Symposium: Endocrinology of male reproduction and infertility

Influence of endocrine disruptors on human male fertility

Yvonne Lundberg Giwercman defended her thesis ‘Studies of androgen receptor gene mutations in patients phenotypically ranging from complete androgen insensitivity to men with preserved fertility’ at the Department of Clinical Genetics, Karolinska Institute, Stockholm, Sweden in 2000 and since 2005 has been Associate Professor in Experimental Urology at Lund University. Her current research includes molecular studies on gene-environment interaction in order to understand cellular responses under physiological conditions as well as in disease. Of special interest is the impact of sex steroid action on the aetiology and pathogenesis of disorders of the male genital tract.

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Abstract

It has been suggested that during the past five decades human sperm counts have declined and the incidence of testicular cancer, hypospadias and cryptorchidism has increased. Furthermore, geographical differences, with respect to these markers of male reproductive function, have been reported. According to a recent hypothesis, all these abnormalities of the male genital system do have a common cause, namely exposure to endocrine disruptors affecting the male in early fetal life. Reduced sperm production as well as congenital abnormalities of male genitalia can be evoked in laboratory animals by exposing them to chemicals with endocrine-disrupting effect, and in humans similar effects have been seen following accidental exposures to very high concentrations of these environmental toxicants. However, the evidence for association between levels of exposure found in the general population and serious adverse effects on male reproduction, including fertility, is still lacking. A recent European Union-supported study, on the effect of persistent organohalogen pollutants on human reproduction, failed to show any correlation between post-natal exposure levels and fertility. Future studies will reveal whether prenatal exposure does more strongly affect male fertility and whether genetic predisposition regulates the susceptibility of an individual to the adverse effects of endocrine disruptors.

Keywords: endocrine disruptors, gene–environment interaction, male fertility, persistent organohalogen pollutants

Introduction

In 1977, Whorton and colleagues reported on azoospermia or oligozoospermia in 14 of 24 men occupationally exposed to the chemical 1,2-dibromo-3-chloropropane (Whorton et al., 1977). However, most other later attempts to link workplace exposure to chemicals with such dramatic effects on male fertility have more or less failed. The discussion regarding a potentially negative impact of environment on male reproductive function was renewed following a meta-analysis by Carlsen and co-workers (Carlsen et al., 1992). This paper, indicating an almost 50% reduction in sperm counts during the period 1940–1990, has been subject to discussion, criticism and several re-analyses, some of them being in support of its original conclusions Swan et al., 1997, 2000), whereas others have claimed no time-related trend in sperm numbers (Olsen et al., 1995). Several other papers have subsequently analysed other sets of data with regard to time-related changes in sperm concentration, some concluding no such trend (Fisch et al., 1996; Vierula et al., 1996) and others confirming the finding of Carlsen and colleagues (Auger et al., 1995; Irvine et al., 1996).

Whereas the question of falling sperm counts was, and still is, debatable, there are solid data showing that the incidence of testicular germ cell cancer (TGCC), which can also be
considered as a male reproductive organ failure, has rapidly increased during recent decades (Richiardi et al., 2004). Increasing prevalence of congenital abnormalities such as cryptorchidism and hypospadias has also been indicated in several studies (Czeizel et al., 1986; John Radcliffe Hospital Cryptorchidism Study Group, 1986; Boisen et al., 2004).

Because these changes have occurred over a relatively short time span, it could be argued that they only reflect adverse changes in the environment or in lifestyle. However, it cannot be excluded that some individuals may be more susceptible or, in contrast, more resistant to these adverse effects than others, indicating that genetic factors also play a role. In this context, the possible adverse effects of environmental chemicals acting as endocrine disruptor compounds (EDC) have been brought into focus, both regarding their effects on the reproductive system and with respect to differential susceptibility to these compounds (Toppari et al., 1996). Concomitantly, the discussion regarding male reproductive function impairment has switched from time-related trends to geographic differences. In recent reports, significant discrepancies in semen quality and in the incidence of other abnormalities of male reproductive organs have been found between socially and geographically closely related areas, thereby adding lifestyle as another actor on the stage of male reproductive dysfunction (Richthoff et al., 2002; Boisen et al., 2004; Jensen et al., 2004; Richiardi et al., 2004).

This article is a summary of the current information on epidemiological trends in male reproductive function, with a focus on fertility and semen quality, and considers the extent to which such trends might be related to EDC exposure.

Epidemiological trends in male reproductive function

Time-related trends

Whereas there is no doubt that an increase in the incidence of TGCC has taken place, at least among Caucasians, there is not sufficient evidence yet to support or dispute the hypothesis of a general deterioration of male reproductive function. With respect to the two most common congenital malformations of male genital organs, cryptorchidism and hypospadias, the diagnostic criteria are not very well-defined and it may, therefore, be difficult to compare data collected over time and at different locations. Semen quality data are based on retrospectively collected materials. In these materials, lack of quality control and risk of discrepancy in selection bias when cohorts included in different centres and at different time points have been compared were used as arguments by the opponents of the hypothesis of a general deterioration of male reproductive function (Olsen et al., 1995). Prospective studies of semen quality in well-defined groups of individuals (military conscripts) have been initiated in Denmark (Andersen et al., 2000; Jorgensen et al., 2002), but it may take years, or even decades, before the issue of a possible decline in sperm counts can be clarified.

Geographical differences

While the issue of a possible time-related trend in male reproductive function remains unresolved, recent research has thrown light on significant geographical differences. The most extensive studies have been performed as a joint venture between research groups in Denmark and in Finland, comparing epidemiological trends in male reproductive disorders between these two countries.

The starting point for performing comparative studies between Finland and Denmark was cancer register data showing that the incidence of TGCC was five times higher among Danish men compared with Finnish men (Richiardi et al., 2004). Studies on semen quality have revealed significantly higher sperm counts in Finland compared with Denmark, regardless of whether proven fertile men (Jorgensen et al., 2001) or military conscripts (Jorgensen et al., 2002) were included. However, despite the differences in sperm numbers found between the fertile men from these two countries, there was no discrepancy with regard to time-to-pregnancy (Jensen et al., 2001), indicating that fertility may be a less sensitive marker of dysfunctional male reproductive function.

Semen studies were followed by assessments of the incidence of congenital abnormalities of male genital organs, showing the same pattern as for TGCC, i.e. higher incidence of cryptorchidism and hypospadias in Danish newborns as compared with their Finnish counterparts (Boisen et al., 2004, 2005). Thus, when comparing male reproductive function between the two geographically and socially closely related countries, a picture emerges showing fewer abnormalities, less TGCC, and better male reproductive function in Finland as compared with Denmark.

However, other countries in the Nordic–Baltic area are also affected by disorders in the genital tract to varying degrees. With respect to TGCC incidence, Norway has reached the Danish level, with these two countries now holding a world leading position, whereas the Baltic states Estonia, Latvia and Lithuania (Richiardi et al., 2004) are at the same low level as Finland (Punab et al., 2002; Tsarev et al., 2005). Sweden is taking an intermediate position with an incidence of 50% of that of Denmark (Richthoff et al., 2002). Interestingly, merging sperm-count data from different studies based on military conscripts gave a very similar picture: the sperm count being as low in Norway as in Denmark (Jorgensen et al., 2002), high in the Baltic countries (Punab et al., 2002; Tsarev et al., 2005), and in Sweden between these two extremes (Richthoff et al., 2002) (Figure 1).

In the context of endocrine disruption and its possible impact on male reproductive function, so far there is no evidence that the above-mentioned differences might be related to variation in exposure to EDC or other environmental toxicants. Although one could argue that men in the Baltic countries and Finland may genetically differ from those in the three other Nordic countries (Rosser et al., 2000), there is no reason to believe that the lower incidence of TGCC and higher sperm count in Sweden, as compared with Norway and Denmark, should be genetically determined. Therefore, it seems more likely that some environmental or lifestyle related factors

Figure 1
are involved in the variation of male reproductive function found in the Nordic–Baltic area, but whether they are related to EDC exposure or not remains to be elucidated.

Differences in sperm counts have not only been seen in comparisons between different countries, but even when looking at cohorts of the same nationality. Thus, significant differences in number of spermatozoa were reported in studies performed within France and the USA (Auger and Jouannet, 1997; Swan et al., 2003a). These reports pointed at lifestyle and environment as important players in regulation of male reproductive function, without defining any specific factor as causative of poor semen quality, TGCC, or male genital abnormalities. In the USA, sperm concentration and motility were significantly reduced in Missouri compared with New York, Minneapolis and Los Angeles, and it was postulated that hampered sperm parameters in Missouri might be related to agricultural pesticides that are commonly used in the Midwest (Swan et al., 2003b).

Testicular dysgenesis syndrome

The above-mentioned studies not only showed common epidemiological trends for congenital abnormalities of male genital organs as well as semen quality and risk of testicular cancer, but also indicated that TGCC and low sperm counts may share aetiology and be the result of factors already operating during fetal life. Based on these observations and on clinical evidence linking cryptorchidism, hypospadias, poor semen quality and testicular malignancy together, Skakkebæk and co-workers introduced the concept of testicular dysgenesis syndrome (TDS), suggesting that poor semen quality, testis cancer, cryptorchidism and hypospadias are symptoms of a common underlying entity (Skakkebæk et al., 2001). TDS was suggested to be a result of disruption of embryonal programming and gonadal development during fetal life and it was concluded that ‘…the aetiological impact of adverse environmental factors such as hormone disrupters, probably acting upon a susceptible genetic background, must be considered’. One of the implications of the concept is that in future human and experimental studies, any of the TDS components might be used as a general marker for the male reproductive system. Furthermore, it clearly pointed to the fetal period as the critical time window for abnormalities of the male reproductive organs and to the importance of genetic susceptibility.

Endocrine disruptors

A large number of environmental compounds have been shown to mimic the actions of hormones such as oestrogens, anti-oestrogens, androgens and anti-androgens (Kelce and Wilson, 1997; Sohoni and Sumpter, 1998). The term EDC has been widely used to describe chemicals possessing any of these hormone-like actions. Persistent organohalogen pollutants (POP) are an important group of EDC compounds, consisting of accidental by-products from various chemicals and combustion processes, such as polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDF), as well as manufactured compounds, such as polychlorinated biphenyls (PCB) used for example in electronic equipment as well as the insecticide 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). These compounds are persistent to both abiotic and biotic degradation and accumulate in the food chain (Brouwer et al., 1995). The main human exposure to POP occurs through diet of animal origin. Another important group of compounds are phthalates, which have been used as additives in industrial products since the 1930s (Latini et al., 2006). Accordingly, phthalates are universally considered to be ubiquitous environmental contaminants. The present paper focuses on these two groups of compounds, POP and phthalates, but there are also a number of other EDC candidates, such as different

Figure 1. Relative sperm counts and incidence of testicular cancer in military conscripts from Denmark, Norway, Sweden and Finland. The levels in Denmark are set to 100%. The figure shows a high incidence of testicular cancer, and corresponding low sperm counts, in Denmark and Norway. Sweden occupies an intermediate position (Jorgensen et al., 2002; Punab et al., 2002; Richthoff et al., 2002; Tsarev et al., 2005).
herbicides and fungicides, and other industrial chemicals such as bisphenol A (Chalubinski and Kowalski, 2006). Examples of different chemical compounds considered as EDC are presented in Table 1.

**Cellular mechanisms of action of endocrine disruptors**

There is growing evidence that environmental exposure to some EDC may result in disruption of endocrine systems in human and wildlife populations (Sanderson, 2006). Those of concern are primarily compounds that interfere with the actions of endogenous hormones in the body (Table 2). Given the complexity of endocrine systems, there are many ways in which EDC can affect the body’s signalling system, and this makes the mechanisms of action of these chemicals difficult to unravel. A major concern is that some of these EDC appear to be biologically active at extremely low concentrations. Hence, the guiding principle of traditional toxicology that ‘the dose makes the poison’ may not always be the case, because some EDC do not induce the classical dose–response relationships.

It has been shown that some EDC can bind to sex-hormone receptors and mimic, agonise or antagonise their effects. Crosstalk has been observed among the aryl hydrocarbon receptor (AHR), which is involved in xenobiotic metabolism and in mediating the toxic effects of dioxin-like compounds, and other nuclear receptors (Matthews and Gustafsson, 2006). The transcriptional activity of the androgen receptor (AR) was recently shown to be modulated by association with activated AHR (Kollara and Brown, 2006), but the interaction between AHR and other receptors has been most well-studied with respect to oestrogen-receptor (ER) signalling.

Oestrogens regulate processes such as development and function of the reproductive system, maintenance of bone mass and protection against cardiovascular disease in both males and females. The oestrogenic effects are mediated by ER, of which two variants, α and β, are present. The ER and the AHR are ligand-activated transcription factors and members of the nuclear receptor and the bHLH–PAS–ARNT–SIM (basic helix–loop–helix Period)–ARNT (AHR nuclear translocator)–SIM (single minded) superfamilies, respectively. Upon ligand binding, the AHR translocates from the cytoplasm to the nucleus where it binds to its dimerization partner, ARNT. The activated AHR/ARNT heterodimer complex binds to DNA sequences, termed xenobiotic response elements, and activates the expression of AHR target genes, such as cytochrome P4501A1 (CYP1A1) and CYP1B1. Different AHR agonists, including 3-methylcholanthrene and dioxin, modulate oestrogen-dependent ER-transactivation through the association of activated AHR/ARNT with ER (Brunnberg et al., 2003) (Figure 2). A novel AHR/ER interaction was recently suggested, implying a redirection of ER from ER target genes to AHR target genes, whereby AHR could regulate ER protein concentrations and consequently oestrogenic responses (Matthews et al., 2006).

It cannot be excluded that AR-mediated responses also can be regulated in a similar manner, or by the recruitment of co-activators. In the absence of a ligand, the AR is present in a non-activated form, together with co-repressors that inhibit constitutive transcriptional activity. Binding of agonists induces release of associated proteins and subsequent interaction with co-activators. Of these, nuclear receptor co-activator 4, also known as AR-associated protein 70, was demonstrated to interact with and amplify AHR action (Kollara and Brown, 2006). However, in those experiments, dihydrotestosterone treatment abolished the activity of AHR, consistent with the possibility that activated AR may effectively compete with AHR for transcription factors and modulators, other than nuclear receptor co-activator.

**Animal studies on EDC**

Exposure during gestational as well as neonatal periods to single dioxin congeners has been shown to adversely affect sperm morphology and sperm production and fertility in male rats and mice (Smits-van Prooije et al., 1996; Faqi et al., 1998; Huang et al., 1998). Also exposures of non-dioxin-like PCB have resulted in a decrease in sperm motility and sperm capability to penetrate hamster oocytes in adult rats (Hsu et al., 2003). Moreover, studies on male rats have indicated that 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE) has strong anti-androgenic properties that result in reduced sperm counts (Kelce et al., 1995). Thus, in animal studies, a number of different POP have clearly shown that they might affect semen quality and male fertility (Table 2).

Based on animal studies, there is mounting evidence that phthalates might also negatively affect male fertility. Most studies have focused on pre- or perinatal exposure, but there is also some evidence that pubertal and adult exposure to phthalates might be of importance (Parmar et al., 1986; Srivastava et al., 1990). Compared with sexually mature animals, pubertal animals have been shown to be more sensitive to phthalates (Dostal et al., 1988; Higuchi et al., 2003). Moreover, exposure to phthalates, consistent with the anti-androgenic mechanism, resulted in an increased proportion of malformations such as hypospadias and reduced sperm production, as well as histological testicular changes indicative of testicular dysgenesis in these animals (Gray et al., 2000; Foster et al., 2001; Fisher et al., 2003). Finally, recent studies on rats have reported significant reductions in anogenital distance after prenatal exposure to different phthalates (Gray et al., 2000; Barlow and Foster, 2003; Tyl et al., 2004).

However, although animal studies have shown negative effects of EDC exposure on male reproductive function, including fertility, exposure levels far above those found in humans have been needed in general to evoke reproductive toxicity of these compounds. On the other hand, experimental studies to date have mainly been based on exposure to a single compound, not mimicking the multi-agent exposure of humans.

**Human studies on EDC**

The main focus regarding EDC and semen quality has been on POP exposure. An accidental episode in 1979 in Taiwan (Yucheng) where rice oil was contaminated resulted in extremely high exposures to PCB and PCDF. Small studies within the ‘Yucheng population’ showed that in utero as well as post-natal exposure increased the proportion of morphologically abnormal sperm and decreased the capacity of oocyte penetration.
Table 1. Chemical categories and subcategories of compounds acting as endocrine disruptors (adapted from Botham and Holmes, 2005).

<table>
<thead>
<tr>
<th>General anthropogenic chemicals</th>
<th>Biogenic substances</th>
<th>Biocides and derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols and glycols</td>
<td>Anthraquinones</td>
<td>Carbamates</td>
</tr>
<tr>
<td>Aromatic hydrocarbons</td>
<td>Flavanones</td>
<td>Fungicides</td>
</tr>
<tr>
<td>Anilines and derivatives</td>
<td>Isoflavonoid compounds</td>
<td>Organochlorines</td>
</tr>
<tr>
<td>Benzene and derivatives</td>
<td>Lignans</td>
<td>Organophosphates</td>
</tr>
<tr>
<td>Benzophenones and derivatives</td>
<td>Phenolic acids</td>
<td>Pyrethroids</td>
</tr>
<tr>
<td>Biphenyls and metabolites</td>
<td>Plant-derived</td>
<td>Miscellaneous</td>
</tr>
<tr>
<td>Dioxins and metabolites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furans and metabolites</td>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Naphthalens and naphthalenes</td>
<td>Miscellaneous</td>
<td></td>
</tr>
<tr>
<td>Phenols and derivatives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siloxanes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Styrene and derivatives</td>
<td></td>
<td></td>
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<tr>
<td>Miscellaneous</td>
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</table>

General anthropogenic chemicals: man-made chemicals (excluding biocides, pharmaceuticals, consumer products, and inorganic compounds and organometallic complexes) used, for example, for industrial purposes (as intermediates), are waste products from disposal or use of industrial chemicals (e.g. dioxins), or are metabolites or degradation products of industrial chemicals.

Biogenic substances: substances occurring naturally in the environment that are derived from plants (including lignans, produced by the digestion of plant material) or as fungal metabolites. Natural steroids from animals are also listed.

Biocides: insecticides, herbicides and fungicides.

Table 2. Examples of chemicals (and their mechanisms of action) shown to possess reproductive toxicity in animal studies, following pre- or perinatal exposure (adapted from International Programme on Chemical Safety, 2002).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol 17-β</td>
<td>ER agonist</td>
</tr>
<tr>
<td>17α-ethinyl oestradiol</td>
<td>ER agonist</td>
</tr>
<tr>
<td>DES</td>
<td>ER agonist</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Weak ER agonist</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>Weak ER agonist</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>Metabolite is ER agonist, AR antagonist</td>
</tr>
<tr>
<td>DDT</td>
<td>ER agonist</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>Weak AR antagonist</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>Metabolites AR antagonists</td>
</tr>
<tr>
<td>Procymidone</td>
<td>AR antagonist</td>
</tr>
<tr>
<td>Linuron</td>
<td>AR antagonist</td>
</tr>
<tr>
<td>Dibutyl phthalate</td>
<td>Reduced synthesis of testosterone in fetal testis</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
<td>AHR agonist</td>
</tr>
</tbody>
</table>

ER = oestrogen receptor; AR = androgen receptor; AHR = arylhydrocarbon receptor; p,p'-DDE = 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene; DDT = 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane; DES = diethylstilbestrol; 2,3,7,8-TCDD = 2,3,7,8-tetrachlorodibenzodioxin.
(Guo et al., 2000; Hsu et al., 2003). In addition, in-utero exposure to PCB/PCDF also resulted in decreased sperm motility (Guo et al., 2000). Although not that clear, there are a number of examples where background exposure to POP has been negatively associated with sperm motility (Dallinga et al., 2002; Hauser et al., 2003; Richthoff et al., 2003; Aneck-Hahn et al., 2007). In some epidemiological studies sperm concentration has also been shown to be negatively affected by relatively low POP exposure (Bush et al., 1986; Ayotte et al., 2001; Dallinga et al., 2002).

From 2002 to 2006, a European Union-financed project under the acronym INUENDO was conducted, with the objective of identifying and characterizing the impact of dietary pollutants on human fertility and providing epidemiological evidence on possible health impacts of environmental exposure to xenobiotics with hormone-like actions (see http://www.inuendo.dk). The project had as its specific objective the study of fertility in populations with high or low POP exposure, such as the Greenland Inuit (n = 258), with the highest body burdens of POP in the world; Swedish fishermen from the east coast as well as from the west coast (n = 184), the former consuming highly POP-polluted Baltic fish; Ukrainians (n = 194), who are mostly exposed by use of pesticides; and a Polish population representing a group supposed not to be exposed to high POP concentrations. POP have for many years been a source of worry regarding interference with normal hormonal release and effect, and many of them have also been shown to have oestrogenic, androgenic or anti-androgenic effects. The chemicals 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and p,p'-DDE were used as biomarkers for POP exposure.

The most consistent finding was a decrease in progressive sperm motility with increasing CB-153 serum concentration in all regions (Toft et al., 2006). Within the Caucasian populations, but not in the Inuit men, a strong CB-153-related effect on sperm chromatin integrity was seen (Spano et al., 2005). Statistically significant, but weak, positive association was seen between the p,p'-DDE concentrations and serum FSH. However, POP exposure level was not related to sperm concentration or morphology (Toft et al., 2006). Fecundity measured by time-to-pregnancy in couples that eventually conceived was not related to CB-153 among Caucasians, but among Inuit the fecundity was reduced among intermediately and highly exposed men compared with less exposed, although no obvious exposure–response relations were found and findings were of borderline significance (Axmon et al., 2006). However, it should be kept in mind that this study only addressed the issue of post-natal

**Figure 2.** Androgen receptor (AR) and aryl hydrocarbon receptor (AHR) signalling in the cell, with competition of co-factors in the presence of androgen. AHRR = aryl hydrocarbon receptor repressor; ARE = androgen response element; DBD = DNA-binding domain; DHT = dihydrotestosterone; HSP = heat shock protein; LBD = ligand-binding domain; NTD = N-terminal domain; P = phosphate; RNAP II = RNA polymerase II; T = testosterone; TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin; XRE = xenobiotic response element.
exposure, whereas, at least according to the TDS hypothesis, the fetal period represents the critical time-window for deleterious effects of EDC on male reproductive function.

The proportion of male births is fairly constant throughout the world at about 51.4% (James, 1996). However, two populations with extreme accidental exposures to POP both showed a lower proportion of male offspring among men heavily exposed before the age of 20 (Mocarelli et al., 2000; del Rio Gomez et al., 2002). On the other hand, in populations exposed to moderate levels of POP, both increased (Karmaus et al., 2002) and decreased (Rylander et al., 1995; Weisskopf et al., 2003) male-to-female ratios have been reported. The underlying mechanisms of the effect of POP exposure on offspring sex ratio are not yet known, but could be due to skewed production of Y-chromosome-bearing spermatozoa. However, among Swedish fishermen a positive correlation between POP levels and the proportion of Y-chromosome-bearing spermatozoa was reported (Tiido et al., 2005).

To date, there have been only a limited number of studies that have investigated potential associations between phthalates and sperm function. Negative associations have been observed between concentrations of different phthalates and sperm motility as well as sperm concentration (Duty et al., 2004; Jonsson et al., 2005; Hauser et al., 2006), but the pattern is far from consistent. In a recent publication, an association between urinary levels of phthalate monoesters and DNA damage in human spermatozoa was observed (Hauser et al., 2007). Moreover, an interesting finding deserving further attention was the interaction between phthalate and PCB exposure in relation to sperm motility (Hauser, 2005). This finding could, however, not be confirmed in another study population (Jonsson et al., 2005). In a study from the USA, the anogenital distance was decreased among male infants with prenatal phthalate exposure (Swan, 2005).

**Gene environment interaction – human evidence**

For many human disorders and diseases, the simple partitioning of variance into environmental and genetic causes is not applicable. In most cases, parents give their children both their genes and their environment. This heritability is essential and leads to the question: how many of the differences in disorders of the male reproductive tract between people are caused by their genetic differences, and how many by their different environments and lifestyles? In this context, polymorphisms in genes can lead to differences in the susceptibility of individuals to potentially adverse effects of environmental influences, such as chemical exposure, on prenatal development or male or female reproductive function.

Spermatogenesis is an androgen-dependent process, requiring high intra-testicular hormone concentrations as well as adequate AR function. The AR gene contains two polymorphic sequences: a glutamine repeat encoded by (CAG), and a glycine repeat encoded by (GGT), (GGG)(GGT), (GGC), commonly referred to as the CAG and the GGN repeat, respectively. As a part of the INUENDO study (see above), the impact of polymorphisms in the AR gene on the association between POP exposure and male reproductive function was investigated.

In all INUENDO cohorts the CAG repeat was normally distributed, varying between 10 and 30 repeats (Giwer cman et al., 2007). A direct association between the CAG number and sperm count was not found, but the polymorphic repeats were investigated regarding their ability to modify the effect of POP exposure on human sperm characteristics in the INUENDO cohorts. The following semen characteristics were determined: volume, sperm concentration, total count, proportion of progressively motile, and morphology. A statistically significant interaction was found between CB-153 and the CAG repeat category in relation to sperm concentration and total sperm count (P = 0.03 and 0.01, respectively). For CAG <20, sperm concentration and total sperm count were 35 and 42% lower, respectively, when the group with CB-153 exposure above the median was compared with that below the median (Figure 3; Giwer cman et al., 2007). Interestingly, the impact of CB-153 exposure on sperm motility was also restricted to the subjects with the shortest CAG lengths and the same was true for the association between p,p′-DDE concentrations and sperm DNA integrity. The study indicated that the AR CAG repeat length might modify the susceptibility of an individual to the adverse effects of POP exposure on semen quality.

**Future lines of research**

During the past 10–15 years a lot of attention has been paid to possible links between EDC exposure and impairment of male reproductive function. New research, within a previously...
more-or-less neglected area of science, has been initiated, although final proof of negative time-related trends in male fertility and the possible role of exposure to EDC in the proposed deterioration is still lacking. However, taking the serious consequences of environmentally related impairment of reproduction into consideration, any warning signals should be taken seriously and a high priority given to this area of research. Based on the TDS hypothesis, future research should focus on two-generation studies linking measurement of fetal exposure to post-natal reproductive outcomes such as semen quality, cryptorchidism, hypospadias and TGCC. Interpretation of human studies is hampered by numerous confounding factors such as mixed exposure, impact of lifestyle and genetic heterogeneity. Such studies therefore need to be backed-up by animal experiments as well as in-vitro studies, which may provide a clue to understanding the mechanisms behind possible adverse effects of EDC exposure. Furthermore, since in animal studies relatively large doses of toxicants need to be used in order to evoke adverse reproductive effects, it needs to be investigated whether mixed exposures at human-relevant doses can, by synergistic action, lead to negative effects on male genital organs.

Investigation of gene-environment interactions may help to discern subjects who are susceptible to negative effects of environmental chemicals, and also to understand the mechanisms behind their harmful action and male infertility.

Finally, although EDC may have serious harmful effects on male fertility, we should not neglect the impact of already well-known lifestyle-related factors such as maternal smoking during pregnancy, which could well be one of the most harmful actions to which the developing and growing fetus is subjected. With respect to the developing reproductive system, maternal smoking has been linked to reduced sperm counts in male offspring in several studies (Jensen et al., 2004, 2005).

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