Reproductive Endocrinology

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Major achievements in reproductive biology and endocrinology have been made during the last year. Two major areas show intense activity and have revised our concepts. There has been continuous high activity in the stem cell field and the advances of stem cell biology are now creating novel possibilities of assisted-reproduction technologies for human use. Another area of interesting development is that of reproductive behavioral sciences, where advanced genetic, molecular and neurophysiological technologies have developed the field. Many of the findings reported in this chapter are still at the experimental level, but ongoing translational approaches are expected to result in rapid applicability in human medicine.

Concepts revised

Biology behind the increased number of children in related human couples

An association between the kinship and fertility of human couples

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Science 2008;319:813–816

Background: Related human couples have been known to produce more children than unrelated couples, but whether this difference depends on biological or socioeconomic factors has not been possible to determine.

Methods: The studies were performed on all known couples in Iceland between 1800 and 1965, defined as those males and females who had at least one common child but also those registered as married without offspring. The data are available from the deCODE genealogical database. This database contains all contemporary Icelanders and their ancestors back to 1650. Presently a total number of over 730,000 persons are recorded in this genealogical database. Completed fertility was defined as the number of offspring for a couple registered in the genealogical database. The reproductive success was studied with two endpoints: (1) the number of children who reproduced, and (2) the number of grandchildren descending from these.

Results: The greatest reproductive success was found in couples related at the level of third or fourth cousins. Indications for impaired reproductive outcome in the offspring of parents related at the degree of second cousins or closer were found.

Conclusion(s): Given the relative homogeneity regarding socioeconomic status among Icelanders, the association between kinship and fertility is likely to be dependent on biological factors.

Previous studies on kinship and reproduction have focused on the close relationship between couples, which is known to increase the risk of miscarriage and infant mortality and morbidity. In other species a lower fitness between distantly related individuals has been shown, explained by a breakdown of co-adapted gene complexes [1]. In this report the evaluation of relationships more distant than second cousins has also been possible, and the authors find that reproductive success is greatest in couples related at the degree of third and fourth cousins. Thus, a certain degree of similarity/common interests also at the genetic level makes the marriages more successful – at least at the reproductive level.

Developmental programming: deficits in reproductive hormone dynamics and ovulatory outcomes in prenatal, testosterone-treated sheep

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Biol Reprod 2008;78:636–647

Developmental programming: impact of prenatal testosterone excess on pre- and postnatal gonadotropin regulation in sheep

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Biol Reprod 2008;78:648–660

Background: Female sheep exposed to excessive testosterone levels during fetal life exhibit hypersecretion of LH and progressive loss of cyclicity. Androgen excess also leads to neuroendocrine, ovarian and metabolic disruptions, culminating in reproductive phenotypes mimicking that of women with polycystic ovary syndrome (PCOS). The mechanism behind this is not known.

Methods: To generate prenatal testosterone-treated females, pregnant sheep were injected intramuscularly (days 30–90 of gestation, term = 147 days) with 100 mg of testosterone propionate in cottonseed oil semi-weekly. Female offspring born to untreated control females and prenatal testosterone-treated females were then studied during their first two breeding seasons.

Results: As compared to controls, females exposed during fetal life to excess androgen exhibited enhanced LH but not FSH responses to intermittent delivery of GnRH boluses under conditions in which endogenous LH (GnRH) pulses were suppressed. LH hypersecretion was more evident in adults than in prepubertal females, and it was associated with development of acyclicity. During the first breeding season, prenatal testosterone-treated females manifested disruptions in the timing and magnitude of primary gonadotropin surges, luteal defects, and reduced responsiveness to progesterone negative feedback. There were also severe disruptions in the peri-ovulatory sequence of events during the second breeding season including, e.g., luteal responses that ranged from normal to anovulatory.

Conclusion(s): Taken together, the results imply that prenatal androgen excess amplifies GnRH-induced LH response, leading to LH hypersecretion and acyclicity in adulthood, and that this programming involves developmental changes in expression of pituitary genes involved in LH and FSH release. These outcomes are likely to be of relevance to the developmental origin of infertility disorders and suggest that differences in fetal exposure or fetal susceptibility to androgens may account for the variability in reproductive phenotypes.

Female sheep prenatally exposed to excess androgens exhibited LH-hypersecretion, leading to acyclicity in adulthood. This programming seems to include developmental changes in expression of pituitary hormones involved in LH and FSH release. These two papers by the group of Padmanabhan, using a sheep model, provide further knowledge on how prenatal conditions alter postnatal fertility. Their results also support studies from several species indicating that prenatal exposure to androgens produces reproductive and metabolic effects that are similar to those of PCOS, an important cause of human infertility but also a cause of the metabolic syndrome in women.

New mechanisms

β -Defensin 126 – an important fertility factor

β -Defensin 126 on the surface of macaque sperm mediates attachment of sperm to oviductal epithelia

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Biol Reprod 2008;78:400–412

Background: β -Defensin 126 coats the entire surface of macaque sperm until the sperm becomes capacitated, and the removal of β -defensin 126 from the sperm head is required for sperm-zona recognition. Viable sperm collected from the cervix and uterine lumen of mated female macaques had β -defensin 126 coating the entire surface, suggesting that the protein is retained on sperm in the oviduct. β -Defensin 126 plays a major role in the attachment of sperm to epithelial cells in the oviduct.

Methods: Sperm were treated to either remove or alter β -defensin 126, thereafter sperm were co-incubated with explants of oviductal epithelial cells. Sperm were treated with either: (A) caffeine + dibutyryl cyclic adenosine monophosphate (dbcAMP) which induces capacitation and complete release of β -defensin 126 from sperm; (B) caffeine which removes β -defensin 126 from over the head and midpiece but does not induce capacitation; (C) anti- β -defensin 126 immunoglobulin, or (D) neuraminidase, which cleaves sialic acid from terminal positions on glycosylation sites of β -defensin 126.

Results: All four of the above treatment regimens resulted in similar and significant levels of inhibition of sperm-oviductal epithelial cell binding. Treatments that induce either removal, masking, or alteration of β -defensin 126 result in loss of sperm-epithelial cell binding that is independent of changes in sperm motility.

Conclusion(s): β -Defensin 126 is directly involved in the formation of a reservoir of sperm in the oviduct of macaques.

Defensins belong to an expanding family of innate immunity factors with antimicrobial activity, found to be produced by epithelial cells lining various organs such as the genitourinary tract. Defensins have been implicated in the host-defense of epithelial surfaces to microbial colonization but other roles have also been proposed. In this study defensin (β 126) is shown to induce formation of sperm reservoirs within the oviduct. This is likely of importance for selection of sperm, and for optimizing timing of fertilization. β -Defensin 126 has previously been shown to be of importance for capacitation and binding of sperm to zona pellucida of the oocyte. Thus, this defensin seems to be an important part of successful fertilization, and as such a target for development of future contraceptive agents.

Mechanism of the year

A potential key to follicle activation is found

Oocyte-specific deletion of *Pten* causes premature activation of the primordial follicle pool

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Science 2008;319:611–613

Background: In the mammalian ovary, activation of primordial follicles from the dormant pool serves as the source of fertilizable ova. The end of female reproductive life, menopause in women, occurs when the primordial follicle pool is exhausted. In a small group of women the ovaries stop producing eggs prematurely, resulting in infertility and early aging. The knowledge of the molecular mechanisms behind follicle activation is poor. In previous studies PI3K (phosphatidylinositol 3-kinase) has been shown to play a role in the activation of primordial follicles [2, 3]. PTEN (phosphatase and tensin

homolog deleted on chromosome 10) is a lipid phosphatase that functions as a major negative regulator of PI3K action [4].

Methods: The *Pten* gene was deleted from oocytes by crossing *Pten*^{loxP/loxP} mice with transgenic mice expressing a Gdf-9 promoter-mediated Cre recombinase, which is specifically active in oocytes. The *Pten*^{loxP/loxP}:GCre⁺ females produced a maximum of one normal sized litter and then became infertile in early adulthood.

Results: In mice lacking PTEN in oocytes the entire primordial follicle pool becomes activated, subsequently resulting in depletion of all primordial follicles in early adulthood, causing premature ovarian failure.

Conclusion(s): The mammalian oocyte serves as the headquarters for programming of follicle activation. In addition, the oocyte PTEN-PI3K pathway governs follicle activation via control of oocyte growth initiation.

The duration of female fertility is determined by the initial size of the primordial follicle pool and by the rate of its activation and depletion. The activation of the primordial follicles is strictly regulated. When the ovaries stop producing eggs maturely, it results in infertility and aging. Premature ovarian failure affects a number of young women who become infertile in early age. Definition of a responsible factor, as in this report an enzyme that could be activated or inhibited, increases the chances for finding a treatment for premature ovarian failure. It could also be a tool for regulating follicle maturation in vitro, when ovaries have been obtained before chemotherapy to preserve the possibility of biological offspring.

Food for thought

Secondary sex ratio variation

1. Don't skip breakfast if you want a son

The contrasting effects of ad libitum and restricted feeding of a diet very high in saturated fats on sex ratio and metabolic hormones in mice

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Biol Reprod 2007;77:599–604

Background: Skewing of the sex ratio towards males occurs among pups born to mice fed a very high saturated fat (VHF) diet. In the present study, the authors tested whether the fat content of the VHF diet rather than the number of calories consumed is responsible for this effect.

Methods: Eight-week-old mice were randomized to a VHF diet either ad libitum (VHF) or in a restricted manner (VHF-R). The VHF-R mice gained weight at a similar rate to controls fed a standard chow diet. Mice were bred at 15 weeks and subsequently at 26 and 35 weeks of age.

Results: The VHF, VHF-R, and control groups delivered 244, 242, and 274 pups, with male proportions of 0.60, 0.43, and 0.48, respectively. The pup sex ratios of the VHF group (favoring males) and VHF-R group (favoring females) were both significantly different from 0.5. The sex ratios also differed between the VHF and control groups, and between the VHF and VHF-R groups. Within the diet groups, maternal body weight had no effect on sex ratio. Serum leptin concentrations among the dams were similar in the VHF and VHF-R groups but higher than in the control group, while the IGF-1 and corticosterone levels were comparable in all 3 groups.

Conclusion(s): The atypical sex ratios of offspring born to dams on the VHF diet seem to be influenced by the amount of fat consumed. Ready access to food high in calories, and not body composition per se, prompts the sex ratio in favor of males. Since males fed the VHF diet had neither more Y-sperm nor sired more sons than daughters, the dietary effects are manifested exclusively through the female.

These results show that free access to food of a murine mother can influence the sex ratio of the offspring to favor males. In the wilderness, females best skilled to compete for food are the ones most likely to give birth to litters skewed to a higher male ratio. This finding was later followed by a paper by Mathews et al. [5] showing similar results in humans with mothers having a higher proportion of male

children when their caloric intake was high prior to conception. Women having sons consumed more breakfast cereals than those having daughters. The small but consistent decline in the proportion of males born over the past 40 years [6] could thus be a sign of the changes in diets among young women.

2. When mum has the final say

Sex of bovine embryos may be related to mothers' preovulatory follicular testosterone

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Biol Reprod 2008;78:812–815

Background: Although the sex of the offspring in mammals is commonly viewed as a matter of chance (depending on whether an X or a Y chromosome-bearing spermatozoon reaches the ovum first), evolutionary biologists have shown that offspring sex ratios are often significantly related to maternal dominance, a characteristic that has been shown to be linked to testosterone in female mammals, including humans. The authors hypothesized that variations in female testosterone might be related to reproductive mechanisms associated with sex determination, with higher levels of follicular testosterone being associated with a greater likelihood of conceiving a male.

Methods: Follicular fluid and cumulus-oocyte complexes were collected from bovine antral follicles. Individual matched samples of follicular fluid were assayed for testosterone, whereas the oocytes were matured, fertilized, and cultured in vitro. The resultant embryos were sexed by PCR. The level of testosterone in the follicular fluid was compared with the sex of the embryo.

Results: Follicular testosterone levels were significantly higher for subsequent male embryos. When embryos from follicles in which the estradiol-to-testosterone ratio was more than 1 were excluded, the same result held.

Conclusion(s): Bovine ova that developed in follicular fluid with high concentrations of testosterone in vivo were significantly more likely to be fertilized by Y chromosome-bearing spermatozoa (in vitro).

There is growing evidence that dominant human females conceive more sons. In relation to the previously cited papers by Alexenko et al. and Mathews et al., one could wonder if dominant females with higher testosterone levels are also those most competent at finding food. The possibility that women with higher testosterone levels are also more likely to have a large appetite and less likely to skip breakfast seems most likely. The question then remains: which factor is most important for the skewing of sex variation in high calorie diets and dominant, androgen-prone females? Chronic stress is known to elevate testosterone in females and stressed mothers would therefore be more prone to give birth to males. Although the stress on mothers is increasing in the Western world, the proportion of sons is decreasing, and therefore the testosterone increase (as seen after stress) seems less likely to have a major impact.

Food for thought

Male flies chase anything with wings

Recurring ethanol exposure induces disinhibited courtship in *Drosophila*

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PLoS ONE 2008;3:e1391

Background: Alcohol has a strong causal relationship with sexual arousal and disinhibited sexual behavior in humans; however, the physiological support for this notion is largely lacking and thus a suitable animal model to address this issue is instrumental.

Methods: The effect of ethanol on sexual behavior was investigated in *Drosophila*.

Results: Wild-type males who typically court females but not males were investigated; however, upon daily administration of ethanol, they exhibited active inter-male courtship, which represents a novel type of behavioral disinhibition. The ethanol-treated males also developed behavioral sensitization, a form of plasticity associated with addiction, since their inter-male courtship activity was progressively increased with additional ethanol experience. We identified three components crucial to the ethanol-induced courtship disinhibition: the transcription factor regulating male sex behavior *Fruitless*, the ABC guanine/tryptophan transporter *White*, and the neuromodulator dopamine. The *fruitless* mutant males normally display conspicuous inter-male courtship, but their courtship activity was not enhanced under ethanol. Likewise, *white* males showed negligible ethanol-induced inter-male courtship, which was not only reinstated but also augmented by transgenic *White* expression. Moreover, inhibition of dopamine neurotransmission during ethanol exposure dramatically decreased ethanol-induced inter-male courtship. Chronic ethanol exposure also affected a male's sexual behavior toward females: it enhanced sexual arousal but reduced sexual performance.

Conclusion(s): These findings provide novel insights into the physiological effects of ethanol on sexual behavior and behavioral plasticity.

The study suggests that the link between sex and alcohol may be the neurotransmitter dopamine. The authors found that lowering dopamine concentrations in drunken flies reduced male-to-male courtship. Dopamine is associated with general activity levels, which makes it unclear whether the reduced dopamine levels may have simply caused the flies to become less active. Chronic boozing sends male flies chasing after any and every potential mate. Also towards females the arousal was enhanced although performance reduced. As stated in a highlight by Heidi Ledford in *Nature* [3 January 2008, doi:10.1038/news.2007.402], 'heavy boozing has been shown to send male fruit flies, like their human counterparts, into a lusty fog'. Thus, flies do seem more like humans than could be expected.

Mechanism of the year

Be cool to become a man by activation of sex-determining genes

Response of candidate sex-determining genes to changes in temperature reveals their involvement in the molecular network underlying temperature-dependent sex determination

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Mol Endocrinol 2007;12:2750–2763

Background: Sex determination is the process of forming an ovary or a testis from a bipotential gonad. This process is critical to the development of sexually reproducing adults. The molecular pathways behind vertebrate gonadogenesis are fairly well known in organisms exhibiting genotypic sex determination but are less well understood in vertebrates whose sex is determined by environmental factors such as temperature.

Methods: The authors examined the response of six candidate sex-determining genes (*FoxL2*, *Wnt4*, *Dmrt1*, *MIS*, *Dax1*, *Sox9*) to sex-reversing temperature shifts in a turtle with temperature-dependent sex determination (TSD).

Results: The results are the first to show that *FoxL2*, *Wnt4*, *Dmrt1*, and *MIS* are regulated by temperature, demonstrating their involvement in the molecular pathway underlying TSD and placing them downstream of the action of temperature. Further, *FoxL2* plays an ovarian-specific role in development, whereas *Wnt4* appears to be involved in both testis and ovary formation. *Dmrt1* expression showed rapid activation in response to a shift to male-producing temperature, whereas *MIS* upregulation was delayed. Furthermore, early repression of *MIS* appeared critical to ovarian development. *Dax1* and *Sox9* of gene expression in response to temperature was found to appear later in gonadogenesis.

Conclusion(s): The authors conclude that by examining the role of candidate genes in TSD, findings of conservation and divergence between sex-determining mechanisms in distant close and species may lead us to better understanding of the genes and mechanisms involved in gonadogenesis.

In turtles with temperature-dependent sex determination, males are generally produced at lower temperatures than females. A temperature change as little as 1–2°C is of importance in this process. At temperatures below 27°C mostly male turtles are produced whereas at warmer temperatures, around 30°C, only female turtles are formed [7]. This study shows for the first time that genes of importance for gene regulated mammalian sex determination are also driven by environmental (temperature) signals. By using such comparative biology one might be able to come closer to a better understanding of what factors are crucial for ovarian development in females and what genes are orchestrated by SRY in males. The important role of FoxL2 for ovarian development was supported by this study.

New mechanism Beyond SRY

Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer

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Nature 2008;453:930–934

Background: Sry acts in sex determination to initiate testis rather than ovary development from early bipotential gonads. The cellular mechanism is to trigger the differentiation of Sertoli cells from the supporting cell lineage that would otherwise give rise to ovarian follicle cells in the absence of Sry. The related autosomal gene Sox9 is also known from disrupting mutations in mice and humans to be essential for Sertoli cell differentiation. Further, abnormal expression of Sox9 in an XX gonad can lead to male development in the absence of Sry. These genetic data, together with the finding that Sox9 is upregulated in Sertoli cell precursors just after SRY expression begins, has led to the proposal that Sox9 could be directly regulated by SRY. However, the mechanism by which SRY may affect Sox9 expression is not understood.

Methods and Results: The authors show that SRY binds to multiple elements within a Sox9 gonad-specific enhancer in mice, and that it does so along with the orphan nuclear receptor gene steroidogenic factor 1 (SF1). Using various analyses including mutation, co-transfection and sex-reversal studies it was shown that SF1 and SRY cooperatively upregulate Sox9 and then, together with SF1, SOX9 also binds to the enhancer to help to support its own expression after that of SRY expression has declined.

Conclusion(s): These results permit further characterization of the molecular mechanisms regulating sex determination and how they have evolved, as well as how they fail in cases of sex reversal.

SRY is an intronless gene encoding a transcription factor that is a member of the high mobility group (HMG)-box family of DNA-binding proteins. It acts by wrestling other DNA out of shape, changing the properties and expression of a number of genes, and resulting in testis formation [8]. Despite extensive search the downstream mechanisms of SRY action in sex determination are largely unknown although several of the molecular players involved have been described. A substantial number of XX males lack SRY expression and increasing data point to Sox9 as the crucial factor behind male development in these cases. This study in mice delivers a mechanistic explanation of how SOX9 expression is maintained in male gonadogenesis and opens up possibilities to investigate these mechanisms in human cases of aberrant sex determination.

New concept

New role for old player: androgen produced by and acting on embryonic stem cells

Evidence that androgens regulate early developmental events, prior to sexual differentiation

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Endocrinology 2008;149:5–14

Background: It is well known that androgen signaling is critical for normal male fetal development but is not thought to regulate events in early embryogenesis. Given the interest in factors controlling the differentiation of embryonic stem (ES) cells, the authors explored the possibility that androgens play a role in ES function.

Methods: Studies at transcriptional, translational, transfectional and functional levels investigating androgen receptor expression and function in murine ES cells.

Results: The authors show expression of androgen receptor (AR) RNA and protein in four independent mouse ES (mES) cell lines, and demonstrate that the AR is functional in an assay involving transfected androgen response elements. AR mRNA was detected throughout 10-day differentiation in embryoid bodies (EBs). Exposure of EBs to testosterone (T) or dihydrotestosterone promoted formation of beating cardiomyocytes. Flow cytometric analyses demonstrated a significant increase in the number of α -actinin and tropomyosin (cardiac markers) positive cells after these treatments. Addition of AR inhibitor flutamide to T-treated EBs suppressed the T-induced proliferation of cardiomyocytes, confirming that the androgens act via the classical AR-mediated genomic pathway. It was also shown that mES cells express key steroidogenic enzymes, as detected by RT-PCR, and during 24-hour incubations secrete T at concentrations levels comparable to those secreted by cultured Leydig cells.

Conclusion(s): This paper presents novel data demonstrating the capacity of androgens to stimulate increased differentiation of mouse ES cells to cardiomyocytes. The findings support recent observations that AR-deficient mice exhibit cardiac impairment in adulthood.

This paper points to novel sources and differentiating effects of androgen in early embryogenesis. These are surprising findings that need to be verified in other species including human. Nevertheless, the results indicate that androgen production and action may occur much earlier than the time when Leydig cells appear and become active in the testis. It is yet also unclear whether the observations are gender dimorphic, although there is no clear reason why they should be. The observations also open new targets for the action of endocrine-disrupting environmental chemicals (EDCs). In the present model one would anticipate that androgen receptor active EDCs would adversely affect early cardiac organogenesis. See also Lee and Teixeira [9].

New methodology

It is all about timing: rat DSD model finds a time window of defective masculinization

Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism

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J Clin Invest 2008;118:1479–1490

Background: Male differentiation in fetal life is determined by androgen-induced masculinization. Disorders of fetal masculinization including hypospadias or cryptorchidism are common, but their

cause remains unclear in most cases. Although masculinization is well studied, no unifying concept explains normal male reproductive development and its abnormalities.

Methods: Rat fetuses of both sexes were exposed to either anti-androgens or androgens during various times of prenatal development and the phenotype of the progeny was investigated.

Results: It was found that masculinization of all reproductive tract tissues was programmed by androgen action during a common fetal programming window. This preceded morphological differentiation, when androgen action was, surprisingly, unnecessary. Only within the programming window did blocking androgen action induce hypospadias and cryptorchidism and altered penile length in male rats, all of which correlated with anogenital distance (AGD). Androgen-driven masculinization of females was also confined to the same programming window.

Conclusion(s): This work has identified in rats a common programming window in which androgen action is essential for normal reproductive tract masculinization. It has also shown that measuring AGD in neonatal humans could provide a noninvasive method to predict neonatal and adult reproductive disorders. Translation of the timings in rats indicates that the programming window in humans is likely to be 8–14 weeks of gestation.

Most newborn boys with signs of defective masculinization in fetal life, commonly presenting as hypospadias or cryptorchidism, lack obvious gene mutations and hormone synthesis defects. One likely explanation behind such a phenotype is temporary inhibition of androgen action during a sensitive time window of male androgen-dependent development. Models suitable for studying such a possibility are highly warranted. This paper presents a disease model in rats that may be useful for such studies of mechanisms behind DSD in humans, in particular those including aberrations of the masculinization of the male reproductive tract. Translation of the most sensitive window detected in rats into human prenatal development indicated a time frame corresponding to the end of the 1st trimester and the beginning of the 2nd trimester of human pregnancy.

Important for clinical practice

Consensus statement from Nordic countries on management of undescended testes: operate early!

Nordic consensus on treatment of undescended testes

Ritzén EM, Bergh A, Bjerknes R, Christiansen P, Cortes D, Haugen SE, Jörgensen N, Kollin C, Lindahl S, Läckgren G, Main KM, Nordenskjöld A, Rajpert-De Meyts E, Söder O, Taskinen S, Thorsson A, Thorup J, Toppari J, Virtanen H
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Acta Paediatr 2007;96:638–643

Background: Although undescended testis is the most common malformation in boys there is still no consensus how and when to treat this disorder. The aim of this work was to reach a consensus among specialists from the Nordic countries on the present state-of-the-art in the treatment of undescended testicles.

Methods: A group of specialists in testicular physiology, pediatric surgery/urology, endocrinology, andrology, pathology and anesthesiology from all the Nordic countries met for 2 days. Before the meeting, reviews of the literature had been prepared by the participants.

Results: The authors came to the following unanimous conclusions. (1) In general, hormonal treatment is not recommended, considering the poor immediate results and the possible long-term adverse effects on spermatogenesis. Thus, surgery is to be preferred. (2) Orchiopexy should be done between 6 and 12 months of age, or upon diagnosis, if that occurs later. (3) Orchiopexy before age 1 year should only be done at centers with both pediatric surgeons/urologists and pediatric anesthesiologists. (4) If a testis is found to be undescended at any age after 6 months, the patient should be referred for surgery to pediatric rather than general surgeons/urologists if the boy is less than 1 year old or if he has bilateral or non-palpable testes, or if he has a relapse of cryptorchidism.

Conclusion(s): Early surgery is the preferred treatment of undescended testes. Such strategy increases the demands on healthcare resources including access to pediatric specialists for this group of patients.

This consensus statement is accompanied by four review papers in the same issue of *Acta Paediatrica* [10–13] presenting the state of the science of etiology, animal disease models, surgical treatment and hormonal treatment of undescended testes. Given the common incidence of this disorder and its negative consequences for adult testis function if untreated, this review provides an important evidence base for modern treatment. The main message of the paper is that surgery is preferred treatment and that such action should be performed early, before 12 months of age. This opinion is backed by recent studies [14] demonstrating that testicular size (a proxy for Sertoli cell number) is negatively affected very early in undescended testes.

Important for clinical practice

Long-term follow-up of CAH women – did we do the right things as pediatricians?

Impaired sexual and reproductive outcomes in women with classical forms of congenital adrenal hyperplasia

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J Clin Endocrinol Metab 2007;92:1391–1396

Background: There is need for long-term follow-up studies of pediatric disorders; in particular those with possibilities of alternative treatments in early life. The objectives of this work were a detailed description of sexual and reproductive outcomes in adult women with congenital adrenal hyperplasia (CAH) of different phenotypic severity at birth, and a comparison of these outcomes among CAH subtypes and between CAH women and non-CAH control women.

Methods: Cross-sectional study using a face-to-face interview, a written questionnaire, the Female Sexual Function Index, and a gynecological examination. Patients included 35 women with CAH, representing Prader stages I–V at birth, aged 18–43 years, who had been treated from birth to adolescence in the same pediatric endocrine clinics. 69 non-CAH healthy control women were selected from hospital-staff families.

Results: None of the CAH women expressed doubts about their gender assignment. 20% (7 of 35) had homosexual inclinations; 23% (8 of 35) were married; 3 reported a complete lack of sexual activity, and 37% (13 of 35) said they never had heterosexual intercourse with vaginal penetration. Sexual functioning as assessed by the Female Sexual Function Index was much lower in CAH women than controls and lowest in CAH women with high Prader stages. 81% (18 of 22) experienced pain during vaginal penetration. Only 8 women became pregnant, and 17% (6 of 35) had children.

Conclusion(s): Despite expert medical and surgical care by physicians dedicated to this rare disease, women with CAH still suffer major limitations in their sexual function and reproductive life.

This study revealed a very poor adult outcome of women with CAH, affecting their quality of life and sexual function. This was not due to outdated treatment protocols for their initial management as some of the women were age 18 years at follow-up. All CAH women showed female gender identity. A minority of the affected women had children and a relatively high percentage was homosexual, confirming previous observations. Together these data demonstrate that exposure to androgens in female fetal life may affect the future sexual orientation but not the gender identity. The present type of long-term outcome study is important for pediatricians as a quality measure of the treatment offered to young patients. The problem we face with evaluation of such treatment is, in addition to its immediate efficacy and safety, the long relay before we can assess the final results.

Old knowledge explained

Female neuronal sensitivity dependent on hormonal status: higher sensitivity at high estrogen/progesterone levels

Convergence of nociceptive information in the forebrain of female rats: reproductive organ response variations with stage of estrus

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Exp Neurol 2008;210:375–387

Background: Neurons in the preoptic area (POA) of the hypothalamus and the bed nucleus of stria terminalis (BST) play an important role in the neuroendocrine control of the reproductive cycle, mating behaviors and nociception.

Methods: Single unit extracellular recordings were performed in the POA and BST region of 20 urethane anesthetized female rats during either the proestrus (elevated levels of estrogen/progesterone) or metestrus (low circulating hormones) stage of the estrous cycle. A total of 118 neurons in the POA and 65 neurons in the BST responded to the search stimuli, bilateral electrical stimulation of the viscerocutaneous branch of the pelvic nerve and/or sensory branch of the pudendal nerve (i.e., dorsal nerve of clitoris).

Results: Most of the neurons responding to the electrical search stimuli received a high degree of somatovisceral convergence, including inputs from the abdominal branches of the vagus, cervix, vagina, colon and skin areas on the perineum and trunk. Mean neuronal response thresholds for vaginal and cervical stimulation but not colon distension were significantly higher for animals tested during proestrus. Also, there was a shift in POA and BST neuronal responsiveness towards more inhibition and less excitation during proestrus for a variety of somatovisceral inputs.

Conclusion(s): The results demonstrate that the changes in hormonal status affect the properties of POA and BST neurons, which likely relates not only to the functional importance of these inputs for reproductive behaviors but also for nociceptive processing as well.

This study offers a neurophysiological correlate to what has been known for long; women have increased sensitivity during menstrual phases with higher sex steroid levels. The general theme of the present findings was more inhibition in the brain nuclei that were studied when hormonal levels were higher. Interestingly one of the nuclei investigated (bed nucleus of the stria terminalis – BST) has been implicated as a potential locus for gender identity. BST has been found to be gender dimorphic and to display morphological differences in transsexual vs. unaffected human individuals [15]. For comparison, it would therefore also be of interest to investigate males in the same model. Since transsexualism cannot be studied in experimental animals and the employed experimental technology cannot be used in humans, one has to await studies using functional MRI or similar methodology to verify the results in human subjects.

New concept

No need for females: derivation of sperm and oocytes from male embryonic stem cells

In vitro differentiation of male mouse embryonic stem cells into both presumptive sperm cells and oocytes

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Cloning Stem Cells 2007;9:535–548

Background: Recent work has demonstrated that male mouse embryonic stem (ES) cells may differentiate into germ cells (GC) showing generations of male or female gametes in separate experiments, by using

genetically manipulated or preselected ES cells. The present study attempted to produce both types of gametes from male mouse ES cells without any genetic manipulation or preselection.

Methods: The authors induced the differentiation by retinoic acid (RA) within nonadherent XY embryoid bodies (EB) and studied formation of gametes by analysis of expressed cell markers.

Results: Gamete-like cell formation occurred in the correct manner based on the expression of early and late GC-specific genes such as Oct-4, Mvh, Stella, Dazl, Piwil 2, Pdrd 1, Rex 14, Rnf 17, Bmp8b, Acrosin, Stra-8, Haprin, LH-R, Gdf9, Zp3, Zp2, Sycp1, and Sycp3. Immuno-fluorescence analysis of morphologically well-formed GC and presumptive gametes showed positive labeling for SSEA1, Oct-4, EMA-1, FE-J1, Dazl, Fragilis, Mvh, Acrosin, and acetylated α -tubulin. Conventional cytogenetic and FISH analysis indicated a chromosome reduction in ES-derived GC.

Conclusion(s): These data suggest that ES cells with XY chromosomes can produce both types of presumptive gametes under the same experimental conditions. This production depends on their positional and temporal information within the embryoid body context.

This paper demonstrates that murine ES cells have the capability to differentiate into both male and female gametes, when subjected to general differentiation signals. Although the cells were identified by their expression of specific markers and functionality in fertilization experiments was not tested, these results open fascinating possibilities for future use of this technology. The obvious advantage of employing a male source of ES cells is the possibility to obtain both X and Y gametes, in contrast to a female source that can only produce X gametes. The methodology used is simple and relatively low-tech and one should therefore expect rapid further development of this field.

New hope I

Important gender equality issue: both ovary and testis may be sources of pluripotent stem cells. Ladies first!

Expression of pluripotent stem cell markers in the human fetal ovary

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Hum Reprod 2008;23:589–599

Background: Human primordial germ cells (PGCs) can give rise to pluripotent stem cells such as embryonal carcinoma cells (ECCs) and embryonic germ cells (EGCs). The study was undertaken to explore whether PGCs express markers associated with pluripotency in EGCs and ECCs in the human fetal ovary.

Methods: The authors examined the expression patterns of multiple pluripotent markers in 5.5–15 weeks post-fertilization (pF) human fetal ovaries and related this expression with the ability to derive pluripotent EGCs in vitro.

Results: Specific subpopulations of cells were identified which included OCT4(+)/Nanog(+)/cKIT(+)/VASA(+) PGCs and oogonia. These cells also expressed SSEA1 and alkaline phosphatase (AP) and SSEA4 expression occurred throughout the entire gonad. Isolation of SSEA1(+) cells from the gonad resulted in AP(+) EGC colony formation. The number of OCT4(+) or Nanog(+) expressing cells peaked by week 8 and then diminished after week 9 pF, as oogonia entered meiosis. In addition, the efficiency of EGC derivation was associated with the number of OCT4(+) cells. TRA-1-60 and TRA-1-81 were only detected in the lining of the mesonephric ducts and occasionally in the gonad.

Conclusion(s): These results demonstrate that PGCs, a unipotent cell, express most, but not all, of the markers associated with pluripotent cells in the human fetal ovary.

Expression of pluripotent stem cell markers in the human fetal testis

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Stem Cells 2008;26:412–421

Background: Human primordial germ cells (PGCs) have proven to be a source of pluripotent stem cells called embryonic germ cells (EGCs), but the developmental potency of these cells in the fetal gonad still remains elusive. The study was undertaken to perform a comprehensive analysis of pluripotent and germ cell marker expression in human fetal testis 7–15 weeks post-fertilization (pF) and compare this expression to the ability of these cells to derive EGCs.

Methods and Results: The majority of germ cells expressed the stem cell markers stage-specific embryonic antigen (SSEA) 1, SSEA4, EMA-1, and alkaline phosphatase but only a small percentage of those (<1%) expressed OCT4, CKIT, and NANOG. Specifically, the number of OCT4(+)/CKIT(+)/NANOG(+) cells significantly increased in the developing cords during weeks 7–9, followed by a gradual decline into week 15 pF. By week 15 pF, the remaining OCT4(+)/CKIT(+)/NANOG(+) cells were found in the cords surrounding the periphery of the testis, and the predominant germ cells, CKIT(+) cells, no longer expressed OCT4 or NANOG. Based on morphology and early germ cell marker expression, including VASA, PUM2, and DAZL, the authors suggested these cells to be mitotically active gonocytes or pre-spermatogonia. Importantly, the number of OCT4(+) cells correlated with an increase in the number of EGC colonies derived in culture. Interestingly, two pluripotent markers, Tra-1-60 and Tra-1-81, although highly expressed in EGCs, were not expressed by PGCs in the gonad.

Conclusion(s): The results suggest that male PGCs maintain expression of pluripotent stem cell markers during and after sexual differentiation of the testis, albeit in very low numbers.

These two papers by Kerr and co-workers demonstrate that human fetal gonads of both sexes harbor pluripotent stem cell-like cells. This is an extension of previous findings in mice in which the functionality of these stem cells already have been proven (see paper by Guan et al. in this chapter, and references in a previous chapter in this series [16]). Although still at an experimental stage and also restricted by ethical concerns, the present findings give new hope that pluripotent stem cells to be used for tissue repair purposes in humans can be easily accessible from peripheral tissue sources. Still, the functionality of the detected cells and safety of the proposed procedures (risk for tumor formation!) have to be proven. Survivors of childhood cancers constitute one expanding group of patients that may benefit from these technologies in the future.

New hope II

Your testis can be your new heart

Generation of functional cardiomyocytes from adult mouse spermatogonial stem cells

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Circ Res 2007;100:1615–1625

Background: Stem cell-based therapy is a promising approach for the treatment of tissue and organ failure including that of the heart. Adult stem cells with the pluripotency of embryonic stem cells (ESCs) would be an ideal cell source. The authors have previously reported on the successful establishment of multipotent adult germline stem cells (maGSCs) from mouse testis. These cultured maGSCs showed phenotypic characteristics similar to ESCs and did spontaneously differentiate into cells from all three germ

layers. In the present study the authors differentiated maGSCs into cardiomyocytes and analyzed their functional properties.

Methods and Results: A hanging drop method was used to culture the cells. Differentiation efficiency of beating cardiomyocytes from maGSCs was similar to that from ESCs. The maGSC-derived cardiomyocytes expressed cardiac-specific L-type Ca^{2+} channels and responded to Ca^{2+} channel-modulating drugs. Cx43 was expressed at cell-to-cell contacts in cardiac clusters, and fluorescence recovery after photobleaching assay showed the presence of functional gap junctions among cardiomyocytes. Action potential analyses demonstrated the presence of pacemaker-, ventricle-, atrial-, and Purkinje-like cardiomyocytes. Stimulation with isoproterenol resulted in a significant increase in beating frequency, whereas the addition of cadmium chloride abolished spontaneous electrical activity. Confocal microscopy analysis of intracellular Ca^{2+} in maGSC-derived cardiomyocytes showed that calcium increased periodically throughout the cell in a homogenous fashion, pointing to a finely regulated Ca^{2+} release from intracellular Ca^{2+} stores. By using line-scan mode, the authors found rhythmic Ca^{2+} transients. When transplanted into normal hearts of mice, maGSCs were found to proliferate and differentiate. No tumor formation was found up to 1 month after cell transplantation.

Conclusion(s): MaGSCs may provide a new source of distinct types of cardiomyocytes for basic research and potential therapeutic application.

These authors have previously demonstrated that adult mouse testis is a source of multipotent stem cells that can differentiate into cell types of all three germ layers. In this work well characterized functional cardiomyocytes were produced from the same source. This is another proof-of-concept of the rapidly expanding stem cell field aiming at identifying sources of stem cells in adult tissues that can be used for tissue repair purposes. Again the findings have to be reproduced in humans and the safety of the procedures has to be ascertained before this technology can be transferred to human medicine. However, at the present stage there are no obvious obstacles that have to be overcome before this becomes a reality.

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