Reproductive Endocrinology

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Reproductive biology and endocrinology is a lively research area attracting considerable attention as the implications concern the everyday life of human beings. Last year's chapter focused somewhat on the rapidly expanding stem cell field which, also this year, has shown continuous high activity, and some contributions adding novel possibilities for fertility preservation have been included. Genetics offers interesting discoveries adding new genes to the scene and revealing novel functions of old friends. Basic cell biology still delivers novel concepts by contributing 3 papers on the role of micro RNAs in reproduction. There is also interesting work linking endocrinology with behavioral sciences. Not everything can be covered and other chapters of this book also deal with subjects linked to reproduction. This chapter aims to present a mix of experimental and clinical papers advancing the field with positive implications for human beings.

New hope Fertility preservation

Ovary and uterus transplantation

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Reproduction 2008;136:671–680

Background: The survival rates for children treated for cancer have increased. Therefore a concern for preservation of fertility is an important aspect to consider in the follow-up, when the children enter adolescence and adulthood. In these cases ovarian and uterine transplantation could have potential applications.

Methods: Cryopreservation of tissue is providing opportunities for banking, e.g., ovaries for indefinite periods before transplanting them back to restore fertility. The natural plasticity of the ovary facilitates grafting to different sites where they can be revascularized and rapidly restore the normal physiology of secretion and ovulation. Uterine transplantation by vascular anastomosis provides potentially longer function but is technically much more demanding and riskier for the recipient.

Results: In humans, autografts have functioned for up to 3 years before needing replacement. Uterine transplantation has enabled successful pregnancies in several species, but not yet in humans. Neither ovary nor uterus is privileged immunologically and allografts become rapidly rejected except in hosts whose immune system is deficient or suppressed pharmacologically.

Conclusions: Cryopreservation and uterine transplantation, with their limitations, raise hope for the preservation of fertility in the increasing number of survivors after childhood cancers.

Production of offspring from a germline stem cell line derived from neonatal ovaries

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Nat Cell Biol 2009;11:631–636

Background: The idea that females of most mammalian species have lost their capacity for oocyte production at birth has recently been challenged by the finding that juvenile and adult mouse ovaries possess mitotically active germ cells. The existence of female germ line stem cells (FGSCs) in postnatal mammalian ovaries is still a controversial issue among reproductive biologists and stem cell researchers.

Methods: In the present study a neonatal mouse FGSC line was established, with normal karyotype and high telomerase activity, by immunomagnetic isolation and cultured for more than 15 months.

Results: FGSCs from adult mice were isolated and cultured for more than 6 months. These FGSCs were infected with GFP virus and transplanted into the ovaries of infertile mice. Transplanted cells underwent oogenesis and the mice produced offspring that had the GFP transgene.

Conclusions: These findings contribute to basic research into oogenesis and stem cell self-renewal and open up new possibilities for use of FGSCs in biotechnology and medicine. Thus, there is a possibility for oocyte renewal after birth!

Long-term spermatogonial survival in cryopreserved and xenografted immature human testicular tissue

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Hum Reprod 2008;23:2402–2414

Background: Preservation of the male germ line in prepubertal boys undergoing gonadotoxic treatment is a crucial consideration in terms of their future quality of life. As these patients do not yet produce spermatozoa for freezing, only immature tissue is available for storage.

Methods: The survival, proliferation and differentiation capacity of spermatogonia were studied after cryopreservation and long-term transplantation of immature testicular tissue pieces. Single pieces of testicular tissue (2–8 mm) from prepubertal boys (7–14 years) were cryopreserved, thawed and transplanted into the scrotum of mice for 6 months. Upon removal, histological, immunohistochemical and ultrastructural analyses and testicular sperm extraction (TESE) were used to evaluate the tissue.

Results: Histology showed $55 \pm 42\%$ of tubules to be intact. MAGE-A4 immunostaining showed mean spermatogonial recovery to be $3.7 \pm 5.5\%$, with 35% of these cells expressing Ki67, giving evidence of proliferation in tissue from boys <14 years of age. No apoptosis was found, as demonstrated by the absence of active caspase-3 and TUNEL staining. Numerous premeiotic spermatocytes, a few spermatocytes at the pachytene stage and spermatid-like cells were observed. No immunostaining was observed for lactate dehydrogenase C, ACE or proacrosin, indicating that the spermatid-like structures observed by histology did not express the meiotic and post-meiotic markers characteristic of normal spermatids. No ultrastructural alterations of the tissue were encountered.

Conclusions: The present study demonstrates that spermatogonia are able to survive and proliferate after cryopreservation and long-term transplantation. Complete regeneration of normal spermatogenesis was not observed since, beyond the pachytene stage, no adequate characterization of germ cells was obtained. Further studies are thus required to investigate the differentiation potential of cryopreserved germ cells.

The 3 publications above are selected from the rapidly emerging literature opening up new avenues to prevent and treat infertility in both sexes after damage to the reproductive organs. Such adverse events are common in children and young adults undergoing aggressive treatments for cancer. The expanding field of late-effects research aims at helping this growing group of new survivors to a better life after cure, employing novel techniques and tools. New survivors subscribing to endocrine services are found not only among cancer patients but also in children born at an extremely premature gestational age, in those with severe metabolic disorders and congenital malformations, and in other patient groups. The stem cell field holds great promise for these patients and is illustrated by the paper on germ line stem cells isolated from postnatal ovaries. This paper challenges the dogma that females lose their capacity for oocyte production at birth, a concept that has held considerable controversy for some time. The authors show convincingly that a female germ line stem cell line established from the postnatal ovary could produce offspring when transplanted into the ovaries of infertile mice. The review by Gosden extends the discussion beyond ovaries and germ cells by discussing the possibility of uterus transplantation. This may be of interest in cases where uterine tissue has been permanently damaged by irradiation to the pelvis. Although technically demanding such an approach has proven successful in experimental animals, and gives hopes for the future also in humans. Testicular damage to prepubertal males before spermatogenesis is established may result in infertility due to azoospermia. It is now 15 years since Brinster et al. [1] showed a prophylactic cure for such an event by demonstrating that mouse hosts receiving spermatogonial stem cells as trans-

plants into the seminiferous tubules were able to produce donor-derived offspring. This breakthrough has since then been replicated in many species but still not in humans. There are still many technical obstacles to be overcome before such a procedure can be established as a safe method in the clinic. The paper by Wyns et al. deals with such attempts focusing on human testis tissue samples.

Spermatogonia survived cryopreservation and transplantation and the few remaining spermatogonia found in long-term orthotopic xenografts of immature human testicular tissue from boys <14 years of age proliferated, suggesting that some SGSCs could be present in this cell population. However, complete regeneration of normal spermatogenesis was not observed, and spermatogenesis stopped at the pachytene spermatocyte stage. The question arises whether cryopreservation with a view to fertility restoration is appropriate in peripubertal boys.

New paradigms

Reviews on non-genomic effects of progesterone

Non-genomic progesterone actions in female reproduction

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Hum Reprod Update 2009;15:119–138

Honey, we need to talk about the membrane progesterin receptors

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Steroids 2008;73:942–952

Background: Progesterone controls key female reproductive events that range from ovulation to implantation, maintenance of pregnancy and breast development. In addition to activating the ‘classical’ progesterone receptors (PRs)-A and B, progesterone also elicits a variety of rapid signaling events independent of transcriptional or genomic regulation. The combination of rapid non-genomic events and the slower transcriptional actions is thought to determine the functional response to progesterone in the female reproductive system in a cell-type- and environment-specific manner.

Methods: The first of the 2 reviews covers current knowledge (PubMed up to August 2008) on the mechanisms and relevance of non-genomic progesterone signaling in female reproduction.

Results: Several putative progesterone-binding moieties have been implicated in rapid signaling events, including the nuclear PR and its variants, PR membrane component 1, and the novel family of membrane progesterin receptors. Progesterone and its metabolites have also been implicated in regulation of e.g. γ -aminobutyric acid type A, oxytocin and sigma-1 receptors. Identification of the mechanisms and receptors that mediate rapid progesterone signaling is an area of research burdened with difficulties and controversy. The recent discovery of 3 closely related cell surface receptors that bind to progesterone and mediate its actions on various cytoplasmic signaling cascades has been regarded as a major breakthrough. The novel membrane progesterin receptors (mPR α , β , γ) are reported to be similar to, and function as, G-protein-coupled receptors. However, studies by these authors failed to confirm that mPRs are expressed on the cell surface. In addition, they were also not able to verify that mPRs mediate rapid progesterone signaling events, not even that they are real progesterin-binding moieties.

Conclusions: While the reason for these startling opposing results remains unclear, these critical reviews of existing data can help to shed some light onto the controversial mPRs. The controversy around membrane-bound PR still remains.

The concept of membrane receptors for steroid hormones exerting non-genomic actions by employing rapid cytoplasmic signaling pathways has been an interesting but controversial subject for many years. All classes of steroid hormones have been implicated within this context but a major

focus has been on the sex steroids [2]. For progestins the existence of such receptors is best shown by studies in lower species such as fish [3], but convincing data from higher organisms are sparse. The selected reviews update the field to the current status. The recent identification of the membrane progestin receptor has not provided the long sought after solution to the unresolved issues in the mechanism of rapid non-genomic progestin actions. Rather, it has sparked a new debate as to the function of these novel proteins. The very promising features of these molecules raised hopes that specific agonists and antagonists could be developed, capable of dissecting nuclear from extra-nuclear, slow from rapid, genomic from non-genomic actions. It is unclear at this stage where the journey will lead. This information may be useful for a pediatric endocrinologist, but the feeling is still that more questions than answers are provided.

Food for the eye Paparazzo of the year

Laparoscopic observation of spontaneous human ovulation

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Fertil Steril 2008;90:833–834

A surgeon, scheduled to perform a laparoscopic hysterectomy on a woman on day 16 of the menstrual cycle, observed spontaneous ovulation in the left ovary, as illustrated by protrusion of the mature follicle and oocyte release into the peritoneal cavity. The release of an egg was considered a sudden, explosive event, but it took place over a period of at least 15 min [see also *New Scientist* of June 11, 2008].

Being at the right time at the right place with a camera the surgeon was able to catch human ovulation at the very moment when the egg was released from the ovarian surface. Must be seen! Comments are superfluous.

Mechanisms explained ER α – a key player both in the female and male reproductive tract

An estrogen receptor- α knock-in mutation provides evidence of ligand-independent signaling and allows modulation of ligand-induced pathways in vivo

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Endocrinology 2008;149:2970–2979

Estrogen-dependent and -independent estrogen receptor- α signaling separately regulate male fertility

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Endocrinology 2009;150:2898–2905

Background: Estrogen receptor- α (ER α) plays a critical role in reproductive tract development and fertility. To determine if estrogen-dependent and/or -independent ER α mechanisms are involved in fertility, the authors examined estrogen non-responsive ER α knock-in (ENERKI) mice.

Methods: ENERKI mice were generated to distinguish between ligand-induced and ligand-independent ER α actions in vivo. These mice have a mutation in the ligand-binding domain of ER α , which significantly reduces ER α interaction with and response to endogenous estrogens, whereas not affecting growth factor activation of ligand-independent pathways.

Results: ENERKI mice have hypoplastic uterine tissues and rudimentary mammary gland ductal trees. Females are infertile due to anovulation, and their ovaries contain hemorrhagic cystic follicles because of chronically elevated levels of LH. The ENERKI phenotype confirmed that ligand-induced activation of ER α is crucial in the female reproductive tract and mammary gland development. Growth factor treatments induced uterine epithelial proliferation in ovariectomized ENERKI females, directly demonstrating that ER α ligand-independent pathways were active. The synthetic ER α selective agonist propyl pyrazole triol (PPT) treatment initiated at puberty stimulated ENERKI uterine development, whereas neonatal treatments were needed to restore mammary gland ductal elongation, indicating that neonatal ligand-induced ER α activation may prime mammary ducts to become more responsive to estrogens in adult tissues. In the male reproductive system it was found that ligand-independent ER α signaling is essential for concentrating epididymal sperm via regulation of efferent ductule fluid reabsorption. On the other hand, estrogen-dependent ER α signaling is required for germ cell viability, most likely through support of Sertoli cell function. By treating ENERKI mice with PPT it was discovered that male fertility requires neonatal estrogen-mediated ER α signalling.

Conclusions: These results indicate both estrogen-dependent and -independent pathways to play separable roles in male murine reproductive tract development. Thus, the ENERKI mouse model is a valuable tool to investigate ER α ligand dependent and independent pathways in the reproductive tract of both males and females.

These are interesting novel data on the role of ER α in male (and female) reproduction. There are both estrogen-dependent and independent actions of ER α , the latter exerted by ER activation by growth factors independent of the traditional ligand. The knock-in model was designed to distinguish between these 2 pathways. In the male the importance of ER α for fluid homeostasis in the testis and epididymis was verified and deducted to be an estrogen-independent effect. The importance of estrogen (or ER) for male germ cell survival was also supported, adding to previous data in other species [4, 5]. The data suggest that the role of ER α in human infertility should be examined more closely. Because only 1 human male has been reported to have an inactivating ER α mutation, it is unlikely that mutations leading to ER α deletion account for a significant percentage of human male infertility cases. However, the role of ER α polymorphisms in male infertility remains to be established.

New Mechanisms

Pubertal hormones restructure the brain

Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions

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Nature Neurosci 2008;11:995–997

Background: Structural sexual dimorphism of the brain results in behavioral differences between the sexes that are well recognized. However, the regulation and timing of the establishment of these differences are not well understood.

Methods: Young male and female rats were labeled with BrdU and the number and location of newly formed cells determined in sexually dimorphic brain regions in the presence and absence of sex steroids.

Results: New cells are added to the rat brain during puberty, and sex differences in the number of newly added cells correspond to sex differences in adult volume in each region. Pubertal sex steroids influence the addition of new cells, presumably by promoting cell genesis and/or survival in sexually dimorphic structures in a sex-specific manner.

Conclusions: Hormone-modulated addition of cells to sexually dimorphic brain regions is an active mechanism for maintaining functional sex differences during adolescence and into adulthood.

Testosterone programs adult social behavior before and during, but not after, adolescence

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Background: The pubertal and adolescent brain is a major target for sex hormones but our understanding of such hormonal influences on adolescent behavioral development is not well understood.

Methods: The authors investigated how variations in the timing of testosterone exposure, relative to adolescence, alters the strength of steroid-sensitive neural circuits underlying social behavior in male Syrian hamsters. Animals were exposed to early, on-time, and late pubertal development by treating gonadectomized males with testosterone implants for 19 days before, during or after adolescence. Effects of preadolescent testosterone treatment on behavior and brain regional volumes within the mating neural circuit of juvenile males were also investigated.

Results: Androgen treatment before or during adolescence, but not after, facilitated mating behavior in adulthood. In addition, pre-adolescent testosterone treatments most effectively increased mating behavior overall, indicating that the timing of exposure to pubertal hormones contributes to individual differences in adult behavior. Pre-adolescent testosterone treatment did not induce reproductive behavior in juvenile males but did increase volumes of the BST, SDN, MePD, and MePV to adult-typical size. In contrast, juvenile MeAD and VMH volumes were not changed by preadolescent testosterone treatment, suggesting that further maturation of these brain regions during adolescence is required for the expression of male reproductive behavior.

Conclusions: Adolescent maturation of social behavior may involve both steroid-independent and steroid-dependent processes, and adolescence marks the end of a postnatal period of sensitivity to steroid-dependent organization of the brain. These results have important translational implications for human pubertal development and are in line with recent data from the same group referred to herein (see above) demonstrating sexually dimorphic additions of new cells in certain brain areas during adolescence.

These papers link exposure to sex steroids, particularly androgens, at appropriate time points of development to sexual dimorphism in brain morphology and behavior. Although the present studies were performed in experimental animals, modern brain imaging techniques associated with metabolic and functional elements now available in human medicine [6] may soon be used to ask similar questions in pediatric and adolescent patient groups. Results to come are expected to add important implications for our management of children with DSD, pubertal and behavioral disorders, and other conditions. One wonders about brain organization in children with early virilization, and whether the timing of pubertal onset affects such organization. Although this research did not directly model a psychiatric disease state, the finding that adult behaviors vary depending on the timing of hormone exposure across the adolescent period speaks for the possibility that direct hormone–brain interactions moderate the effects of pubertal timing on many behaviors, even in humans.

Old genes What can they do for puberty?

Association studies of common variants in 10 hypogonadotropic hypogonadism genes with age at menarche

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J Clin Endocrinol Metab 2008;93:4290–4298

Background: Although the timing of puberty is a highly heritable trait, little is known about the genes that regulate pubertal timing in the general population. Several genes have been identified that, when mutated, cause disorders of delayed or absent puberty such as hypogonadotropic hypogonadism (HH). Because severe variants in HH-related genes cause a severe puberty phenotype, we hypothesized that common subtle variation in these genes could contribute to the population variation in pubertal timing.

Methods: The common genetic variation in 10 HH-related genes in 1,801 women from the Hawaii and Los Angeles Multiethnic Cohort with either early (age <11 years) or late (age >14 years) menarche and in other replication samples was assessed. In addition to these common variants, the most frequently reported HH mutations were also determined to assess their role in the population variation in pubertal timing. Within the general community, 1,801 women from the Hawaii and Los Angeles Multiethnic Cohort participated. The association of genetic variation with age at menarche was assessed.

Results: No significant association between any of the variants tested and age at menarche was detected; although it cannot be ruled out that a modest effect of these variants or of other variants may operate at long distances from the coding region. In several self-reported racial/ethnic groups represented in the study, an association between estimated genetic ancestry and age at menarche was observed.

Conclusions: The results suggest that common variants near 10 HH-related loci do not play a substantial role in the regulation of age at menarche in the general population.

The nature and function of the 'pubertal clock' regulating the onset of puberty in humans is not well understood, although this timing is highly heritable. Contrary to expectations, the laborious approach taken by these authors did not pay off since none of the tested HH-related genes was found to be associated with the age at menarche in a large cohort of women. This underlines the complexity of this issue and the need for a more genome-wide approach to make progress in this important research. Such efforts are underway in ongoing studies by other investigators [see also the selected paper by Ong et al. below], but negative results over a decade seem to indicate that the timing of puberty is not in the gene sequence, but rather in their expression.

Mechanism of the year Now proven: females make a difference ... for males!

Female mice delay reproductive aging in males

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Biol Reprod 2009;80:1009–1014

Background: Reproductive aging of the male is characterized by decreasing reproductive function and fertility but factors that protect against this process in the aging male are largely unknown. Previous work has demonstrated that both female presence and aging have a dramatic effect on fertility in the male; yet, the effect of female presence on fertility in the aging male mouse is unknown. The objective of this work was to determine the effect of long-term isolation or cohabitation with females on fertility in aged male mice.

Methods: Male mice were housed with or without females until between 16 and 32 months of age. Males were subjected to fertility tests at specific ages, after which serum and testes were isolated for radioimmunoassay and histological analysis.

Results: Male mice continuously housed with females remain fertile longer (approximately 20% of the reproductive lifespan) than male mice housed alone. Fertility became significantly reduced 6 months sooner for males housed alone compared with males housed with females; however, the rate of decline was the same for males housed with or without females once fertility began to decrease. Testis weight decreased as the mice aged, and a nearly significant positive effect of female presence was observed. Additionally, histological analysis indicated that abnormal spermatogenesis occurred sooner in isolated males, suggesting that defects in spermatogenesis may play a role in the greater decrease in fertility in isolated males.

Conclusions: These results have significant implications for the maintenance of male fertility in wildlife, livestock, and human populations.

These are fascinating and provocative data demonstrating that the physical presence of females delays reproductive aging in males. Although the evidence comes from mice, the possible translation of these results into human biology has obvious and important societal and psychosocial implications. In evolutionary terms, where fitness is measured in fecundity, this makes a lot of sense: for a species longevity increases fitness only if a reproductive partner is around. However whether this 'mechanism' works both ways, i.e. females vs. males, as demonstrated here, and vice versa males vs. females, remains to be established. The findings may have particular relevance in societies with increasing ageing populations, with demands for maintained high quality of life. Exactly how the medical profession will adapt and transform these novel findings into medical advice and practice will be interesting to follow.

Genetics

New gene and novel functions of old friends

Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the CBX2 gene

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Am J Hum Genet 2009;84:658–663

Background: A specific molecular diagnosis is identified in only 20% of disorders of sex development cases. SRY alone is insufficient to induce testicular development and several other components of the male pathway have been identified, including Sox9, Dmrt1, Fgf9, Dhh, Sox8, and Dax1. WNT4 appears to be essential for sexual differentiation in women. Mice with targeted ablation of M33, an ortholog of *Drosophila* polycomb, show male-to-female sex reversal. Apart from sterility, most M33 knockout Sry-positive mice were phenotypically perfect females. A human counterpart of murine M33 deletion has so far not been identified.

Methods: A girl with a prenatal 46,XY karyotype was born with a completely normal female phenotype, including uterus and histologically normal ovaries. The human homolog of M33, Chromobox homolog 2 (CBX2), was analyzed.

Results: A loss-of-function mutation of CBX2 was found, identifying the present case as a human counterpart of the murine model.

Conclusions: By placing CBX2 upstream of SRY, an additional component of the still incomplete cascade of factors regulating human sex development has been added.

Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome

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Am J Hum Genet 2008;83:511–519

Background: CHARGE syndrome and Kallmann syndrome (KS) are two distinct developmental disorders sharing overlapping features of impaired olfaction and hypogonadism. KS is a genetically heterogeneous disorder consisting of idiopathic hypogonadotropic hypogonadism (IHH) and anosmia, and is most commonly due to KAL1 or FGFR1 mutations. CHARGE syndrome, a multisystem autosomal-dominant disorder, is caused by CHD7 mutations. The authors hypothesized that CHD7 would be involved in the pathogenesis of IHH and KS (IHH/KS) without the CHARGE phenotype and that IHH/KS represents a milder allelic variant of CHARGE syndrome.

Methods: Mutation screening of the 37 protein-coding exons of CHD7 was performed in 101 IHH/KS patients without a CHARGE phenotype. In an additional 96 IHH/KS patients, exons 6–10, encoding the conserved chromodomains, were sequenced. RT-PCR, SIFT, protein-structure analysis, and in situ hybridization were performed for additional supportive evidence.

Results: Seven heterozygous mutations, 2 splice and 5 missense, which were absent in 180 or more controls, were identified in 3 sporadic KS and 4 sporadic normosmic IHH patients. Three mutations affect chromodomains critical for proper CHD7 function in chromatin remodeling and transcriptional regulation, whereas the other 4 affect conserved residues, suggesting that they are deleterious. CHD7's role is further corroborated by specific expression in IHH/KS-relevant tissues and appropriate developmental expression. Sporadic CHD7 mutations occur in 6% of IHH/KS patients.

Conclusions: CHD7 represents the first identified chromatin-remodeling protein with a role in human puberty and the second gene to cause both normosmic IHH and KS in humans. The findings indicate that both normosmic IHH and KS are mild allelic variants of CHARGE syndrome and are caused by CHD7 mutations.

Mutations in NR5A1 associated with ovarian insufficiency

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N Engl J Med 2009;360:1200–1210

Background: The genetic causes of nonsyndromic ovarian insufficiency are largely unknown. A nuclear receptor, NR5A1 (better known as SF1), is a key transcriptional regulator of genes involved in the hypothalamic-pituitary-steroidogenic axis. Mutation of NR5A1 causes 46,XY disorders of sex development, with or without adrenal failure, but growing experimental evidence from studies in mice suggests a key role for this factor in ovarian development and function as well.

Methods: To test the hypothesis that mutations in NR5A1 cause disorders of ovarian development and function, the authors sequenced NR5A1 in four families with histories of both 46,XY disorders of sex development and 46,XX primary ovarian insufficiency and in 25 subjects with sporadic ovarian insufficiency. None of the affected subjects had clinical signs of adrenal insufficiency.

Results: Members of each of the 4 families and 2 of the 25 subjects with isolated ovarian insufficiency carried mutations in the NR5A1 gene. In-frame deletions and frameshift and missense mutations were detected. Functional studies indicated that these mutations substantially impaired NR5A1 transactivational activity. Mutations were associated with a range of ovarian anomalies, including 46,XX gonadal dysgenesis and 46,XX primary ovarian insufficiency. These mutations were not observed in more than 700 control alleles.

Conclusions: NR5A1 mutations are associated with 46,XX primary ovarian insufficiency and 46,XY disorders of sex development.

These 3 papers reveal novel genetic contributions to DSD and pubertal disorders. The first human case (XY sex reversal) with a mutation of the CBX2 gene, a homolog of the murine M33 gene, was

described by Biason-Lauber et al. The human phenotype matches that of the M33 knockout mouse adding an interesting contribution to the array of genes involved in mammalian sex differentiation. The CBX2 gene is believed to be located upstream of SRY. CDH7 is a chromatin remodeling protein which, when mutated, is known to cause CHARGE syndrome. When analyzing a large group of IHH/Kallmann patients without genetic diagnosis, CDH7 was shown to be involved in 3–5% of cases thus representing a milder phenotype of CHARGE. Even if its precise function is uncertain, CDH7 appears to be important in GnRH and olfactory neuron migration to their embryologic destination in the hypothalamus. Although providing proof-of-concept the paper shows that genes other than CDH7 must be involved in the pathogenesis of IHH/Kallmann in most cases.

Important work by Lourenço et al. demonstrated that the NR5A1 gene (SF1) is linked to primary ovarian failure and DSD in several independent familial cases without adrenal involvement. This is a major contribution since the background to most cases of such ovarian insufficiency is unknown.

Important for Clinical Practice

Consensus statement on the use of gonadotropin-releasing hormone analogs in children

Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR; ESPE-LWPES GnRH Analogs, Consensus Conference Group, Antoniazzi F, Berenbaum S, Bourguignon JP, Chrousos, GP, Coste J, Deal S, de Vries L, Foster C, Heger S, Holland J, Jahnukainen K, Juul A, Kaplowitz P, Lahlou N, Lee MM, Lee P, Merke DP, Neely EK, Oostdijk W, Phillip M, Rosenfield RL, Shulman D, Styne D, Tauber M, Wit JM

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Background: Gonadotropin-releasing hormone analogs revolutionized the treatment of central precocious puberty. However, questions remain regarding their optimal use in central precocious puberty and other conditions.

Methods: The Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology convened a consensus conference to review the clinical use of gonadotropin-releasing hormone analogs in children and adolescents.

Participants: When selecting the 30 participants, consideration was given to equal representation from North America (United States and Canada) and Europe, an equal male/female ratio, and a balanced spectrum of professional seniority and expertise. Preference was given to articles written in English with long-term outcome data. The US Public Health grading system was used to grade evidence and rate the strength of conclusions. When evidence was insufficient, conclusions were based on expert opinion. Participants were put into working groups with assigned topics and specific questions. Written materials were prepared and distributed before the conference, revised on the basis of input during the meeting, and presented to the full assembly for final review. If consensus could not be reached, conclusions were based on majority vote. All participants approved the final statement.

Results and Conclusions: The efficacy of gonadotropin-releasing hormone analogs in increasing adult height is undisputed only in early-onset (girls <6 years old) central precocious puberty. Other key areas, such as the psychosocial effects of central precocious puberty and their alteration by gonadotropin-releasing hormone analogs, need additional study. Few controlled prospective studies have been performed with gonadotropin-releasing hormone analogs in children, and many conclusions rely in part on collective expert opinion. The conference did not endorse commonly voiced concerns regarding the use of gonadotropin-releasing hormone analogs, such as promotion of weight gain or long-term diminution of bone mineral density. Use of gonadotropin-releasing hormone analogs for conditions other than central precocious puberty requires additional investigation and cannot be suggested routinely.

Puberty, contraception, and hormonal management for young people with disabilities

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Background: Assessment and management of a young person with a severe disability is multifaceted and complex. Variations of puberty can cause major concerns for parents and carers, with fears of imminent menstruation, peer and personal differences, concern for height outcome, as well as grief for a loss of childhood. Addressing physical, emotional, and social issues assists in optimizing outcomes.

Methods and Results: This article outlines specific evaluation and detailed management strategies for female and male pubertal problems in the context of disability, including treatments for extreme pubertal delay or acceleration, menstrual management at different ages, contraceptive issues, and sexual function and choices for both sexes.

Conclusions: This paper gives valuable advice and recommendations for the management of this important and difficult subject in young people.

Two papers contributing to good clinical practice were selected. Although the use of gonadotropin-releasing hormone analogs to treat children with hypergonadotropic precocious puberty is daily routine for every pediatric endocrinologist there are still unresolved questions concerning optimal protocols, combination with growth hormone treatment and other issues. The consensus statement by LWPES/ESPE on this topic after a well-structured conference is therefore much warranted and recommended as evidence base.

The 2nd paper gives valuable advice on how to manage problems with puberty, contraception and sexual function in adolescents with disabilities. This is a sensitive and not much discussed topic in adults with a handicap and even more so in the present age group. The paper is recommended to all colleagues seeing such patients in their clinics including pediatric endocrinologists, pediatricians gynecologists, general practitioners and others.

New paradigms Big roles for small RNAs

Deletion of Dicer in somatic cells of the female reproductive tract causes sterility

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Background: Dicer is a ribonuclease enzyme necessary for microRNA (miRNA) processing and the synthesis of siRNAs from long double-stranded RNA. Dicer plays important roles in the mammalian germ line and early embryogenesis but its functions in somatic cells of the female reproductive tract are largely unknown.

Methods: Employing conditional knockout technology mice were made deficient in Dicer1 in the mesenchyme of the developing Müllerian ducts and postnatally in ovarian granulosa cells and mesenchyme-derived cells of the oviducts and uterus.

Results: Deletion of Dicer in these cell types results in female sterility and multiple reproductive defects including decreased ovulation rates, compromised oocyte and embryo integrity, and other structural changes. Sequencing of small RNAs in the oviduct revealed downregulation of specific miRNAs in Dicer conditional knockout females compared with wild-type. These miRNAs are predicted to mainly regulate genes important for Müllerian duct differentiation and mesenchyme-derived structures, several of which were significantly upregulated upon conditional deletion of Dicer1.

Conclusions: Dicer and its miRNA products have critical roles in the development and function of the female reproductive tract.

Dicer1 is required for differentiation of the mouse male germline

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Biol Reprod 2008;79:696–703

Background: MicroRNAs (miRNAs) are small noncoding RNAs that posttranscriptionally regulate gene expression. A great number of miRNAs are expressed in mammals but their specific functions are yet to be uncovered. The enzyme Dicer1 is required for miRNA processing, and mouse knockouts of Dicer1 are embryonic lethal before 7.5 days postcoitus.

Methods: The function of miRNAs was examined specifically in the germ line using a conditional knock-out of Dicer1 in a mouse model.

Results: Removal of Dicer1 from germ cells resulted in male infertility. Germ cells were present in adult testes, but few tubules contained elongating spermatids. Germ cells that did differentiate to elongating spermatids exhibited abnormal morphology and motility. Rarely, sperm lacking Dicer1 could fertilize wild-type eggs to generate viable offspring.

Conclusions: Dicer1 and miRNAs are essential for proper differentiation of the male germ line. The results point to novel potential mechanisms behind male infertility.

Genetic variation in LIN28B is associated with the timing of puberty

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Background: The timing of puberty is highly variable but the genetic background of this phenomenon is largely unknown.

Methods: The authors carried out a genome-wide association study for age at menarche in 4,714 women.

Results: An association in LIN28B on chromosome 6 was reported. In independent replication studies in 16,373 women, each major allele was associated with 0.12 years earlier menarche. This allele was also associated with earlier breast development in girls; earlier voice breaking and more advanced pubic hair development in boys; a faster tempo of height growth in girls and boys, and shorter adult height in women and men in keeping with earlier growth cessation. All associations mentioned were highly statistically significant.

Conclusions: These studies identify variation in LIN28B, a potent and specific regulator of microRNA processing, as the first genetic determinant regulating the timing of human pubertal growth and development.

MicroRNAs are small species of non-coding RNA molecules actively produced and processed by the cell to control gene expression at the posttranscriptional level. This intrinsic control system has recently become a hot topic in many areas of human biology. Experimental manipulation of miRNA expression and processing at the systemic level results in severe phenotypes and embryonic lethality. Tissue-specific deletions cause various disturbances dependent on the affected organ system. The dependence of an intact miRNA system for proper development of the male and female reproductive systems is illustrated here by 2 papers selected from the recent literature. Tissue-specific deletion of Dicer1, a key enzyme in miRNA processing, in cells of male and female reproductive organs results in maldifferentiation and infertility as reported by Nagaraja et al. for females and by Maatouk et al. for males.

The importance of miRNA for reproduction is further strengthened by the recent paper by Ong et al. (>40 authors) searching for a genetic background to the timing of puberty. By employing a genome-wide approach with high power, the authors unexpectedly identified a highly significant association in LIN28B on human chromosome 6 with the timing of human pubertal growth and development. LIN28B is a marker of embryonic stem cells and an important regulator of miRNA processing [7] involved in the regulation of IGF-2 expression. A recent genome-wide association study for adult

height identified another LIN28B polymorphic mark as 1 of 10 loci with robust association. Different LIN28B isoforms could therefore contribute to the heritability of the timing of growth and development.

New concerns **Earlier start of puberty without obesity**

Recent decline in age at breast development: the Copenhagen Puberty Study

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Background: Recent publications showing unexpectedly early breast development in American girls have created debate worldwide. However, secular trend analyses are often limited by poor data comparability among studies performed by different researchers in different time periods and populations.

Methods: The authors present new European data systematically collected from the same region and by 1 research group at the beginning and end of the recent 15-year period. Girls ($n = 2,095$) aged 5.6–20.0 years were studied in 1991–1993 (1991 cohort; $n = 1,100$) and 2006–2008 (2006 cohort; $n = 995$). All girls were evaluated by palpation of glandular breast, measurement of height and weight, and blood sampling (for estradiol, luteinizing hormone, and follicle-stimulating hormone). Age distribution at entering pubertal breast stages 2–5, pubic hair stages 2–5, and menarche was estimated for the 2 cohorts.

Results: Onset of puberty, defined as mean estimated age at attainment of glandular breast tissue (Tanner breast stage 2+), occurred significantly earlier in the 2006 cohort (estimated mean age 9.86 years) when compared with the 1991 cohort (estimated mean age 10.88 years). The difference remained significant after adjustment for BMI. Estimated ages at menarche were 13.42 and 13.13 years in the 1991 and 2006 cohorts, respectively. Serum follicle-stimulating hormone and luteinizing hormone did not differ between the 2 cohorts at any age interval, whereas significantly lower estradiol levels were found in 8- to 10-year-old girls from the 2006 cohort compared with similarly aged girls from the 1991 cohort.

Conclusions: The authors found significantly earlier breast development among girls born more recently. Alterations in reproductive hormones and BMI did not explain these marked changes, which suggests that other factors yet to be identified may be involved.

Studies from the US have convincingly demonstrated an earlier start of puberty in girls, particularly in certain ethnic groups. This recent trend, adding an additional component to the secular trend that has been operating for centuries, coincides with the obesity explosion in the US, also mainly affecting the same ethnic groups. Direct correlations between higher BMI and earlier breast development have also been demonstrated in these groups. Despite considerable search, similar trends have been difficult to demonstrate in Europe, which has been suggested to be due to a later onset and less severe obesity trend than that in the US. It is therefore of concern to read the report by Aksglaede et al. on 2 Danish cohorts of girls, one investigated recently and the other 15 years earlier, by the same research group. The authors demonstrate a recent earlier breast development in girls but fail to show a correlation with BMI and the levels of reproductive hormones. Yet, the age at menarche was little affected. In fact, it is surprising that as late as 2006, the average age at menarche was 13.13 years; we thought it was earlier in northern Europe. This points to an influence of environmental factors, the nature of which are still to be determined.

Maternal levels of perfluorinated chemicals and subfecundity

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Background: The flame retardants perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are ubiquitous manmade compounds that are possible hormonal disruptors. These chemicals are used, e.g., in impregnation of clothes and household fabrics, like carpets.

Methods: In this study the authors examined whether exposure to these compounds may decrease fecundity in humans. Plasma levels of PFOS and PFOA were measured at weeks 4–14 of pregnancy in 1,240 women. For this pregnancy, women were asked to report time to pregnancy (TTP) in five categories (<1, 1–2, 3–5, 6–12 and >12 months). Infertility was defined as having a TTP of >12 months or receiving infertility treatment to establish pregnancy.

Results: Longer TTP was associated with higher maternal levels of PFOA and PFOS. Compared with women in the lowest exposure quartile, the adjusted odds of infertility increased among women in the higher three quartiles of perfluorinated compound levels. Fecundity odds ratios (FORs) were also estimated. The adjusted FORs were virtually identical for women in the 3 highest exposure groups of PFOS compared with the lowest quartile.

Conclusions: The findings from the study suggest that PFOA and PFOS exposure at plasma levels seen in the general population may reduce fecundity. Such results deserve attention, since these substances are all around us and might not only affect us but also generations to come.

Endocrine disruptors (EDs) are manmade chemicals in the environment reported to affect hormonal actions at various levels in the organism. Most convincing data of such effects are from wildlife and proof-of-concept studies in experimental animals. Direct correlation studies in humans are sparse, and causative effects nonexistent. The selected paper demonstrates a correlation between plasma levels of two flame retardants known as EDs and decreased fertility in women. These findings have to be substantiated by more data and confirmation by other groups but still need attention as exposure to these and similar compounds is not easy to avoid in a modern society. Precautionary principles must therefore rule!

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