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Estrogens and phytoestrogens in male infertility

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Purpose of review
A time-related negative trend in male reproductive function has been suggested. It has been hypothesized that this is due to exposure to chemicals interfering with the action of sex hormones. Also a negative effect of phytoestrogens on male fertility has been postulated. This review aimed to review the epidemiological evidence of deteriorating male reproductive function and summarize the most recent literature on exposure to endocrine disrupters and phytoestrogens in relation to male fertility and/or semen quality.

Recent findings
There is no doubt that the incidence of testicular cancer has increased through the past 50 years, a decline in sperm counts, if any, may have leveled off during the past decade. There are some reports indicating negative association between exposure to certain chemicals and sperm parameters such evidence has not been found for phytoestrogens. The majority of these studies have been limited to assessing postnatal exposure.

Summary
Although possible negative impact of industrial chemicals and male fertility is an important issue on the research agenda, so far, it has no clinical implications. The future research should focus on looking at the impact of low dose exposure to a mixture of chemicals, two generation studies and gene-environment interaction.

Keywords
endocrine disruptors, male fertility, male reproductive function, phytoestrogens, semen quality

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Introduction

Androgens are traditionally considered as the male sex hormones whereas estrogens as the female sex hormones. However in both sexes, the androgens are metabolized to estrogens and there is an increasing evidence indicating that both types of hormones play an important role for regulation of physiological functions in both sexes. It has, however, been suggested that an excessive exposure to estrogens may be deleterious to normal male reproductive function [1]. This may be in particular true if the exposure takes place in early fetal life at the time of sexual differentiation [2]. It has also become evident that a number of environmental toxicants may mimic the effect of estrogens and thereby disturb the male fertility. Such effect may also be exerted by the so-called phytoestrogens, a group of substances that are derived from plants and have biological effects on animals similar to those of estrogen.

Much of the current research focusing on the impact of estrogens and phytoestrogens on male fertility has been a consequence of epidemiological data pointing to possible time related and geographical trends in male reproductive function.

In this review, these data are described and subsequently some of the recent research in relation to estrogens/phytoestrogens and male fertility is summarized.

Male reproductive function – epidemiologic trends

In 1992, a meta-analysis by Carlsen *et al.* [3] indicated a decline in mean sperm count from 113 mill/ml in 1940 to 66 mill/ml in 1990. These results are still an object of discussion and statistical re-analyses performed on the same material have led to somewhat diverging conclusions. One confirmed a statistically significant decline both in USA and in Europe, whereas others stated that the decline was not present at all [4–6]. Recently, in a study of semen quality in military conscripts in Southern Sweden, no significant difference in sperm number or motility was seen between 2000–2001 and

2009–2010 [7••]. Furthermore, in Denmark, where monitoring of sperm counts in adolescent men has taken place during the past 15 years, no negative trend in sperm numbers has been observed [8]. In contrary, in a Finish study of army conscripts during the same period, such decrease was reported [9]. These are the first prospective data regarding secular trends in semen quality. However, these findings do not exclude that in some parts of the world a decline had already happened but was leveled off after 2000.

The other interesting point is whether the male fertility potential has decreased during the past years. A recent Swedish study has shown a time related decrease in the proportion of couples with more than 1 year's time to pregnancy [10]. However, this finding might reflect a country-specific decrease over time in sexually transmitted diseases, changes in sexual behaviour induced by socioeconomic conditions, or educational trends, rather than being a result of improvement in testicular and/or ovarian function.

The possible decrease in sperm number has been linked to possible deterioration in other indices of male reproductive function. Although a significant 2–4 times increase in the incidence of testicular cancer during the past 50 years has been observed globally, at least among Caucasians, the data indicating same trend for the risk of hypospadias and cryptorchidism are less reliable [11–15].

Furthermore, apart from the time-related trend some interesting geographical differences in regard to the male reproductive function have been observed.

Cancer register data show that the incidence of testicular cancer is five times higher among Danish as compared with Finnish men [11]. Studies on semen quality performed in 1990s have revealed significantly higher sperm counts in Finland as compared with Denmark. However, according to the most recent data, the Finish sperm counts have decreased to the Danish level [9]. Furthermore, despite the differences in sperm numbers found between the fertile men from these two countries, no discrepancy with regard to time-to-pregnancy has been observed [16], indicating that fertility may be a less sensitive marker of male reproductive dysfunction.

Regional differences in sperm numbers have also been found in USA and across the Europe [17,18].

The explanation for this geographical trend is not known. The reports showing that incidence of testicular cancer in the second generation immigrants in Sweden and Denmark, who have the risk of this malignancy at the magnitude of males in the country in which they are living and not at that of the background population from which their parents are pointing to a strong effect of environment and/or lifestyle [19,20].

Key points

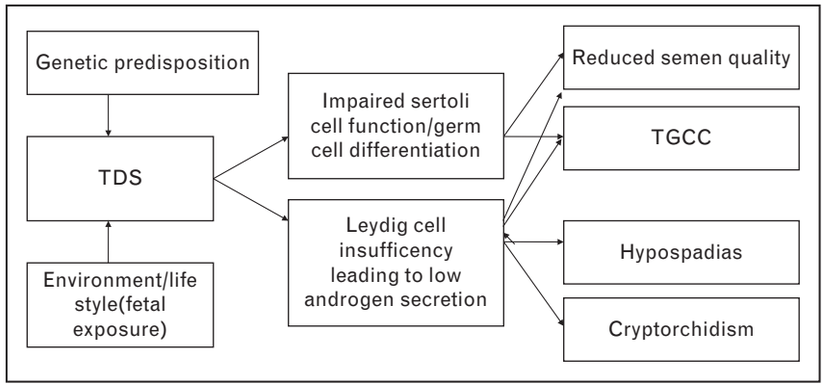
- Epidemiological data show a time-related increase in the incidence of testicular cancer and it has also been suggested that even sperm counts have been declining, but the evidence for such trend is not strong.
- It has been suggested that fetal exposure to chemicals interfering with sex hormone action – endocrine disruptors – may lead to impairment of male reproductive function.
- Some association between adult levels of exposure to endocrine disruptors and semen quality parameters has been reported but the results are not unequivocal and the human data on fetal exposure are lacking.
- There are no convincing data showing that phytoestrogens in humans may be deleterious to male fertility/semen quality.

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 testicular germ cell cancer (TGCC) and low sperm counts may share causes and be the result of factors operating already in fetal life. On the basis of these observations as well as clinical evidence linking cryptorchidism, hypospadias, poor semen quality, and testicular malignancy together, Skakkebaek *et al.* in 2001 coined the concept of testicular dysgenesis syndrome (TDS), suggesting that poor semen quality, testis cancer, cryptorchidism, and hypospadias are symptoms of a common underlying entity [21] (Fig. 1). TDS was suggested to be a result of disruption of embryonal programming and gonadal development in fetal life due to environmental or lifestyle related factors combined with genetic susceptibility of the individual. AQ1

Originally, it was hypothesized that the fetal disturbances in the development of male reproductive system is related to an increased exposure to endogenous or exogenous compounds with estrogenic properties [1]: However, subsequently, the concept of 'estrogen hypothesis' was changed to an imbalance between the androgenic and estrogenic action in favor of the latter [22]. Interestingly, a recent review of the available human data concluded that 'with the possible exception of testicular cancer there is no strong epidemiological evidence to indicate that prenatal exposure to estrogen are linked to

Figure 1 Schematic illustration of the hypothesis of testicular dysgenesis syndrome



Environmental and/or lifestyle related factors acting in fetal life cause dysfunction of Leydig cells and Sertoli cells leading to testicular dysgenesis including poor semen quality, testicular germ cell cancer (TGCC), hypospadias and/or cryptorchidism.

disturbed development of the male reproductive organs' [23].

Sex hormones in male and cellular mechanisms of action

Testosterone is transported to target tissues in a protein bound state, mainly bound to albumin (50–55%) or to sex hormone binding globulin (40–45%). Normally, the fraction of free testosterone is only 2% in the circulation of men. It dissociates from carrier proteins and enters the cell via passive diffusion. In the cytoplasm, testosterone can be converted to the more potent androgen DHT by the enzyme 5α-reductase as well as to estradiol by the enzyme aromatase. Both testosterone and DHT exert their actions through the intracellular androgen receptor (AR). Failure of the AR to activate its target genes in the presence of hormone results in target organ resistance to the hormone, phenotypically ranging from complete feminization to mild under virilization in males [24].

Estrogens are exerting their action through two types of estrogen receptor (ER): ERα and ERβ. Both receptors are expressed in the human testis. ERα mostly found in the rete testis whereas ERβ has been found in both Sertoli cells and the germ cells. More recently, it has become clear that estradiol actions can also rapidly be mediated through a membrane associated or intra cellular estrogen receptor [25]. Recent investigations show that the cellular activities of estrogens and xenoestrogens – products of industrial or chemical processing that have estrogen like effects – are the result of a combination of both pathways [26].

The impact of estrogens on spermatogenesis is only poorly understood. Thus, administration of estrogens to prostate cancer patients and to male to female transsexuals, results in atrophy of the seminiferous tubules [27,28]. However this effect might be indirect, mediated through a negative feed-back on the secretion of gonadotropins. Disturbances in spermatogenesis were also observed in aromatase knock-out mice [29], this finding indicating a functional role of these hormones in relation to normal sperm production. Thus, in principle, both an excess and a lack of estrogenic action may be deleterious for spermatogenesis.

Endocrine disrupters – molecular actions

The endocrine system can be considered as a closed feedback loop that functions to maintain homeostasis. Many of the industrially produced chemicals were shown *in vitro* and in animal studies to act like hormones in the endocrine system and disrupt the physiologic function of endogenous hormones, so called endocrine disrupting compounds (EDCs) [2].

Many EDCs identified do in fact interfere with estrogenic actions (see below), modifying the synthesis or actions of estradiol or interfering with the estrogen receptors. These chemicals include drugs, natural products, and manufactured chemicals.

Although animal studies have raised concern that the influence of EDCs would obstruct the development of the male reproductive system, in general, exposure levels far above those found in humans have been needed to evoke reproductive toxicity in the animal models. Human data are inconclusive and have evoked the question whether EDCs can have any impact on hormonal function and thus health consequences when natural hormones are present. Indeed, many contaminants with hormone-like activity are much less potent than

endogenous hormones themselves [30]; 17β-estradiol was for instance estimated to be 17 000 times more potent than p,p'-DDT. However, humans are exposed to a multitude of agents, and when present in sufficient number and/or concentration, they might in principle act together to impact on the actions of endogenous hormones. Whether such impacts will be physiologically relevant is still not known, but in worst case scenario, there are no threshold levels below which there are no effects at all.

A number of environmentally derived chemicals were shown to have an estrogenic effect or to interfere with the synthesis or metabolism of this hormone. Some of those are listed in Table 1 (http://en.wikipedia.org/wiki/Xenoestrogen).

During the recent period, a lot of research has been focusing on finding possible associations between levels of certain EDC and semen quality. Originally most of those studies focused on persistent organohalogen pollutants as polychlorinated biphenyl (PCB) and p,p'DDE [31]. During the most recent years, in particular phthalates [32,33] and Bisphenol A [34,35•] have been on the research agenda. Some of the most recent of these studies are summarized in Table 2. In general, those reports point to some negative association between the exposure levels and sperm number/quality. However, it should be kept in mind that these studies have been focusing on exposure during the adult life, there being no data on fetal exposure which according to the TDS hypothesis (see above) should be the most deleterious to the male reproductive function. Furthermore, some publication bias in favor of studies concluding association between levels of EDC and semen quality/fertility cannot be excluded.

Apart from chemicals interfering directly with estrogenic action, synthesis and/or metabolism, it can be speculated that those affecting the AR or aryl hydrocarbon receptor – as 2,3,7,8-TCDD (dioxin) – may indirectly have an impact on the activity of the estrogen receptor. Interestingly, deterioration in semen quality has recently been reported in sons to mothers exposed to dioxin after the accident in Seveso [39•] (Fig. 2).

Phytoestrogens

Phytooestrogens are nonsteroidal compounds that can bind to both ERα and ERβ because of their ability to mimic the conformational structure of estradiol [41]. Phytooestrogens are found in many vegetables and are particularly abundant in soy products. Genistin and daidzin, two major soy isoflavone glucosides, are present at high concentrations in soybeans and soybean-derived products and are a major source of xeno-oestrogen exposure in both humans (e.g., soy-based formula for infants; tofu) and animals (most commercially available diets). Many foods contain phytoestrogens, such as walnuts, soybeans, cereals (oats, barley, wheat, rice), legumes (beans, lentils), berries, apples, carrots, ginseng, fennel, and anise used to barboun and beer. The oils of seeds, soya beans and tofu are foods that contain the highest concentration of phytoestrogens. AQ3

The mechanism of action of phytoestrogens in relation to male reproductive function is not only limited to their interaction with estrogen receptors. Genistein has also

AQ9 **Table 1 Some examples of endocrine disrupting chemicals potentially interfering with the estrogenic pathway**

Alkylphenols	Intermediate chemicals used in the manufacture of other chemicals
Atrazine	Weedkiller
4-Methylbenzylidene camphor	Sunscreen lotions
Butylated hydroxyanisole, BHA	Food preservative
Bisphenol A	Monomer for polycarbonate plastic and epoxy resin; antioxidant in plasticizers
Dichlorodiphenyldichloroethylene	One of the breakdown products of DDT
Dieldrin	Banned insecticide
DDT	Banned insecticide
Endosulfan	Widely banned insecticide
Erythrosin	FD&C Red No. 3
Ethinylestradiol	Combined oral contraceptive pill
Heptachlor	Restricted insecticide
Lindane, hexachlorocyclohexane	Restricted insecticide
Metalloestrogens	A class of inorganic xenoestrogens
Methoxychlor	Banned insecticide
Nonylphenol and derivatives	Industrial surfactants; emulsifiers for emulsion polymerization; laboratory detergents; pesticides
Pentachlorophenol	Restricted general biocide and wood preservative
Polychlorinated biphenyls (PCBs)	Banned; formerly used in electrical oils, lubricants, adhesives, paints
Parabens	Lotions
Phenosulfothiazine	A red dye
Phthalates	Plasticizers
DEHP	Plasticizer for PVC
Propyl gallate	Used to protect oils and fats in products from oxidation

Table 2 Recent (2010–2011) human studies focusing on association between exposure to endocrine disruptors and semen quality/fertility

Exposure	Association found	Reference
Bisphenol A	Negative association between urine levels and sperm number and motility	[35•]
Bisphenol A	Negative association with Inhibin B and positive association with FSH levels	[36•]
Bisphenol A	Negative association with sperm number, motility, morphology and DNA integrity	[37••]
Phthalates	Negative association with sperm motility – *in vivo* and *in vitro*	[38]
Dioxin[a]	Negative association between perinatal exposure and sperm number and motility	[39•]
Organochlorine pesticides[b] and polychlorinated biphenyls (PCBs)	Concentration of several of these compounds in adipose tissue did not differ between infertile and fertile men. However, in the former group higher levels of PCB52 and PCB 180	[40]

[a] The effect on the estrogen pathway, if any, might be indirect through interaction between aryl hydrocarbon and estrogen receptors.
[b] For example: p,p'-DDE, and p,p'-DDT.

been reported to act through other mechanisms, including acting as a tyrosine kinase inhibitor or an antioxidant [42,43]. Furthermore, it has been suggested [44••] that phytooestrogen exposure might interfere with the androgen receptor pathway and affect the late steps of spermatogenesis.

Generally, soy isoflavones have received much attention as a result of their potential health benefits. Soy consumption in oriental countries correlates with low incidences of breast and invasive prostate cancers, improvements in metabolic parameters, and amelioration of age-related diseases, for example, cardiovascular diseases, osteoporosis [45,46]. However, exposure to high levels of phytoestrogens, either from a lifetime of exposure or during a critical period of development, could also have potentially detrimental effects on fertility and reproductive functions, through the molecular mechanisms described above.

However, so far, there has been a lack of consistency in human and animal studies examining the effects of soy and phytooestrogens on reproductive parameters (Table 3).

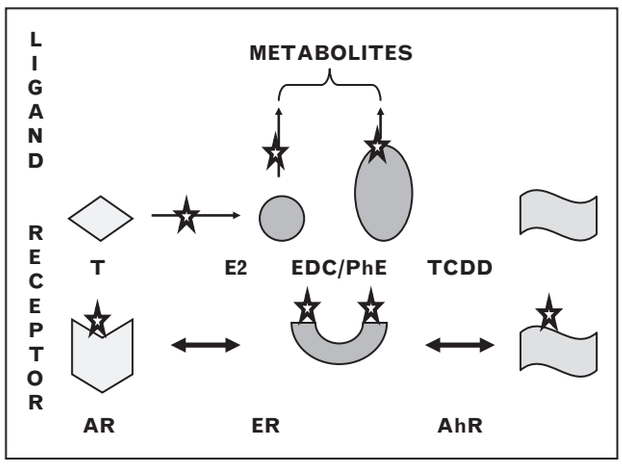
These discrepancies certainly reflect inadequate descriptions or insufficient sample size to permit confidence in the observed results and also the variety of experimental designs, in some cases the exposure levels applied in animal studies being at significantly higher level than dose expected to be found in humans.

Semen data from Asia, where the consumption of phytoestrogens is more pronounced than in other parts of the world, are rather scarce. However, a recent review [51] might indicate that sperm numbers, in this part of the world, are about the same level as the lowest ones seen in Europe. Whether this is due to the diet, other lifestyle related factors and/or genetic diversity cannot be concluded.

Another intriguing issue is the timing of the exposure an important question being whether exposure during infancy causes detrimental male reproductive effects in adulthood. A study that has assessed the adult reproductive effects of soy formula consumption during infancy in humans reports that no significant differences in onset of puberty or in reproductive functions were found in comparison with those fed with milk formulas [52]. However, the true relevance of this study has been the subject of controversy, as result of the lack of direct measurements of hormone levels and reproductive functions [53,54].

Other important aspects to be taken into consideration are the impact of mixed exposure. An intriguing question is whether a mixture of different EDCs, a scenario which

Figure 2 Possible mechanisms of action ☆ of endocrine disrupting compounds and/or phytoextrogens affecting the estrogen regulating mechanisms



The action may be mediated through some of the estrogen receptors, which are receptor for androgens or arylhydrocarbon, some of the two latter interacting with the estrogen receptor. The ligand for arylhydrocarbon is dioxin (TCDD). Other possible effects are through affecting synthesis of the estradiol from testosterone or the metabolism of estradiol, EDS and/or phytoextrogens.

AQ8

Table 3 Human studies evaluating the effects of gestational and postnatal exposure to isoflavones on fertility and male hormones

Ethnic group	Diet	Isoflavone intake (mg/day)	Duration of exposure	No. of patients	Plasma isoflavone levels (μm)	Urine isoflavone levels (ng/μmol creatinine)	Sperm production	Sperm motility	Seminal volume	Blood hormone levels	Reference
Caucasian											
Caucasian	Tofu	70	4 weeks	21 (21)	ND	G: 201 D: 401	ND	ND	ND	T: No effect	[47]
Caucasian	Soy extract	40	2 months	15	G: 1 D: 0.5	ND	No effect	No effect	No effect	T, LH, FSH: No effect	[48]
Japanese	Soy food	<22.3	Less and more than 2 months	100	ND	ND	Decreased	No effect	No effect	ND	[49]
Japanese	Soy milk	76.8	8 weeks	17 (17)	ND	ND	ND	ND	ND	T: No effect	[50]

For control groups, the number of individuals (*n*) is shown in parentheses.
D, daidzein; G, gensitein; ND, not determined; T, testosterone. Adapted from [44••].

AQ10

reflects the daily day situation, can be deleterious at exposure doses at which no effect of single compound can be measured. Recently, it was reported that the relatively weak alterations in reproductive function of males exposed to low doses of genistein (1 mg/kg/day) were exacerbated when co-exposed with a low dose of the fungicide vinclozolin (1 mg/kg/day), a ubiquitous antiandrogenic food contaminant [55]. Indeed, the genistein/vinclozolin mixture induced greater alterations on the male reproductive tract and fertility endpoints when compared with the exposure to each compound in isolation, at the same dose.

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Gene-environment interaction

During the past few years an increasing amount of epidemiological and in-vitro data have indicated possible gene-environment interaction in relation to the effect of EDC on male reproductive system. Thus, we have previously reported that the negative effect of PCB exposure in relation to the sperm numbers may only be seen in males with certain variant of the androgen receptor gene [56]. This might also be true in relation to polymorphisms in the ERα and ERβ as well as genes involved in the synthesis or metabolism of estrogens as well as those encoding for receptors interfering with the action of estrogen receptor as, for example, aryl hydrocarbon receptor. Thus, recently a negative association between obesity and sperm counts was only found in patients with the genetic variants of the aromatase gene resulting in higher estrogen levels [57].

Gene-environment interaction might also encounter for regional differences in sperm numbers.

Conclusion

Phytoestrogens as well environmental chemicals, directly or indirectly interfering with the physiological effect of estrogens, might in principle interfere with the function of male reproductive system. However, convincing human evidence that such exposures, at levels to which general population is exposed has an impact on male fertility, is still lacking. On the contrary, due to a serious potential consequences of such scenario, intensive research is needed in order to elucidate whether there is any reason to worry or not. Thus, the future research agenda should focus on studies related to fetal exposure, the impact of mixed exposure of low doses of different chemicals, and gene-environment interaction [58]. For the clinician, this issue is interesting but yet, without any clear clinical implications. Although aromatase inhibitors have been tested in the treatment of male infertility [58], evidence for their effects are still lacking.

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Acknowledgement

Conflicts of interest

AQ6 The study was supported by grants from the European Commission (5th Framework Programme Quality of Life and Management of Living Resources, Key Action 4: Environment and Health, contract no. QLK4-CT-2001–00202), the Swedish Cancer Society (grant no: CAN 2009/817), the Swedish Governmental Research Fund (ALF), the Cancer Research Fund of Malmö University Hospital, the Research Fund of Skåne University Hospital, and the Swedish Childhood Cancer Fund (grant no: PROJ10/030).

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

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