Changes in male reproductive health and effects of endocrine disruptors in Scandinavian countries

Alterações na saúde reprodutiva masculina e efeitos dos desreguladores endócrinos nos países escandinavos

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Abstract *Male reproductive health has deteriorated in many ways during the last decades. The incidence of testicular cancer has rapidly increased in Europe and European-derived populations. Sperm concentrations have declined and sperm motility and morphology have worsened in many areas. Both adverse trends have been shown to be associated with year of birth. Older birth cohorts have better reproductive health than the younger generations. Incidences of cryptorchidism and hypospadias have also increased according to several studies. The reasons for secular trends are unknown, but the rapid pace of the change points to environmental causes. Endocrine disrupting chemicals have been hypothesized to influence male reproductive health.*
Key words *Testicular Neoplasms; Cryptorchidism; Hypospadias; Semen; Endocrine Disruptors*

Resumo *A saúde reprodutiva masculina tem-se deteriorado de diversas maneiras nas últimas décadas. A incidência do câncer de testículo aumentou rapidamente na Europa e nas populações descendentes de europeus. A concentração de espermatozóides tem decaído, e a motilidade e morfologia têm piorado em muitas regiões. Ambas tendências adversas foram correlacionadas ao ano de nascimento. As coortes mais velhas apresentam melhor saúde reprodutiva do que as gerações mais jovens. De acordo com vários estudos, as incidências de criptorquidia e de hipospadia vêm aumentando. Não se conhecem os fatores envolvidos nessas tendências, mas a velocidade da mudança sugere alterações ambientais. Foi levantada a hipótese da influência dos desreguladores endócrinos sobre a saúde reprodutiva masculina.*
Palavras-chave *Neoplasias Testiculares; Criptorquidismo; Hipospadia; Sêmen; Desreguladores Endócrinos*

Introduction

During the past decade, male reproductive health has been in the focus of discussion on endocrine disrupting chemicals. Since sexual development and reproductive functions are hormonally regulated, it was natural to suggest that developmental disorders in sexual differentiation and reproduction could be caused by disruption of the endocrine system. The meta-analysis of semen studies of healthy men from a 50-year period suggested a major decline in sperm counts, which prompted a vivid discussion and epidemiologic research on male reproductive health. A concurrent increase in the incidence of testicular cancer in several countries further increased concerns about environmental factors that could contribute to these trends. Some data also suggested that the incidence of congenital abnormalities of male genitals had increased. While the reasons for the trends have not been identified, search for agents that could disturb the endocrine system have continued, and at the same time genetic factors have been actively pursued. This review briefly describes secular trends in male reproductive health and the effects of endocrine disruptors.

Testicular cancer

The incidence of testicular cancer varies greatly in different populations. It is highest in Europeans and European-derived populations, with age-standardised rates of between 2 to 9 per 100,000 World Standard Population (Parkin et al., 1997). In non-European populations the incidence rates are usually below 2 per 100,000. Maori population of New Zealand is an exception with an incidence rate of 7.1 per 100,000.

Testicular cancer occurs typically in young adults (20 to 35 years), and it is the most frequent malignancy in this age group in several countries. The incidence of testicular cancer has increased rapidly in populations of European origin (Adami et al., 1994; Forman & Møller, 1994). Interestingly, this is associated with the time of birth (Bergström et al., 1996). The risk of testicular cancer has increased many-fold for successive birth cohorts during the last century. The second World War caused an interruption in this development in Scandinavian countries, but after the War the increasing trend has continued (Bergström et al., 1996). These epidemiological findings have been interpreted to support the hypotheses that testicular cancer may have its origin already in fetal development and environmental effects may be crucial in etiology.

Etiology of testicular cancer is unknown, but there is a lot of evidence that it already originates during early development (Skakkebæk et al., 1998). Carcinoma *in situ* (CIS) is a local malignant lesion that precedes testicular cancer (seminomas and non-seminomas) (Skakkebæk et al., 1981). CIS-cells resemble closely fetal germ cells, and the prevalence of CIS findings in testicular biopsies of different populations is in good correlation with their risk to develop testicular cancer (Dieckman & Loy, 1998; Giwercman et al., 1991). Early disruption of fetal germ cell differentiation may start the malignant development that becomes apparent only after hormonal stimulation in young adults.

Genetic factors in testicular tumorigenesis have been searched actively, but causative factors have not been identified. Frequently the dosage of the sex chromosomes is altered in the tumors (Peltomäki et al., 1991). Another typical cytogenetic finding in testicular germ cell cancers is excess material of the short arm of chromosome 12, an isochromosome of 12 (i12p) (Bosl et al., 1994). In intersex conditions and gonadal dysgenesis, the cancer risk is very high: 10 to 50 % of patients develop germ cell cancer (Rutgers & Scully, 1987). This further lends support to the hypothesis that testicular cancer already has its origin in developing gonad.

Epidemiological studies have revealed several prenatal risk factors for germ cell cancer:

- treated or persistent cryptorchidism (Møller & Skakkebæk, 1997; Prener et al., 1996).
- neonatal jaundice (Akre et al., 1996; Wanderås et al., 1998).
- low birth weight due to intrauterine growth retardation (Akre et al., 1996; Møller & Skakkebæk, 1997; Wanderås et al., 1998).
- low parity of mother (Akre et al., 1996; Møller & Skakkebæk, 1997; Sabroe & Olsen, 1998; Wanderås et al., 1998).
- high maternal age of the first-born boy (Akre et al., 1996, Møller & Skakkebæk, 1997; Swerdlow et al., 1987).

The risk factor profile, association of the incidence rate to birth cohort and biological data on testicular tumorigenesis are consistent with fetal origin of testicular tumors.

Semen quality

Semen quality has been studied since the 1930s. Nelson & Bunge (1974) reported that semen quality might have deteriorated during

the 1950s and 1960s. Similar findings followed, but only after a meta-analysis by Niels E. Skakkebæk's group (Carlsen et al., 1992) an extensive debate and research on this topic started. The meta-analysis revealed a decline in sperm concentrations of healthy men from 113 million/ml in 1938 to 66 million/ml in 1990 (Carlsen et al., 1992). The analysis was repeated by Olsen et al. (1995) and Swan et al. (1997), and the main conclusion remained the same: in the 1950s sperm concentrations were higher than in the 1970s. Several laboratories published reports on declining sperm counts, deteriorating sperm motility and morphology on the basis of their archival data (Adamopoulos et al., 1996; Auger et al., 1995; Irvine et al., 1996; Menchini-Fabris et al., 1996; Van Waeleghem et al., 1996; Younglai et al., 1998). However, regional variation was observed, and there was no observable deterioration of semen quality in some areas (Bujan et al., 1996; Fisch et al., 1996; Itoh et al., 2001; Paulsen et al., 1996; Rasmussen et al., 1997; Suominen & Vierula, 1993; Vierula et al., 1996). Swan and coworkers extended the meta-analysis to include studies until 1996, confirming the declining trend in the United States and Europe (Swan et al., 2000). Several multinational studies were started to solve the problem of inter-laboratory variation in methodology. Finnish-Danish comparison showed that Finnish men had much higher sperm concentrations than Danish men (Jensen et al., 2000). In a larger study including partners of pregnant women from Denmark, Finland, France, and Scotland, Finnish men were found to have higher sperm counts than the others (Jørgensen et al., 2001). Quality control program of semen laboratories was used to eliminate technical differences as a source of variation.

A common interesting feature in the declining sperm concentrations is that it is associated with the year of birth of men. The younger birth cohorts have lower sperm concentrations than the older generations (Auger et al., 1995; Irvine et al., 1996; Licht, 1998; Thierfelder et al., 1999). Unfortunately, most of the semen studies have ignored this variable in the analyses. Interestingly, the lowest sperm counts were found in 18 to 19 year-old healthy men in Denmark during 1996-97 (Andersen et al., 2000). The median sperm concentration was only 41 million/ml, and 18 percent of men had concentration below 20 million/ml that is considered a threshold for possible subfertility by World Health Organization (WHO). These kinds of studies are needed for follow-up of male reproductive health in the future.

Hypospadias

The International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS) collects data of hypospadias from countries that have acceptable malformation registries. The systematic collection and analysis of data is requested for comprehensive monitoring of congenital malformations. Data that relates only health statistics from administrative sources does not fullfill these requirements. However, even the acceptable registries differ from each other, and ICBDMS discourages any comparisons of data from different registers. Within the same registry, trends can be followed if the ascertainment, reporting and registering have not changed over time. However, variable reporting remains a major problem in malformation registers (Hemminki et al., 1993).

ICBDMS is the major data source for follow-up of secular trends in the birth rate of hypospadias. An increased rate has been reported in Hungary (Czeizel, 1985), England and Wales (Matlai & Beral, 1985), Italy (ICBMDS, 1998), the United States (Paulozzi et al., 1997), and in Scandinavian countries Denmark, Norway, and Sweden (ICBMDS, 1998; Källen & Winberg, 1982; Källen et al., 1986). The adverse trend appeared to end in the 1980s in most European countries, but in the United States the increasing trend continued until 1990s (Paulozzi, 1999; Paulozzi et al., 1997). The rate of hypospadias doubled in the Metropolitan Atlanta area between 1968 and 1993, and the ratio of severe to mild forms of hypospadias increased from three- to five-fold (Paulozzi et al., 1997). The observed increase in the rate of hypospadias in the U. S. National Birth Defects Monitoring Program (BDMP) was from 20.2 to 39.7 per 10,000 births during 1970-1993 (Paulozzi et al., 1997). It is unlikely that the increase would have been caused by improved reporting of mild cases, because the ratio of severe to mild forms of hypospadias in the Atlanta region increased.

Registry data are problematic for epidemiological studies when diagnostics and reporting vary. According to Källen et al. (1986) one third of the boys with hypospadias requiring surgery were primarily not registered in Sweden and almost two thirds remained un-reported in Denmark in the 1970s. Similarly, a recent study from Finland (Aho et al., 2000) showed that only one third of hypospadias cases were reported in the 1970s and 1980s. In this study, which was based on hospital discharge registers, no time trend in the rate of hypospadias was observed between 1970 and 1986 (Aho et al.,

2000). Under-reporting had already been noticed earlier (Hemminki et al., 1993) and the Finnish Malformation Registry changed its system starting an active surveillance of hospital registers in 1993. It is obvious that well-coordinated international birth cohort studies are necessary to obtain accurate and reliable data on mild malformations such as hypospadias. Comparisons between countries will be possible only when the diagnostic criteria, ascertainment of cases and reporting are similar.

We performed a cohort study in Finland during 1997 to 1999 in collaboration with a Danish study group (Virtanen et al., 2001). During the study period, 11,162 children were born in Turku University Central Hospital. The birth rate of hypospadias was 17 per 10,000 births which was close to the current figures from the Finnish Birth Malformation Register (14.2 per 10,000 in 1996; ICBDMS 1998), suggesting that the registry is now finding all the cases reliably. Furthermore, the recently published study based on Finnish hospital discharge registers also indicated a similar rate of hypospadias in the boys born between 1970 and 1986 as the rate in the cohort study (Aho et al., 2000). In Denmark, the prevalence rate seems to be significantly higher than in Finland (our unpublished results), although the current Finnish figures are close to the previous register figures from Denmark (ICBDMS, 1991). However, as stated earlier, the data from ICBDMS (1991) is based on registers with severe under-reporting, and it seems obvious that there is a genuine difference in the rate of hypospadias between Denmark and Finland.

Cryptorchidism

Secular trends in the incidence of cryptorchidism have been difficult to assess, because there are only few comparable studies that would allow any temporal analysis. Registry data are unreliable concerning cryptorchidism, and the reported incidence rates vary from 4 to 42 per 10,000 births (ICBDMS, 1991; Paulozzi, 1999; Toppari & Kaleva, 1999). Under-reporting is apparent, since eg. in the United States, ICBDMS gave a rate of 40 per 10,000 births (i.e. 0.8% of boys) during the 1980s, whereas according to a cohort study of 6,935 male infants in New York (Berkowitz et al., 1993) 19.8% of the boys with birth weight less than 2.5kg and 2.2% of those with birth weight more than 2.5kg were cryptorchid. In this study, clear diagnostic definitions were given and examination methods were described well. Two similar studies have been performed in England, and they also provide us with some longitudinal data (John Radcliffe Hospital Cryptorchidism Study Group, 1992; Scorer, 1964). Scorer's study was performed in London in the late 1950s, whereas the John Radcliffe Hospital study took place in Oxford in the late 1980s. More than 3,000 male infants were included in the London study and more than 7,000 in the Oxford study. The incidence of cryptorchidism in boys weighing more than 2.5kg was 2.7% at birth and 0.9% at three months in the 1950s, whereas in the 1980s, the corresponding figures were 4.1 and 1.6%, suggesting a clear increase. Studies based on hospital discharge registers from England, Wales, and Scotland had earlier suggested an increase in the incidence rate in Great Britain (Campbell et al., 1987; Chilvers et al., 1984).

In Denmark the incidence of cryptorchidism in the late 1950s was between 1 and 1.8% at birth in boys who weighed more than 2.5kg (Buemann et al., 1961). On the basis of hospital registers the corresponding figure was approximately 2% in the early 1980s (Thorup & Cortes, 1990), although some school surveys suggested much higher incidence, i.e. 7 % (Blom, 1984). In an on-going cohort study in Denmark and Finland, an increasing incidence rate of cryptorchidism is emerging in Denmark, whereas in Finland, the incidence is comparable to that in the 1950s in England (unpublished results, Toppari et al., 2001).

Effects of endocrine disruptors

The reasons for declined semen quality, increased incidence of genital abnormalities and testicular cancer are unknown. Endocrine disruptors have been hypothesized to play a role, because sex steroids are important in the physiology of spermatogenesis, testicular descent and urethral development. The best example of interference of steroid action by endocrine disruptor is diethylstilbestrol (DES) that has been studied extensively both in humans and experimental animals. In the 1950's double-blind, placebo-controlled trials have already demonstrated that the treatment was not efficacious for prevention of miscarriage or premature birth (Dieckmann et al., 1953), but despite that, it was used for millions of women until early 1970s. Boys exposed to DES in utero had an increased number of several structural and functional genital abnormalities, such as epididymal cysts (20.8% of 307 DES-exposed boys vs. 4.9% of 307 placebo-exposed boys), meatal stenosis (12.9% vs. 1.8%), hypospadias (4.4%

vs. 0%), testicular abnormalities, including cryptorchidism, hypoplastic testis and capsular induration (11.4% vs. 2.9%) (Gill et al., 1979; Stillman, 1982). Early exposure to DES was associated with higher frequency of abnormalities than late exposure (Wilcox et al., 1995). Semen quality was found to be worse in men exposed to DES than in placebo-exposed men (Gill et al., 1978; Schumacher et al., 1981; Stenchever et al., 1981). However, the sperm concentrations were compatible with normal fertility, and no difference in fertility has been shown (Wilcox et al., 1995). In contrast, the incidence of testicular cancer may have slightly increased after DES exposure, since the odds ratio was 2.6 with 95% confidence limits of 1.1-6.1 on the basis of six case-control studies (Toppari et al., 1996).

DES has been shown to induce similar effects in experimental animals as in human (Newbold & McLachlan, 1985). Male offspring of DES-exposed mice had increased incidence of epididymal cysts, cellular atypia in the prostate, cryptorchidism, testicular hypoplasia, poor semen quality and subfertility (Newbold & McLachlan, 1985). Sertoli cell hyperplasia, interstitial testicular tumors, squamous metaplasia of the seminal vesicles, and rete testis adenocarcinoma have also been found in these animals (Newbold et al., 1986).

Progestins have been reported to cause genital abnormalities, but a meta-analysis of studies on the fetal genital effects of first-trimester sex hormone exposure (progestin or estrogen), excluding diethylstilbestrol (DES), did not show an increased risk (Raman-Wilms et al., 1995).

Exposure to DES should be non-existent at the present time, and there are no other as potent endocrine disruptors of which we are aware. However, a growing number of chemicals has been shown to possess weak hormonal activities resembling estrogens, androgens, or their antagonists. Many of these have been shown to cause adverse effects in experimental animals when large doses are used (for review, see Toppari & Skakkebæk, 1998). An open question is whether mixtures of these could cause problems, although the level of individual compounds would be on a "safe" level. Chemicals that are in large-scale use have been suspected. These include eg. bisphenol-A, alkylphenol ethoxylates (nonylphenol, octylphenol), and some phthalates (butylbenzyl phthalate, di-n-butyl phthalate, and di(2-ethylhexyl)phthalate). There is not yet any human evidence of the association of exposure to these compounds to male reproductive health problems.

Antiandrogenic pesticides and other agrochemicals have been shown to cause feminization and demasculinization of experimental animals. Vinclozolin and DDE are examples of such compounds (Gray Jr. et al., 1994; Kelce et al., 1995). It is not known whether fetal exposure to these compounds could cause adverse effects in humans, but adult exposure to levels that were well below no observable adverse effect level was associated with increased FSH level (Zober et al., 1995), suggesting testicular damage. However, the authors of the study interpreted the increased gonadotropin level as a sign of previous gonadal damage in childhood (Zober et al., 1995). In contrast, another agrochemical, dibromochloro propane, caused undeniable testicular damage in exposed workers resulting in sterility of some men (Potashnik et al., 1978; Whorton et al., 1977). These effects were similar to those described in experimental animals almost two decades earlier (Torkelson et al., 1961). This has been a warning example of negligence of experimental data in human risk assessment.

Adverse trends in male reproductive health are of concern. Prospective international studies are needed to analyze regional variation and temporal trends. While etiology of the reproductive health problems is largely unknown, environmental causes, including endocrine disruptors, should be extensively studied. Genetic susceptibility to endocrine disruption may vary, but identification of any new risk factors would give us possibilities for prevention. Since the putative time gap between exposure in utero and outcome in adulthood is long, all preventive actions would show effects decades later. Similarly, adverse effects of today may be apparent only after two or three decades.

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