This review summarizes the current task force recommendations for the assessment and management of female reproductive complications after treatment for childhood, adolescent, and young adult cancers. Experimental pretreatment as well as posttreatment fertility preservation strategies, including barriers and ethical considerations, which are not included in the COG-LTFU Guidelines, are also discussed.

Conclusion: Ongoing research will continue to inform COG-LTFU Guideline recommendations for follow-up care of female survivors of childhood cancer to improve their health and quality of life.

This review is an attempt to give an important update on the situation for female patients who survived childhood cancer. The authors state that the risk of gonadal, reproductive, and sexual complications in female cancer survivors varies according to cancer type, age at diagnosis, and therapeutic exposure. A summary of pre- and posttreatments in fertility preservation strategies, which are not included in the COG-LTFU Guidelines, is given. They also conclude that it is vital for the oncologists to be aware of potential complications that may affect long-term reproductive and sexual health and discuss them in an appropriate way with patients and families before treatment. The final message is that all patients should have regular assessments of pubertal development, sexual health and function, and pregnancy attempts and outcomes. The patients should be cared for by specialists in dedicated late-effects clinics.

The ovarian reserve in mammals: a functional and evolutionary perspective

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Mol Cell Endocrinol 2012;356:2–12

Background: The constitution and the control of the ovarian reserve are of importance in mammals and women. In particular, the number of primordial follicles at puberty is positively correlated with the number of growing follicles and their response to gonadotropin treatments. The size of this ovarian reserve depends on genes involved in germ cell proliferation and differentiation, sexual differentiation, meiosis, germ cell degeneration, formation of primordial follicles, and on a potential mechanism of self-renewal of germ stem cells.

Methods and Results: This review presents state-of-the-art knowledge of genes and factors involved in all these processes. Almost 70 genes identified mainly by mouse invalidation models are in focus. The most plausible hypothesis concerning the possibility of the existence of germ cell self-renewal by neo-oogenesis in animal species and humans, with a special interest for the role of corresponding genes in evolutionary distinct model species, is discussed.

Conclusion: All of the genes pointed out are candidates susceptible to explain fertility defects, such as the premature ovarian failure in humans.

This review summarizes the present knowledge of genes and factors involved in the maintenance of the ovarian reserve in mammals. Almost 70 genes from mouse models were identified and the possibility of self-renewal of germ cells by neo-oogenesis in mammals was discussed. Several conclusions were drawn: (1) The key hormones for terminal follicular growth in mammals, FSH and LH, do not seem to play any role in oogenesis, nor in the formation of the pool of primordial follicles. (2) For estimation of the ovarian reserve, systematic investigation of the number of primordial follicles is required at key stages of development. If the phenotype is not drastic, there will be no consequences for fertility. (3) It is uncertain whether conclusions drawn on the basis of gene invalidation in mouse models can be fully extrapolated to other species. From the 120 genes studied by Liao and Zhang [5], several examples of discrepancies between mice and humans were found. Some genes (e.g. WT1 and FOXL2) seem to play very similar roles in these species, but there are several mutations in Kit and Kit ligand genes that have dramatic consequences on germ cells in the mouse, whereas women carrying mutations on orthologous loci do not display any fertility defect. (4) Concerning the existence of neo-oogenesis in mammalian ovaries, the hypothesis based on the data by Tilly and colleagues [6] remains to be fully confirmed. The model of depletion of the ovarian reserve during female reproductive life remains the most plausible. (5) The new era of high throughput genotyping and whole-genome association studies will make it possible in the near future to identify candidate genes responsible for the high variability of the ovarian reserve size and other parameters of fertility. The authors end with this promising fifth perspective, possibly enabling in vitro models of neo-oogenesis in the not so distant future.

Challenges of disorders of sex development: diverse perceptions across stakeholders

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Background: Disorders of sex development (DSD) are congenital conditions in which chromosomal, gonadal, or anatomic sex development is atypical. Optimal management is patient- and family-centered and delivered by interdisciplinary teams. The present pilot study elicits concerns held by important stakeholders on issues affecting young patients with DSD and their families.

Methods: Content from focus groups with expert clinicians (pediatric urologists (n = 7), pediatric endocrinologists (n = 10), mental health professionals (n = 4), DSD patient advocates (n = 4), and interviews with parents of DSD-affected children (newborn to 6 years; n = 11)) was coded and content-analyzed to identify health-related quality of life issues.

Results: Key stressors varied across stakeholder groups. In general, family-centered issues were noted more than child-centered. In the child-centered domain, providers worried more about physical functioning; family and advocates emphasized gender concerns and body image. In the family-centered domain, parental concerns about medication management outweighed those of providers. Advocates reported more stressors regarding communication/information than other stakeholders.

Conclusions: Variability exists across stakeholder groups in the key concerns affecting young children/families with DSD. Interdisciplinary DSD healthcare team development should account for varying perspectives when counseling families and planning treatment.

The development of the best care for DSD patients and their families is a challenging issue in pediatric endocrinology. Multidisciplinary efforts are now routine in centers with high standards of health-care. This paper presents the views and perspectives of various groups of stakeholders on key issues for DSD patients. For a pediatric endocrinologist it is important to notice the variable perceptions by stakeholders often diverging from the views of the medical team. Although this is a pilot study with low numbers and therefore limited representation of views, the approach is important and should be complemented with follow-up investigations at an older age of the DSD patients to see if the views and differences among stakeholders persist.

Castrati singers – all for fame

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Background: The phenomenon of castration is very closely associated with the baroque era. In a period that placed emphasis on pure sensual pleasure, castrati singers, with their angelic voices, were a perverted outcome of this ambition. It was the intention that these castrato voices with their supernatural sound would mesmerize audiences. At that time, it could be said that within certain society circles, an addiction to these voices existed. Equally, they were oblivious to the spiritual side of the lives of the castrati. Farinelli, Caffarelli, and Senesino, three of the most famous castrati, were the first musical superstars of the 18th century. Their voices moved decadent audiences to tears and standing ovations. The price for this fame was, however, high. The aim of this review is to provide an overview of castrati singers, especially in the baroque era, their sexuality and the effects of castration on their physical development.

Methods: A literature search of relevant databases, books, and articles in journals was performed and compared with current data concerning androgen suppression and endocrine aspects of male sexual dysfunction.

Results: The effects of castration on physical development were notoriously erratic. Much depended on the timing of the operation: boys pruned before the age of 10 or so very often grew up with feminine features – smooth, hairless bodies, incipient breasts, infantile penis, and often a complete lack of sex drive.

Conclusions: The success of the castrati faded with the end of the 18th century. The last castrato was Alessandro Moreschi, a solo singer in the choir of the Sistine Chapel of the Vatican. Following the ban on castration, Pope Pius X sent him into retirement in 1912, thus putting an end to a very impressive era in the history of music.

A castrati is a male singer who had been castrated before puberty to keep his voice equivalent to that of a soprano or mezzo soprano, in contrast to a eunuch who typically was castrated after puberty not affecting the voice to a major extent. Prepubertal castration was banned and made illegal in Italy in 1870 and it is now close to 100 years ago since the last professional castrati singer was sent to retirement by the Pope. The cited paper deals with an important part of the medical history describing the lives and 'phenotypes' of the famous castrati singers in the 18th century, based on available old literature and comparison with the current level of knowledge in endocrinology. Despite lack of deeper insights of endocrine physiology at the time of writing the presentations of the castrati singers as males with complete hypogonadism are well described.

Food for thought

Testosterone levels and its relation to male parenthood

Testosterone and relationship quality across the transition to fatherhood

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Biol Psychol 2012;90:186–191

Background: Recent research points to decreasing testosterone (T) levels as well as decreasing relationship quality during the transition to fatherhood, and it has been suggested that T reflects and affects motivation and behavior with respect to mating or paternal effort. The authors hypothesized that decreases in T are associated with decreasing relationship quality in new fathers.

Methods: 37 fathers and 38 men in committed romantic relationships without children (controls) were recruited. All subjects participated actively by collecting saliva samples for T assessment three times a

day on 2 assessment days, 4 weeks prior to birth (day 1) and 8 weeks after birth (day 2) for fathers, and 3 months after the first assessment for controls and by filling out questionnaires on relationship quality. *Results:* Significantly lower T levels (AUCg-T) were found in fathers compared with in controls at day 2 and significant decreases in relationship quality from day 1 to day 2 in fathers, but not in controls. In particular, the new fathers reported tenderness in their relationship to have significantly decreased from pre to post birth in comparison to the controls. These results were partially moderated by T levels at day 1.

Conclusion: The results are in line with the ‘challenge hypothesis’ in humans, according to which T levels are positively associated with mating effort and negatively related to paternal activities.

Does co-sleeping contribute to lower testosterone levels in fathers? Evidence from the Philippines

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Background: Because cross-species evidence suggests that high testosterone (T) may interfere with paternal investment, the relationships between men’s transition to parenting and changes in their T are of growing interest. Studies of human males suggest that fathers who provide childcare often have lower T than uninvolved fathers, but no studies to date have evaluated how nighttime sleep proximity between fathers and their offspring may affect T.

Methods: Using data collected in 2005 and 2009 from a sample of men ($n = 362$; age 26.0 ± 0.3 years in 2009) residing in metropolitan Cebu, Philippines, the fathers’ T was evaluated based on whether they slept on the same surface as their children (same surface co-sleepers), slept on a different surface but in the same room (room sharers), or slept separately from their children (solitary sleepers). A large majority (92%) of fathers in this sample reported practicing same surface co-sleeping.

Results: Compared to fathers who slept solitarily, same surface co-sleeping fathers had significantly lower evening (PM) T and also showed a greater diurnal decline in T from waking to evening (both $p < 0.05$). Among men who were not fathers at baseline (2005), those who were co-sleeping fathers at follow-up (2009) experienced a significantly greater longitudinal decline in PM T over the 4.5-year study period ($p < 0.01$) compared to solitary sleeping fathers. Among these same men, baseline T did not predict fathers’ sleeping arrangements at follow-up ($p > 0.2$).

Conclusions: These results are consistent with previous findings indicating that daytime father-child interaction contributes to lower T among fathers. These findings specifically suggest that close sleep proximity between fathers and their offspring results in greater longitudinal decreases in T as men transition to fatherhood and lower PM T overall compared to solitary sleeping fathers.

Studies in humans suggest that fathers who provide childcare have lower testosterone (T) levels than uninvolved fathers [7]. This hypothesis is tested in these two studies. Perini et al. have collected saliva samples from 75 men, 4 weeks before and 8 weeks after fatherhood, or 3 months apart for the controls. They did find significantly lower T levels in fathers compared to controls in sample 2, and a significant decrease in relationship quality from sample 1 to 2 in fathers, but not in controls. In the second paper the fathers were evaluated either as co-sleepers or solitary sleepers. The same surface co-sleepers had lower evening T levels than solitary sleepers, and also a greater diurnal decline in T from waking up to evening. There is though a rather skewed balance in the groups sizes, with 17 for solitary sleepers and 345 for co-sleepers. The strength of the paper is the results of a follow-up in 2009 when those that were not fathers in 2005 were studied after they had become fathers. Among these men it was shown that the baseline T values did not predict their sleeping arrangements as fathers. The results are consistent with previous findings from this cohort, where father-child interaction contributes to lower T levels among fathers [8]. There was a strong association between self-reported involvement in childcare and lower evening T, supporting the idea that the evening nadir in T is related to social interactions across the day [8]. Thus, when men are closely interacting in childcare their T levels decline. What about females then? In the studies on the Philippian cohort, the mothers showed lower waking T as compared to the non-mothers [9]. Thus, in child-caring fathers, and mothers, the T levels decline.

These results indicate that the infants reduce T in the fathers rather than low T fathers being better at parenting. This could be physiologically relevant for the situation when intercourse more often resulted in new babies. The decline in T levels could then reduce the 'risk' of a new pregnancy too close to the previous and therefore increase the chance for the baby to survive the first years, i.e. when the parents are caregivers of dependent babies their libido is put on hold. Interpretations on the social and societal impact of these observations are up to the reader.

– and its impact in utero on female offspring...

Perinatal testosterone exposure and maternal care effects on the female rat's development and sexual behavior

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J Neuroendocrinol 2013;25:528–536

Background: Natural variations in maternal care have profound influences on offspring behavior, brain activity, and hormone release. Measuring the amount of time a rat dam spends licking/grooming (LG) her pups during their first week of life allows for characterization of distinctive low, mid, and high LG phenotypes. It was previously shown that female offspring of high LG mothers are less sexually receptive, less motivated to mate, and show a later onset of puberty relative to low LG offspring. Given that high LG females are exposed to greater levels of testosterone in utero, the hypothesis that differences in sexual behavior between high and low LG female offspring are driven in part by differences in prenatal hormone exposure was raised.

Methods: To test this hypothesis, pregnant dams pre-characterized as low, mid, or high LG mothers were implanted with testosterone or placebo on gestational day 16. Offspring body weight and anogenital index were assessed at gestational day 21 and in adulthood. Age of vaginal opening and estrous cyclicity were assessed to determine timing of pubertal onset.

Results: Testosterone exposure removed the effects of maternal LG phenotypes on pubertal onset in the offspring by delaying vaginal opening and appearance of first proestrus. In adulthood, sexual behavior in a paced mating chamber following sham surgery or ovariectomy with steroid replacement was examined. Low, mid, and high LG female offspring were found to be differentially affected by perinatal testosterone exposure, and that this exposure removes low LG offspring's precocial pubertal onset and increases high LG's sexual proceptivity and receptivity.

Conclusion: These results suggest that maternal programming of the female reproductive system may be mediated, in part, through differences in perinatal testosterone exposure, instead of solely through maternal behavior.

In rodents there is a natural variation in maternal care which has a strong effect on the offspring. The dams are regarded as low, mid or high licking/grooming (LG) phenotypes, and it is known that the offspring of high LG mothers are less sexually active, less interested in mating and have a later onset of puberty as compared the offspring of low LG mothers. High LG mothers are found to be exposed to higher levels of testosterone (T) in utero. Therefore the idea that differences in sexual behavior between high and low LG females offspring are driven by the different prenatal hormone exposure was raised. In this study Borrow et al. studied the effect of T exposure in utero, during late pregnancy, on the offspring. The mothers of all LG phenotypes were included and the results showed that the T exposure eliminated the differences in the offspring. Thus, T removed the low LG offspring's precocious onset of puberty and increased the proceptivity and receptivity of the high LG offspring. It was concluded that the T level in utero influences the reproductive behavior and development of the female offspring more than the maternal care itself. The implications for human development, if any, remain to be elucidated. The exposure to androgens in female fetuses with severe CAH may be of interest in this respect.

Is premature thelarche in the first two years of life transient?

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Background: Premature thelarche (PT) refers to isolated onset of thelarche in girls younger than 8 years of age. Most cases have an onset under 2 years of age. The authors aimed to establish whether the onset of thelarche under 2 years of age certifies a transient clinical course, as suggested by several other authors.

Methods: 67 girls with an onset of PT under 2 years of age were classified as having early puberty (EP) or classical PT after 1 year of follow-up. Progression of pubertal findings or absolute growth velocity (GV) standard deviation score (SDS) >1 SDS constituted the criteria for a diagnosis of EP.

Results: 20 (29.1%) girls were classified as having EP and 47 (70.1%) as having classical PT. Basal serum luteinizing hormone (LH; ICMA) values above a cut-off level of 0.3 IU/l were found to be a significant risk factor for having an atypical course (OR 7.8, 95% CI 2.04–30.4; $p = 0.003$).

Conclusions: Onset of thelarche under 2 years of age does not assure a transient course in a remarkable proportion of girls with PT. An absolute GV value of >1 SDS or a basal LH level ≥ 0.3 IU/l are suggested as indicators for close follow-up.

Thelarche in young girls is a common clinical problem for pediatricians and these patients are frequently referred to the pediatric endocrinologist for consultation. The problem is to distinguish simple thelarche from precocious puberty and this subject has been the matter of a number of previous publications. The present paper presents the clinical course of 67 otherwise healthy girls with early onset (<2 years of age) breast development to see if it was a transient development or turned into a true precocious puberty (PP). As many as 30% of the girls were diagnosed with PP, which is a greater number than anticipated from previous studies. Suggested indicators for PP development were high basal LH and increased growth velocity. The message from this study is that girls with onset of thelarche at age <2 years should be followed up closely.

Maternal menopause as a predictor of anti-mullerian hormone level and antral follicle count in daughters during reproductive age

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Background: The association between menopausal age in mothers and daughters has been established through several epidemiological studies. In this study the authors investigate if the ovarian reserve in a woman at a given age is associated with her mother's age at menopause.

Methods: Participants were recruited from female healthcare workers aged 20–40 years employed at Copenhagen University Hospital, Rigshospitalet, and were enrolled in the study between September 2008 and February 2010. Cross-sectional data were obtained from a prospective cohort study of 863 women. The study comprised 527 participants from this prospective cohort whose mothers' age at natural menopause was known. The response rate was 52.1%. Endocrine and ovarian parameters related to reproductive ageing: anti-mullerian hormone (AMH) and antral follicle count (AFC) were assessed by serum AMH analyses and transvaginal ovarian sonography on cycle days 2–5. Data on reproductive history, including age at natural maternal menopause, were obtained through an internet-based questionnaire. An analysis of covariance model was used, with serum-AMH and AFC as outcomes, age as the quantitative predictor and onset of maternal menopause as the categorical predictor, with further adjustments for BMI, use of oral contraceptives, participants' smoking habits and prenatal smoking exposure.

Results: There was a significant association between age at maternal menopause and both serum AMH levels ($p < 0.001$) and AFC ($p = 0.005$). Median serum-AMH concentration declined by 8.6% per year

in the group with early maternal menopausal age (≤ 45 years), by 6.8% per year in the group with normal maternal menopausal age (46–54 years) and by 4.2% per year in the group with late maternal menopausal age (≥ 55 years). Median AFC declined by 5.8% per year in the group with early maternal menopausal age, by 4.7% per year in the group with normal maternal menopausal age and by 3.2% per year in the group with late maternal age at menopause.

Conclusion: A significant, positive association between age at maternal menopause and AMH levels and AFC in daughters was demonstrated. The rate of decline in serum-AMH level and AFC is also associated with age at maternal menopause. This paper shows that early maternal menopause is related to an advanced depletion of the ovarian reserve and that late maternal menopause is related to a delayed depletion.

The age of menopause is genetically determined and the daughters' age is associated with the mothers, but is the ovarian reserve in a woman between 20 and 40 years of age associated with her mother's age at menopause? Bentzen and co-workers (as highlighted in *Biology Reproduction* [10]) answer this question in a large cohort of Danish women, where the mothers' age at natural menopause is known. The fertility was evaluated by two methods on cycle days 2–5: (1) the serum concentration of AMH, which correlates with the number of viable eggs remaining in the ovaries, and (2) ultrasonography to determine the number of antral follicles (AFC), a parameter known to correlate with the ovarian reserve. The authors found that the age of maternal menopause did affect the measurements of ovarian reserve. A positive association between age at maternal menopause and AMH levels and AFC in daughters was demonstrated. However, the results may not be surprising, since an earlier age of menopause of the mothers (associated with a similar age for the daughters) also would indicate a faster decline of the ovarian reserve and vice versa. These findings may be of interest when counseling young women concerning their fertility prospects in certain cases.

Important for clinical practice Adolescent gynecology

Menstrual disorders in adolescence: a marker for hyperandrogenemia and increased metabolic risks in later life? Finnish general population-based birth cohort study

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Background: Hyperandrogenism per se has been suggested to be a significant metabolic risk factor in women and a cause of physical and psychological morbidity in adolescent girls. A weak positive correlation has been described between hyperandrogenemia and obesity in adolescent girls, but the clinical consequences are still poorly understood. Hyperandrogenism and insulin resistance are also key features of polycystic ovary syndrome (PCOS), and women with PCOS are consequently at an increased risk of developing type 2 diabetes mellitus and/or metabolic syndrome, and may have increased cardiovascular morbidity. Are self-reported menstrual disorders associated with hyperandrogenemia and metabolic disturbances as early as in adolescence?

Methods: A population-based, cross-sectional study on postal questionnaires targeting 15- to 16-year-old girls in the Northern Finland Birth Cohort 1986 ($n = 4,567$). There were 3,669 girls who answered the postal questionnaire and out of 3,373 girls who also underwent clinical examinations and blood tests, 2,448 were included in the analyses. The questionnaire included one question about the regularity and length of the menstrual cycle: 'Is your menstrual cycle (the interval from the beginning of one menstrual period to the beginning of the next period) often (more than twice a year) longer than 35 days?' The girls who answered 'yes' to this question were considered to be suffering from menstrual disorders and were classified as 'symptomatic'. The girls who answered 'no' were defined as 'non-symptomatic'.

Results: There were 709 (29%) girls who reported menstrual disorders (symptomatic girls) and 1,739 who had regular periods (non-symptomatic girls). In the whole population and in both study groups, there were significant correlations between body mass index (BMI) (and waist-to-hip ratio), hyperandrogenemia and metabolic parameters. Symptomatic girls exhibited significantly higher serum concentrations of testosterone ($p = 0.010$), lower levels of sex hormone-binding globulin ($p = 0.042$) and higher free androgen indices (FAIs). The two groups had comparable BMI and insulin sensitivity, and serum levels of glucose, insulin and lipids. There was a significant linear trend towards higher FAI values in the higher BMI quartiles in both symptomatic and non-symptomatic girls. In the whole population, there was a statistically significant linear decrease in high-density lipoprotein concentrations ($p < 0.001$) and higher triglyceride concentrations ($p = 0.004$) in the upper FAI quartile.

Conclusion: The results confirm that the association between menstrual disorders, hyperandrogenism, obesity and metabolic risks is already evident in adolescence. Information regarding menstrual disorders in adolescence is a good marker of hyperandrogenemia and may be an early risk factor for the development of PCOS in adulthood. The association between obesity, hyperandrogenism and metabolic risks is already evident in adolescence, which strengthens the importance of noting menstrual disorders at an early stage.

Period problems: disorders of menstruation in adolescents

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Background: Adolescence is a time of great psychological and physical change. In the UK, girls enter puberty around the age of 10 years with a median age of menarche of 12.9 years; thereafter, it may be several years before regular menstrual cycles are established. Variations in the type and the frequency of periods may create anxiety regarding ill health or serious underlying disorders. With the increase in childhood obesity and subsequent polycystic ovary syndrome, there is a greater awareness and presentation of girls with disorders of menstruation.

Methods and Results: The review focuses on normal variations of menses and common pathological causes of menstrual problems, including amenorrhea, dysmenorrhea and menorrhagia. Further consideration is given to the variations of presentation of polycystic ovary syndrome.

Conclusion: The review provides a guide to evaluate the various symptoms, investigations and management options.

The normal menstrual cycle is a coordinated order of events involving the hypothalamus, pituitary, ovary and uterus. The onset of first menstrual period (menarche) is an important landmark, reflecting health and signifying the maturation of a reproductive potential for the young girl. These two articles present data on menstrual disorders in adolescence. Pinola et al. present a large Finnish birth cohort study on girls born 1986. The question they raised was: Are self-reported menstrual disorders associated with hyperandrogenemia and metabolic disturbances as early as in adolescence? The question 'Is your menstrual cycle often (more than twice a year) longer than 35 days?' could be answered with yes or no. The girls responding 'yes' were considered to be suffering from menstrual disorders and therefore symptomatic, whereas the 'no' responding group was considered non-symptomatic. Nearly one third of the girls were symptomatic, while the rest had regular periods. In both groups of girls there was a correlation with BMI, hyperandrogenemia and metabolic parameters. Symptomatic girls had significantly higher testosterone levels in serum, lower levels of SHBG and higher free androgen indices, than the non-symptomatic. The authors conclude that the results confirm that the association between menstrual disorders, hyperandrogenism, obesity and metabolic risks is already evident in adolescence. Information regarding menstrual disorders in adolescence is a good marker of hyperandrogenemia. Peacock et al. review normal variation of menses and pathological causes of menstrual problems. Their paper provides a guide to evaluate the symptoms, investigations and management options. It is concluded that disorders of menstruation can be due to physical or environmental factors or a combination of these. Adolescent gynecology is a neglected area of pediatric endocrinology and these papers show that it is important to evaluate the background causes for the young woman who has problems with her periods, especially because these may have implications for reproductive, endocrine and metabolic disturbances in later life.

Androgen insensitivity syndrome

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Background: Androgen insensitivity syndrome in its complete form is a disorder of hormone resistance characterized by a female phenotype in an individual with an XY karyotype and testes producing age-appropriate normal concentrations of androgens. Pathogenesis is the result of mutations in the X-linked androgen receptor gene, which encodes for the ligand-activated androgen receptor – a transcription factor and member of the nuclear receptor superfamily.

Methods and Results: In this review the authors describe the clinical manifestations of androgen insensitivity syndrome from infancy to adulthood, review the mechanism of androgen action, and show examples of how mutations of the androgen receptor gene cause the syndrome.

Conclusions: Management of androgen insensitivity syndrome should be undertaken by a multidisciplinary team and include gonadectomy to avoid gonadal tumors in later life, appropriate sex-hormone replacement at puberty and beyond, and an emphasis on openness in disclosure.

This paper is an excellent up-to-date review of the genetics, pathophysiology, clinical manifestations and management of patients with androgen insensitivity syndrome. Although these patients are rare their clinical presentations may vary if not detected at birth. This diagnosis should therefore be made known to a wider audience in addition to the pediatric endocrinologist, who should be the primary care specialist in charge.

Aberrant gene expression profiles in pluripotent stem cells induced from fibroblasts of a Klinefelter syndrome patient

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Background: Klinefelter syndrome (KS) is the most common male chromosome aneuploidy. Its pathophysiology is largely unexplained due to the lack of adequate models.

Methods: The authors report the derivation of induced pluripotent stem cell (iPSCs) lines from a KS patient with a karyotype of 47,XXY.

Results: Derived KS-iPSCs met all criteria of normal iPSCs with the potential for germ cell differentiation. Although X chromosome inactivation occurs in all KS-iPSCs, genome-wide transcriptome analysis identifies aberrantly expressed genes associated with the clinical features of KS.

Conclusions: The derived KS-iPSCs can serve as a cellular model for KS research. Identified genes may become biomarkers for early diagnosis or potential therapeutic targets for KS and significantly accelerate the understanding, diagnosis, and treatment of Klinefelter syndrome.

This is the first report of the derivation of an induced pluripotent stem cell (iPSC) line from a XXY patient with Klinefelter syndrome (KS). Transcriptome analysis showed aberrant gene expression compatible with the clinical features of KS. Analysis of these genes may form a basis for biomarkers and possible treatment targets in KS. The cell line itself constitutes an in vitro cellular model for the syndrome.

Impact of estrogen replacement throughout childhood on growth, pituitary-gonadal axis and bone in a 46,XX patient with CYP19A1 deficiency

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Background: The adequate replacement dose of estrogens during infancy and childhood is still not known in girls. Aromatase deficiency offers an excellent model to study how much estrogens are needed during infancy, childhood and adulthood.

Methods: The impact of oral 17 β -estradiol treatment on longitudinal growth, bone age maturation, pituitary gonadotropin feedback, multicystic ovaries and bone mass in the long-term follow-up of a girl compound heterozygote for two point mutations of the CYP19A1 gene was investigated.

Results: Low doses of 17 β -estradiol were needed to achieve normal height velocity and adequate bone age maturation from early childhood onwards. Serum estradiol levels needed for breast development and for the appearance of an endometrial reflex were not sufficient to achieve physiological gonadotropin levels. Without 17 β -estradiol treatment the ovaries of the patient showed a multicystic appearance, which reversed on 17 β -estradiol replacement. Bone mass was within normal ranges during the whole follow-up period.

Conclusions: The authors demonstrated that estradiol is needed not only in puberty but also in childhood for normal growth, bone maturation and achievement of normal bone mass. Particularly, this observation underscores the importance of early low-dose estrogen replacement also in other estrogen-deficient conditions, as for instance in Turner's syndrome.

The authors describe a female patient with aromatase deficiency and her detailed needs of endocrine treatment to achieve optimal growth, pubertal development, ovarian morphology, menstrual cycles and bone health during her childhood and adolescent development. Although based on a single patient, this paper is worthwhile studying as the principles of treatment and dosing of the hormones are well described and possible to translate to other cases.

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