Reproductive endocrinology is a wide field encompassing areas such as germ stem cell biology, assisted reproduction technologies, reproductive behavior, gonadal physiology, puberty, endocrine pharmacology, and many other topics of great interest for pediatric endocrinologists. This year’s chapter includes references to recent papers on reproductive concerns of parental obesity, possible adverse effect caused by use of aromatase inhibitors for short stature, guidelines on endocrine treatment of transsexual adolescents, and many more. There were of course several other excellent papers published in these areas during the past year, some which we might have missed in our search of the area and yet others which were not possible to include due to space limitation. Although the present selection of papers obviously represents our own bias, we hope you will find them enjoyable to read and some provocative and helpful for your activity in the pediatric endocrinology arena.

Important for clinical practice/new concerns in obesity

Maternal obesity, inflammation, and fetal skeletal muscle development

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Biol Reprod 2010;82:4–12

Background: Maternal obesity coupled with Western-style and high-energy diets represents a special problem that can result in poor fetal development, leading to harmful, persistent effects on offspring, including predisposition to obesity and type 2 diabetes.

Methods: Since skeletal muscle is the principal site for glucose and fatty acid utilization and composes 40–50% of total body mass, changes in the properties of offspring skeletal muscle and its mass resulting from maternal obesity may be responsible for the increase in type 2 diabetes and obesity. There is no net increase in the muscle fiber number after birth, therefore the fetal stage is crucial for skeletal muscle development. Its development involves myogenesis, adipogenesis, and fibrogenesis, which are all derived from mesenchymal stem cells. Shifting commitment of mesenchymal stem cells from myogenesis to adipogenesis and fibrogenesis will result in increased intramuscular fat and connective tissue, as well as reduced numbers of muscle fiber and/or diameter, all of which have lasting negative effects on offspring muscle function and properties.

Results: Maternal obesity leads to low-grade inflammation, changing the commitment of mesenchymal stem cells in fetal muscles through several possible mechanisms: (1) inflammation down-regulates wingless and int (WNT) signaling, which attenuates myogenesis; (2) inflammation inhibits AMP-activated protein kinase, which promotes adipogenesis, and (3) inflammation may induce epigenetic modification through polycomb group proteins.

Conclusion: More studies are needed to further explore the underlying mechanisms associated with maternal obesity, inflammation, and the commitment of fetal mesenchymal stem cells.
Maternal metabolism and obesity: modifiable determinants of pregnancy outcome

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Background: Obesity among pregnant women is highly prevalent worldwide and is associated in a linear manner with markedly increased risk of adverse outcome for mother and infant. The role of maternal metabolism in determining these outcomes and the potential for lifestyle modification are largely unknown.

Methods: Studies were identified by searching PubMed, the metaRegister of clinical trials and Google Scholar without limitations. Sensitive search strategies were combined with relevant medical subject headings and text words.

Results: Maternal obesity and gestational weight gain have a significant impact on maternal metabolism and offspring development. Insulin resistance, glucose homeostasis, fat oxidation and amino acid synthesis are all disrupted by maternal obesity and contribute to adverse outcomes. Modification of lifestyle is an effective intervention strategy for improvement of maternal metabolism and the prevention of type 2 diabetes and, potentially, gestational diabetes.

Conclusion: Maternal obesity requires the development of effective interventions to improve pregnancy outcome. Strategies that incorporate a detailed understanding of the maternal metabolic environment and its consequences for the health of the mother and the growth of the child are likely to identify the best approach.

Maternal and paternal obesity has a known negative impact on fertility and pregnancy outcome. The two selected papers show a need of pre-maternity advice on lifestyle factors, since a reduction in weight will reduce the risk of negative effects on the child, but also increase the likelihood of becoming pregnant [1]. The impact of obesity and the low-grade inflammation on mesenchymal stem cells with potentially negative effects on offspring muscle function and metabolism need further investigation.

A meta-analysis aimed at determining if paternal factors like semen parameters and reproductive hormones are affected by obesity [2]. The authors found no relation between increased BMI and semen parameters, but strong evidence of a negative relationship for testosterone, SHBG and free testosterone with increased BMI. A significantly higher number of embryos with a normal karyotype were found in miscarriages of overweight and obese women as compared to normal weight women. The results indicate that the excess risk of miscarriages in the overweight and obese population is independent of embryonic aneuploidy [3].

A recent comment in Biology of Reproduction [4] stated that the high incidence of obesity may aggravate adverse effects of environmental pollutants. Many of these environmental toxicants are lipophilic and thus stored and accumulated in fat tissue. An increased fat mass will therefore increase the toxic dose in obese individuals. Paradoxically, such toxic effects may increase during weight loss when compounds stored in fat are released to the systemic circulation. Combined interaction of reproductive toxicants and obesity is indicated to be additive [5], which predicts an increased need for assisted reproductive technologies (ART) in the future. Of great concern with such development is that more children may be at risk to be born with birth defects and possibly imprinting disorders [6]. A likely explanation for the abnormalities in children born after ART is the link to an altered embryonic epigenome [7]. Preventive measures at the population level and with focus on risk groups, like education in the importance of lifestyle factors, are of great importance for future reproductive health and favorable pregnancy outcome.
Endocrine treatment of transsexual persons: an Endocrine Society clinical practice guideline

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J Clin Endocrinol Metab 2009;94:3132–3154

Background: The aim was to formulate practice guidelines for endocrine care and treatment of transsexual persons including children.

Methods: An evidence-based guideline was developed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system to describe the strength of recommendations and the quality of evidence, which was low or very low.

Results: Committees and members of The Endocrine Society, European Society of Endocrinology, European Society for Paediatric Endocrinology, Lawson Wilkins Pediatric Endocrine Society, and World Professional Association for Transgender Health commented on preliminary drafts of these guidelines.

Conclusions: Transsexual persons seeking to develop the physical characteristics of the desired gender require a safe, effective hormone regimen that will (1) suppress endogenous hormone secretion determined by the person’s genetic/biologic sex and (2) maintain sex hormone levels within the normal range for the person’s desired gender. A mental health professional must recommend endocrine treatment and participate in ongoing care throughout the endocrine transition and decision for surgical sex reassignment. Because a diagnosis of transsexualism in a prepubertal child cannot be made with certainty, the authors do not recommend endocrine treatment of prepubertal children. They recommend treating transsexual adolescents (Tanner stage 2 or later) by suppressing puberty with GnRH analogues until age 16 years, after which cross-sex hormones may be given under strict criteria. They suggest suppressing endogenous sex hormones, maintaining physiologic levels of gender-appropriate sex hormones and monitoring for known risks in adult transsexual persons.

Psychosexual identity is probably the most important part of the individual’s sex and gender complex. Still, we know very little about its underlying biology and disorders, and the diagnostic criteria of gender identity disorders (GID) are based on a weak evidence base. This guidelines paper is therefore an important tool for pediatric endocrinologists caring for children with GID. An increasingly high number of adolescents with GID are referred to many centers, probably due to greater awareness of the condition and changes in healthcare-seeking behavior. Although the diagnostic work-up of GID is mainly psychiatric, the pediatric endocrinologist has an important role as a team member not only in the pharmacological treatment but also in the initial phase to exclude a cryptic DSD. GID patients and particularly adolescents create many challenges for the physician and it is therefore especially important to have evidence- and experience-based guidelines to consult for the daily routine.

Food for fertility...

Childhood nutrition and later fertility: pathways through education and pre-pregnant nutritional status

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Demography 2010;47:125–144

Background: Better childhood nutrition is associated with earlier physical maturation during adolescence and increased schooling attainment. However, as earlier onset of puberty and increased schooling can have opposing effects on fertility, the net effect of improvements in childhood nutrition on a woman’s fertility are uncertain.
Methods: Using path analysis, the strength of the pathways was estimated between childhood growth and subsequent fertility outcomes in Guatemalan women followed prospectively since birth.

Results: Height for age z score at 24 months was positively related to body mass index (BMI) and height in adolescence and to schooling attainment. BMI was negatively associated and schooling was positively associated with age at first birth. Total associations with the number of children born were positive with BMI and negative with schooling. Height was not related to age at first birth or the number of children born.

Conclusions: In summary, childhood nutrition, as reflected by height at 2 years, was positively associated with delayed age at first birth and fewer children born. If schooling is available for girls, increased growth during childhood will most likely result in a net decrease in fertility.

This investigation links early childhood nutritional aspects with later fertility parameters in an underprivileged society. The paper shows that a well-nourished girl (as determined by height at 2 years) spends more time at school, postpones the birth of her first child and also that she will have fewer children. If the girl has an increased BMI (is overnourished) she will give birth earlier and also have more children, and spend less time at school. Although there are obvious questions as to who is the hen and who is the egg for several associations described, the long-term prospective nature of this paper makes it quite valuable.

An old concern maintained – bisphenol-A

Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons

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Biol Reprod 2009;81:690–699

Background: Developmental exposure to endocrine-disrupting compounds (EDCs) is hypothesized to adversely affect female reproductive physiology by interfering with the organization of the hypothalamic-pituitary-gonadal axis.

Methods: The effects of neonatal exposure to two environmentally relevant doses of bisphenol-A (BPA) was compared with the ERα-selective agonist PPT on the development of the female rat hypothalamus and ovary. Oil vehicle and estradiol benzoate (E2) were used as negative and positive controls, respectively.

Results: Exposure to E2, PPT, or the low dose of BPA advanced pubertal onset. A total of 67% of females exposed to the high BPA dose were acyclic by 15 weeks after vaginal opening compared with 14% of those exposed to the low BPA dose, all of the E2- and PPT-treated females, and none of the control animals. Ovaries from the E2-treated females were undersized and showed no evidence of folliculogenesis, whereas ovaries from the PPT-treated females were characterized by large antral-like follicles, which did not appear to support ovulation. Severity of deficits within the BPA-treated groups increased with dose and included large antral-like follicles and lower numbers of corpora lutea. Fos induction in hypothalamic gonadotropic (GnRH) neurons after hormone priming was impaired in the E2- and PPT-treated groups but neither of the BPA-treated groups.

Conclusion: These data suggest that BPA disrupts ovarian development but not the ability of GnRH neurons to respond to steroid-positive feedback.

The research field of endocrine-disrupting compounds is controversial and the risks of exposure to bisphenol-A have been particularly debated. This paper adds novel hard data to the field. Previously, bisphenol-A has been shown to affect the regulation of vascular endothelial growth factor in rat uterine endothelial cells thus affecting fertility. In addition, the severity of reproductive tract deficits within neonatally bisphenol-A-treated animals increased with the dose of bisphenol-A and included large antral-like follicles and lower numbers of corpora lutea. The present results demonstrate that
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bisphenol-A disrupted ovarian development but not the ability of GnRH neurons to respond to steroid-positive feedback. Thus, this endocrine-disrupting compound is primarily affecting fertility at the gonadal level. A recent review by Hunt et al. [8] discusses, in light of the ‘bisphenol-A saga’, the importance to analyze environmental factors to determine their potential effects on reproduction in mammals – in order to preserve reproductive health.

Food for thought
Sex without reproduction – reproduction without sex

Sex and reproduction: an evolving relationship
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Hum Reprod Update 2010;16:96–107

Background: Technological advances now allow for both sex without reproduction and reproduction without sex. This review summarizes social and ethical commentaries on the new relationship between sex and reproduction.

Methods: This is a literature study where a systematic search was made using PubMed, Medline, ScienceDirect, classic books, Google and/or religious websites. The search focused on publications between 1975 and 2009, but some older materials were also utilized.

Results: The classic picture of sex for reproduction and bonding between mating partners is increasingly being replaced by reproduction separate from sexual activity. Although not every advance in assisted reproduction resulted in a further separation from sexual intercourse, these two fundamental human activities are today increasingly carried out independently. Thus, reproduction is possible, not only without sex, but even through the intervention of more than two partners. The possibility of reproduction with only one or even no gametes, although highly controversial and not yet feasible, is nonetheless being investigated.

Conclusion: Technological advances in the field of reproductive biology have enabled couples considered infertile to conceive and have healthy babies, causing a revolution in culture and customs. The independence of sex and reproduction is now established and in the future human reproduction may move even further away from the sexual act, an option definitely unacceptable to some ethicists.

Since more and more children are conceived in a laboratory setting rather than during parental intercourse, a need for increasing awareness is needed in examination of these children for adverse effects from advanced ART. The effect on the genome, transferred though generations, is a risk that should not be ignored [6, 7]. As fantastic as the prospect to give infertile couples the possibility of parenthood, the awareness of potentially adverse, maybe subtle, effects in their children must be brought to attention and any deviations reported.

As a consequence of obesity as discussed above, an increased need for ART is evolving in parallel with the increased incidence of obesity in a large part of the world. The accumulation of endocrine disrupting compounds (EDCs) in fat and their potentially detrimental effect on fertility point to the risk of the combination of obesity, EDCs and ART to set the scene for a dim scenario for future generations.
The androgen receptor governs the execution, but not programming, of male sexual and territorial behaviors

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Background: Testosterone and estrogen are essential for male behaviors in vertebrates. How these two sex steroid and their signaling pathways interact to control masculinization of the brain and behavior is not known. Circulating testosterone activates the androgen receptor (AR) and also serves as a substrate for local production of estrogen in the brain.

Methods: AR was specifically deleted in the mouse nervous system. This approach permitted determination of the function of AR in sexually dimorphic behaviors in males while maintaining circulating testosterone levels within the normal range.

Results: The AR mutant males were found to exhibit masculine sexual and territorial displays, but to have striking deficits in specific components of these behaviors. The mutant mice were for example less likely to initiate mating and spent less time fighting to protect their home cage, as compared to the wild-type mice.

Conclusions: Taken together with the very limited expression of AR in the developing brain, these results indicate that testosterone most probably acts as a precursor to estrogen to masculinize the brain and behavior. The AR mutant mice exhibited striking deficits in the pattern and extent of male sexual and territorial behaviors. AR is not essential for the masculinization of mating, aggression and urine marking, but rather serves to amplify the display of this behavioral repertoire in males.

For decades the dominating concept has been that the male fetus is masculinized by a prenatal androgen surge [9]. This new evidence using genetically modified mice shows that animals lacking AR in the brain do develop male sexual and territorial behaviors. Thus, there is always an imposing female (factor) behind every man! On the same theme, see the paper by Wu et al. analyzed page 2 in the neuroendocrinology chapter.

A signaling principle for the specification of the germ cell lineage in mice

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Cell 2009;137:571–584

Background: Specification of the germ cell lineage is vital to development and heredity. In mice, the germ cell fate is induced in pluripotent epiblast cells by signaling molecules, yet the underlying mechanism remains unknown.

Methods and Results: The authors demonstrate that germ cell fate in the epiblast is a direct consequence of BMP4 signaling from the extraembryonic ectoderm (ExE), which is antagonized by the anterior visceral endoderm (AVE). BMP8b from the ExE restricts AVE development, thereby contributing to BMP4 signaling. In addition, Wnt3 in the epiblast ensures its responsiveness to BMP4. Serum-free, defined cultures revealed that, in response to BMP4, competent epiblasts uniformly expressed key transcriptional regulators Blimp1 and Prdm14 and acquire germ-cell properties, including genome-wide epigenetic reprogramming, in an orderly fashion.

Conclusions: Induced cells contributed to both spermatogenesis and fertility of offspring. By identifying a signaling principle in germ cell specification, this study presents a strategy for reconstituting the mammalian germ cell lineage in vitro.
A novel approach for the derivation of putative primordial germ cells and Sertoli cells from human embryonic stem cells

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Stem Cells 2009;27:68–77

Background: In vitro gametogenesis established from stem cells without use of viral vectors and genetic manipulation is highly warranted for regenerative medicine purposes in humans, e.g. to preserve fertility in children receiving gonadotoxic chemotherapy for cancer. A number of recent studies have shown that gametes can be derived from murine embryonic stem cells (mESCs) but simple approaches to for derivation of such cells in humans are yet to be demonstrated.

Methods: Using human ESCs, the authors describe a novel method for rapid derivation and enrichment of primordial germ cells (PGCs) and Sertoli cells. The methodology does not require genetic manipulation or complex three-dimensional culture.

Results: The authors determined that simply reducing the size of cultured ESC colonies and manipulating the number of feeding cycles, results in the rapid emergence of cells that are comparable to migratory PGCs. Importantly, these cells can be monitored and purified on the basis of the expression of the chemokine receptor CXCR4. Under more stringent differentiating conditions, these cells mature and upregulate the expression of specific germ cell markers. Importantly, this process is accompanied by the development of Sertoli-like cells, known to provide trophic support and immunoprotection to developing germ cells.

Conclusions: The putative Sertoli-germ cell co-cultures generated in this study may ultimately be developed to establish autologous human gametogenesis as a mean to rescue male fertility in selected cases with gonadal damage.

The above two studies cited are examples of the continuous contribution and impact of the stem cell field to our understanding of the early steps of sexual differentiation and germ cell development. This also keeps open and widens the roads to future exploitation in human reproductive medicine. Results first obtained in mouse models are now being increasingly translated into human systems, with success in most cases so far. Although it is still a long journey before the efficacy and safety of these novel cell therapy methods will be ascertained for clinical use in human medicine, the relatively simple and straightforward approaches used will facilitate their clinical implementation. It is therefore important for the clinical pediatric endocrinologist to be well informed and updated on these continuous developments.

New genes – sex determination

New candidate genes identified for controlling mouse gonadal sex determination and the early stages of granulosa and Sertoli cell differentiation

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Biol Reprod 2010;82:380–389

Background: Mammalian gonadal sex-determining (GSD) genes are expressed in a unique population of somatic cells that differentiate into granulosa cells in XX gonads or Sertoli cells in XY gonads. The ability to efficiently isolate these somatic support cells (SSCs) during the earliest stages of gonad development would facilitate identifying (1) new candidate GSD genes that may be involved in cases of unexplained abnormal gonad development and (2) genes involved in the earliest stages of granulosa and Sertoli cell differentiation.

Methods: A unique mouse model was developed, carrying two transgenes that allow XX and XY mice to be distinguished as early as embryonic day 11.5 (E11.5) and allow SSCs to be isolated from undifferen-
tiated (E11.5) and early differentiated (E12.5) fetal gonads. The Mouse Genome 430v2.0 GeneChip (Affymetrix) was used to identify transcripts exhibiting a sexual dimorphic expression pattern in XX and XY isolated SSCs.

Results: The analysis revealed previously unidentified sexually dimorphic transcripts, including low-level expressed genes such as Sry, a gene not identified in other microarray studies. Multi-gene real-time PCR analysis of 57 genes verified that 53 were expressed in fetal gonads in a sexually dimorphic pattern, and whole-mount in situ hybridization analysis verified 4930563E18Rik, Pld1, and Sprr2d are expressed in XX gonads, and Fbln2, Ppargc1a, and Scrn1 are expressed in XY gonads.

Conclusion: The data provide a comprehensive resource for the spatial-temporal expression pattern of genes that are part of the genetic network underlying the early stages of mammalian fetal gonadal development, including the development of granulosa and Sertoli cells.

In XY mammalian embryos, including humans, initial expression of Sry occurs in Sertoli cell precursors in the gonadal anlagen, which thereafter develop into testes. In the absence of functional Sry the same precursor cells develop into granulosa cells determining the ovary. However, there are still other molecular events and genes involved in sex determination yet to be discovered. Most DSD cases with gonadal dysgenesis not caused by numerical chromosome aberrations have still an unknown origin. In this study employing an elegantly created mouse model, the authors detected more than 50 genes that were expressed in early development in fetal gonadal tissue in a sexually dimorphic fashion. Some of these identified genes can be studied for a role in the pathogenesis of human DSDs.

New mechanisms

The ovary
Basic biology and clinical implications

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Background: The classical view of ovarian follicle development is that it is regulated by the hypothalamic-pituitary-ovarian axis, in which gonadotropin-releasing hormone (GnRH) controls the release of the gonadotropic hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and that ovarian steroids exert both negative and positive regulatory effects on GnRH secretion. More recent studies in mice and humans indicate that many other intra-ovarian signaling cascades affect follicular development and gonadotropin action in a stage- and context-specific manner.

Methods: Review paper with update on current status of our understating of ovarian function and folliculogenesis and their translational implications.

Results and Conclusions: Evidence from mutant mouse models and clinical observations indicate that some of the most powerful intra-ovarian regulators of follicular development include the TGF-β/SMAD, WNT/FZD/β-catenin, and RAS/ERK1/2 signaling pathways and the FOXO/FOXL2 transcription factors.

Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation

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Cell 2009;139:1130–1142

Background: In mammals, the transcription factor SRY, encoded by the Y chromosome, is normally responsible for triggering the indifferent gonads to develop as testes rather than ovaries. However, testis differentiation can occur in its absence.

Methods: Inducible deletion of Foxl2 in adult ovarian follicles.
**Results:** The forkhead transcriptional regulator FOXL2, as a single factor, was shown to be required to prevent transdifferentiation of an adult ovary to a testis. Deletion of Foxl2 leads to immediate upregulation of testis-specific genes including the critical SRY target gene Sox9. Concordantly, reprogramming of granulosa and theca cell lineages into Sertoli-like and Leydig-like cell lineages occurs with testosterone levels comparable to those of normal XY male littermates.

**Conclusions:** Maintenance of the ovarian phenotype is an active process throughout life. They might also have important medical implications for the understanding and treatment of some disorders of sexual development in children and premature menopause in women.

These two papers on the ovary are excellent updates on important basic mechanisms and clinical implications of ovarian development and function. The original paper on FOXL2 published in *Cell* presents seminal work demonstrating that the ovarian-specific gene FOXL2 has a role to suppress testis transdifferentiation in the adult ovary, i.e. that females continue to fight against their inner males throughout life [10]. Thus, a single gene deletion could induce male transformation – the old dogma that the testis and ovary are terminally differentiated tissues is not any longer valid. This paper was also commented on in *Nature Medicine* [11], where Foxl2's possible impact on the polycystic ovary syndrome (PCOS) is discussed. The transient knockout of FOXL2 expression results in ovaries that, as in PCOS, produce primarily androgens rather than estrogens.

**New methodology – can you be too sensitive?**

**Assessment of circulating sex steroid levels in prepubertal and pubertal boys and girls by a novel ultrasensitive gas chromatography-tandem mass spectrometry method**

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*J Clin Endocrinol Metab* 2010;95:82–92

**Background:** Estrogens and androgens play key roles for pubertal onset and sexual maturation. Most currently used immunoassays are not sensitive enough to accurately measure the low circulating levels of sex steroids in children without any signs of puberty. However, this does not exclude that sex steroids have important biological roles in prepubertal children. The aim of the study was to accurately determine levels of sex steroid hormones and their metabolites in serum of healthy children before any physical signs of puberty and to evaluate possible sex differences.

**Methods:** Total (unconjugated plus conjugated) serum levels of 17β-testosterone, 17α-testosterone, 5α-dihydrotestosterone, 5β-dihydrotestosterone, androsterone, etiocholanolone, estradiol, and estrone measured by an ultrasensitive method based on gas chromatography-tandem mass spectrometry in samples from 81 healthy school children (42 boys) without any signs of puberty. For comparison, 48 pubertal children were studied.

**Results:** 17β-Estradiol levels in prepubertal boys were undetectable or extremely low (median <3.7 pmol/l), whereas levels in prepubertal girls were significantly higher (median 9.6 pmol/l, p < 0.001). Among the older prepubertal children (>8 years), girls had significantly higher androsterone (4.07 vs. 1.45 nmol/l, p < 0.05), etiocholanolone (5.45 vs. 1.95 nmol/l, p < 0.0001), 5α-dihydrotestosterone (0.11 vs. <0.10 nmol/l, p < 0.01), and 17β-testosterone concentrations (0.69 vs. 0.47 nmol/l, p < 0.05) compared with similarly aged prepubertal boys.

**Conclusions:** Using an accurate and sensitive method, significantly higher levels of estrogens as well as androgen metabolites were found in prepubertal girls compared with age-matched boys. The higher prepubertal sex steroid levels in girls may contribute to their earlier onset of puberty including pubic hair development.

The authors used ultrasensitive methodology with high accuracy to determine circulating sex steroid levels in prepubertal and pubertal children of both sexes and found subtle sexual dimorphisms for...
the levels of estrogens at the low picomolar level for the levels of androgens at the subnanomolar level. This paper may serve as an important source of normative data for several sex steroids in children. However, it remains to be determined whether at these very low levels, these sex steroids exert any biological function and whether the differences observed have any phenotypic relevance.

**Concerns – earlier start of puberty**

### Trends in puberty timing in humans and environmental modifiers

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*Mol Cell Endocrinol* 2010;324:339–344

**Background:** Secular trends in timing of puberty appear to continue although undernutrition has not been any longer a limiting factor for pubertal development. Now obesity and other environmental reasons have been suspected to cause this trend, and endocrine disrupting chemicals have become into focus as possible contributors.

**Methods:** Epidemiological studies on endocrine disrupters are still scarce and show only weak associations between exposures and timing of puberty.

**Results:** Since genetic background explains 50–80% of variability in the timing of puberty, it is not surprising that the observed environmental effects are rather modest when individual exposures are assessed. Despite that, some exposures have been reported to be associated with early (e.g., polybrominated biphenyls) or delayed (e.g., lead) puberty.

**Conclusions:** The authors review the available data on recent trends in timing of puberty and the possible role of endocrine disrupters.


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*Paediatr Perinat Epidemiol* 2010;24:222–231

**Background and Methods:** The association between birth weight and the odds ratio (OR) of pubertal status in girls aged between 8 and 11 and in boys aged between 8 and 12 was examined using the 1988-94 Third National Health and Nutrition Examination Survey (NHANES III). Girls (n = 956) and boys (n = 1,199) who had data on birth weight and Tanner staging were included. Maternal-reported birth weight, smoking in pregnancy and other information were provided in a home interview, while Tanner staging to assess pubertal status was part of a medical examination. Multiple logistic regression models were computed for the endpoints of the OR of being Tanner stage 2+ vs. 1 or being 2+ vs. 1 in an asynchronous pubertal pathway after adjustment for the complex sampling design of NHANES, age, race, height and body mass index (BMI).

**Results:** Birth weight was not associated with the OR of Tanner stage 2+ among girls, however boys who were low birth weight (<2,500 g) and boys born higher than average birth weight (3,500–3,999 g) were more likely to be Tanner stage 2+ than 1. Childhood BMI was associated with the OR of having entered puberty among girls, but not boys. In an analysis of asynchronous maturation, girls born at high birth weight (>4,000 g) were more likely to have breast development 3+ than girls of normal birth weight, OR = 3.18 (95% CI 1.39, 8.25).

**Conclusions:** The birth weight-puberty association varies by gender and by pubertal pathway. The findings need replication in prospective longitudinal studies and research to understand the mechanisms underlying the relation of early life exposures to cancer risk.
The concept of an earlier start of puberty at the population level during the past few decades, particularly in girls, continues to provoke discussions and scientific debate. These two studies report on and discuss the possible association of earlier onset of puberty with environmental factors and with birth weight. It is clear that there is still limited data to associate specific endocrine disrupting chemicals with an earlier onset of puberty although ongoing prospective studies may add more insights into this issue. The role of body weight, BMI and the obesity epidemic as triggers of the onset of puberty has been convincingly demonstrated for certain ethnic groups but whether such mechanisms operate at the wider population level needs further attention. Longitudinal studies with prospective cohorts employing more accurate methods to determine puberty onset will add to this. Still, reports from several continents now seem to agree that there is a recent trend to start puberty earlier, which is difficult to explain as a consequence of a continuing secular trend or genetic alterations. Thus, there is urgent need to look more closely into possible environmental factors behind this phenomenon.

Follow-up on a Yearbook 2009 paper (Ong et al. [12])

LIN28B in constitutional delay of growth and puberty
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J Clin Endocrinol Metab 2010;95:3063–3066

Background: Recently, variation in LIN28B, a human ortholog of the gene regulating processing of micro-RNAs (miRNAs) controlling the timing of major developmental events in the nematode Caenorhabditis elegans, was reported to be associated with timing of puberty in humans. In C. elegans, a gain-of-function allele of lin-28 causes a retarded phenotype. The objective of the study was to evaluate the variation in the LIN28B gene in 145 subjects with constitutional delay of growth and puberty (CDGP).

Methods: For this study, 115 males and 30 females with CDGP were included. CDGP was defined by Tanner genital or breast stage II and pubertal growth spurt taking place 2 SD later than average. The four coding exons (exons 1–4) and exon-intron boundaries, as well as the fragment of 3’ untranslated region containing miRNA recognition elements A and B, of LIN28B were PCR amplified from genomic DNA obtained from peripheral blood leukocytes of the subjects and bidirectionally sequenced.

Results: No variation in the coding region of LIN28B in the 145 subjects with CDGP was found. However, 16 of 145 subjects carried a 2-nucleotide deletion immediately 5’ from miRNA recognition element A. These patients did not differ in phenotypic features as compared with non-carriers, and this variant was present in 100 controls with the same frequency.

Conclusions: These results show that mutations in the coding region or 3’ untranslated region miRNA recognition elements A and B of LIN28B do not underlie CDGP. Lack of any variation in the coding region of the gene suggests that LIN28B in developmental timing is so crucial that any changes in the conserved protein would probably be lethal.

Last year we reported on the importance of microRNAs, and as one example of LIN28B, in the timing of puberty [12]. This year, Tommiska et al. tried to find variation in the LIN28B gene in individuals with constitutional delay in growth and puberty (CDGP). No variation in the coding region of the gene was found in CDGP subject. It should be remembered that the common variant of LIN28B detected by several groups [12] only modulated the age at menarche by a few weeks. The data presented here clearly indicate that variations in LIN28B are not a common cause of CDGP but leave open several possibilities. Given the sample size, it remains possible that variations in LIN28B are rare causes of CDGP not detected in the sample studied here. Alternatively, other genes in the LIN28B pathway could be involved in CDGP, a transmitted condition with so far no mechanistic basis. The last severe alterations of LIN28B might be lethal or lead to different phenotypes than the one analyzed here.
Important for clinical practice

Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes

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Background: Idiopathic hypogonadotropic hypogonadism (IHH) with normal smell (normosmic IHH) or anosmia (Kallmann syndrome) is associated with defects in the production or action of GnRH. Accordingly, most IHH patients respond to physiological pulsatile GnRH replacement by normalizing serum LH, FSH, and testosterone (T) levels and achieving gametogenesis; some patients, however, show atypical responses. Interestingly, several IHH-associated genes are expressed in multiple compartments of the hypothalamic-pituitary-gonadal axis. The aim of the study was to investigate whether the clinical, biochemical, or genetic characteristics of IHH men with atypical responses to GnRH indicate alternative or additional defects in the hypothalamic-pituitary-gonadal axis.

Methods: 90 IHH men undergoing long-term pulsatile GnRH treatment over 30 years were studied. A retrospective study of response to GnRH at a Clinical Research Center was conducted. Physiological regimens of pulsatile sc GnRH were administered for at least 12 months. Dose-response studies using iv GnRH pulses assessed the pituitary LH response. Serum T, LH, FSH, and inhibin B levels, sperm in ejaculate, and sequence of IHH-associated genes was measured.

Results: 26% of subjects displayed atypical responses to GnRH: (1) 10 remained hypogonadotropic and hypogonadal, demonstrating pituitary and testicular defects; (2) 8 achieved spermatogenesis and normal T but only with hypergonadotropism, indicating impaired testicular responsiveness to gonadotropins, and (3) 5 remained azoospermic despite achieving adult testicular volumes and normal hormonal profiles, suggesting primary defects in spermatogenesis. Mutations were identified only in KAL1 across groups.

Conclusions: In addition to hypothalamic GnRH deficiency, IHH men can have primary pituitary and/or testicular defects, which are unmasked by GnRH replacement.

TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood

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Background: Mutations in TAC3 and TACR3 (encoding neurokinin B and its receptor) have been identified in Turkish patients with idiopathic hypogonadotropic hypogonadism (IHH), but broader populations have not yet been tested and genotype-phenotype correlations have not been established. A broad
cohort of normosmic IHH probands was screened for mutations in TAC3/TACR3 to evaluate the prevalence of such mutations and define the genotype/phenotype relationships.

Methods: The study consisted of sequencing of TAC3/TACR3, in vitro functional assays, and neuroendocrine phenotyping conducted in tertiary care centers worldwide. 345 probands, 18 family members, and 292 controls were studied. Reproductive phenotypes throughout reproductive life and before and after therapy were examined. Rare sequence variants in TAC3/TACR3 were detected.

Results: In TACR3, 19 probands harbored 13 distinct coding sequence rare nucleotide variants (3 nonsense mutations, 6 non-synonymous, 4 synonymous (1 predicted to affect splicing)). In TAC3, one homozygous single base pair deletion was identified, resulting in complete loss of the neurokinin B decapetide. Phenotypic information was available on 16 males and 7 females with coding sequence variants in TACR3/TAC3. Of the 16 males, 15 had microphallus; none of the females had spontaneous gynecomastia. Seven of the 16 males and 5 of the 7 females were assessed after discontinuation of therapy; 6 of the 7 males and 4 of the 5 females demonstrated evidence for reversibility of their hypogonadotropism.

Conclusions: Mutations in the neurokinin B pathway are relatively common as causes of hypogonadism. Although the neurokinin B pathway appears essential during early sexual development, its importance in sustaining the integrity of the hypothalamic-pituitary-gonadal axis appears attenuated over time.

These two papers represent a large body of work to better understand the etiology and pathophysiology as well as the clinical phenotypes in central hypogonadotropic hypogonadism. This is a relatively common diagnosis in the pediatric endocrinology clinic thus adding a substantial number of patients to the records. General conclusions from these studies are that it is worth looking more closely into the molecular defects when working up these cases, and that it is not uncommon that these patients suffer from several gene defects that may result in poor functional outcome with respect to fertility despite state-of-the-art endocrine treatment. One important practical implication is the recognition that some of the patients recognized in adolescence as having hypogonadism actually have reversible phenotypes and later in life have normal gonadotropic function and can reproduce without medical assistance [13]. So far, a small proportion of such patients has been identified among the vast number of patients with hypogonadism, without any clue on the factors involved in this reversible phenotype. Although not definitive, the data on patients with TAC3/TACR3 mutations suggest that these are the patients with the reversible phenotype suggesting that molecular analysis could help predict the course of the disease.

New Concerns – safety of endocrine treatment

Cognitive effects of aromatase inhibitor therapy in peripubertal boys

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Background: Aromatase inhibitors, blockers of estrogen biosynthesis, have emerged as a new potential treatment modality for boys with short stature. The cognitive effects of such therapy are unknown. In this study, we explored the effects of aromatase inhibition on cognitive performance in peripubertal boys.

Methods: Prospective, double-blind, randomized, placebo-controlled clinical study. 28 boys, aged 9.0–14.5 years, with idiopathic short stature were treated with the aromatase inhibitor letrozole (2.5 mg/day), or placebo, for 2 years. During the treatment, the progression of physical signs of puberty and the concentrations of sex hormones were followed up. A selection of cognitive tests, focusing on memory function, was administered to the participants at entry, at 12 months, and at 24 months after the start of the treatment.

Results: Letrozole effectively inhibited the conversion of androgen to estrogen, as indicated by high serum testosterone and low serum estradiol concentrations in letrozole-treated boys who progressed into
puberty. In both groups there was a gain in performance during the follow-up period in tests of verbal performance, in most of the tests of visuospatial performance, and in some tests of verbal memory. No significant differences between the letrozole and placebo-treated boys in development of cognitive performance were found in any of the tests during the follow-up period.

Conclusions: These results suggest that blockade of estrogen biosynthesis with an aromatase inhibitor does not influence cognitive performance in peripubertal males.

Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty

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Background: Aromatase inhibitors (AIs), blockers of estrogen biosynthesis, delay bone maturation and are therefore increasingly used to promote growth in children and adolescents with growth disorders. The effects of treatment on skeletal health are largely unknown.

Methods: As estrogen deficiency is associated with various detrimental skeletal effects, we evaluated in this cross-sectional post-treatment study vertebral body morphology, dimensions and endplates, and intervertebral discs by the use of magnetic resonance imaging (MRI) in two cohorts of males previously treated with the AI letrozole, or placebo. Males with idiopathic short stature received treatment with letrozole or placebo for 2 years during pre-puberty or early puberty; males with constitutional delay of puberty received letrozole or placebo in combination with low-dose testosterone for 1 year during early or mid-puberty.

Results: In males with idiopathic short stature, mild vertebral body deformities were found in 5 of 11 (45%) letrozole-treated subjects while in the placebo group no deformities were detected (p = 0.01). In the cohort of males with constitutional delay of puberty a high prevalence of endplate and intervertebral disc abnormalities was observed in both letrozole and placebo-treated males.

Conclusions: The authors conclude that AI therapy during pre-puberty or early puberty may predispose to vertebral deformities, which probably reflect impaired vertebral body growth rather than impaired bone quality and compression fractures. If AIs are used in growth indications, follow-up of vertebral morphology is indicated.

These two papers deal with important safety issues in boys treated with aromatase inhibitors in order to stimulate pubertal growth. Given the common diagnosis of boys with short stature at the pediatric endocrinology clinic and the poor arsenal of tools available to stimulate their growth and increase final height, aromatase inhibitors have been increasingly tried for such purposes. It is therefore important to address the safety concerns. It is reassuring to learn that there were no observable negative effects on the cognitive performance of the treated teenager boys. However, when it comes to bone health there were clear indications that the spinal skeleton could be adversely affected by the treatment. The group of Leo Dunkel should be commended for this long-term commitment to evaluate several aspects of anti-aromatase therapy and not only the primary outcomes. These data re-emphasize the need for formal and comprehensive evaluation of new medications in the context of clinical trials before physicians even consider using them in their daily practice. It is unfortunate that this is not the case for the use of aromatase inhibitors [14].
Rescue of defective G-protein-coupled receptor function in vivo by intermolecular cooperation

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Background: G-protein-coupled receptors (GPCRs) are ubiquitous mediators of signaling of hormones, neurotransmitters, and sensing. The old dogma is that a one-ligand/one-receptor complex constitutes the functional unit of GPCR signaling. However, there is mounting evidence that some GPCRs form dimers or oligomers during their biosynthesis, activation, inactivation, and/or internalization.

Methods: This evidence has been obtained exclusively from cell culture experiments, and proof for the physiological significance of GPCR di/oligomerization in vivo is still missing. In the present study a transgenic mouse model was used.

Results: The authors demonstrate, using the mouse luteinizing hormone receptor (LHR) as a model GPCR, that transgenic mice co-expressing binding-deficient and signaling-deficient forms of LHR can re-establish normal LH actions through intermolecular functional complementation of the mutant receptors in the absence of functional wild-type receptors.

Conclusions: These results provide compelling in vivo evidence for the physiological relevance of intermolecular cooperation in GPCR signaling.

This paper demonstrates that a G-protein coupled receptor (GPCR) made deficient in its hormone-binding capacity, but with a still functional signaling capability, can cooperate successfully with a GPCR moiety with a reciprocal deficiency, lacking signaling activity but retaining hormone binding, to exert a normal receptor action. This is compelling evidence for the existence of interaction and functional cooperation between protein receptors at the molecular level within the cell membrane. These findings have important implications for better understanding of hormone actions and clinical phenotypes in endocrine disorders.

References