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Is human fecundity declining?

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Summary

The decreasing trends in fertility rates in many industrialized countries are now so dramatic that they deserve much more scientific attention. Although social and behavioural factors undoubtedly play a major role for these trends, it seems premature, and not based on solid information, to conclude that these trends can be ascribed to social and behavioural changes alone. There is evidence to suspect that changing lifestyle and increasing environmental exposures, e.g. to endocrine disrupters, are behind the trends in occurrence of male reproductive health problems, including testis cancer, undescended testis and poor semen quality. These biological factors may also contribute to the extremely low fertility rates. However, the necessary research is complex and requires non-traditional collaboration between demographers, epidemiologists, clinicians, biologists, wild life researchers, geneticists and molecular biologists. This research effort can hardly be carried out without major support from governments and granting agencies making it possible to fund collaborative projects within novel research networks of scientists.

Introduction

Human fertility rates are declining all over the world (Fig. 1). In some Western countries the rates are far below the point at which the population can be maintained at the current level (Lutz *et al.*, 2003; World Bank, 2005). It is generally assumed that the trends towards lower fertility rates are because of social and economic changes, such as better contraception, women's working careers, their education, etc. The possibility that biological factors may now contribute to the declining fertility rates is rarely considered. However, human infertility rates are currently very high (Andersen & Erb, 2006) and male subfertility may be increasing. In this article we shall, therefore, focus on male reproductive health aspects, which should also be considered as possible contributing factors to the recent changes in fertility rates. Our analysis suggests that we may, in fact, be seeing the signs of modern lifestyle influence on our reproductive capacity.

Fertility rates

Until the 1990s global overpopulation was a major international health issue (Diczfalusy, 1996) with fertility rates as high as 8–10 in some developing countries. However, during the recent two decades a remarkable drop in fertility rates has been noticed all over the world, including both developed and developing countries (Fig. 1) (Lutz *et al.*, 2003; World Bank, 2005). It is generally assumed that these trends are because of changed behaviour related to altered socio-economic policies and women's participation in the work force. However, although social factors without doubt play a role in the changed fertility rates, the possible contribution of synchronous adverse biological factors causing a deterioration of reproductive health has not been ruled out. In fact, the dramatic fall in fertility rates in several developing countries (Rosenfield & Schwartz, 2005) seems difficult to explain by increased use of contraceptives alone. It was suggested that the falling pregnancy and abortion rates among teenagers in the US were due to better use of contraception and increased sexual abstinence among teenagers (Donovan, 1998). However, similar trends in developed European countries are difficult to explain as there were no apparent changes in use of contraception or sexual habits among these young age groups during the same period (Jensen *et al.*, 2002). In some eastern European countries, like Russia and some Baltic states, the recent low fertility rates may

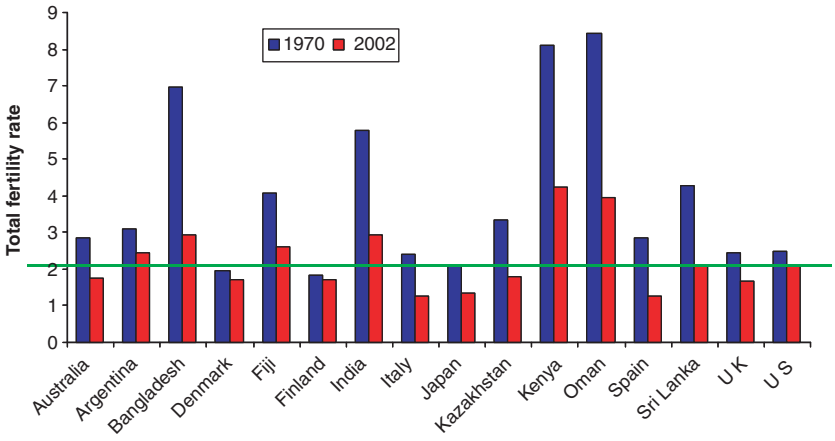

Figure 1 Changes in fertility rates in developing and industrialized countries. The green line is the 2.1 fertility rate, which is necessary to sustain a population at its current level (from World Bank, 2005).

be explained by very high abortion rates. However, in industrialized countries, where the fertility rates in some areas are as low as 1.1–1.5, there has been a concomitant fall in abortion rates during the period of falling fertility rates. Thus, we cannot exclude that reduced fecundity can contribute to the extremely low fertility rates.

High demand for infertility treatment

A possible sign of decreasing reproductive health is the high demand for infertility treatment, which includes intracytoplasmatic sperm injection (ICSI), in vitro fertilization (IVF), and insemination with partner's or a donor's semen. In Denmark, assisted reproduction contributed significantly to the number of children born during recent years. During 2002–2004 more than 6% of Danish children were born after assisted reproduction (Andersen & Erb, 2006). In addition, many couples adopted foreign children. Thus, a total of approximately 7% of all Danish children born during the last few years were not conceived 'naturally'. The underlying pathologies include female as well as male factors. However, a high demand for ICSI (Andersen & Erb, 2006) reflects the existence of a significant number of couples with predominantly male infertility factor, caused in most cases by testicular failure and poor semen quality.

Poor semen quality of young men in the general population in some countries

Results of a coordinated European research effort revealed that Danish men were among those with the poorest semen quality in Europe (Andersen *et al.*, 2000; Jørgensen *et al.*, 2001; Jørgensen *et al.*, 2002). These studies attracted attention of the Danish Government, which supports ongoing systematic surveillance studies of semen quality of young men from the general population. Using WHO criteria and other international standards we have concluded that as many as 30% of young Danish men may have semen quality in a subfertile range, and more than 10% may be in the infertile range (Jørgensen *et al.* 2006; Fig. 2). The Danish studies were coordinated with similar studies in other Baltic countries, including Finland and remarkable differences in semen quality were found; the Danish men had lower sperm counts and less sperms with normal morphology. Our recent data are in concert with earlier studies from our and other groups suggesting that there has been a trend towards lower sperm counts during the last century (Carlsen *et al.*, 1992), although the topic is complex (Aitken *et al.*, 2004) and still considered controversial by some investigators (Jouannet *et al.*, 2001).

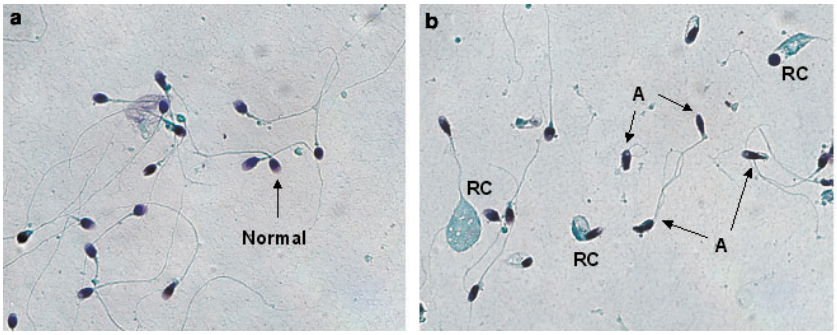

Figure 2 Examples of smears of semen samples with (a) good quality (18.5% morphologically normal spermatozoa) and (b) poor quality (0.05% normal spermatozoa). Men having <9% morphologically normal spermatozoa are considered to be in a subfertile range (Guzick *et al.*, 2001). 67% of normal young Danish men have <9% normal spermatozoa. RC, residual cytoplasma; A, abnormal sperm head.

Increasing rates and evidence for a developmental origin of testis cancer

Testis cancer, which is the most common malignancy in young males between 20 and 45 years of age has been rising in incidence in western industrialized countries during the past half century (Møller, 1993). The trend has been very consistent among Caucasian populations although significant geographical gradients exist, e.g. the Danish incidence is four to five times higher than the Finnish rate (Richiardi *et al.*, 2004). The most recent epidemiological studies report that incidence rates may begin to level off in some developed countries (Purdue *et al.*, 2005). These observations may be of great importance in relation to other reproductive health aspects in industrialized populations, as testicular cancer seems to be an important biomarker of testicular development and fecundity. Although the aetiology of testicular cancer remains unknown, there is growing evidence for the importance of impaired foetal gonadal development and function. Our previous studies on the common precursor cell, carcinoma in situ (CIS), suggested that aetiological factors would operate during development of the primordial germ cell in foetal testes (Skakkebæk *et al.*, 1987). During the subsequent years, this hypothesis was supported by numerous studies showing a gonocyte-resembling phenotype and stem cell like properties of the CIS cell (Rajpert-De Meyts & Skakkebæk, 1994; Rajpert-De Meyts *et al.*, 1996; Rajpert-De Meyts *et al.*, 1999; Almstrup *et al.*, 2002; Looijenga *et al.*, 2003; Almstrup *et al.*, 2004; Honecker *et al.*, 2004; Høi-Hansen *et al.*, 2004).

Early studies by Berthelsen (1984) demonstrated that the contralateral testis in patients with testis cancer often showed signs of malfunction both with regard to spermatogenesis and hormone production. Both ipsilateral and contralateral testes had frequently impaired spermatogenesis, such as Sertoli cell-only pattern, spermatogenic arrest, microcalcifications, seminiferous tubule atrophy or tubules with CIS. Several subsequent reports confirmed the association between testis cancer and poor semen quality (Fosså *et al.*, 1993; Giwercman *et al.*, 1993; Petersen *et al.*, 1999; Jacobsen *et al.*, 2000) Finally, a case–control study showed that males with testis cancer had significantly lower fertility rates 2 years prior to development of the testicular tumour (Møller & Skakkebæk, 1999). Taken together, these studies also may explain the apparent synchrony of frequencies of testicular pathologies, such as testis cancer and poor semen quality and also explain the remarkable difference in male reproductive health between Denmark and Finland. They are also in line with the assumption that testis cancer and oligozoospermia may be symptoms of one underlying syndrome caused by a common pathogenetic factor.

Evidence of increasing rates in congenital genital abnormalities

The striking difference in the incidence of testis cancer between Denmark and Finland inspired us to launch systematic birth cohort studies in both countries in order to establish the prevalence of cryptorchidism and hypospadias, conditions which carry an inherent increased risk of testicular neoplasia and for which no good registry data existed (Toppari *et al.*, 2001). As we expected, we not only found significantly higher incidence rates of these disorders in Denmark than in Finland, but also, surprisingly, the Danish rates turned out to be higher than those found in a previous, similar study in Denmark in the 1960s (Boisen *et al.*, 2004; Boisen *et al.*, 2005b; Figs 3 & 4). This finding was consistent with the simultaneous rise in the incidence of testicular cancer and suggested that a common pathogenic factor associated with both diseases increased its impact during the most recent four decades.

Testicular dysgenesis syndrome

Recently, we proposed that testicular cancer, male infertility, hypospadias and undescended testis may all be symptoms of one underlying pathology, namely a testicular dysgenesis syndrome (TDS) (Skakkebæk *et al.*, 2001). The evidence for the existence of the syndrome is partly of biological nature, partly epidemiological.

Biological evidence

During our microscopy work on testicular biopsies from patients with fertility problems, undescended testis, and on contralateral testicular biopsies from men with testis cancer, we have detected changes in the testicular architecture that most likely represent dysgenesis (Fig. 5). These changes include dysplastic tubules with

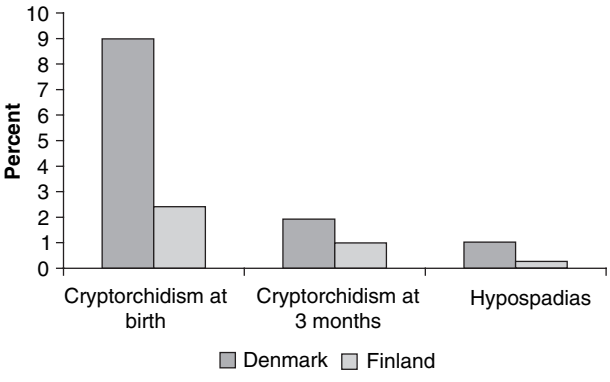

Figure 3 Differences in prevalence of genital abnormalities between Denmark and Finland (from Boisen *et al.*, 2004; Boisen *et al.*, 2005a).

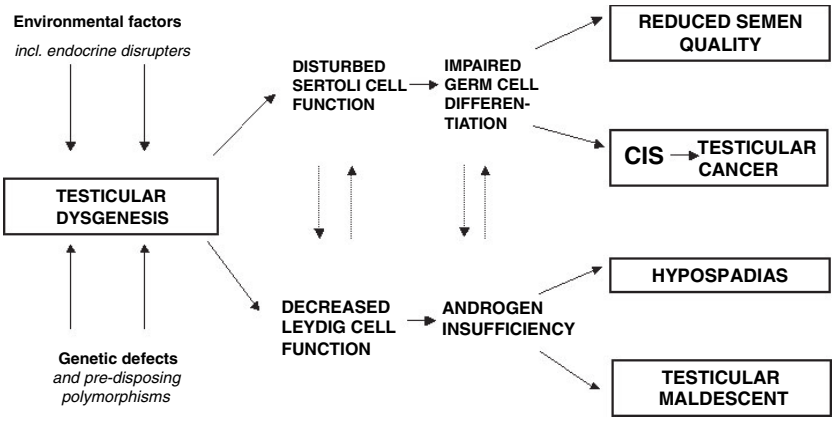

Figure 4 Schematic representation of pathogenetic links between the components of the clinical manifestations of testicular dysgenesis syndrome (from Skakkebæk *et al.*, 2001).

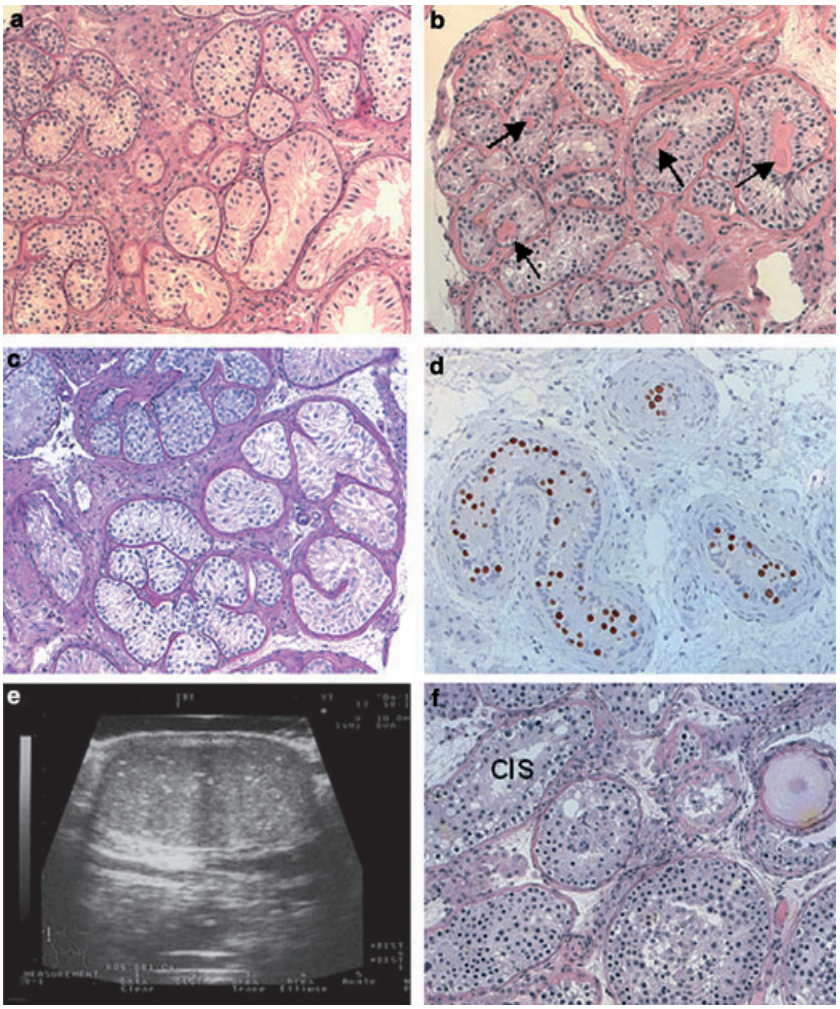

Figure 5 Examples of testicular dysgenesis syndrome in andrological patients. The three first images show distored and undifferentiated tubules with immature Sertoli cells in different patients with infertility: note variable grade of differentiation of Sertoli cells in (a), formation of microliths in (b) (marked with arrows) and the lack of proper differentiation of tubular membranes in (c) (PAS staining). (d) Tubules with undifferentiated Sertoli cells and CIS cells (CIS nuclei marked with immunohistochemical staining for AP-2γ). The two bottom images show a patient with testicular microlithiasis in the scrotal ultrasound (e), who was diagnosed with CIS in the surgical biopsy (f): note a large microlith in the vicinity of tubules with CIS.

undifferentiated Sertoli cells, Sertoli cell-only tubules, intratubular microliths (microcalcifications), and sometimes CIS cells (Høi-Hansen *et al.*, 2003). The changes are somewhat similar to those present in gonadoblastoma in patients with 46,XY pure gonadal dysgenesis (Scully, 1953; Scully, 1970). The demonstration of histological association between microcalcifications and CIS cells has turned out to be of clinical value, as the presence of ultrasonic microliths in the contralateral testis of a patient with testis cancer is associated with increased risk of testicular neoplasia and therefore strengthens the indication for testicular biopsy for early detection of CIS (Lenz *et al.*, 1987; Rajpert-De Meyts & Skakkebæk, 1999; Holm *et al.*, 2002; Holm *et al.*, 2003).

Epidemiological evidence

The epidemiological evidence concerning the geographical trends in male reproductive health problems has already been mentioned. There is also overwhelming epidemiological evidence that testis cancer, undescended testis, hypospadias and poor semen quality are risk factors for each other (Sharpe & Skakkebæk, 2003). Obviously, male infertility is a very heterogeneous disorder and can be caused by a number of factors, including postnatal infections, accidents, chemotherapy, genetic aberrations causing meiotic arrest and many others. However, in most cases of male infertility no apparent known aetiological factor can be revealed (Skakkebæk *et al.*, 1994). We suspect that the impairment of spermatogenesis in this subgroup of infertile men may, in fact, often be the result of testicular dysgenesis during foetal life.

A few studies have examined the effect of mothers' smoking in pregnancy on semen quality in the offspring (Ratcliffe *et al.*, 1992; Storgaard *et al.*, 2003; Jensen *et al.*, 2004b). Two of them found reduced semen quality of the sons (Storgaard *et al.*, 2003; Jensen *et al.*, 2004b) although, in utero exposure to smoking could not explain the geographical difference in semen quality between the countries.

The epidemiological literature is in agreement with biological evidence that testicular cancer may also originate from pre- or perinatal pathological events (Skakkebæk *et al.*, 1987). There are several pieces of evidence that foetal events are of utmost importance for development of testicular cancer: (i) the peak incidence of testis cancer is in young men suggesting an early onset of the disease (Møller, 1993), (ii) perinatal environmental events (like World War II) have been shown to be related to the occurrence of the disease (Møller, 1989) and (iii) pre- and perinatal exposures and mothers pregnancy health problems increase the risk of developing testicular cancer in young adulthood (Weir *et al.*, 2000; Hemminki & Li, 2002; Hemminki *et al.*, 2002) and, (iv) maternal exposures to environmental chemicals have been associated with testis cancer in sons (Hardell *et al.*, 2003).

All these facts, biological as well as epidemiological, gathered over the past decades of research, are in line with the hypothesis that testis cancer (seminoma as well as non-seminoma) is of foetal origin and, importantly, testis cancer may serve as a most useful biomarker of male reproductive health. As a matter of fact, societies with high and increasing rates of testis cancer should consider the testis cancer statistics as a 'writing on the wall' that a significant and increasing subgroup of that population may be suffering from bad reproductive health. The quite dramatic increase in testis cancer over a couple of generations suggests that the adverse trends are caused by environmental exposures associated with modern life rather than genetic changes. In our search for these factors we should focus on environmental effects on pregnant women and the developing foetus.

TDS and endocrine disrupters

There is growing concern that exposure to endocrine disrupters may contribute to rising prevalence of TDS in the male population. Although the endocrine disrupter hypothesis was initially established on basis of a possible mechanism by which oestrogenic compounds might affect the developing gonad, more recent research have disclosed that agents with anti-androgenic effects might significantly contribute to TDS-like symptoms in animal experiments (Foster *et al.*, 2001; Fisher *et al.*, 2003). Examples include phthalates and some pesticides (Table 1). Particularly the data on phthalates are currently of concern as we know that newborn babies sometimes are exposed to considerable daily doses of phthalates via breast milk (Calafat *et al.*, 2004; Mortensen *et al.*, 2005),

However, the search for the possible role of environmental factors, including endocrine disrupters in pathogenesis of TDS is extremely complex. Modern life includes many uses and misuses of a number of consumer items, which may be contaminated with a mixture of a very high number of chemicals and natural products with adverse effects. Many of these exposures are obscure, e.g. residuals of pesticides, phthalates and other chemicals in breast milk. Some of these chemicals, e.g. PCBs, DDT and its metabolites accumulate in the body, often in fat tissue and are persistent, while other agents, such as some phthalates seem to have a rather short half-life in the body.

Thus, although there are quite convincing experimental animal data and wild life data that endocrine disrupters may cause disruption of the male reproductive system (Jobling & Sumpter, 1993; Guillette, 1994), hitherto few

Table 1 Examples of endocrine disrupters

Plasticizers (Mahood *et al.*, 2006, Foster, 2006)	DBP (dibuthyl phthalate), DEHP (di(2-ethylhexyl) phthalate), DINP (di-isononyl phthalate)
Denaturants in cosmetics (evidence for endocrine disruption still weak; Lottrup *et al.,* 2006)	DEP (diethylphthalate), MEP (dimethylphthalate)
Preservatives (in food and cosmetics) (Oishi, 2002; Gomez *et al.*, 2005)	Buthylparaben, propylparaben
Surfactants (Toppari *et al.*, 1996)	Bisphenol-A (and isomers), Nonyl- and octylphenol (and isomers)
Resins used in dental sealings (Wada *et al.*, 2004)	HMBP (2-hydroxy-4-methoxy-benzophenone), DMPA (2,2-dimethoxy-2-phenyl-acetophenone)
UV-filters (sunscreens) (Schlumpf *et al.*, 2004; Morohoshi *et al.*, 2005; Suzuki *et al.*, 2005)	3-(4-methylbenzylidene)-camphor, hydroxylated benzophenones
Pesticides and other agrochemicals (Toppari *et al.*, 1996; Earl Gray Jr *et al.*, 2006; Vinggaard *et al.*, 2006)	DDT and DDE, dieldrin, methoxychlor, endosulfan, vinclozolin, prochloraz
Phytoestrogens*	Genistein, coumestrol, chrysin, lignans

*Although they are fairly potent oestrogens, phytoestrogens have properties that distinguish them from the synthetic estrogenic and anti-androgenic chemicals, and investigations suggest that they may have beneficial effects and therefore probably should not be considered as endocrine disrupters (Almstrup *et al.*, 2002; Cornwell *et al.*, 2004).

reports on adverse effects of endocrine disrupters in humans have been published (Hardell *et al.*, 2003).

Genetic aspects of TDS

In our opinion, environmental factors must play a primary role behind the observed adverse trends in the male reproductive health problems, which have occurred over a relatively short period of one or two generations. However, there are marked geographical and ethnic differences in prevalence of testis cancer and other reproductive problems (Clemmesen, 1981; Møller, 1993). The presence of familial cases speaks also for the importance of genetic background (Weissbach & Widmann, 1986; Lutke Holzik *et al.*, 2004). Compelling evidence has proven that Y chromosome aberrations, including chromosome aneuploidy and interstitial deletions cause male infertility or subfertility (Reijo *et al.*, 1996; Krausz *et al.*, 2001; McElreavey & Quintana-Murci, 2003). Some genetic polymorphisms of the Y chromosome have been linked to decreased spermatogenesis and it is possible that these polymorphisms may render the carriers more susceptible to develop symptoms of TDS (McElreavey & Quintana-Murci, 2003). Also mutations in the androgen receptor gene have been associated with poor testicular development and function, including hypospadias, subfertility and germ cell neoplasia (Giwercman *et al.*, 2000). Some frequently occurring polymorphisms of the androgen receptor gene have been associated with male genital malformations (Giwercman *et al.*, 2000; Lim *et al.*, 2001; Aschim *et al.*, 2004) and it is possible that they may contribute to the increased risk of testis cancer, although our own study has not found such an association in the Danish population (Frydelund-Larsen *et al.*, 2003).

In addition, there is no doubt that 46, XY pure gonadal dysgenesis and 45,X/46,XY are linked to persistence of gonocytes in the abnormal gonads with risk of development into CIS, gonadoblastoma and even frank germ cell tumour in these rare cases of genetic diseases. It is also well documented that undescended testis in rare cases may be the result of mutations in the *INSL3* gene (Ferlin *et al.*, 2003; Wilson *et al.*, 2004) Thus, there are without question also genetic components in the pathogenesis of all components of TDS, including testis cancer, male infertility, cryptorchidism and hypospadias.

Obesity and other lifestyle factors

Besides possible environmental effects causing dysgenesis of the developing testis there is growing evidence that obesity and sedentary occupation (Andersen *et al.*, 2000; Jensen *et al.*, 2004a; Magnusdottir *et al.*, 2005) may play a role for male infertility. We found evidence that semen quality among over- and underweight young men from the general population was lower than among normal weight men (Jensen *et al.*, 2004a). As there has been an epidemic increase in obesity in the western world during the past decade, obesity may be a contributing explanation to the decline in semen quality.

In addition, modern civilization has changed many other aspects of daily life, which potentially could affect the development and function of the male gonads. One report has suggested that the use of disposable plastic lined nappies can increase the scrotal temperature of baby boys significantly and thereby potentially interfere with

testicular function in adult life. Only well-planned long-term follow-up studies will be able to test this hypothesis (Partsch *et al.*, 2000).

Conclusions

- The decreasing trends in fertility rates in many industrialized countries are now so dramatic that they deserve much more scientific attention.
- Although social and behavioural factors undoubtedly play a major role for these trends, it seems premature, and not based on solid information, to conclude that these trends can be ascribed solely to social and behavioural changes.
- There is evidence to suspect that changing lifestyle and increasing environmental exposures, e.g. to endocrine disrupters, are behind the trends in occurrence of male reproductive health problems, including testis cancer, undescended testis and poor semen quality. These biological factors may also contribute to the low fertility rates.
- However, the necessary research is complex and requires non-traditional collaboration between demographers, epidemiologists, clinicians, biologists, wild life researchers, geneticists and molecular biologists.
- Such a research effort can hardly be carried out without major support from governments and granting agencies making it possible to fund collaborative projects within novel research networks of scientists.

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References

Aitken, R. J., Koopman, P. & Lewis, S. E. (2004) Seeds of concern. *Nature* 432, 48–52.

Almstrup, K., Fernández, M. F., Petersen, J. H., Olea, N., Skakkebæk, N. E. & Leffers, H. (2002) Dual effects of phytoestrogens result in U-shaped dose-response curves. *Environmental Health Perspectives* 110, 743–748.

Almstrup, K., Høi-Hansen, C. E., Wirkner, U., Blake, J., Schwager, C., Ansorge, W., Nielsen, J. E., Skakkebæk, N. E., Rajpert-De Meyts, E. & Leffers, H. (2004) Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. *Cancer Research* 64, 4736–4743.

Andersen, A. N. & Erb, K. (2006) Register data on assisted reproductive technology(ART) in Europe. Including a detailed description of ART in Denmark. *International Journal of Andrology* 29, 12–16.

Andersen, A. G., Jensen, T. K., Carlsen, E., Jørgensen, N., Andersson, A.-M., Krarup, T., Keiding, N. & Skakkebæk, N. E. (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Human Reproduction* 15, 366–372.

Aschim, E. L., Nordenskjold, A., Giwercman, A., Lundin, K. B., Ruhayel, Y., Haugen, T. B., Grotmol, T. & Giwercman, Y. L. (2004) Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. *Journal of Clinical Endocrinology and Metabolism* 89, 5105–5109.

Berthelsen, J. G. (1984) Thesis. Andrological aspects of testicular cancer. *International Journal of Andrology* 7, 451–483.

Boisen, K. A., Kaleva, M., Main, K. M., Virtanen, H. E., Haavisto, A.-M., Schmidt, I. M. *et al.* (2004) Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 363, 1264–1269.

Boisen, K., Chellakooty, M., Schmidt, I., Kai, C., Damgaard, I., Suomi, A. M., Toppari, J., Skakkebæk, N. E. & Main, K. (2005a) Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at three months of age. *Journal of Clinical Endocrinology and Metabolism* 90, 4041–4046.

Boisen, K. A., Chellakooty, M., Schmidt, I. M., Kai, C. M., Damgaard, I. N., Suomi, A.-M., Toppari, J., Skakkebæk, N. E. & Main, K. (2005b) Hypospadias in a cohort of 1072 Danish newborn boys; prevalence and relationship to placental weight, anthropometrical measurements at birth, and pituitary-gonadal hormones at three months of age. *Journal of Clinical Endocrinology and Metabolism* in press.

Calafat, A. M., Slakman, A. R., Silva, M. J., Herbert, A. R. & Needham, L. L. (2004) Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* 805, 49–56.

Carlsen, E., Giwercman, A., Keiding, N. & Skakkebæk, N. E. (1992) Evidence for decreasing quality of semen during past 50 years. *BMJ* 305, 609–613.

Clemmesen, J. (1981) Testis cancer incidence-suggestion of a world pattern. *International Journal of Andrology Supplement* 4, 111–122.

Cornwell, T., Cohick, W. & Raskin, I. (2004) Dietary phytoestrogens and health. *Phytochemistry* 65, 995–1016.

Diczfalusy, E. (1996) The third epoch, the third world and the third millennium. *Contraception* 53, 1–7.

Donovan, P. (1998) Falling teen pregnancy, birth rates: what's behind the declines? *Gutmacher Report on Public Policy* 5, 6–9.

Earl Gray Jr L., Wilson, V. S., Stoker, T., Lambright, C., Furr, J., Noriega, N. *et al.* (2006) Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. *International Journal of Andrology* 29, 96–104.

Ferlin, A., Simonato, M., Bartoloni, L., Rizzo, G., Bettella, A., Dottorini, T., Dallapiccola, B. & Foresta, C. (2003) The

INSL3-LGR8/GREAT ligand-receptor pair in human cryptorchidism. *Journal of Clinical Endocrinology and Metabolism* 88, 4273–4279.

Fisher, J. S., Macpherson, S., Marchetti, N. & Sharpe, R. M. (2003) Human ''testicular dysgenesis syndrome'': a possible model using in-utero exposure of the rat to dibutyl phthalate. *Human Reproduction* 18, 1383–1394.

Fosså, S. D., Aabyholm, T., Vespestad, S., Norman, N. & Ous, S. (1993) Semen quality after treatment for testicular cancer. *European Urology* 23, 172–176.

Foster, P. M. D. (2006) Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. *International Journal of Andrology* 29, 140–147.

Foster, P. M. D., Mylchreest, E., Gaido, K. W. & Sar, M. (2001) Effects of phthalate esters on the developing reproductive tract of male rats. *Human Reproduction Update* 7, 231–235.

Frydelund-Larsen, L., Vogt, P. H., Leffers, H., Schadwinkel, A., Daugaard, G., Skakkebæk, N. E. & Rajpert-De Meyts, E. (2003) No AZF deletion in 160 patients with testicular germ cell neoplasia. *Molecular Human Reproduction* 9, 517–521.

Giwercman, A., von der Maase, H., Rørth, M. & Skakkebæk, N. E. (1993) Semen quality in testicular tumour and CIS in the contralateral testis. *Lancet* 341, 384–385.

Giwercman, A., Kledal, T., Schwartz, M., Giwercman, Y. L., Leffers, H., Zazzi, H., Wedell, A. & Skakkebæk, N. E. (2000) Preserved male fertility despite decreased androgen sensitivity caused by a mutation in the ligand-binding domain of the androgen receptor gene. *Journal of Clinical Endocrinology and Metabolism* 85, 2253–2259.

Gomez, E., Pillon, A., Fenet, H., Rosain, D., Duchesne, M. J., Nicolas, J. C., Balaguer, P. & Casellas, C. (2005) Estrogenic activity of cosmetic components in reporter cell lines: parabens, UV screens, and musks. *Journal of Toxicology and Environmental Health. Part A* 68, 239–251.

Guillette, L. J. Jr (1994) Endocrine-disrupting environmental contaminants and reproduction: lessons from the study of wildlife. In: Women's Health Today: Perspectives on Current Research and Clinical Practice (eds D. R. Popkin & L. J. Peddle), pp. 201–207. Parthenon Publication Group, New York.

Guzick, D. S., Overstreet, J. W., Factor-Litvak, P., Brazil, C. K., Nakajima, S. T., Coutifaris, C. *et al.* (2001) Sperm morphology, motility, and concentration in fertile and infertile men. *New England Journal of Medicine* 345, 1388–1393.

Høi-Hansen, C. E., Holm, M., Rajpert-De Meyts, E. & Skakkebæk, N. E. (2003) Histological evidence of testicular dysgenesis in contralateral biopsies from 218 patients with testicular germ cell cancer. *Journal of Pathology* 200, 370–374.

Høi-Hansen, C. E., Nielsen, J. E., Almstrup, K., Sonne, S. B., Graem, N., Skakkebæk, N. E., Leffers, H. & Rajpert-De Meyts, E. (2004) Transcription factor AP-2 g is a developmentally regulated marker of testicular carcinoma in situ and germ cell tumors. *Clinical Cancer Research* 10, 8521–8530.

Hardell, L., van Bavel, B., Lindstrom, G., Carlberg, M., Dreifaldt, A. C., Wijkstrom, H., Starkhammar, H., Eriksson, M., Hallquist, A. & Kolmert, T. (2003) Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. *Environmental Health Perspectives* 111, 930–934.

Hemminki, K. & Li, X. (2002) Cancer risks in second-generation immigrants to Sweden. *International Journal of Cancer* 99, 229–237.

Hemminki, K., Li, X. & Czene, K. (2002) Cancer risks in first-generation immigrants to Sweden. *International Journal of Cancer* 99, 218–228.

Holm, M., Rajpert-De Meyts, E., Daugaard, G. & Skakkebæk, N. E. (2002) Re: The prevalence of testicular microlithiasis in a asymptomatic population of men 18 to 35 years old (letter). *Journal of Urology* 168, 1108–1109.

Holm, M., Høi-Hansen, C. E., Rajpert-De Meyts, E. & Skakkebæk, N. E. (2003) Increased risk of carcinoma in situ in patients with testicular germ cell cancer with ultrasonic microlithiasis in the contralateral testicle. *Journal of Urology* 170, 1163–1167.

Honecker, F., Stoop, H., De Krijger, R. R., Chris Lau, Y. F., Bokemeyer, C. & Looijenga, L. H. (2004) Pathobiological implications of the expression of markers of testicular carcinoma in situ by fetal germ cells. *Journal of Pathology* 203, 849–857.

Jørgensen, N., Andersen, A.-G., Eustache, F., Irvine, D. S., Suominen, J., Petersen, J. H. *et al.* (2001) Regional differences in semen quality in Europe. *Human Reproduction* 16, 1012–1019.

Jørgensen, N., Carlsen, E., Nermoen, I., Punab, M., Suominen, J., Andersen, A.-G. *et al.* (2002) East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Human Reproduction* 17, 2199–2208.

Jacobsen, R., Bostofte, E., Engholm, G., Hansen, J., Olsen, J. H., Skakkebæk, N. E. & Møller, H. (2000) Risk of testicular cancer in men with abnormal semen characteristics: cohort study. *British Medical Journal* 321, 789–792.

Jensen, T. K., Carlsen, E., Jørgensen, N., Berthelsen, J. G., Keiding, N., Christensen, K., Petersen, J. H., Knudsen, L. B. & Skakkebæk, N. E. (2002) Poor semen quality may contribute to recent decline in fertility rates. *Human Reproduction* 17, 1437–1440.

Jensen, T. K., Andersson, A.-M., Jørgensen, N., Andersen, A.-G., Carlsen, E., Petersen, J. H. & Skakkebæk, N. E. (2004a) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertility and Sterility* 82, 863–870.

Jensen, T. K., Jørgensen, N., Punab, M., Haugen, T. B., Suominen, J., Zilaitiene, B. *et al.* (2004b) Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770

young men from the general population in five European countries. *American Journal of Epidemiology* 159, 49–58.

Jobling, S. & Sumpter, J. P. (1993) Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxicology* 27, 361–372.

Jouannet, P., Wang, C., Eustache, F., Jensen, T. K. & Auger, J. (2001) Semen quality and male reproductive health: the controversy about human sperm concentration decline. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 109, 48–61.

Jørgensen, N., Asklund, C., Carlsen, E. & Skakkbæk, N. E. (2006) Coordinated European investigations of semen quality: results from studies of scandinavian young man is a matter of concern. *International Journal of Andrology* 29, 54–61.

Krausz, C., Quintana-Murci, L., Rajpert-De Meyts, E., Jørgensen, N., Jobling, M. A., Rosser, Z. H., Skakkebæk, N. E. & McElreavey, K. (2001) Identification of a Y chromosome haplogroup associated with reduced sperm counts. *Human Molecular Genetics* 10, 1873–1877.

Lenz, S., Giwercman, A., Skakkebæk, N. E., Bruun, E. & Frimodt-Møller, C. (1987) Ultrasound in detection of early neoplasia of the testis. *International Journal of Andrology* 10, 187–190.

Lim, H. N., Nixon, R. M., Chen, H., Hughes, I. A. & Hawkins, J. R. (2001) Evidence that longer androgen receptor polyglutamine repeats are a causal factor for genital abnormalities. *Journal of Clinical Endocrinology and Metabolism* 86, 3207–3210.

Looijenga, L. H., Stoop, H., de Leeuw, H. P., Gouveia Brazao, C. A., Gillis, A. J., van Roozendaal, K. E. *et al.* (2003) POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Research* 63, 2244–2250.

Lottrup, G., Andersson, A.-M., Leffers, H., Mortensen, G. K., Toppari, J., Skakkebæk, N. E. *et al.* (2006) Possible impact of phthalates on infant reproductive health. *International Journal of Andrology* 29, 172–180.

Lutke Holzik, M. F., Rapley, E. A., Hoekstra, H. J., Sleijfer, D. T., Nolte, I. M. & Sijmons, R. H. (2004) Genetic predisposition to testicular germ-cell tumours. *Lancet Oncology* 5, 363–371.

Lutz, W., O'Neill, B. C. & Scherbov, S. (2003) Demographics. Europe's population at a turning point. *Science* 299, 1991–1992.

Mahood, I. K., Mckinnell, C., Walker, M., Hallmark, N., Scott, H., Fisher, J. S. *et al.* (2006) Cellular orgins of testicular dysgenesis in rats exposed in utero to di(*n*-butyl) phthalate. *International Journal of Andrology* 29, 148–154.

Møller, H. (1989) Decreased testicular cancer tisk in men born in wartime. *Journal of the National Cancer Institute* 81, 1668–1669.

Møller, H. (1993) Clues to the aetiology of testicular germ cell tumours from descriptive epidemiology. *European Urology* 23, 8–15.

Møller, H. & Skakkebæk, N. E. (1999) Risk of testicular cancer in subfertile men: case-control study. *British Medical Journal* 318, 559–562.

Magnusdottir, E. V., Thorsteinsson, T., Thorsteinsdottir, S., Heimisdottir, M. & Olafsdottir, K. (2005) Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Human Reproduction* 20, 208–215.

McElreavey, K. & Quintana-Murci, L. (2003) Y chromosome haplogroups: a correlation with testicular dysgenesis syndrome? *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 111, 106–113.

Morohoshi, K., Yamamoto, H., Kamata, R., Shiraishi, F., Koda, T. & Morita, M. (2005) Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by in vitro assays. *Toxicology In Vitro* 19, 457–469.

Mortensen, G. K., Main, K. M., Andersson, A.-M., Leffers, H. & Skakkebæk, N. E. (2005) Determination of phthalate monoesters in human breast milk, consumer milk and infant formula by tandem mass spectrometry(LC/MC/MS). *Analytical Bioanalytical Chemistry* 382, 1084–1092.

Oishi, S. (2002) Effects of propyl paraben on the male reproductive system. *Food and Chemical Toxicology* 40, 1807–1813.

Partsch, C. J., Aukamp, M. & Sippell, W. G. (2000) Scrotal temperature is increased in disposable plastic lined nappies. *Archives of Disease in Childhood* 83, 364–368.

Petersen, P. M., Giwercman, A., Hansen, S. W., Berthelsen, J. G., Daugaard, G., Rørth, M. & Skakkebæk, N. E. (1999) Impaired testicular function in patients with carcinoma in situ of the testis. *Journal of Clinical Oncology* 17, 173–179.

Purdue, M. P., Devesa, S. S., Sigurdson, A. J. & McGlynn, K. A. (2005) International patterns and trends in testis cancer incidence. *International Journal of Cancer* 115, 822–827.

Rajpert-De Meyts, E. & Skakkebæk, N. E. (1994) Expression of the c-kit protein product in carcinoma-in-situ and invasive testicular germ cell tumours. *International Journal of Andrology* 17, 85–92.

Rajpert-De Meyts, E. & Skakkebæk, N. E. (1999) Testicular microlithiasis and carcinoma in situ. *Urology* 53, 455–456.

Rajpert-De Meyts, E., Jørgensen, N., Müller, J., Giwercman, A. & Skakkebæk, N. E. (1996) Origin of germ cell tumours. In: Sex Differentiation: Clinical and Biological Aspects (eds I. A. Hughes), pp. 45–54. Frontiers in Endocrinology, Serono Symposia Publications, Rome.

Rajpert-De Meyts, E., Giwercman, A. & Skakkebæk, N. E. (1999) Carcinoma in situ of the testis: a precursor of testicular germ cell cancer: biological and clinical aspects. In: Comprehensive Textbook of Genitourinary Oncology (eds N. J. Vogelzang, P. T. Scardino, W. U. Shipley, D. S. Coffey & B. J. Miles), pp. 897–908. Lippincott Williams & Wilkins, Philadelphia, PA.

Ratcliffe, J. M., Gladen, B. C., Wilcox, A. J. & Herbst, A. L. (1992) Does early exposure to maternal smoking affect future fertility in adult males? *Reproductive Toxicology* 6, 297–307.

Reijo, R., Alagappan, R. K., Patrizio, P. & Page, D. C. (1996) Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet* 347, 1290–1293.

Richiardi, L., Bellocco, R., Adami, H. O., Torrang, A., Barlow, L., Hakulinen, T. *et al.* (2004) Testicular cancer incidence in eight northern European countries: secular and recent trends. *Cancer Epidemiology, Biomarkers and Prevention* 13, 2157–2166.

Rosenfield, A. & Schwartz, K. (2005) Population and development – shifting paradigms, setting goals. *New England Journal of Medicine* 352, 647–649.

Schlumpf, M., Schmid, P., Durrer, S., Conscience, M., Maerkel, K., Henseler, M. *et al.* (2004) Endocrine activity and developmental toxicity of cosmetic UV filters–an update. *Toxicology* 205, 113–122.

Scully, R. E. (1953) Gonadoblastoma. A gonadal tumor related to the dysgerminoma (seminoma) and capable of sex-hormone production. *Cancer* 6, 455–563.

Scully, R. E. (1970) Gonadoblastoma. A review of 74 cases. *Cancer* 25, 1340–1356.

Sharpe, R. M. & Skakkebæk, N. E. (2003) Male reproductive disorders and the role of endocrine disruption: advances in understanding and identification of areas for future research. *Pure and Applied Chemistry* 75, 2023–2038.

Skakkebæk, N. E., Berthelsen, J. G., Giwercman, A. & Müller, J. (1987) Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *International Journal of Andrology* 10, 19–28.

Skakkebæk, N. E., Giwercman, A. & De Kretser, D. (1994) Pathogenesis and management of male infertility. *Lancet* 343, 1473–1479.

Skakkebæk, N. E., Rajpert-De Meyts, E. & Main, K. M. (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction* 16, 972–978.

Storgaard, L., Bonde, J. P., Ernst, E., Spanò, M., Andersen, C. Y., Frydenberg, M. & Olsen, J. (2003) Does smoking during pregnancy affect sons' sperm counts? *Epidemiology* 14, 278–286.

Suzuki, T., Kitamura, S., Khota, R., Sugihara, K., Fujimoto, N. & Ohta, S. (2005) Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. *Toxicology and Applied Pharmacology* 203, 9–17.

Toppari, J., Larsen, J. C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L. J. Jr *et al.* (1996) Male reproductive health and environmental xenoestrogens. *Environmental Health Perspectives* 104, 741–803.

Toppari, J., Kaleva, M. & Virtanen, H. E. (2001) Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Human Reproduction Update* 7, 282–286.

Vinggaard, A. M., Hass, U., Dalgaard, M., Andersen, H. R., bonefeld-Jørgensen, E., Christiansen, S. *et al.* (2006) Prochloraz: an imidazole fungicide with multiple mechanisms of action. *International Journal of Andrology* 29, 186–192.

Wada, H., Tarumi, H., Imazato, S., Narimatsu, M. & Ebisu, S. (2004) In vitro estrogenicity of resin composites. *Journal of Dental Research* 83, 222–226.

Weir, H. K., Marrett, L. D., Kreiger, N., Darlington, G. A. & Sugar, L. (2000) Pre-natal and peri-natal exposures and risk of testicular germ-cell cancer. *International Journal of Cancer* 87, 438–443.

Weissbach, L. & Widmann, T. (1986) Familial tumor of the testis. *European Urology* 12, 104–108.

Wilson, V. S., Lambright, C., Furr, J., Ostby, J., Wood, C., Held, G. & Earl Gray, L Jr. (2004) Phthalate ester induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. *Toxicology Letters* 146, 207–215.

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