Gonadal development and reproductive hormones in infant boys
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Abstract
Background: The intrauterine milieu plays a crucial role for cardiovascular and metabolic diseases in adulthood, but little is known about its impact on gonadal development and reproduction. Impaired testis development in fetal life can lead to cryptorchidism, hypospadias, impaired semen quality, and testicular cancer, disorders that may present symptoms of a testicular dysgenesis syndrome. The prevalence of these disorders appears to increase in many areas, probably due to environmental factors acting in utero. Denmark has a significantly higher incidence of testicular cancer and lower sperm quality than Finland.

Methods: We conducted a population-based study of newborn boys from Denmark and Finland, in order to examine whether this geographic difference was reflected in the reproductive health of newborns.

Results: Danish boys had a lower testis volume at birth, a smaller testis growth up to 18 months of age and lower serum inhibin B and FSH levels at 3 months than Finnish. Danish boys also had a higher prevalence of both cryptorchidism and hypospadias than Finnish boys. In boys with cryptorchidism and hypospadias subtle changes in hormonal levels occurred, towards increased gonadotropins and lower inhibin B in cryptorchidism. Both types of congenital malformations were more frequent in children born small for gestational age, indicative of a relationship between growth and reproductive development.

Conclusions: These early postnatal findings suggest that the previously observed population differences in reproductive health between young Danish and Finnish men are of fetal origin. The differences may originate as a result of gene–environment interactions, where endocrine disrupters may also play a role.

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Introduction
The intrauterine milieu and the postnatal phase of human fetal development play a crucial role for subsequent morbidity in adult life, i.e. the risk of cardiovascular diseases, diabetes, dyslipidemia, and obesity (1, 2). Little is currently known about the impact of fetal life on gonadal function and reproduction in humans (3).

In 2001, a hypothesis of a testicular dysgenesis syndrome (TDS) was suggested, linking adult male reproductive disorders, i.e. impaired semen quality and testicular cancer, with congenital malformations, such as hypospadias and cryptorchidism (4), thereby emphasizing the role of fetal life for reproductive function throughout life. While a small percentage of these reproductive diseases has a detectable genetic or endocrine origin, in the majority of patients the etiology remains unknown. It has been speculated that life style and environmental factors during intrauterine life may play a role (5–7). Prenatal exposure to environmental chemicals may also play a role, i.e. a picture similar to the human TDS could be induced in rats by in utero exposure of male offspring to dibutyl phthalate (8), a chemical ubiquitously used as an additive in household and industrial products.

The following review summarizes present evidence that the fetal and postnatal periods are important for reproductive development by using Denmark and Finland as a model of two countries with a large difference in male reproductive health in adult men; Denmark having a considerably higher incidence of testicular cancer (9) and lower semen quality (10) than Finland.

Postnatal reproductive hormones and genital growth in healthy boys
Shortly after birth, a brief activation of the hypothalamus–pituitary–gonadal axis with an increase of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) leads to a peak in reproductive hormones,
such as testosterone, inhibin B, and anti-Müllerian hormone (AMH) (11–17). It has been known for many decades, that this feature can be utilized clinically, i.e. in diagnosing hypogonadotropic hypogonadism (18, 19) and studying primary testicular dysfunction, i.e. in Klinefelter syndrome (20, 21). Measurement of inhibin B and AMH during this period can also be used as a marker of the presence of testicular tissue in conditions like bilateral cryptorchidism with non-palpable testes and disorders of gender differentiation (22).

The biological significance of the postnatal hormonal surge in healthy boys is not yet fully understood. As serum levels of sex hormone-binding globulin increase parallel to the gonadotropins and testosterone, it was assumed that very little free testosterone was available and thus biologically active. As a consequence, there is some dispute as to whether the free fraction of testosterone in humans increases during this postnatal peak (23) or not (24). It has, however, been shown in three patients with gonadotropin insufficiency that the postnatal development of scrotum and penis was compromised if the testosterone surge did not occur, and testes ascended (25). These symptoms could be reversed with androgen or gonadotropin treatment (18).

During the postnatal phase, testis development continues (26). Leydig cell proliferation and germ cell differentiation are dependent on an intact gonadotropin and androgen action (27). In primates, manipulation of the postnatal testosterone levels has shown distinct effects on phallic growth, sexual behavior, and reproduction (28–30). The postnatal rise in inhibin B is associated with an increase in the number of Sertoli cells (31, 32), which in turn may reflect future potential for sperm production (33, 34). Also, the number of Leydig and germ cells increases transiently during the first 3 months of life, and the development of the testicular cell lines, Sertoli and Leydig cells, are closely interlinked (35, 36).

Whereas the efficacy of exogenous testosterone in the treatment of micropenis has been established for some time (37), it was only recently shown that endogenous testosterone secretion was also important for penile growth. In 1962 healthy Danish and Finnish boys born at term with normal birth weight, penile length increased approximately 1 cm from birth to 3 years of age. Both penile length at 3 months and penile growth rate between birth and 3 months of age were significantly correlated with testosterone and free testosterone, in addition to other factors, such as body height and weight. Body mass index was negatively correlated to penile length, which could indicate technical difficulties with measurement accuracy or an altered estrogen–androgen balance through increased aromatase activity in fat tissue (38).

Penile length measurements did not differ significantly between healthy Danish and Finnish boys and corresponded well to previous standards for penile length in Caucasian populations (39, 40). This binational Nordic study, however, revealed a significant population difference in testis size and the serum level of Sertoli cell marker inhibin B at 3 months (32, 41). Testis volume was measured by ultrasound, which provided a better sensitivity than an orchidometer. Testicular volume in Danish boys was approximately 3% lower than in Finnish boys at birth, a difference that increased to 36% at 3 months and 28% at 18 months. This was still true when testis volume measurements were adjusted for body surface area to correct for differences in body size between populations. At the same time, both FSH and inhibin B levels were 9 and 16% lower respectively in Danish than in Finnish boys. There was a positive correlation between the serum concentration of inhibin B and testicular volume, as seen previously in adults and pubertal boys (42), and the ratio between inhibin B and FSH did not differ between the countries. The hormonal findings and differences in testis volume taken together were indicative of a higher set point of the pituitary–gonadal axis in Finnish boys due to a larger pool of seminiferous tubules. During the postnatal period, Sertoli cells still replicate in humans (26, 43, 44). Postnatal testicular growth consists of an increase in the length of seminiferous tubules, and total number of germ and Sertoli cells, and a decreased apoptosis of Sertoli cells (43–47). Postnatal reduction in Sertoli cell number in rats leads to a decreased Sertoli cell pool in adult animals and reduced sperm production (48). Disturbance of the postnatal gonadotropin surge compromised replication of Sertoli cells in the Marmoset monkey (49). Thus, the findings suggested that the Danish boys were born with a smaller Sertoli cell pool, which in addition did not grow to the same extent as in the Finnish boys.

Danish adult men have a lower testis volume and lower semen quality than Finnish men, and there is a correlation between testis size and semen quality (10). Danish men also have a higher risk of testicular cancer than Finnish men (9, 50). Thus, the observations in newborn boys are in line with findings in adult men and indicate a fetal origin of the geographic difference. Although the long-term biological consequence of the smaller testis size in Danish boys is yet unknown, it may be a subtle sign of a perinatal testicular dysgenesis. This, in turn, may lead to a higher risk of impaired semen quality and development of testicular cancer in adulthood (51).

Prevalence of cryptorchidism and hypospadias

Highly variable prevalence rates of cryptorchidism (2–7%) have been reported in western countries on the basis of registry studies (52, 53). Such studies are, however, seriously hampered by a lack of standardization in diagnostic procedures and stringency in reporting to registries (54). Cryptorchidism is often regarded as a minor malformation, and therefore not systematically reported. In consequence, prevalence
data from cohort studies tend to be higher than from registries (55–58). Due to spontaneous testicular descent and changes in treatment standards, the prevalence of orchidopexy cannot be applied as a substitute for population-based data (59–61) to study geographic or temporal trends.

There appear to be regional differences in the prevalence of cryptorchidism and an increase over time (52). Our binational study revealed a large difference in the prevalence of cryptorchidism between Denmark and Finland (62). Both at birth and at 3 months, Danish boys had a significantly higher rate of cryptorchidism than Finnish boys. The prevalence of congenital cryptorchidism in Denmark was higher, and in Finland lower, than reported from population studies of other countries (55, 58, 63, 64). In the Danish population, a temporal increase from 1.8 in the 1960s to 8.5% in the present cohort was observed for boys born with normal birth weight (65). This corresponded with some previous studies from other countries (52, 59, 66), which also showed an increase of cryptorchidism over time. As cryptorchidism is a main risk factor for testicular cancer and reduced sperm quality (4), the difference in prevalence of congenital cryptorchidism between Denmark and Finland corroborated further the hypothesis of a common prenatal origin of reproductive disorders.

Hypospadias are another relatively common malformation with a reported prevalence of 0.7–1.8 out of 1000 newborn boys (67, 68). Some reports suggest that there is a rise in the prevalence of hypospadias in several, but not all, regions (52, 54, 69–73). However, most studies were based on registries, which have the same limitations as for cryptorchidism data (54, 74). In addition, the mildest and most frequent form of hypospadias, the glanular form, is not registered at all in many registries. Glanular hypospadias may be associated with a normal foreskin, and thus first become detectable after the dissolution of the physiological phimosis (75) and often remain undiagnosed throughout life.

Denmark had a significantly higher prevalence of hypospadias at birth than Finland (74, 75) and also a higher number of severe cases. The systematic follow-up of the Danish boys allowed the detection of a substantial number of additional cases of mild hypospadias after the dissolution of the physiological phimosis. This resulted in a point prevalence of 4.6% at 3 years of age. This number could potentially increase further with age, as the prepuce could only be retracted in two-thirds of the boys at 3 years. The Danish prevalence, even at birth, was considerably higher than previously reported for Denmark or other countries (67–69, 76), but due to the above-mentioned imprecision of registry data and differences in cohort study designs, no temporal trends could be determined with certainty. Again, the geographic difference in the prevalence of hypospadias between the two Nordic countries was in favor of Finland, and thus in line with data on congenital cryptorchidism, semen quality, and testicular cancer incidence.

Low birth weight, either defined as <2500 g or as weight for gestational age <−2 S.D., is a known strong risk factor for both cryptorchidism and hypospadias (3, 77). In the joint Danish–Finnish cohort study, low birth weight increased the risk of cryptorchidism two- to threefold. Prematurity was an additional risk factor for cryptorchidism, although boys were examined corrected for the expected date of birth. However, the country difference in the prevalence of cryptorchidism was not explained by birth weight or frequency of prematurity, as the largest geographical difference was observed for children with normal birth weight and born at term.

In boys with hypospadias, birth weight for gestational age and placenta weight were significantly lower than in control boys, with an association with the severity of hypospadias. In coronal and penile hypospadias also, birth length and head circumference were significantly smaller. Such associations with size at birth have also been reported from previous register and population studies (77–82). The longitudinal follow-up revealed that this difference in body size tended to persist throughout infancy, as body weight and height were still significantly smaller than in controls at 3 years of age. It has previously been hypothesized that the gender dimorphism of birth weight and length was caused by fetal androgens (83, 84). In our study, boys with severe forms of hypospadias had a birth weight and length similar to or even lower than girls from the same cohort (85), which is similar to another previous report (86). In healthy boys, weight for gestational age was positively correlated with testicular volume (41). This association was most pronounced at birth, but persisted through infancy. There therefore appears to be an interaction between fetal growth and genital development, the direction of which is yet unclear.

### Postnatal reproductive hormones in boys with cryptorchidism and hypospadias

A normal function of the hypothalamic–pituitary–gonadal axis is necessary for normal testicular development and descent (87, 88), including gonadotropins, testosterone, and insulin-like hormone 3 (89). In return, persistent malposition of the testes themselves leads to increased loss of germ cells (90). Congenital cryptorchidism increases the risk for fertility problems and testicular cancer in adulthood (91–94). While Leydig cell function and loss of germ cells appear to benefit to some degree from early treatment (95, 96), the risk of testicular cancer appears to remain unaltered after orchidopexy (91), which indicates a prenatal, irreversible onset of the testicular dysgenesis (4). There was some controversy in the literature as to when in life hormonal changes related to maldescended testes were
detectable, i.e. the increase in gonadotropin drive due to reduced testosterone and inhibin B secretion, and whether they are the cause or consequence of the disorder (97–100).

In our cohort (101), the Finnish boys with cryptorchidism showed significantly higher FSH and lower inhibin B levels than healthy controls. Changes were most pronounced in severe cryptorchidism, which persisted from birth to 3 months of age. In this group also, a significant effect on Leydig cell function was seen, with increased LH and LH:testosterone ratio. However, boys with very mild forms of cryptorchidism, i.e. high scrotal testis, or transient forms, i.e. with spontaneous descent between birth and 3 months of age, also showed a subtle impairment of Sertoli cell function. In the Danish cohort, inhibin B concentrations did not differ between controls and cryptorchid boys of any severity, probably due to the lower set point of the pituitary–gonadal axis in the Danish boys as mentioned earlier. However, also in the Danish cohort, an increase in gonadotropins was found in persistent severe cryptorchidism. These findings were in line with previous studies (95, 102–106). As boys with congenital cryptorchidism already showed a subtle impairment of primary testicular function at 3 months of age, this was likely to be of fetal origin. An increased drive of gonadotropins was also found in mild and transient forms of cryptorchidism, i.e. a high scrotal position was also indicative of a subtle testicular dysgenesis, and spontaneous descent did not ameliorate testicular function. These novel findings will have to be followed up into adulthood to establish whether they are of biological significance.

Previous studies on hormonal parameters or fertility in patients with hypospadias are very limited (107–110). In our study (75), a significantly increased FSH and FSH:inhibin B ratio were found in boys with hypospadias. We have previously demonstrated that there is an interrelation between hormones related to Sertoli and Leydig cell functions during the postnatal surge, i.e. between inhibin B and LH or testosterone respectively. Although urethral fusion itself is thought to be predominantly dependent on testosterone (111), this finding was thus in line with the hypothesis of a TDS affecting all testicular cell types simultaneously.

| Table 1 Regional differences in male reproductive health between Denmark and Finland. |
|---------------------------------|----------------|----------------|
| Incidence of testicular cancer  | Higher | Lower |
| Prevalence of cryptorchidism and hypospadias | Higher | Lower |
| Semen quality                   | Lower | Higher |
| Infant testis volume            | Lower | Higher |
| Postnatal serum inhibin B       | Lower | Higher |

boys, which may be of consequence for adult life. This enables research into disruptive factors for testicular development that are active during pregnancy without having to wait for full maturation.

Significant population differences in the reproductive health of newborn boys were seen between Denmark and Finland. Danish boys did not only show a significantly higher rate of congenital cryptorchidism and hypospadias than Finnish, but there was also a significant difference in testis size and in the serum level of the Sertoli cell marker inhibin B in healthy boys. These findings were consistent with the well-characterized differences in adult male reproductive health between the two countries, with Denmark having a higher rate of impaired semen quality and testicular cancer than Finland (Table 1). The findings support the hypothesis of an intrauterine TDS that appears to be more frequent in Denmark than in Finland. In addition to possible genetic differences between these two populations, the lifestyle or environmental factors seem to play a role.

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Summary and conclusions

Fetal life and the immediate postnatal period are important for the development of the testes. Malformations, such as cryptorchidism and hypospadias are well-known indicators of disturbed testicular development and are closely related to fetal growth. However, neonatal testicular size and testes growth, as well as the level of reproductive hormones during the postnatal hormonal surge, can also reveal disturbances of perinatal testicular development in otherwise healthy

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