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OPINION

Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects

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Numerous reports have recently focused on various aspects of adverse trends in male reproductive health, such as the rising incidence of testicular cancer; low and probably declining semen quality; high and possibly increasing frequencies of undescended testis and hypospadias; and an apparently growing demand for assisted reproduction. Due to specialization in medicine and different ages at presentation of symptoms, reproductive problems used to be analysed separately by various professional groups, e.g. paediatric endocrinologists, urologists, andrologists and oncologists. This article summarizes existing evidence supporting a new concept that poor semen quality, testis cancer, undescended testis and hypospadias are symptoms of one underlying entity, the testicular dysgenesis syndrome (TDS), which may be increasingly common due to adverse environmental influences. Experimental and epidemiological studies suggest that TDS is a result of disruption of embryonal programming and gonadal development during fetal life. Therefore, we recommend that future epidemiological studies on trends in male reproductive health should not focus on one symptom only, but be more comprehensive and take all aspects of TDS into account. Otherwise, important biological information may be lost.

Keywords: environmental disrupters/infertility/male reproduction/testicular cancer/ testicular development

Introduction

A considerable number of recent medical reports have focused on various adverse trends in male reproductive health which have been observed in many countries during the last decades of the 20th century (Toppari *et al*., 1996). These health problems include the increasing incidence of testicular cancer (Adami *et al*., 1994; Møller 1998); low and probably declining semen quality in several regions of the world (Carlsen *et al*., 1992; Auger *et al*., 1995; Irvine *et al*., 1996; Fisch *et al*., 1996; Swan *et al*., 1997; Andersen *et al*., 2000); high and possibly increasing frequencies of undescended testis and hypospadias (Chilvers *et al*., 1984; Matlai and Beral, 1985; Campbell *et al*., 1987; Paulozzi *et al*., 1997); and an apparently growing demand for assisted reproduction. Professional specialization has led to doctors and scientists concentrating on issues relevant to their own field, e.g. andrologists have been interested in sperm counts, paediatric endocrinologists have focused on hypospadias and undescended testis, and oncologists on testicular cancer. Similarly, epidemiologists traditionally analyse the incidence and risk factors separately for each disorder.

This paper applies a comprehensive approach to reproductive disorders by reviewing the evidence for the hypothesis that poor semen quality, testis cancer, undescended testis and hypospadias are symptoms of one underlying entity, the testicular dysgenesis syndrome (TDS). This syndrome may be more common than anticipated if each symptom is analysed separately. In other words, the published adverse trends in these symptoms may, in reality, reflect an increasing number of males suffering from various degrees of TDS. The rapid pace of the increase of reproductive disorders suggests that environmental or life-style factors, rather than an accumulation of genomic structural defects, are the most likely causes. This does not exclude that certain genetic aberrations or polymorphisms may predispose to augmented effects by environmental factors. Therefore, the article will also discuss the potential role of endocrine disrupters (environmental oestrogenic and anti-androgenic compounds) in the aetiology of TDS and review relevant data from animal studies as well as from investigations of humans. A crucial question from a clinical perspective is the following: what effects may be expected in humans, if endocrine disrupters, ubiquitous in small amounts in food and water, have an impact on the male reproductive system? In order to answer this question we need to consider some animal evidence first.

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Evidence from animal studies and wildlife

There is a wealth of data showing that male animals exposed in utero or perinatally to exogenous oestrogens (diethylstilboestrol, ethinyl oestradiol, bisphenol A) and anti-androgens [flutamide, vinclozolin, 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)] develop hypospadias, undescended testis, low sperm counts or, in the worst case, intersex conditions, teratomas and Leydig cell tumours (Viguier-Martinez *et al*., 1983; Newbold and McLachlan, 1985; Yasuda *et al*., 1985; Luthra and Hutson, 1989; Walker *et al*., 1990; Kelce *et al*., 1997). A recent report provided experimental evidence that ubiquitous phthalates, can also hamper testicular descent in rats when administered prenatally (Shono *et al*., 2000).

While hypospadias has been long known as a symptom of decreased action of androgens during the development of the reproductive system, the molecular mechanisms behind experimental cryptorchidism, in particular concerning the regulation of the transabdominal phase of testicular descent, have only recently began to be elucidated. An insulin-like hormone (Insl3), expressed by Leydig cells in the developing testes (Adham *et al*., 1993), was shown to regulate growth and differentiation of the gubernaculum, and the targeted deletion of the *Insl3* gene led to bilateral cryptorchidism in mice (Nef and Parada, 1999; Zimmermann *et al*., 1999). Importantly, two recent independent studies subsequently demonstrated that the *Insl3* gene is most probably regulated by oestrogens, as the prenatal exposure leading to experimental cryptorchidism causes a specific down-regulation of this gene (Emmen *et al*., 2000; Nef *et al*., 2000).

Unfortunately, it is frequently overlooked that the above-mentioned side-effects of prenatal exposure to hormones or endocrine disrupters are not evenly distributed among animals in the exposed groups. The same animal may show more than one of the symptoms, e.g. hypospadias, undescended testis, and low sperm counts, whereas other animals may be completely normally developed and have normal fertility, even at the same level of exposure. This phenomenon has contributed considerably to the dispute over the real effects of exogenous hormones on the reproductive system. However, differences in genetic background of inbred laboratory animals affecting their susceptibility to exogenous hormones may provide one possible explanation (Spearow *et al*., 1999).

The co-existence of several reproductive problems in one animal should be seen in light of the knowledge about normal sex differentiation and subsequent male fetal development. If this sequence of events is disturbed at an early stage by exposure to endocrine disruptors which affect differentiation of Sertoli cells and Leydig cells, germ cell proliferation and testosterone production will be impaired (Figure 1). As these processes are necessary for testicular descent and normal development of the external genitalia, the end result will frequently be a genital abnormality and/or cryptorchidism in the newborn animal, followed by fertility problems later in life. Even though clinically detectable symptoms appear postnatally, the underlying cause is irreversible testicular dysgenesis during early fetal development. In contrast, adult males exposed to similar or even higher dose of the same agents may be asymptomatic or only demonstrate reversible symptoms.

In summary, all male reproductive problems in humans currently of concern in relation to environmental hazards can be experimentally produced in animals by pre- and perinatal exposure to endocrine disrupters—with the exception of germ cell cancer, for which, unfortunately, there is no suitable animal model as yet.

Substantial evidence of adverse developmental effects caused by endocrine disrupters comes from observations made in wildlife after accidental environmental disasters. A well-known case of demasculinization and reproductive failure of alligators in Lake Apopka (Florida, USA) was caused by a spill of DDT, a weakly oestrogenic compound, which is metabolized to a potent anti-androgen DDE (Guillette *et al*., 1994). In the same region, a dramatic decrease of reproductive fitness among panthers was observed. Initially, this reproductive failure was attributed to inbreeding. However, investigations of serum hormone levels demonstrated that many male panthers had been demasculinized and feminized as a result of prenatal or perinatal exposure to endocrine disrupters (Facemire *et al*., 1995). When a large number of intersex fish began to appear in English rivers attention was drawn to the widespread contamination of the aquatic environment by oestrogenic compounds (Matthiessen and Sumpter, 1998). The population of common seals in Western European coastal areas has also dramatically declined during the past few decades. Field studies provided evidence that impaired reproduction and immune function in seals were caused by the presence of polychlorinated biphenyls (PCB) in the food chain (Reijnders, 1986). Although most of the observed side-effects in wildlife concern heavily polluted areas, widespread occurrence of more subtle effects, e.g. imposex in marine snails, thinning of egg shells in various bird species and reproductive problems of polar bears, suggest that contamination with persistent endocrine disrupters may be a global problem relevant also to humans (Vos *et al*., 2000).

Human experience

Basic, clinical and epidemiological evidence suggests that a large fraction of human male reproductive disorders is of antenatal origin. This is not only true for congenital disorders, such as hypospadias and undescended testis, but also for testicular cancer (Dieckmann and Skakkebæk, 1999; Ottesen *et al*., 1999). In addition, there is evidence that the underlying cause of male infertility often is of fetal origin. The following section will focus on testicular cancer, cryptorchidism, hypospadias and abnormal spermatogenesis as disorders associated with each other and resulting from testicular dysgenesis (Figure 1).

Testicular cancer, cryptorchidism, hypospadias and infertility

Testicular cancer arises from carcinoma in-situ (CIS) cells, which are presumed to derive from primordial germ cells that escaped normal differentiation *in utero* (Skakkebæk *et al*., 1987; Rajpert-De Meyts *et al*., 1998). CIS cells closely resemble gonocytes, their morphology is very similar, and

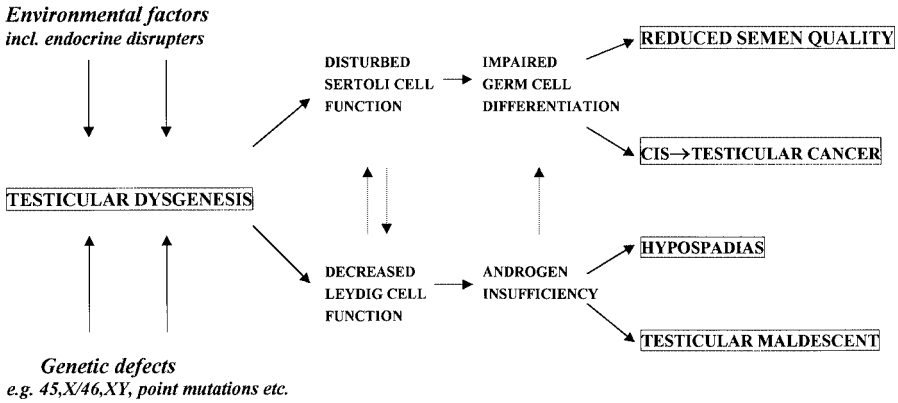

Figure 1. Schematic representation of pathogenetic links between the components and clinical manifestations of testicular dysgenesis syndrome.

both cell types express a number of common immunochemical markers (Figure 2).

It is well documented that rare genetic abnormalities which cause testicular dysgenesis (e.g. 45,X/46XY and androgen insensitivity) are associated with a high risk of testicular cancer, often in combination with undescended testis and hypospadias (Aarskog 1970; Scully, 1981; Savage and Lowe, 1990; Rajpert-De Meyts *et al*., 2000).

Similarly, several studies have documented that men with undescended testis and/or hypospadias are significantly over-represented among patients with testicular cancer (Giwercman *et al*., 1988). It is also well documented that the contralateral testis in men with unilateral testis cancer often is dysgenetic, with tubules containing Sertoli cells only, which are frequently poorly differentiated, spermatogenic arrest, microcalcifications, or even CIS (Berthelsen and Skakkebæk, 1983) (an example is shown in Figure 2). In 1956, Sohval first reported microscopic evidence of testicular dysgenesis in a significant proportion of specimens of testicular parenchyma adjacent to testicular tumours (Sohval, 1956). Since then, more studies have suggested that there is an element of testicular maldevelopment in a major fraction of men with testicular cancer. However, some studies attributed the altered phenotype of Sertoli cells not to the poor development of the testis but rather to a process of de-differentiation stimulated by the presence of proliferating CIS cells (Kliesch *et al*., 1998).

Not only the histology, but also the function of the testis with germ cell neoplasia is altered. Sperm counts in men with testis cancer are often extremely low; much lower than one would expect in a man with one functioning testis only (Petersen *et al*., 1998). Furthermore, it was shown that men with testis cancer had significantly fewer children than controls prior to development of their tumour (Møller and Skakkebæk, 1999) (Figure 3). That study also presented for the first time preliminary evidence that men who later develop testicular cancer have a lower proportion of male children (offspring sex ratio) than other men. The association between testicular cancer and decreased offspring sex ratio was recently corroborated by a study performed on 3530 Danish men with testicular cancer (Jacobsen *et al*., 2000a). Prior to the cancer diagnosis these men fathered a significantly lower proportion of boys (48.9%) compared with the general Danish population (51.3%), and their overall standardized fertility rate ratio was also significantly reduced (0.93). The number of children is, however, a rather poor estimate of fertility, thus the recent study of the same group that documented abnormal semen characteristics in men who later developed testicular cancer (Jacobsen *et al*., 2000b) provided robust evidence which strongly supports our hypothesis that impaired spermatogenesis and testicular cancer are aetiologically linked.

The assumption that common fetal factors are responsible for trends in testicular cancer and semen quality is additionally corroborated by epidemiological evidence. This includes (i) Danish men born during the Second World War had a lower frequency of testicular cancer in adulthood than expected from the overall incidence of the disease (Møller, 1993); and (ii) a strong association between low birth weight and testicular cancer (Depue *et al*., 1986; Møller and Skakkebæk, 1997). Similarly, recent studies have indicated that low birth weight was associated with hypospadias and low sperm counts (Møller and Skakkebæk, 1997; Francois *et al*., 1997). Finally, a role for perinatal factors in reported trends in testicular cancer and semen quality has been suggested by studies from France, Scotland and Denmark (Auger *et al*., 1995; Carlsen *et al*., 1992, 2000; Irvine *et al*., 1996), which provided evidence for a significant correlation between year of birth and semen quality: the younger birth cohorts had the poorest semen quality. In line with these observations, a recent study from Denmark of 18–20 year old men born around 1980 showed the lowest sperm counts ever reported from investigations of normal Danish men (Andersen *et al*., 2000).

There seems to be little doubt that cryptorchidism also forms a part of the TDS. A number of studies showed that undescended testis is associated with some degree of maldevelopment of the seminiferous tubules, including Sertoli cell-only tubules and spermatogenic arrest (Sohval, 1954; Huff *et al*., 1993). As a result, men with a history of undescended testis are over-represented at infertility clinics. Undescended testis may also be associated with microcalcifications and clusters of undifferentiated tubules (Regadera *et al*., 2001). The association of cryptorchidism with testicular cancer is well documented (Campbell, 1942; Giwercman *et al*., 1989). As undescended testis is usually present at birth, the condition is, per definition, of fetal origin, which again is in line with

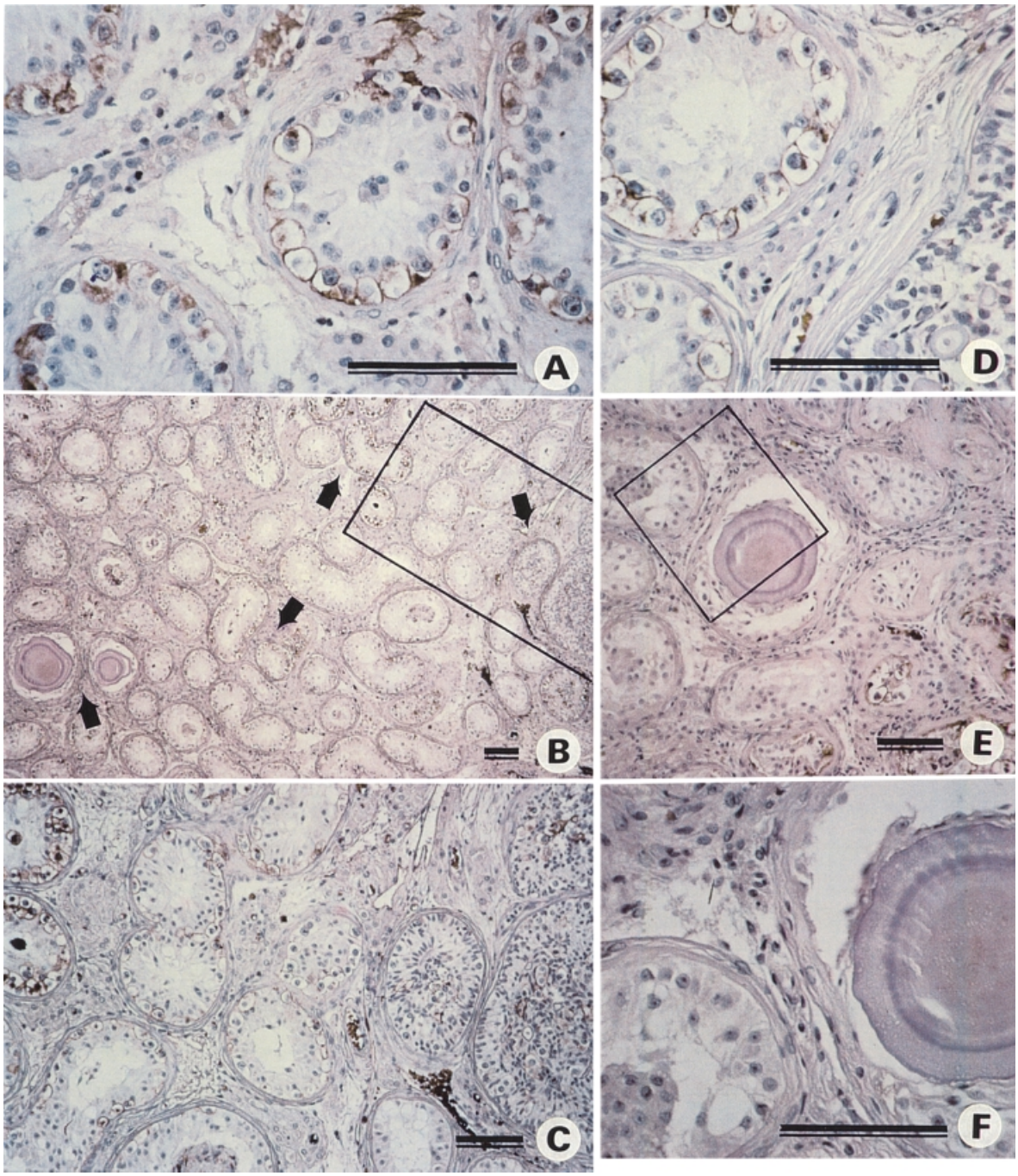

Figure 2. Examples of testicular dysgenesis in two contralateral biopsies of patients with unilateral testicular tumours. Both biopses contain carcinoma in-situ (CIS) cells, which are visualized by immunohistochemical staining for placental-like alkaline phosphatase (dark brown colour). Morphology of CIS cells is shown in detail in (**A**) and (**D**); the latter showing two CIS tubules side by side with a tubule with undifferentiated Sertoli cells and microcalcifications. (**B**) General overview of a biopsy with three dysgenetic features (indicated by arrows): CIS tubules, microliths (hyaline bodies) and undifferentiated Sertoli cells. The marked field contains CIS tubules and dysgenetic tubules resembling gonadoblastoma nests and is shown in higher magnification in (**C**). (**E**) Another biopsy with CIS, a large hyaline body and Sertoli cell-only tubules. The marked field is shown in detail in (**F**). Note poorly differentiated Sertoli cells in a tubule adjacent to the microlith. Scale bar = 100 µm.

reports showing a strong association between low birth weight and the testicular maldescent (Morley and Lucas, 1987). The molecular mechanisms behind the recent rise in the incidence of cryptorchidism are not known, but the influence of environmental factors is a plausible hypothesis. A recent study showed the high proportion of malformations of gubernaculum in association with cryptorchidism (Favorito *et al*., 2000). The fetal development of gubernaculum is regulated in rodents by the oestrogen-inhibited insulin-like Leydig factor (relaxin-like factor). Several recent studies searched for mutations of the human homologue (*INSL3)* in human subjects with cryptorchidism (Koskimies *et al*., 2000; Krausz *et al*., 2000; Lim *et al*., 2001). Although no mutations have been found, several polymorphisms of the gene were detected, and it is not

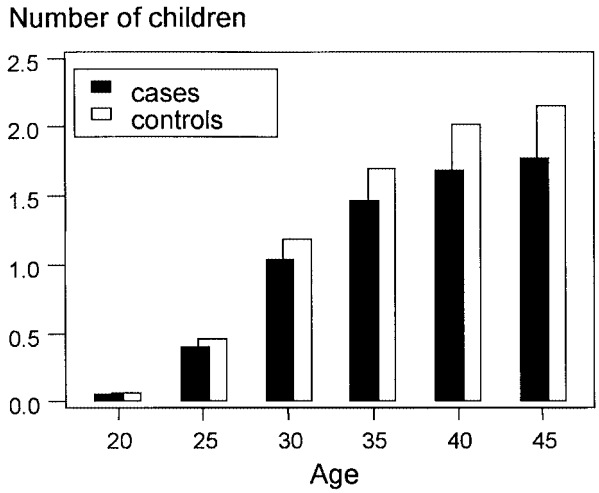

Figure 3. Epidemiological evidence of decreased fertility in men who later developed testicular tumours. The bars represent mean cumulative age-specific fertilities of men with testicular cancer and of control men. [Reprinted with permission from Møller and Skakkebæk, *Br. Med. J.* (1999) **318**, 559–562.]

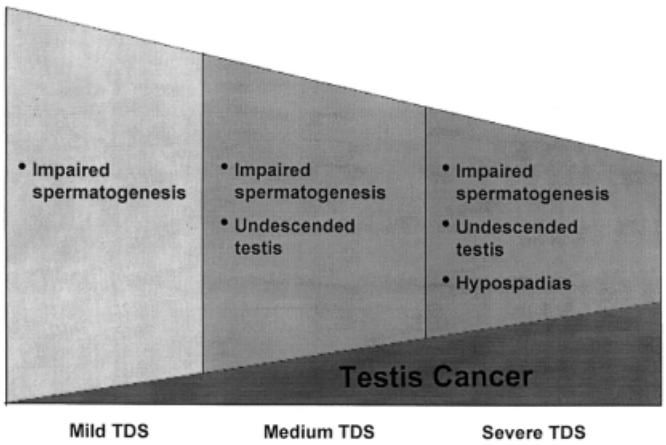

Figure 4. Illustration of the relationship between the relative frequency of various symptoms of the testicular dysgenesis syndrome (TDS). Note that while the overall incidence of TDS decreases with the degree of severity, the relative incidence of testicular cancer increases.

unreasonable to speculate that some of these polymorphisms may render the gene more prone to the transcriptional regulation by oestrogens in the developing reproductive system.

Various forms of testicular dysgenesis syndrome

The clinical expression of symptoms in a given syndrome may vary considerably, even within a syndrome caused by a single gene defect. The same may be true for TDS. As shown in Figure 4, we propose that the presence of symptoms may vary with the severity of the syndrome. The most severe forms of TDS, e.g. in individuals with 45X/46,XY karyotype (and a high percentage of aneuploid cells), often include three or four symptoms, including undescended testis, impairment of spermatogenesis, hypospadias and/or testicular neoplasia. These symptoms will develop successively. On the other hand, individuals with a less severe form may only have one or perhaps two symptoms. Therefore, it may have been overlooked in the past that testicular neoplasia in general may be part of TDS. However, it is unquestionable that there is a link between testicular cancer and undescended testis (Campbell, 1942; Morrison, 1976; Batata *et al*., 1982; Giwercman *et al*., 1989) as well as between maldescent of one testis and poor spermatogenesis of the contralateral, normally descended, testicle (Berthelsen and Skakkebæk, 1983; Petersen *et al*., 1998).

TDS may be more common than immediately apparent because mild forms may present only with slight impairment of spermatogenesis. Our studies on semen quality of young men from the general population suggest that the mild form of the syndrome (sperm concentration $<20\times10^6$/ml) may be as common as 20% (Andersen *et al*., 2000). At the other end of the scale, the most severe forms of TDS, including intersex conditions and testicular cancer, appear to be extremely uncommon. A careful estimate of the frequency of medium severity TDS in Denmark is ~5%, based on the knowledge of the current frequencies of testicular cancer and cryptorchidism.

It should be noted that in addition to dysgenetic forms of testicular insufficiency, several adult (acquired) forms of male reproductive failure exist, for example testicular atrophy caused by mumps orchitis, testicular torsion and other trauma, or effects of drugs and irradiation. However, these aspects will not be considered further in this paper.

TDS and environment

TDS may be caused by genetic or environmental factors, or a combination of both. The advent of modern molecular genetics has expanded the understanding of genetics of endocrine disorders, including dysgenesis of the testis, which may be caused by a range of genetic defects (Hughes 1998; Pinsky *et al*., 1999). However, in a significant number, perhaps the majority, of newborns with malformations of genitalia, no chromosomal or other genetic defect can be demonstrated with our current knowledge. These cases should be subject to exploration for possible environmental aetiology. The relevance of considering a possible role of environment factors for development of TDS is highlighted by epidemiological findings of geographic and temporal synchrony in the symptoms of TDS. In Finland, for example, the rates of testis cancer, undescended testis and hypospadias are much lower than among Danish men, who, in return, also have poorer semen quality. In addition, the rates of all these conditions are rising synchronously in both countries at a speed that strongly suggests that environmental factors are operating.

The possible role of endocrine disrupters in aetiology of TDS

The so-called oestrogen hypothesis (Sharpe and Skakkebæk, 1993) has been expanded to include also environmental antiandrogens as endocrine disrupters with potential adverse effect on male reproductive health (Toppari *et al*., 1996). In our opinion the endocrine disrupter hypothesis is relevant and plausible. However, relatively few chemicals have so far been closely examined for their possible hormone activity. Furthermore, their possible impact on humans has not been documented, except for the effects of DES on human fetuses. In addition, epidemiological studies reported an increased risk

of genital malformations in children of workers exposed occupationally to pesticides (Weidner *et al*., 1998), and the clustering of cryptorchidism in areas of intensive agriculture (Garcia-Rodriguez *et al*., 1996). However, research is needed to delineate the role of endocrine disrupters in humans and to indicate the possible actions for protection of future generations from reproductive problems. The seriousness of these problems is highlighted by recent health statistics from Denmark, where reproductive diseases, including testis cancer, are still increasing. Almost 1% of (mostly young) men are treated for testicular cancer, 5–6% of schoolboys have undescended testis, almost 1% have penile abnormalities at birth, and >40% of young adult men have subnormal sperm counts (Andersen *et al*., 2000). There have also been concerns about a low and decreasing birth rate in many industrialized countries, where up to 4–5% of children today are born after assisted reproduction. The latter phenomenon is usually ascribed to behavioural factors (e.g. increasing numbers of women in the workforce), but it remains to be investigated whether or not the decline in male reproductive health also contributes to the problem.

Conclusions and perspectives

Growing evidence from clinical observations of individual patients and from larger epidemiological studies indicates a synchronized increase in the incidence of male reproductive problems, such as testicular cancer, genital abnormalities, reduced semen quality and subfertility. Temporal and geographical associations, as well as frequent combination of more than one problem in one individual, strongly suggests the existence of a pathogenetic link. The association of male reproductive problems is probably not coincidental but reflects the existence of a common underlying cause resulting in a maldeveloped testis. We named the resulting phenotype the testicular dysgenesis syndrome (TDS). Experimental biological investigations and epidemiological studies leave little doubt that the TDS can be a result of disruption of embryonal programming and gonadal development during fetal life. As the rise in the incidence of the various symptoms of TDS occurred rapidly over few generations, the aetiological impact of adverse environmental factors such as hormone disrupters, probably acting upon a susceptible genetic background, must be considered.

Acknowledgements

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