Chapter 12-EPIDEMIOLOGY OF MALE REPRODUCTIVE DISORDERS

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INTRODUCTION

Ailments of the reproductive organs are common through the whole life span of a man. Undescended testes (cryptorchidism) and hypospadias, abnormally located urethral orifices along the ventral side of the penis, represent the two most common congenital malformations of new-born boys, affecting 2-4% and 0.3-0.7%, respectively. In 20-40 years old men, testicular germ cell tumours are the most common neoplasm, whereas prostate cancer is the overall leading cancer in older men. In addition, in the western societies as many as 15% of all couples experience infertility problems and rough estimates indicate a sole or contributory male cause in at least 50% of the cases.

Reports of a possible deterioration of semen quality over the past 50 years, and an increasing incidence of testicular cancer noted in many countries (1) are a source of concern. A hypothesis that a common cause underlies these abnormalities has been put forward, but is still a subject of controversy (2). However, this debate has generated a great deal of research, which has created new knowledge regarding the impact of genetic, environmental, life-style related, geographic and social factors on male reproductive parameters. Recently, serious life-threatening conditions as atherosclerosis, metabolic syndrome and diabetes have also been linked to testosterone deficiency, which is more prevalent in sub fertile males (3). Thus, failure of the reproductive system comprises a significant proportion of men and is of concern, not only on an individual basis, but also for the society, where the financial burden of management is substantial.

In the current review we summarise current information on epidemiological trends in male reproductive function, with focus on fertility and semen quality, and discuss to what extent such trends may be related to exposure of endocrine disruptors.

SECULAR TRENDS IN MALE REPRODUCTIVE FUNCTION

Traditionally, semen quality is considered as the most significant marker of male fertility. However, several other characteristics can be used for monitoring the function of male reproductive organs including the incidences of congenital malformations of the genital organs or of cancer in the reproductive system, testicular size and fecundity (a couple's chance of conceiving). Interestingly, and to some degree concordant, secular trends have been reported for most of these characteristics.

Semen quality

During the past decades several reports have suggested a time related decline in semen quality (4-6) A meta-analysis of 63 studies, mainly from USA and from Western Europe, published in 1992 by Carlsen et al indicated almost 50% reduction – from 113 x 10^6/mL to 65 x 10^6/mL – in mean sperm concentration, during the period 1940 to 1990 (7). This publication evoked an intensive debate and the main points of criticism were the question of comparability of the methodology for sperm counting with and between laboratories over the period of five decades, and the criteria of subject selection (8, 9). In addition, the validity of the statistical model applied for estimation of the time-related changes was questioned. However, Swan et al (10) performed a careful re-analysis of the data and also included additional data, finally resulting in a total of 101 studies. The conclusion was that there was a significant time-related negative trend in sperm concentration both in North America (0.8% per year) and

Take home points

Through all phases of life, reproductive disorders belong to the most common pathological conditions

There are significant geographic and ethnic differences in male reproductive function;

There is a good evidence for time-related increase in the incidence of testicular cancer. For other disorders of male reproductive function evidence is less solid;

Interactions between genetics, lifestyle and environment play an important role in the aetiology and pathogenesis of male reproductive function

Our knowledge about the above mentioned factors is yet insufficient to allow efficient prevention and treatment.

in the western Europe (2.4% per year) during this period, even if possible confounding factors were taken into consideration.
Nevertheless, the picture is by no means clear. In a report from 1996, no secular trend was found in Finland (11), whereas in France, similarly selected materials of sperm donor candidates from Paris (12) and Toulouse (13) showed quite opposed pictures with a significant decline in Paris, from on average 89 million sperm/mL in 1973 to 60 million/mL in 1992, but no such change in Toulouse. In the sperm donors from Paris, a decline in the proportion of motile and morphologically normal sperm was also noted. Furthermore, it was demonstrated that the sperm parameters were more closely related to the year of birth than the year of sample collection, which indicated that events occurring before birth could have an impact on semen quality. Other studies indicating secular trends in sperm concentration came from Scotland (14) and Belgium (15) whereas two American (16, 17), one Danish (18) and an Australian study (19) did not support such a trend. All these publications were, however, based on retrospective materials.
Based on these results, one could suspect a decline in fertility over time as a consequence of falling sperm concentration since a close correlation between these parameters has been demonstrated (20). However, there is a scarcity of data to show whether male fertility actually has changed over recent decades. Most recent data from Denmark and Sweden indicate no decrease in sperm number during the past decennium (21, 22), whereas the opposite was observed in Finland (23). Thus, it is not possible finally to conclude whether semen quality is deteriorating or not and even if no negative trend currently is evident, it does not exclude a significant reduction in sperm numbers at the end of previous millennium.

Testicular cancer
In contrast to the on-going discussion regarding a possible secular trend in sperm counts, there is a general agreement that – at least among Caucasians – there has been a significant increase in the incidence of testicular germ cell cancer (TGCC), which to date is 2-3 times higher than 30-40 years ago (24). This is the most common form of testicular malignancy, mainly appearing among males aged 25 to 40 years. Since the risk of TGCC has been shown to be strongly correlated to the birth year (25), environmental or life-style related factors were thought to be of importance in the aetiology and pathogenesis of this malignancy possibly already affecting prenatally. This hypothesis has been strengthened by the finding of decreased risk of TGCC among boys born during the Second World War as compared to pre- and post-war birth cohorts (26).

Genital malformations
A time-related increase in the incidence of congenital malformations of the male genital organs – undescended testes and hypospadias has also been suggested (27). However, for both conditions comparisons of studies from different countries may be problematic due to variations in criteria, diagnosis and registration. In general, data on cryptorchidism is less reliable than on hypospadias. Cryptorchidism affects 2–9% of all new-born boys and 1–3% of boys at 3 months of age, decreasing further to 0.7-1% at the age of 1 (28). Hence, the age of the baby at the time of examination and the proportion of prematurely born children, who have a higher prevalence of cryptorchidism, may play a role in determining this defect. Cryptorchidism can also occur due to postnatal retraction of the testes. A study from 1960 considering this problem, showed a higher prevalence of cryptorchidism at 5, 8 and 11 years of age compared to the prevalence at birth, on the same children (29). In addition, the criteria for offering treatment may vary between countries and over time. In a report from the UK, in which attempts were made to apply identical diagnostic and selection criteria on a thirty years old material from the late 1950s as on cohorts 30 years later, in the 1980s, a significant overall increase in the incidence of cryptorchidism from 4.0 to 5.4% was observed over the time period (29). The magnitude of this increase depended partly on the age of the child at the time of investigation and whether or not premature babies were included. Nevertheless, when the analysis was restricted to babies with a birth weight above 2500 g, the increase at the age of 3 months was still significant, from 0.9% in the 1950's to 1.6% in the late 1980's. However, analyses of frequency of cryptorchidism among controls participating in 30 case-control studies revealed no evidence that cryptorchidism became more common during the years 1960-1990 (30).

With respect to hypospadias, by using data from the European Surveillance of Congenital Anomalies (EUROCAT, (http://www.eurocat-network.eu/) network of population-based registries on congenital anomalies in Europe, a trend in increasing incidence of hypospadias was noted over a 10-years

period (31). However, due to a change in definition midways, when glandular hypospadias was no longer excluded as a minor anomaly, this trend may also be subject to uncertainty and follow-up studies are therefore on-going. Apart from differences in the diagnostic criteria and the quality of registers, geographic trends in disorders of the male reproductive system may also be a phenomenon pointing to environmental or life-style differences acting locally, possibly in combination with a genetic predisposition of certain populations

GEOGRAPHIC TRENDS

Trends and geographical differences in congenital malformation rates are almost exclusively derived from hospital-based birth defect registers, which are sensitive to selection bias and incomplete reporting (32). However, according to WHO records, differences in **hypospadias** prevalence globally are striking (www.who.int). In general, this anomaly is much more common among Caucasians than in other ethnic groups. The International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR, http://www.icbdsr.org) also reports prevalence rates for various malformations for different populations around the world. In a recent study combining these databases, highest rates of **cryptorchidism** were found in Australia and New Zealand (55 and 59:10 000 births, respectively), whereas the prevalence was much lower in the USA and Italy (3 and 2:10 000, respectively) (33). Also **hypospadias** rates were highest in Australia (34:10 000 births), followed by Israel and New Zealand (29 and 24:10 000, respectively).

In countries where reliable cancer registries are available, an equal rise in **testicular germ cell tumours (**TGCC) risk has been observed all over the world, the only exception being amongst African Americans in whom the incidence of TGCC has not changed over time. Remarkable regional and ethnic differences have also been observed, with Northern (6.7%) and Western Europe (7.8%) and Australia (6.5%) having the highest age-standardised incidence rates, whereas Asia and Africa were possessing much lower rates (<1%) (24). Not only have Caucasian men significantly higher risk of this cancer than Black men living in the same area (32), but there are also significant differences in the incidence of TGCC between people of the same ethnicity. Among the Nordic countries, the incidence of the usually uncommon malignancy is now almost 10 times the global average in Norway and Denmark, according to the International Agency for Research on Cancer in Lyon (34). As compared to the neighbouring country Sweden, this risk is reduced by 50% and in Finland by 80%. Interestingly, immigrant studies and national cancer registries have documented immigrants retaining their original location's TGCC incidence despite moving to another country (35), suggesting a strong genetic factor in this particular disorder.

The difference between Denmark and Finland in regard to the risk of TGCC is also accompanied by higher **sperm concentrations** in Finnish men as compared to Danes (36). In a study of the partners of pregnant women, Finnish men were not only found to have higher sperm concentrations than those from Copenhagen, Denmark, but also in comparison to men recruited in Edinburgh and Paris (36). This difference was also found when sperm concentrations from Danish and Finnish military conscripts were compared (11). In a 15 year follow-up study, however, an increase in sperm concentration rather than a decrease was recently reported among the Danish conscripts (22), whereas the opposite was found among the Finnish conscripts at a follow-up (37).

Furthermore, 9% of Danish new-born boys were found to have **cryptorchidism** compared to 2.4% in Finish boys (38). This supports the suggestion that the Danish-Finnish difference is due to genetic or environmental factors. However, when the lack of a testis in the scrotum was used as definition for cryptorchidism, the incidence in Denmark was 2%, which also was at the same level as another study from Denmark on more than 1000 new-born boys found (2.4%) when the same definition was used (39). Moreover, the relatively high incidence of cryptorchidism among Lithuanian new-borns (40), despite high sperm numbers found in military conscripts from this country (41), challenge the hypothesis of common underlying causes behind poor semen quality, testicular cancer and some congenital male reproductive disorders (42).

In Southern Sweden, where the population is genetically very similar to that in Denmark, the total sperm counts were found to be 30% higher than among Danish conscripts (Figure 1), whereas the

frequency of self-reported history of cryptorchidism was significantly lower (43).

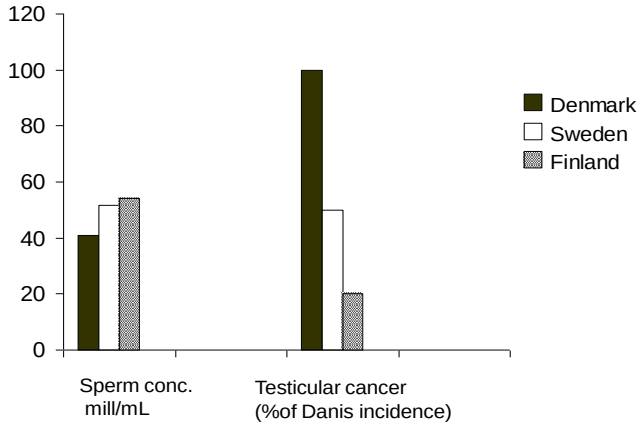

Fig 1. Comparison between relative levels of total sperm counts among military conscripts and the incidence of testicular cancer in Denmark, Finland and Sweden (22;35;39). The figures are given as percentages of the reference level (Finland for sperm concentrations and Denmark for testicular cancer).

In a follow-up 10 years later, sperm concentrations were unchanged (21). It therefore seems as life-style differences, possibly introduced already some decades ago, account for most of the discrepancies between these neighbouring countries (Figure 2).

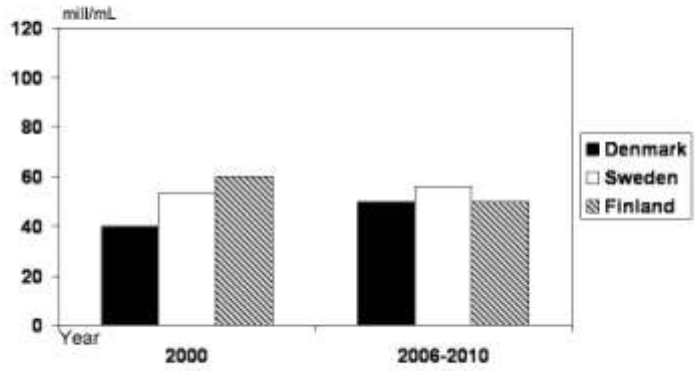

Fig 2. Time-related changes in median sperm concentration among military conscripts in Denmark, Finland and Sweden**.** The columns show data published for 2000 sperm collection and for sampling between 2006 and 2010.

Geographic variations in sperm concentration have not only been found between countries, but also when comparing different regions of France (12, 13) and the USA (16, 44) . In the latter study, based on samples from fertile males, the mean sperm concentration was significantly lower in Columbia, Missouri, than in New York; Minneapolis, Minnesota and Los Angeles, California, ranging from 59-103 million/mL. Even the total number of motile sperm was lower in Missouri than in the other centres, the calculations being made in multivariate models that controlled for abstinence time, semen analysis time, age, race, smoking, history of sexually transmitted diseases, and recent fever. The authors suggested that sperm concentration may be reduced in semi-rural and agricultural areas relative to more urban and less agriculturally exposed areas.

Low sperm concentration has been associated with decreased fecundity (45). It would therefore be expected that regional discrepancies in sperm concentrations should be reflected in corresponding differences in fertility, measured as waiting time to pregnancy (TTP). Joffe reported a significantly higher fertility rate in Finland as compared to Britain (46). However, in the above-mentioned European four-city study, decreased probability of conception was found among French couples as compared to those from Denmark, Finland and Scotland (47), whereas no difference in TTP was found for the three latter countries. Thus, the TTP data did not correspond to sperm concentrations making the picture somewhat blurred. Furthermore, men selected because of being the partners of pregnant women represented a highly selected group and may, therefore not be representative of whole populations as ideally required for studying geographical trends in fertility parameters. Such studies will not reveal changes in the proportion of males with semen quality so poor as to preclude conception. Newer markers of sperm quality e.g. sperm chromatin structure, may perhaps be more useful in prediction of fecundity, at least regarding the male part (48).

LIFESTYLE AND REPRODUCTIVE FUNCTION

Life-style related factors may have a significant impact on male reproductive parameters. It is well known that the length of the period of abstinence affects sperm concentration and total sperm counts (43) and that cigarette smoking (49), alcohol consumption (50, 51) and dietary habits (52) also play a role. Lifestyle related habits are known to be subject to geographic as well as time-related variation, and if associated with male reproductive function, may at least partly explain some of the observed epidemiological trends. Whereas moderate alcohol intake does not seem to affect the fertility potential of a male (53), recent data show that cigarette smoking implies 30% reduction in sperm number and 10% lower seminal volume indicating an anti-androgenic effect of smoking (54). Maternal smoking during pregnancy was also shown to hamper spermatogenesis in the sons (55), a finding which has been confirmed by several other studies (56-59).

Additionally, a recent report indicated 30% lower sperm numbers in sons of men smoking during pregnancy even after adjustment for the smoking habits of the mother (60). Since the effect was more pronounced than if the mother was smoking, this could indicate some pre-conception damage to spermatozoa mediated through genetic or epigenetic mechanisms. In the Nordic countries, the pattern of increased smoking among females mirrors the rise in the incidence of testicular cancer, but so far, no association between maternal smoking during pregnancy and the risk of TGCC among their sons has been disclosed.

With respect to genital malformations, in an epidemiological study to which 47,000 Scandinavian women were invited and their live born sons followed for cryptorchidism; intake of mild analgesics such as acetaminophen was associated with congenital cryptorchidism in boys (61, 62). Later studies have shown that this also applies to malformations in animals (63) and that these drugs inhibit testosterone synthesis in rodents (63, 64). In vitro studies on human testicular tissues showed significantly reduced steroidogenesis in the presence of analgesics (65). Taken together, these compounds act like endocrine disruptors, which may be of concern, since more than 50% of pregnant women in Europe and the USA frequently use weak analgesics.

Endocrine disrupting factors

Because of the rapid increase in TGCC as well as in congenital genital anomalies in boys, it seems reasonable to propose that environmental, rather than genetic factors could play a role in the increase of these disorders. Although many of these compounds have the capability to interfere with hormone synthesis or hormone receptors and animal models convincingly shown their adverse effects on the male reproductive system, it has been debated in decades as to whether they truly are causing genital malformations or TGCC in humans, with one exception - diethylstilbestrol (DES).

The use of the estrogenic compound DES from 1940s to 1971 to prevent abortions and pregnancy complications comprised millions of women in the USA and Europe and it was not banned until it was shown to associate with the uncommon vaginal cancer (vaginal clear cell adenocarcinoma) in *in utero* exposed girls (66). In exposed boys, increased risk of cryptorchidism, epididymal cysts and testicular infection was shown (67), whereas studies on increased risk of TGCC have been inconclusive. In a report from 1983, an increased risk was suggested (68), but this was not confirmed 20 years later in a study on women taking DES at an early stage of pregnancy, which may be the most critical developmental window for the foetus (67). In a multigenerational study on 529 families,

an increased risk of hypospadias in grandsons of women prescribed DES during pregnancy was observed, but no other abnormalities (69).

Regarding effects of exposure to compounds with estrogenic or anti-androgenic effects in the general population, data are scarce. In 2002-2006 an EU-financed project under the acronym INUENDO (www.inuendo.dk) was conducted with the objective to identify and characterize the impact of dietary pollutants on human fertility and to provide epidemiologic evidence on possible health impacts of environmental exposure to xenobiotic with hormone like actions. The study had the specific objective to study fertility in European populations with high or low exposure, such as the Greenland Inuit, with the highest body burdens of persistent organic pollutants (POP) in the world, Swedish fishermen from the polluted east coast as well as from the west coast, Ukrainians, who are mostly exposed by use of pesticides, and a Polish population, as a low exposure group. The POP exposure was negatively correlated to sperm motility and sperm DNA integrity (70). However, no association between the POP exposure level and fecundity or sperm concentrations was found, despite the fact that some of the subjects included in the study presented with extremely high POP levels in serum.
Sera from the same cohorts were recently measured concerning levels of perfluorinated compounds. Apart from a negative association between serum levels of perfluorooctane sulfonate and sperm morphology, no other indications of negative effects in relation to semen quality were observed (71). Exposure to phthalates, another type of compounds belonging to the family of endocrine disrupters, was also reported to be associated with deterioration of classical sperm parameters and sperm DNA integrity (72, 73), as well as ano-genital distance among new-born boys, which is considered as marker of prenatal androgen exposure and was reported to be negatively correlated to the levels of phthalates in the serum of the mothers (74). The same parameter, when evaluated in adult men, was found to be negatively associated with signs of testicular dysfunction, hypogonadism and non-obstructive azoospermia (75, 76).

GENE-ENVIRONMENT INTERACTION

The question of how individual differences arise is fundamental to, for example biology, psychology and personalised medicine. Two biological mechanisms that can underlie individual differences are gene–environment interactions and phenotypic plasticity, which refer to the genotypes' response to environmental variation, and the ability of a genotype to produce different phenotypes in response to changing environmental conditions, respectively. Classical parameters in this context are height and age at menarche. Secular increases in height and decreases in age of menarche over several generations are well-documented in many populations, analysed as parameters before and after the secular change (77, 78). The underlying causes are not known, but socioeconomic improvement, reduction in infections, reduced neonatal mortality, improved nutrition, genetic changes and natural selection have been suggested as contributing factors (79). In order to gain insight into the mechanisms, an investigation on an isolated Mexican community was undertaken and data collected 1968, 1978 and 2000 (80). The study showed that genotype-environment interaction was the predominant causes of the gain in height and age of menarche, rather than natural selection.
The literature on gene-environment interaction and male reproductive disorders is slender. A Dutch study on 712 hypospadias case-parent triads found gene-environment interaction for a variant in the 5α-reductase II gene (SRD5A2), oestrogen exposure and maternal hypertension or preeclampsia (81). However, the effects of environmental exposures could not be studied because of the study design, which relied on information from questionnaires, which may introduce recall problems.

In the INUENDO study, by utilizing the birth register of Greenland, 11 076 live male births during the period 1982-2002 were identified (82). Through the local register on congenital malformations, all reported cases of hypospadias were traced. The incidence of hypospadias in Greenland was compared to that in Scandinavia, based on information obtained from *International Clearinghouse for Birth Defects Monitoring Systems* (www.icbd.org). Only two cases of hypospadias were identified in Greenland among the boys born during the actual period, corresponding to an incidence of 0.02%. This was approximately 10 times lower than among Scandinavian boys, who have an incidence of 0.2%. Interestingly, 85% of the population in Greenland carried a specific androgen receptor variant that *in vitro* was shown to result in a more active androgen receptor than other lengths tested (83). Furthermore, despite a lack of association between POP exposure and sperm numbers, men with short androgen receptor CAG tract and high POP exposure had 40% lower sperm counts than men with low POP levels or other genotypes (84).

CONCLUSION

A growing body of toxicology data based on aquatic and wildlife species as well as on laboratory animal studies, suggests that exposure to endocrine disrupting agents are associated with disorders of the male reproductive system. Although humans seem to be less susceptible to many compounds, as compared to rodents, a still unresolved problem is the issue of mixed exposures. Each of them may be present in rather modest concentration, but the total effect could be additive or even multiplicative. In addition, the effect of the environmental toxicants may be modified by genetically determined susceptibility, which complicates the interpretation of studies focusing solely on the impact of environmental agents on male reproductive function. Finally, many men with fertility problems will ultimately take advantage of intracytoplasmic sperm injection (ICSI). This raises the possibility that underlying genetic disorders may be passed on to the offspring. Indeed there appears to be a slightly higher rate of congenital malformations in children conceived by in vitro fertilisation techniques, compared to naturally conceived offspring. However the data set remains limited and it is unclear as to whether ICSI presents any additional risk risk (85, 86) or whether the additional risk relates to the in vitro fertilisation procedures themselves as opposed to being intrinsic to the infertile couple. A recent study has indicated that the rate of hypospadias and undescended testicles seems directly related to the severity of the infertility in the father (87). Although concerning, these results were not significant even though a national cohort study was utilised, probably due to the sample size. It may therefore take decades before these trends are visible and collaborations regarding this topic are warranted.

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