



Adverse trends in male reproductive health and decreasing fertility rates

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Abstract

Fertility rates have been declining worldwide and most developed countries have fertility rates below the population replacement level. To a large extent, this change can be explained by the socioeconomic development. However, increasing fertility problems might play a role as well as there seems to have been a temporal decline in semen quality and an increase in male reproductive diseases like testicular cancer and genital malformations. These male reproductive problems are associated and in some cases proposed to be part of a testicular dysgenesis syndrome. This syndrome is hypothesized to originate in fetal life due to suboptimal androgen activity during a critical developmental period. Genetics may play a role in the development of the syndrome but the rapid increase in male reproductive disorders indicates that environmental factors, including endocrine disrupting compounds, are likely crucial. It is of great importance that the fertility trends are reversed as a fertility rate only slightly below the replacement level causes a significant decline in the population of women in the reproductive age within only a few generations.

Keywords: endocrine disrupter, fertility rates, semen, testicular cancer, testicular dysgenesis syndrome.

Introduction

Human fertility rates have decreased in most countries (Fig. 1). In some parts of the World, e.g. Africa and Western Asia, they are still above the population replacement level of 2.1 children per woman. However, in most parts of Asia and Europe they are below 2.1 (Frejka and Sobotka, 2008; Frejka *et al.*, 2010). South America has also experienced quite dramatic declines in fertility rates although most countries have a fertility rate around or above the replacement level. Fertility rates and the proportion of childless men and women in a country reflect both the ability to conceive children (fecundity) and the wish to do so. Socioeconomic factors, like access to contraception and more women getting an education, entering the workforce and having career aspirations, have had a large impact on a couples' age for starting a family and their desired number of children (ESHRE Capri Workshop Group, 2010). However, several factors indicate that biology also plays a role in fertility trends.

Interestingly, studies among all Danish women

have shown a birth cohort related decline in natural conceptions. The rate of natural conceptions, which is defined as the total births and abortions but excluding births obtained after assisted reproductive techniques, should to a larger degree than fertility rates reflect fecundity. However, the concept does not account for the potential influence of different use of contraception in various birth cohorts of women. Unfortunately, to our knowledge, sufficient data to elucidate this aspect does not exist. The observed decline, which was explained by a decrease in abortions and increased impact of assisted reproductive techniques, was hypothesized to be influenced by poor semen quality among the younger birth cohorts of Danish men (Jensen *et al.*, 2008; Lassen *et al.*, 2012). This interpretation was supported by a recent similar study among men in Denmark born from 1945 onwards which showed a birth cohort related decline in total male fertility and, noteworthy, also an increase in the proportion of childless men with consecutive birth cohorts (Fig. 2; Priskorn *et al.*, 2012). A higher level of childlessness among men than among women indicates that a higher number of men than women have children with more than one partner. Theoretically, a poor semen quality among a large proportion of Danish men may play a role for the trend although systematic long-term follow up studies on semen quality of childless men remain to be carried out.

Semen quality

Whether human semen quality has been declining during the last century has been debated since Carlsen *et al.* (1992) published a meta-analysis of 61 studies of men without a history of infertility. Included were studies from a variety of countries representing different areas of the world but with a large overrepresentation of studies from the United States. The analysis showed a significant decline in semen quality from a mean sperm concentration of 113 million/ml of men examined in 1940 to 66 million/ml of men examined in 1990 (Carlsen *et al.*, 1992). The paper was widely commented on, with some expressing concern partly about heterogeneity of the men in the different studies with regard to fertility status, age and socioeconomic status and partly due to differences in methods. Furthermore, it was discussed whether the observed trend should be ascribed to regional differences rather than an actual temporal change in semen quality (Jouannet *et al.*, 2001). However, a reanalysis of the data, with thorough adjustment for important confounders and stratification of the studies into three

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regions (the United States, Europe and Australia and other non-Western countries) confirmed an overall decline in semen quality of 1.5% per year in the United States between 1938 and 1988 and 3.1% per year in Europe between 1971 and 1990 (Swan *et al.*, 1997). These findings of overall trends did not rule out the possibility of geographical differences between the countries pooled into the same region. The debate prompted several prospective studies of semen quality within relatively homogeneous groups of men. Some studies reported a decline in semen quality as well, others found no trend and some even a temporal increase in semen quality (for review see Jouannet *et al.*, 2001). However, what these studies demonstrated was that there seemed to be a striking geographical diversity in semen quality that needed to be explored further. A study designed to investigate this among partners of pregnant women showed a geographical difference in Europe with Danish men having the poorest semen quality, followed by French and Scottish men and Finnish men showing the best semen quality (Jørgensen *et al.*, 2001). Another study, based on men from the general population, showed that men from Denmark and Norway had slightly lower sperm counts than men from Finland, the Baltic countries and Sweden (Jørgensen *et al.*, 2002; Punab *et al.*, 2002; Richthoff *et al.*, 2002; Tsarev *et al.*, 2005). Within the United States a large difference in sperm concentration of fertile men has been found between New York (102.9 million/ml) and Missouri (58.7 million/ml). Interestingly, the findings from this study indicate that sperm

concentration may be reduced in semirural and agricultural areas compared to more urban areas (Swan *et al.*, 2003). Thus, even within the same country or regions of similar geography, genetics and history, remarkable differences in sperm counts are found.

In a recent study from Sweden no significant changes in any of the semen variables were found in a comparison of young men examined between 2000-2001 and 2008-2010 (Axelsson *et al.*, 2011). However, a program, monitoring the semen quality among unselected young men in both Finland and Denmark, shows that whereas semen quality seems to have been stable or even slightly increasing during the last 15 yr in Denmark (Jørgensen *et al.*, 2012), in Finland there has been a decreasing trend with average sperm concentration and percentage of morphologically normal spermatozoa reaching a lower level similar to that in Denmark (Jørgensen *et al.*, 2011). When comparing, at the present time young men from the general population in Denmark with a historical cohort of men being part of an infertile couple, the data support a temporal decrease in sperm counts (Fig. 3). Thus, a higher sperm count was observed in the historic cohort despite the fact that this cohort consisted of the male part of barren partnerships (Jørgensen *et al.*, 2012). Importantly, the two studies used similar methods for counting sperm, making a comparison meaningful. Previously, semen quality was shown to be related to the birth cohort with the younger birth cohorts having lower semen quality compared to the older birth cohorts also in Denmark (Bonde *et al.*, 1998b; Jørgensen *et al.*, 2006).

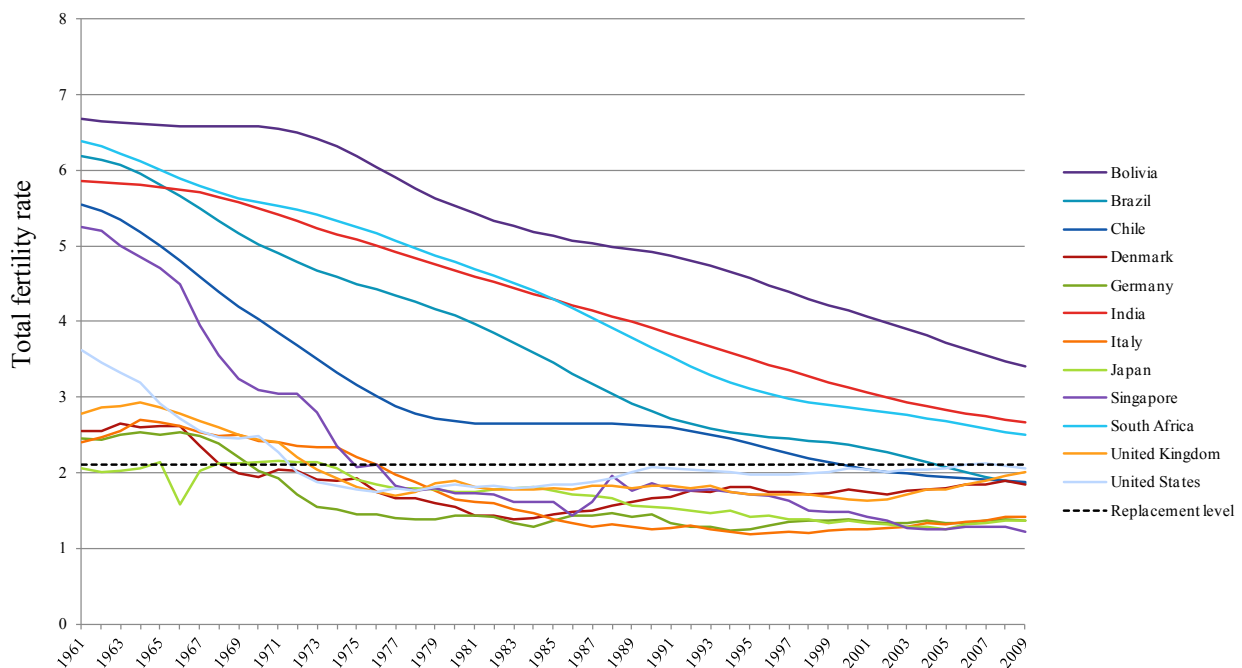


Figure 1. Fertility trends in selected countries from 1961 to 2010. The dotted black line marks the population replacement level of 2.1 children per woman. World DataBank (2012).

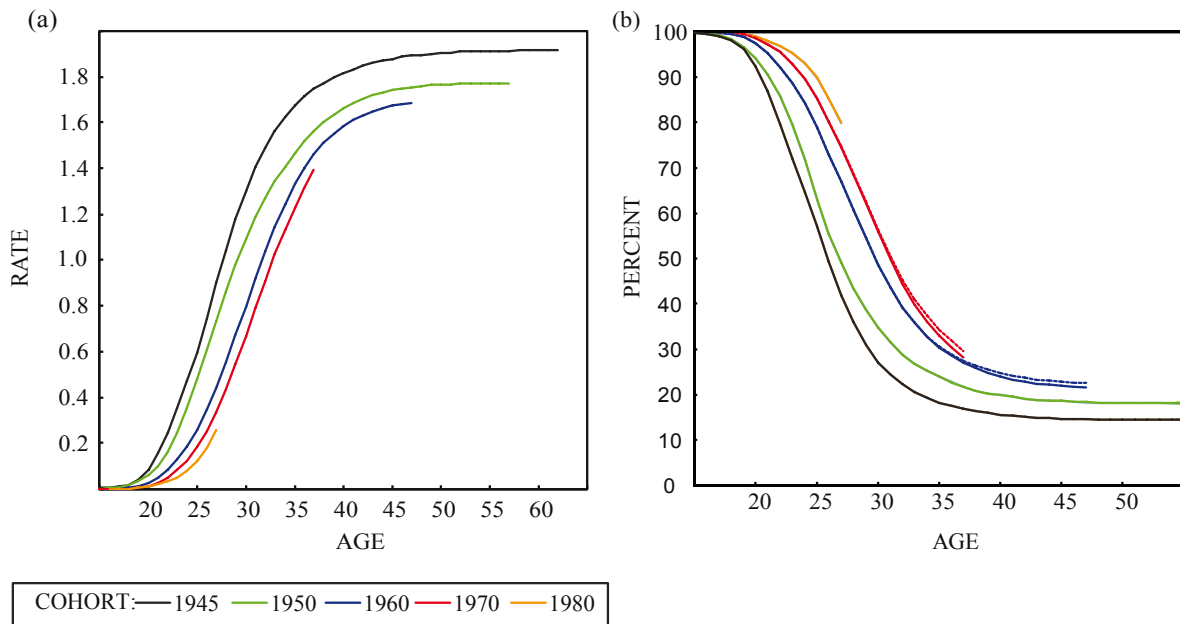


Figure 2. (a) Cumulative fertility rates by age for birth cohorts of native Danish men when excluding children born after assisted reproductive technology. (b) Childlessness among native Danish men (full lines) and when excluding children born after assisted reproductive technology (dotted lines). From Priskorn *et al.* (2012).

The World Health Organization has recently, in their guideline for semen analysis, lowered the cutoff value for normal sperm concentration from 20 million/ml to 15 million/ml. This was based on the definition of a normal range as one that covers 95% of the fertile population (rather than what is physiologically optimal and desirable) (Skakkebak, 2010). A semen sample contains an excess of spermatozoa and a decline in semen quality might therefore not necessarily affect the ability to conceive. A study of pregnancy planners relating semen quality to time to pregnancy found a progressive increase in the likelihood of achieving pregnancy from a sperm concentration of 0 million/ml up to 40 million/ml. Above this threshold there was no further benefit of higher concentrations regarding fertility (Bonde *et al.*, 1998a). Two other studies placed the threshold at 48 million/ml and 55 million/ml, respectively (Guzick *et al.*, 2001; Slama *et al.*, 2002). It is also important that sperms have normal morphology and good motility and besides sperm concentration, sperm morphology is informative when trying to discriminate between fertile and infertile men (Kruger *et al.*, 1986; Guzick *et al.*, 2001). The likelihood of conception is apparently reduced in men with less than 9% morphologically normal spermatozoa (Guzick *et al.*, 2001). Interestingly, in our large study of unselected

men from the general population, we found that the median number of morphological normal spermatozoa was 6.5% (Jørgensen *et al.*, 2012). However, the sperm concentration is a more robust variable when investigating temporal trends, as measures of morphology and motility are more vulnerable to inter observer variation. The studies reporting a change in sperm concentration usually report no change in semen volume; thus, the observed change in concentration must be due to falling total sperm counts. In Denmark and Finland the present average sperm count among young men is 48 million/ml (Jørgensen *et al.*, 2011, 2012). About 40% of young men in these two countries have sperm concentrations below the above mentioned threshold of 40 million/ml, potentially resulting in a prolonged time to pregnancy or need of fertility treatment when trying to have children (Jørgensen *et al.*, 2006, 2011). According to Jørgensen *et al.*, as many as 15% of young Danish men would be at risk of need of fertility treatment if wanting to have children (Jørgensen *et al.*, 2012). As women today are older and are past their most fertile period when starting to try to have children they only have limited time to become pregnant. Poor semen quality in a large group of men may therefore exacerbate the trends determined by social changes resulting in more couples having difficulties achieving their family aspirations.

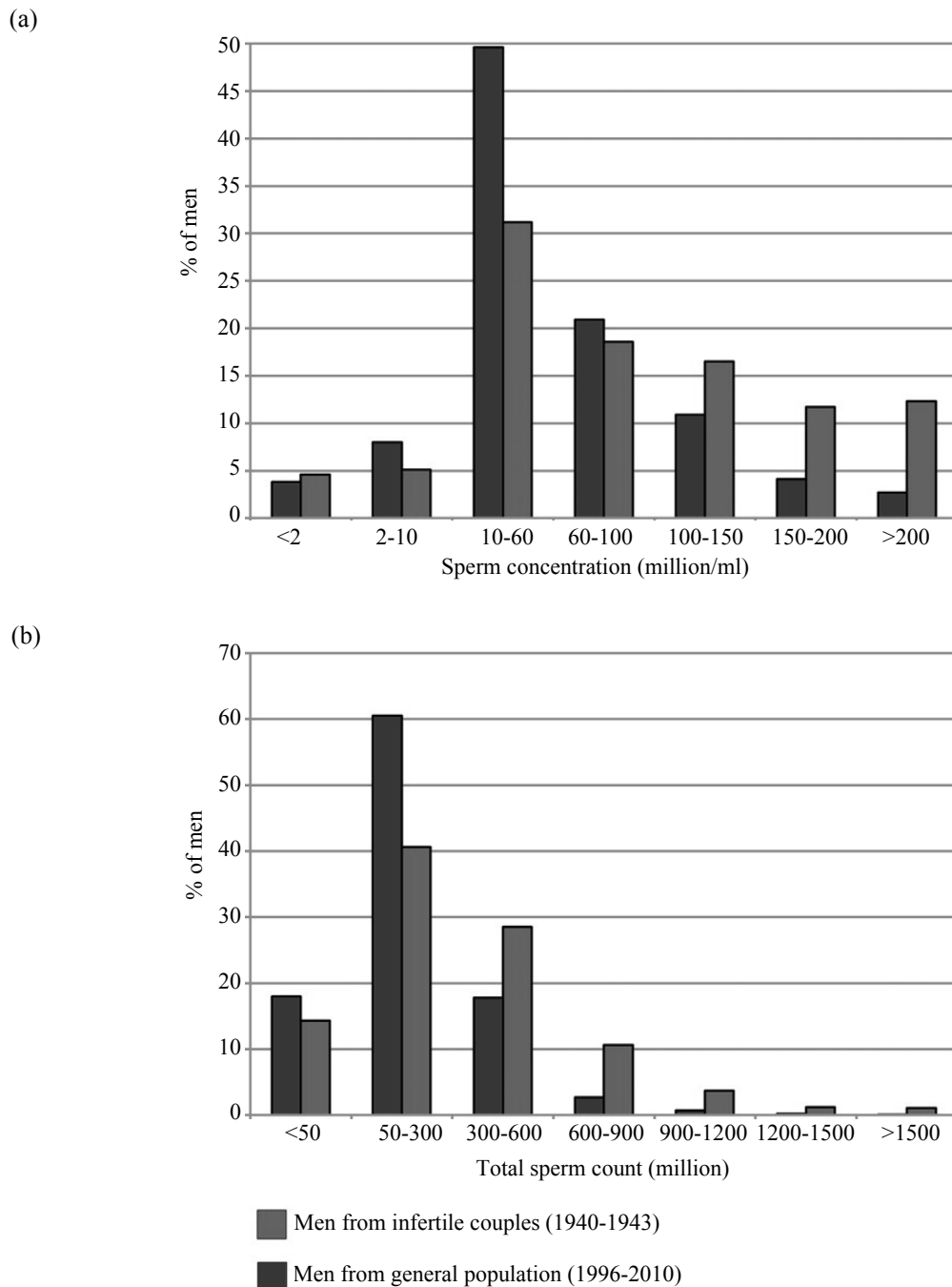


Figure 3. Distributions of sperm counts in Danish men from the general population, examined from 1996 to 2010, and Danish men examined in an infertility clinic in the 1940s. From Jørgensen *et al.* (2012).

Testicular cancer

The occurrence of other male reproductive problems than low semen quality has also increased, including testicular cancer. Testicular cancer is the most common cancer type among young men between the ages of 15 and 39. Almost all cases of testicular cancer (98.9%) can be classified into two large groups, seminomas and non-seminomas, based on histology. The incidence of testicular cancer has shown a steady

increase in most European countries (Richiardi *et al.*, 2004; Bray *et al.*, 2006; Jacobsen *et al.*, 2006), a trend much more established than that of semen quality due to the reliable cancer registries in many Western countries. The regional differences, as observed in semen quality, are also apparent for testicular cancer incidence (Fig. 4; Jørgensen *et al.*, 2006; Chia *et al.*, 2010). In general, the incidence of testicular cancer is higher in the developed than in developing countries, a phenomenon not found to be explained by differences in quality of registration.

However, within regions of developed countries a large difference can be observed as well. When comparing the Nordic countries, traditionally the incidences have been much higher in Denmark and Norway than in Sweden and Finland but an adverse trend in testicular cancer has recently been observed among Finnish men (Jørgensen *et al.*, 2011). Moreover, the incidence of testicular cancer differs with race. In the United States, Caucasian men are having a higher incidence than Afro-American men. Incidences of young white men in the Nordic countries are as high as 11.5 per 100,000 man-year compared to an incidence rate of 1-2 per 100,000 man-year among black and Asian men (Chia *et al.*, 2010). Thus, genetic factors may explain some of these geographical

differences. However, the importance of environmental and lifestyle factors has been supported by immigrant studies. The risk of testicular germ cell cancer in first generation immigrants is similar to that in their country of origin, whereas their children, born in the immigrant country, adopt the risk of men in that country (Hemminki and Li, 2002; Schmiedel *et al.*, 2010). Besides, as with semen quality, there seems to be a strong birth cohort effect in testicular cancer incidence with each subsequent birth cohort having a higher incidence. An exception to this consecutive increase is Scandinavian men born during the Second World War, for whom the incidence was lower than for the surrounding birth cohorts (Møller, 1993; Jacobsen *et al.*, 2006).

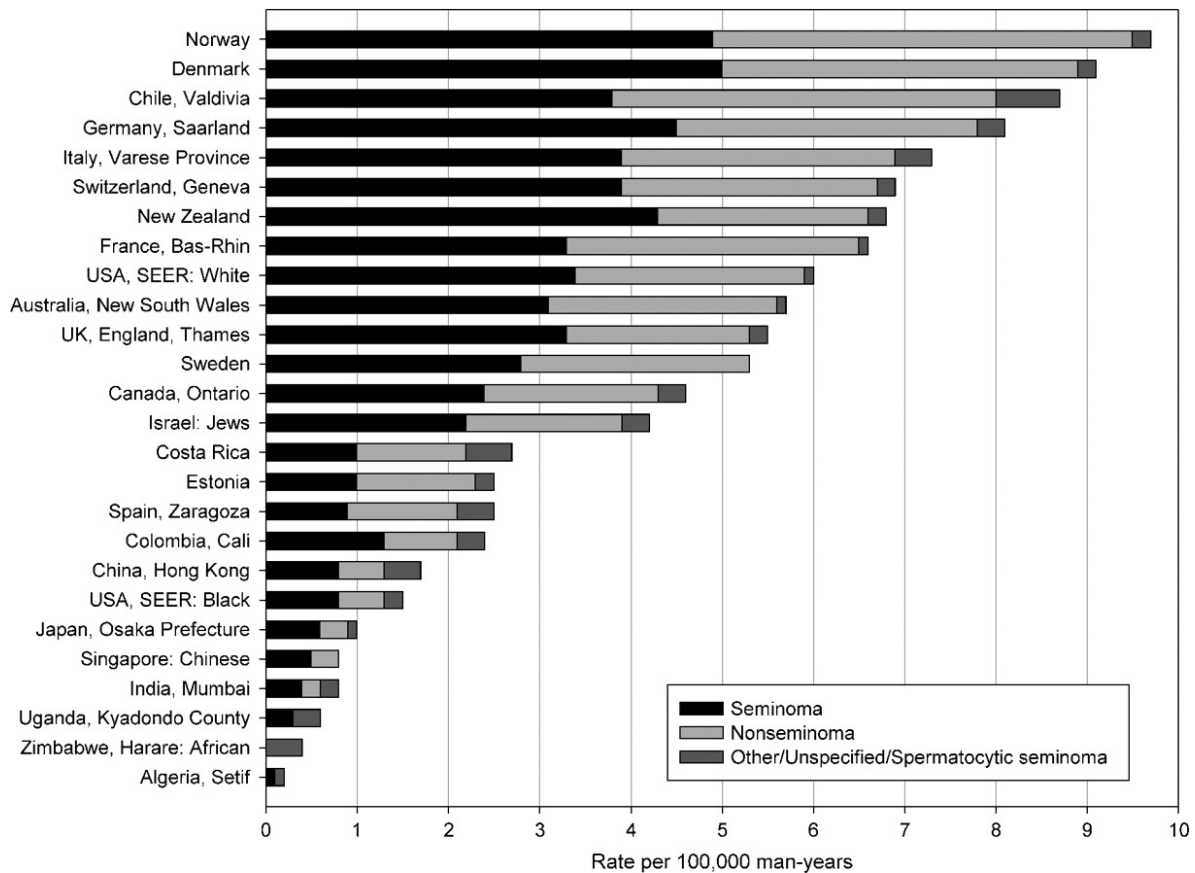


Figure 4. Incidence rates of testicular cancer (per 100,000 man-years) age-standardized to the World's population (1998-2002). From Chia *et al.*(2010).

Congenital malformations of the genitals

Cryptorchidism and hypospadias are the most common genital birth defects among boys and affect 2-9% and 0.2-1% of newborns, respectively (Toppari *et al.*, 2010). Congenital cryptorchidism is undescended testis at birth and hypospadias is an abnormal location of the urethral opening varying in severity from an opening on

the ventral side of the penile glans to the scrotum or perineum. As with semen quality and testicular cancer the incidences of cryptorchidism and hypospadias show a large geographical variation. However, the incidences of these congenital malformations are highly dependent on whether the child was examined in the newborn period or later and whether only severe cases necessitating treatment were registered. A comparison



between Denmark and Finland, using a standardized technique to assess testicular position, showed a significant difference in the prevalence of cryptorchidism registered at birth being 9.0% in Denmark and 2.4% in Finland. At three months of age, however, the prevalence was much lower due to spontaneous descent of the testis and the country difference was reduced to a prevalence of 1.9% in Denmark compared with 1.0% in Finland (Boisen *et al.*, 2004a). The incidences of hypospadias also showed a difference between Denmark and Finland (Virtanen *et al.*, 2001; Boisen *et al.*, 2005). A problem in determining the incidence of hypospadias is that the registries are not very precise and many glanular forms are not detected at birth due to physiologic phimosis. In Denmark the rate is 4.6% among 3-yr-old boys and only 1% at birth (Boisen *et al.*, 2005). As with the other male reproductive health problems mentioned, the incidence of cryptorchidism seems to be rising although the registration over time is subject to some uncertainty due to differences in diagnostic criteria. Increasing trends have been reported from Denmark and the United Kingdom (Boisen *et al.*, 2004b; Acerini *et al.*, 2009) in comparisons of studies using similar diagnostic criteria. Increasing trends in the incidence of hypospadias have been reported from Denmark, Australia and the United States as well (Paulozzi *et al.*, 1997; Boisen *et al.*, 2005; Nassar *et al.*, 2007).

Testicular dysgenesis syndrome

Low semen quality, testicular germ cell cancer and congenital malformations of the genitals are in some cases associated. Epidemiological studies have shown that testicular cancer is associated with risk of poor semen quality and in men with unilateral testicular cancer, semen quality is lower than expected compared with other men with only one functioning testis (Petersen *et al.*, 1998). Furthermore, men with testicular cancer father fewer children than men in the general population, even prior to development of the tumor (Møller and Skakkebak, 1999; Jacobsen *et al.*, 2000). Also cryptorchidism and hypospadias are associated with lower fertility, lower semen quality and increased risk of testicular cancer (Lee, 2005; Asklund *et al.*, 2010; Schnack *et al.*, 2010). This increased risk of men with cryptorchidism is not just related to the undescended testis but also the contralateral 'normal' testis (Pinczowski *et al.*, 1991). Based on the epidemiological evidence and findings in testicular tissue specimens from men with testicular cancer and undescended testis; and some cases of hypospadias and men with oligozoospermia we have proposed the existence of a testicular dysgenesis syndrome (TDS) of fetal origin. Hypospadias and congenital cryptorchidism are per se of fetal origin. But also testicular germ cell cancer and some cases of low semen quality seem to be

due to fetal maldevelopment of the testis, although the latter conditions do not appear until adult life. Recent research has shown strong biological evidence for fetal origin of carcinoma *in situ* testis (CIS), the common precursor cell of both seminomas and non-seminomas. CIS cells resemble fetal gonocytes and express several embryonic markers, including C-kit, NANOG, Oct-4 and AP 2 gamma (Almstrup *et al.*, 2004; Sonne *et al.*, 2009). CIS cells are presumed to derive from transformed gonocytes arrested in their fetal differentiation (Rajpert-De Meyts, 2006). Thus, the TDS hypothesis is not just based on epidemiological associations; it is also anchored in several basic studies on specimens from men with CIS and germ cell cancer. Men with TDS display dysgenesis of the testes expressed as histological changes in parts of the testis, like maldevelopment of the seminiferous tubules including Sertoli-cell-only tubules, spermatogenic arrest, microcalcifications and clusters of undifferentiated tubules (Fig. 5; Skakkebak *et al.*, 2001; Rajpert-De Meyts, 2006).

The number of symptoms in a man with TDS varies with the severity of the syndrome and a mild form may just be expressed in a lower sperm count and a few Sertoli-cell-only tubules whereas more severe but relatively rare cases display all four symptoms. Importantly, although TDS may explain the majority of cases of isolated testicular germ cell cancer and cryptorchidism only part of the cases of isolated hypospadias and impaired spermatogenesis may be due to TDS (Jørgensen *et al.*, 2010).

TDS has been hypothesized to arise due to suboptimal androgen activity during a critical period in utero, the masculinization programming window, resulting in maldevelopment of the Leydig and Sertoli cells. In humans, the window is thought to occur at gestational weeks 8 to 12 (Sharpe, 2012). Studies in rats have shown that the production of androgens in the specific developmental window is crucial for masculinization and normal development of the male reproductive organs, including external genitalia. Insufficient androgen production at this point in time may result in reduced size of the adult reproductive organs of the animals (testes, prostate, seminal vesicles and penis) and increase the risk of TDS disorders, except for testicular germ cell cancer for which no animal model exists (Welsh *et al.*, 2008). Anogenital distance, which is the length between anus and phallus/scrotum, has been shown to be a sensitive marker of androgen activity during the masculinization programming window in both rodents and humans. Anogenital distance seems to be shorter in boys with hypospadias and cryptorchidism and is associated with sperm counts and fertility in adult men (Hsieh *et al.*, 2008; Eisenberg *et al.*, 2011; Mendiola *et al.*, 2011) suggesting a prenatal origin of some of these cases.

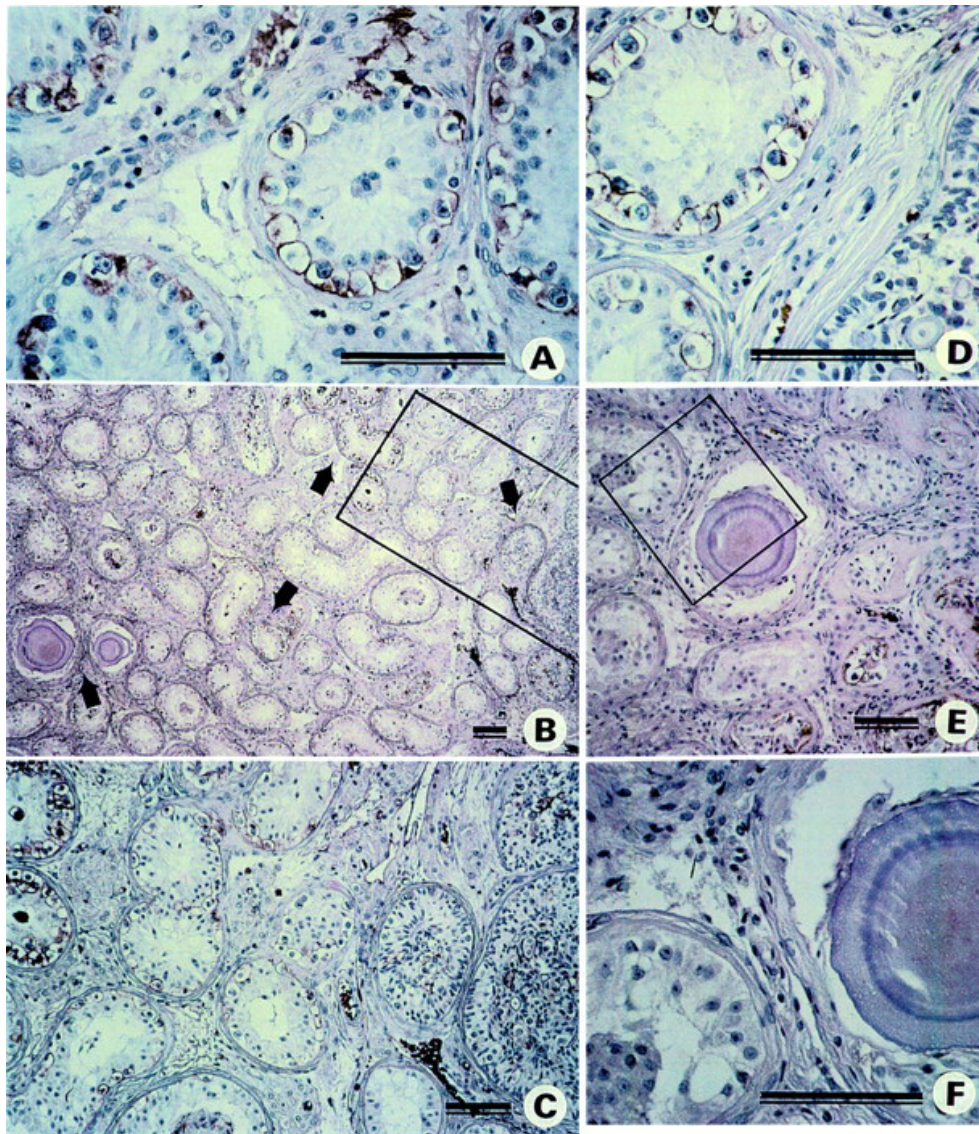


Figure 5. Examples of testicular dysgenesis in two contralateral biopsies of patients with unilateral testicular tumours. Both biopsies contain carcinoma in-situ (CIS) cells, which are visualized by immunohistochemical staining for placental-like alkaline phosphatase (dark brown color). Morphology of 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 μ m. From Skakkebaek *et al.* (2001).

Possible risk factors

The rapid increase in male reproductive problems indicates that the explanations should be found among environmental factors, including lifestyle. Genetics may, however, also play a role, as some individuals and populations may be more susceptible and thereby more disposed to developing TDS at the same level of risk exposure. This makes it important to

combine research disciplines and methods when investigating possible risk factors for development of TDS. The same exposure might cause different effects in different populations due to genetic differences. As TDS is hypothesized to develop during fetal life the causes should hypothetically be sought in the mothers' environment and lifestyle during pregnancy.

During the past 10 to 15 yr several researchers have explored a possible role of endocrine disrupting



compounds, including pesticides and industrial chemicals, for male reproductive health problems (WHO report, expected to be released September 2012). So far most evidence concerning reproductive effects originate from animal studies. However, there are some examples of incontrovertible negative reproductive effects of endocrine disrupters in human. For instance, a negative impact of dibromochloropropane (DBCP) on human spermatogenesis was discovered in the 1970s in groups of DBCP production workers among whom a shortage of children was observed. When investigated further, a striking dose-response association was found between the duration of chemical exposure to DBCP and sperm count. A large part of the men exposed to DBCP due to their occupation were azoospermic or oligospermic. As early as 1961 DBCP had been shown to be a reproductive toxicant in animals but this finding was thought to be irrelevant for humans (Potashnik *et al.*, 1978; Whorton *et al.*, 1979). Another example of endocrine disrupters is the Seveso accident (1976) which showed that human exposure to dioxin resulted in lower semen quality in the males exposed in utero and perinatally via breast milk whereas there was no effect on semen quality in the males exposed as adults (Mocarelli *et al.*, 2008, 2011). Thus, exposure to some compounds can induce azoospermia in adult men but in utero and perinatal exposures seem to be an even more critical time of exposure. In the masculinization programming window, male fetuses are very susceptible to endocrine disruption (Welsh *et al.*, 2008). Animal studies have shown that males exposed perinatally to a range of endocrine disrupting environmental chemicals experience reductions in the reproductive organs, including anogenital distance, and can develop hypospadias, cryptorchidism and poor semen quality (Foster, 2006; Gray *et al.*, 2006). The doses used in these experimental studies, though, are much higher than the ones humans are exposed to. Of great importance, studies have shown that attention should be paid to the aggregate effect of similar compounds as different compounds in combination have adverse effects at doses where the individual compound shows no effect at all (Bellingham *et al.*, 2012). This is termed the 'cocktail effect' and is very relevant to and representative of the human exposure to different compounds through a range of sources like cosmetics, food and pollution.

Besides endocrine disrupting compounds, mother's smoking during her pregnancy has been found to be associated with poor semen quality, hypospadias and cryptorchidism in her son, although controversial results concerning the malformations have been reported (Jensen *et al.*, 2004, 2007; Brouwers *et al.*, 2007; Ramlau-Hansen *et al.*, 2007b; Ravnborg *et al.*, 2011; Virtanen, 2012). Likewise alcohol consumption and mild analgesics during pregnancy has shown a dose-response effect on the risk of cryptorchidism, but this association is not strong either (Damgaard *et al.*,

2007; Kristensen *et al.*, 2011). Obviously, semen quality can also be affected by exposures operating during the man's adolescence and adulthood, e.g. smoking (Jensen *et al.*, 1998; Ramlau-Hansen *et al.*, 2007a), agricultural pesticides, scrotal heating, and obesity (Sharpe, 2010). Unlike the damage induced in utero, causing TDS, the cases of poor semen quality that are due to adult exposures are likely reversible (Sharpe, 2010).

Fertility perspectives

It seems crucial for industrialized countries with low fertility rates to explore the reasons for them. If, as most demographers seem to believe, it is a question about modern women's choices, the trends should be possible to reverse through changes in family and employment politics. Furthermore, a temporary population decrease in some parts of the world could even be seen as a healthy development in an overpopulated World. On the other hand, if non-reversible, biological factors contribute significantly to the extremely low fertility rates, now seen in many European and Asian countries, future generations in these countries may be witnessing severe decreases of their populations to a level where significant impacts may occur for the societies. Denmark, which with European standard has had a relatively high fertility rate, has over the past 40 yr had an average fertility rate of 1.7 children per woman. As it can be seen (Fig. 6) even such a fertility rate only slightly below replacement level will in 40 yr result in a 20% decrease in the population of women in their reproductive age. As people live longer, this decrease will have a delayed effect on the total number of people in a population. What we at first hand will notice is therefore an ageing population, difficulties with budgets for pensions etc. and therefore the economic issues are in the forefront these days. However, eventually the decreasing number of young people, able to reproduce, will without doubt result in a significantly lower number of people, also of the elderly. Hopefully, this fact will soon be realized by authorities in Europe. Particular Asian researchers and politicians, e.g. in Singapore have taken part in the debate. Most focus has been on social-physiological programs to raise fertility rates; albeit there has been little effect of those programs (Xueying, 2011). It seems imperative to also start focusing on the role, population wide changes in fecundity may play in the observed low fertility rates. For 35 yr Singapore has had fertility rates below replacement and the current rate is among the lowest in the world (1.1 children per woman). If the current Singapore rate was to continue for three generations the population of women in the reproductive age will decline to 15% of what it is today. Hopefully, these widespread trends of low fertility rates can be reversed at some point. If not, our grandchildren and their children will experience enormous societal changes.

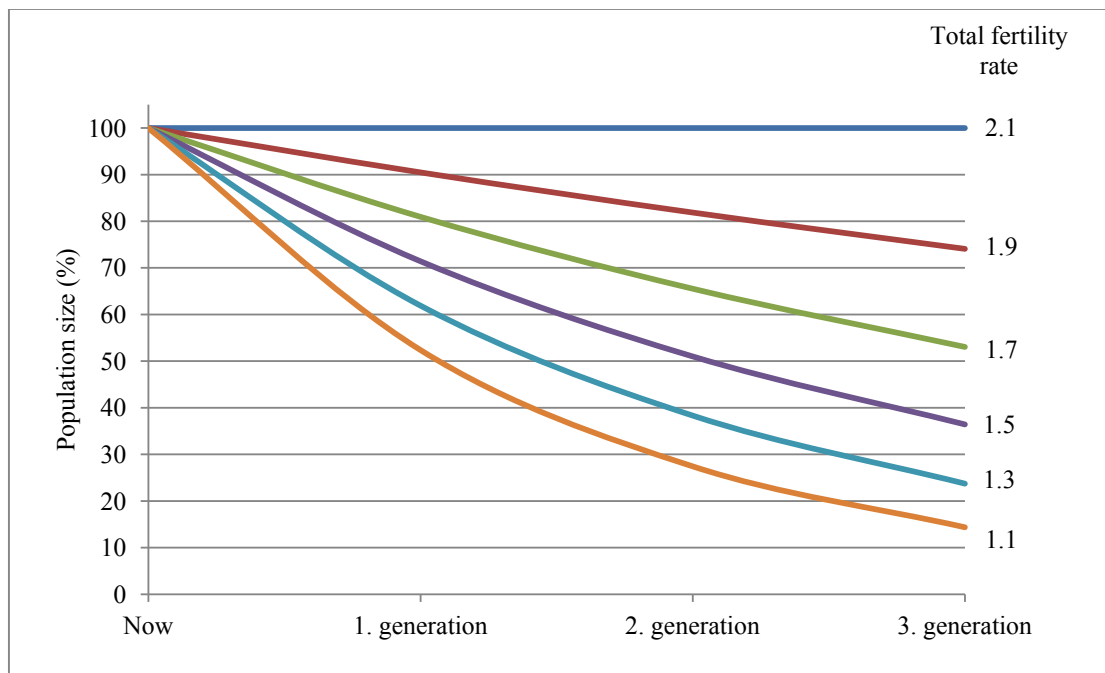


Figure 6. Estimated decrease of the female reproductive population among future generations at different levels of below replacement total fertility rates.

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