



UNIVERZITA KARLOVA V PRAZE
3. LÉKAŘSKÁ FAKULTA



Urologická klinika FNKV

Michal Daniel

**Estrogens in environment, their impact
on male fertility**

*Estrogeny v životním prostředí, jejich vliv
na mužskou potenci*

Diplomová práce/Thesis

Prague, January 2009

Autor práce/Author: Michal Daniel

Studijní program/Study program: Všeobecné lékařství

Vedoucí práce/Adviser: **MUDr. Jiří Heráček Ph.D.**

Pracoviště vedoucího práce/Adviser's clinic: **Urologická
klinika, FNKV**

Datum a rok obhajoby/Date of the defense: 22.1. 2009

Prohlášení/Proclamation

I declare that the given elaborate has been written by myself and only the given sources and literature have been used. I also give a permission that my thesis is to be used for study purposes.

In Prague on January 15th, 2009 Michal Daniel

Content

CONTENT	3
INTRODUCTION	4
1. PHYSIOLOGY OF SITES SENSITIVE TO ENDOCRINE DISRUPTORS ..	6
1.1 SERTOLI CELLS.....	6
1.2 LEYDIG CELLS.....	7
1.3 SPERMATOGENESIS.....	8
1.4 HUMORAL CONTROL OF SPERMATOGENESIS	9
1.5 ESTROGEN IN MALE REPRODUCTIVE SYSTEM	11
1.5.1 <i>Estrogen receptors</i>	12
1.5.2 <i>ERs in male reproductive system</i>	13
2. EPIDEMIOLOGY OF INFERTILITY	15
2.1 FERTILITY AND SEMEN QUALITY	18
2.2 TESTICULAR CANCER	19
2.3 ANOMALIES OF THE MALE GENITALIA	20
3. EFFECTS OF XENOESTROGENS ON MALE REPRODUCTIVE SYSTEM	22
CONCLUSION	26
SOUHRN	27
SUMMARY	28
LIST OF TABLES AND GRAPHS	29
LIST OF LITERATURE	30

Introduction

Build-up of estrogen and other endocrine disruptors in our habitat has been recently an issue widely debated not only amongst health professionals but also by main stream media. Their impact on wild animals has been observed and documented for some time now and several studies on rodents and other mammals have demonstrated a highly probable connection between exposure in early stages of development to endocrine disrupting chemicals and abnormal sexual maturation of males (hypospadias, cryptorchidism), as well as impaired male reproductive functions. Epidemiological studies have been claiming significant drop in sperm count and quality of spermatozoa in last several decades, increased incidence of testicular cancer, which is being among other causes attributed also to pooling of endocrine disruptors in the environment. This reasoning brings a lot of controversy since the respective chemicals are by-products of substances commonly used in agriculture and industry and replacing them is expensive and logistically challenging. It is also quite difficult to determine and quantify the long term effects of specific chemicals on human body since the population is being exposed to not just one substance but to a whole cocktail of them and the symptomatology is subjected to age, length of the exposure, quantity of the chemicals in the environment, their interactions and many other variables.

My ambition is to give a review of what different authors state to be the effects of estrogens on human male reproductive system and to give my assessment to what extent this plays a significant role in growing rate of sexual defects in the human population.

First part will summarize the physiology of reproductive axis in males and how it responds to sexual hormones and estrogens in particular. Further the epidemiology and symptomatology of male infertility with respect to exposure to endocrine disruptors will be introduced in the second part. Finally, studies on rodents which strive to describe the underlining mechanisms of estrogen effects on male reproductive system will be presented.

1. Physiology of sites sensitive to endocrine disruptors

There are several potential target sites in the male reproductive tract that can be affected by endocrine disruptor. The most important are the testes where spermatogenesis and androgen production takes place. The different compartments of the testes are under autocrine and paracrine regulation, which is overall managed by pituitary gland and hypothalamus. About 80% of the testicular tissue consists of highly coiled seminiferous tubules where spermatogenesis takes place. The remaining 20% of the mass are Leydig and Sertoli cells, which are mainly responsible for establishing of normal spermatogenesis. Spermatozoa – haploid germ cells are then the final product of spermatogenesis and are responsible for fertilization and propagation of species.¹ Other potential target sites are prostate gland and corpora cavernosa penis, which are responsible for secretion and erectile function of penis.²

1.1 Sertoli cells

These are cells located within testes that form a continuous lining within the tubular walls which protect and nurture the developing germ cells during spermatogenesis. These cells, also called “nurse cells” form the blood-testis ultimate barrier thanks to desmosomes in between. The luminal environment maintained by Sertoli cells is under the influence of follicle stimulating hormone (FSH) and inhibin. They provide nourishment for the

¹ SIKKA, Suresh C. et. al.: Endocrine disruptors and estrogenic effects on male reproductive axis, *Asian Journal of Andrology*, 2008, vol.10, no.1, p. 135-136.

² GOYAL H.O. et. al.: Role of estrogen in induction of penile dysmorphogenesis: a review, *Reproduction*, 2007, issue 134, p. 200.

developing sperm cells; eliminate defective sperm cells; secrete fluid that enables the transport of sperm into the epididymis and release inhibin, a hormone which is partially responsible for regulation of sperm production.

Sertoli cells must fully differentiate and form a competent blood-testis barrier. This is essential to the establishment of normal spermatogenesis during puberty. Therefore defective spermatogenesis can be often caused by interference of endocrine disruptors with function of the Sertoli cell population and not necessarily by pathology in the germ cells themselves.³

It is also important to mention that fully differentiated Sertoli cell is unable to proliferate any further. Therefore, once spermatogenesis has begun, no more Sertoli cells are being created.

1.2 Leydig cells

Leydig cells arise from interstitial mesenchymal tissue between the tubules during the eighth week of human embryonic development. They are located in the connective tissue between the seminiferous tubules. They function as endocrine cells in the testis and produce insulin-like factor 3, testosterone, androstenedione and dehydroepiandrosterone (DHEA) from cholesterol through series of enzymatic pathways and steroidal intermediates. This is regulated by luteinizing hormone (LH) from the pituitary gland. Androgens are essential for regulating of germ cell maturation as well as for stimulation of development of secondary masculine features.

³ SIKKA, Suresh C. et. al.: Endocrine disruptors and estrogenic effects on male reproductive axis, *Asian Journal of Andrology*, 2008 vol.10, no.1, p.136.

1.3 Spermatogenesis

This process takes about 80 days in men and approximately 40–50 days in the rodents. In this period, the relatively undifferentiated spermatogonia gradually develop into highly specialized spermatozoa. Germ cells undergo several mitotic divisions to generate a large population of primary spermatocytes, which produce haploid spermatids by two meiotic divisions. Spermiogenesis is the transformation of spermatids into elongated flagellar germ cells capable of motility. The release of mature germ cells capable of fertilization of an egg is known as spermiation. These cells form the most of the testicular volume, which diminishes if testicular damage has occurred. During mitotic arrest, germ cells become highly sensitive to toxic agents. Only small doses of toxic chemicals or radiation during the sensitive period can utterly eradicate the whole germ cell population while at the same time the population of Sertoli cells is nearly untouched and thus a Sertoli-cell-only testes is being created.⁴

The testicular parenchyma is consisting of seminiferous tubules and interstitial tissue is enclosed by a capsule called the tunica. The interstitial tissue contains the blood and lymphatic vessels, which are essential for the movement of hormones and nutrients into, and out of, the testis. The most frequently encountered cells type in the interstitium are the Leydig cells which are primarily involved in the secretion of androgens as well as other steroids including estrogen. Within the seminiferous tubules, the Sertoli cells reside on a basement membrane, under which are the lymphatic endothelium and the peritubular myoid cells. The structure of the Sertoli cell is extremely complex, with numerous

⁴ SIKKA, Suresh C. et. al.: Endocrine disruptors and estrogenic effects on male reproductive axis, *Asian Journal of Andrology*, 2008 vol.10, no.1, p. 136.

cup shaped processes encompassing the various germ cell types. Developing germ cells form intimate associations with Sertoli cells, with multiple germ cell types in contact with one Sertoli cell. The various generations of germ cells are not randomly distributed within the seminiferous epithelium, but are arranged in strictly defined cellular associations. It is the unique associations of these germ cells with Sertoli cells that constitute the cycle of the seminiferous epithelium, and each particular association of germ cells is referred to as a stage.

The number of stages of spermatogenesis in a particular species is thus defined by the number of morphologically recognizable germ cell associations within the testis; in the mouse there are 12 stages, in the rat there are 14, and in the human there are 6.⁵

1.4 Humoral control of spermatogenesis

A highly coordinated interaction between a germ cell and the Sertoli cell is essential for its development. The communication between them is carried out by paracrine factors or directly via ligand-receptor-mediated interactions. For many years it was presumed that Sertoli cells were the major controlling factor in the timing of germ cell development; however, recent studies investigating rat-to-mouse spermatogonial transplantation clearly demonstrated that rat germ cells in contact with mouse Sertoli cells develop according to the kinetics of rat spermatogenesis, thus highlighting the role of germ cells in controlling their own fate.⁶

⁵ O'DONELL Liza et al.: Estrogen and Spermatogenesis, *Endocrine Reviews*, 2001, vol.22, no.3, p. 289.

⁶ FRANCA LR et al.: Germ cell genotype controls cell cycle during spermatogenesis in the rat. *Biol Reprod*, 1998, issue 59, p. 1371-1377.

As well as the production of spermatozoa, the testis is involved in the production of hormones that are required for various functions in the body, including maintenance of secondary sexual functions, and feedback on the hypothalamus and the pituitary to control the secretion of the gonadotropins LH and FSH.

Gonadotropins are the major endocrine regulators of spermatogenesis. LH target the Leydig cell to stimulate the secretion of androgens, namely testosterone, which in turn acts on androgen receptors in the seminiferous epithelium to control spermatogenesis.

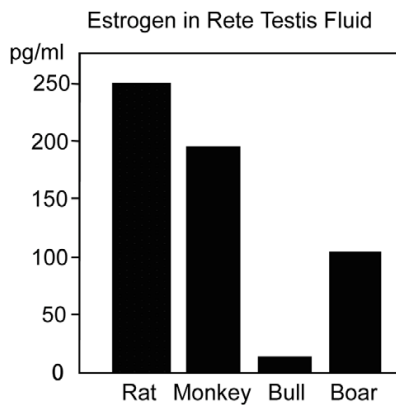
FSH targets receptors within the Sertoli cell to regulate spermatogenesis by stimulating the production of numerous Sertoli cell factors. The roles of testosterone and FSH in the testis have been studied extensively, yet relatively little is known about how these hormones act within the Sertoli cell to stimulate and maintain spermatogenesis. Androgens alone have been shown to stimulate all phases of germ cell development in the hypogonadal mouse, which is congenitally deficient in GnRH and therefore LH and FSH highlighting the requirement of spermatogenesis for androgen.

In terms of the endocrine regulation of spermatogenesis by FSH, LH, and androgens, it is clear that the initiation and maintenance of quantitatively normal spermatogenesis and thus full fertility rely on the delicate balance of the hypothalamo-pituitary-testis axis.

One of the focuses of this paper is to stress the importance of how estrogens are involved in the regulation of spermatogenesis.

1.5 Estrogen in male reproductive system

Estrogen is produced in sizable quantities in the testis, as well as the brain. It is also present in very high concentrations in the semen of several species (see graph 1).



Graph 1: Estrogen in rete testis fluid
(Source: Van der Molen HJ et al, 1981)

Early studies reported that the primary source of estrogen in the immature male was the Sertoli cell. In the adult testis, Leydig cells express aromatase (P450arom) and actively synthesize estradiol at a rate much greater than that seen in the adult Sertoli cell.

Germ cells have been also observed to synthesize estrogen, and possibly they serve as the major source of this steroid in the male reproductive tract. In 1993 it has been reported for the first time that P450arom is present in testicular germ cells of the adult male mouse. The enzyme was localized in the Golgi of round spermatids and throughout the cytoplasm of elongating and late spermatids. Its presence was confirmed by Western and Northern blot analysis of isolated germ cells. Its activity in germ cells was equal to or exceeded the activity found in the interstitial cells. Later, Carreau and others have shown aromatase expression and activity in the human sperm.

The presence of P450arom in male germ cells has been demonstrated in several species, including mouse, rat, brown bear, the bank vole, rooster, and man. The enzyme is located in cytoplasmic droplets of the sperm tail, but the staining becomes less intense as sperm traverse the epididymis. Its presence in germ cells and spermatozoa was recently confirmed and shown to represent approximately 62% of the total testicular aromatase. The concentration of estrogens in peripheral blood is

typically low in the male, but ranges from 2–180 pg/ml depending upon the species. Estrogen concentrations are typically higher in the testicular vein and lymph than in the general circulation. Also, in the reproductive tract, estrogen can reach relatively high concentrations. In one report, estrogen concentration in rete testis fluid of the rat was approximately 250 pg/ml, which is higher than the average serum concentration of estradiol in the female. Estrogens are also abundant in semen and depending upon the species, their concentrations can range from 14 to nearly 900 pg/ml.

1.5.1 Estrogen receptors

Now as it is established that estrogens are indeed physiologically present in the male reproductive system, the consecutive question one must ask is what tissues respond to them. The first piece of a puzzle came in 1970s when Jensen and DeSombre first characterized estrogen receptor (ER). This led in turn to their cloning in the 1980s and it was established that the ER family is like aromatase a member of a large gene superfamily, in this case the ligand-activated nuclear receptor family, which also contains receptors for the other steroid hormones, thyroid hormone, vitamin D, retinoids and others. For several years it was thought that only one form of nuclear ER existed. However, in 1996 a second form was reported in a number of species including rat, mouse, and human.

This newly discovered receptor was termed ER β , resulting in the classical ER being renamed ER α . The two receptors are not isoforms of each other, but rather are the products of distinct genes located on separate chromosomes.

Although ER α and ER β bind estradiol with similar affinity, there is considerable selectivity of the different receptor subtypes in terms of affinities of various ligands. For example some phytoestrogens, such as genistein and coumestrol, have a significantly higher affinity for ER β than ER α .⁷

1.5.2 ERs in male reproductive system

It is important to mention the localization of ER molecules in testes of rodents, since these are the most common model for observing effects of estrogen on male fertility. In the testes of adult rats and mice, ER α immuno-expression has been detected in the Leydig cells and the peritubular myoid cells, but not in the Sertoli and germ cells. The cells within the epithelium of efferent ductules in both species express high levels of ER α . In comparison, ER β protein can be detected in multiple cell types, including the Sertoli cells, and in some but not all germ cells, as well as in epithelial and stromal cells within the male reproductive system. Both types of ER can be also found in prostate tissue, corpora cavernosa penis.⁸

The table below shows the known distribution of both kinds of ER in different cell types in mice.

⁷ O'DONELL Liza et. al.: Estrogen and Spermatogenesis, *Endocrine Reviews*, 2001, vol.22, no.3, p. 292-294.

⁸ GOYAL H.O. et. al.: Role of estrogen in induction of penile dysmorphogenesis: a review, *Reproduction*, 2007, issue 134, p. 199.

Tissue/Cell Type	ER α	ER β
Testis		
Sertoli cells	—	++
Leydig cells	+++	+++
Germ cells	—	Variable
Efferent ductule	E+++/S-	E+++/S+++or-
Epididymis		
Initialsegment	E+++/S-	E+++/S+
Caput/corpus	E*/S-	E+++/S++
Cauda	E*/S+++	E+++/S+++
Vas deferens	E*/S+++	E+++/S+++

NOTE: Data are summarized from Zhou *et al.* E = epithelial cells; S = stromal cells; E* = a very few immunopositive epithelial (clear) cells, which are present in the regions of the epididymis other than the initial segment. In the vas deferens a few ER α -positive basal cells can be detected.

Table 1: Distribution of ERs in testes (Source: Jayne E. Sierens et al, 2005)

Attempts to detect ER α mRNA in human tissue biopsy specimens haven't been successful so far, even though the mRNA can be detected in "testicular" cDNA pools and libraries from commercial sources. This is consistent with the failure to immunolocalize ER α to cells within the human testes even though high levels of ER α are expressed in the efferent ductules.

Messenger RNAs encoding ER β of several subtypes have been detected in tissue extracts of human testes. Furthermore there have been ER β subtypes localized in round spermatids and Sertoli cells. There has been a study by Pentikainen et al. which reported incubation with estrogens reduces levels of germ cell apoptosis in isolated human seminiferous tubules. This suggests that the expression of ER β within germ cells may play a role in germ-cell survival.

Proteins homologous to ER β have also been immunolocalized to sections of testes from primate species and their pattern of expression appears to be identical to that seen in the human.⁹

⁹ SIERENS Jayne E. et al.: Estrogens in Testis Biology, *Ann. N.Y. Acad. Sci.*, 2005, issue 1061, p. 69-73.

2. Epidemiology of Infertility

As it was previously mentioned it is difficult to specify long term effects of xenoestrogens alone on male population for they are just a portion of a whole cocktail of substances to which we are exposed that disrupt endocrine balance in male reproductive system. Most of the gathered data is in form of indirect descriptive epidemiology and case studies of patients professionally or therapeutically exposed to xenoestrogens. There is a general consensus that male fertility has been deteriorating in the last half-century or perhaps even longer and there are some leads suggesting that it is the very exposure to endocrine disruptors what is one of the major causes. Here is a list of some known agents which have shown effects on male fertility but the information on biological processes underlying these effects is rather fragmentary.¹⁰

Principal exogenous substances that may affect sex hormone function

A. Estrogenic effects

1. High potency—pharmacological agents

- DES (diethylstilboestrol)
- ethinyl estradiol (component of contraceptive pill)

2. Medium potency—dietary phytoestrogens

- isoflavones, e.g. genistein, daidzein
- coumestans, e.g. coumestrol
- lignans

3. Low potency—environmental or occupational agents

- bisphenol A
 - octylphenol and nonylphenol
- pesticides, including chlordecone, DDT, dieldrin, endosulphan, p,p'-methoxychlor, toxaphene

B. Anti-androgenic effects

- p,p'-DDE (the major breakdown product of DDT)
- certain phthalates, e.g. DBP, DEHP
- pesticides, including linuran, procymidone, metabolites of vinclozolin
- hydroxyflutamide

C. Others

¹⁰ JOFFE Michael: Infertility and environmental pollutants, *British Medical Bulletin*, 2003, vol.68, p. 47-48.

- dioxins, furans and 'dioxin-like' PCBs

Table 2: Endocrine disruptors (Source: data taken from Joffe, 2003)

Alterations in male reproduction were first observed in wild animals, in studies reporting the effects of accidental exposure of estrogenic chemicals on wildlife in the natural environment. These changes in male reproductive function vary from very subtle changes to permanent alterations, such as feminization or changes in reproductive behavior studied on the male reproductive functions of alligators in two lakes in Florida. These two lakes are located very close to each other geographically, excluding the possibility of climate-based bias in these studies. They found that adult male alligators in Apopka Lake, which was polluted with agricultural waste and experienced a major chemical spill in 1980, had lower testosterone levels and presented micropenis and disorganized testes. A key part of this story is that no chemicals could be detected in the water of the apparently contaminated lake and thus the alligators were being exposed simply by being at the top of the food chain. Other documented disruptions or alterations of reproductive activity and physiology have been correlated with exposure of contaminants in fish, amphibians, reptiles, birds, and mammals. Most of the reported effects on wildlife have been done on aquatic food chain organism making the causal link a direct or an indirect effect of pollutants hard to do.

In humans, there is increasing evidence that the birth sex ratio is altered in areas close to industry and exposed to environmental and industrial chemicals. The findings of the very recent report on the Aamjiwnaang First Nation community in Canada show that the proportion of male live births in this community has been decreasing continually from 1990 to 2003, the sex ratio

(number of male births/total number of births) reaching only 0.3. The epidemiologic data have also shown an increase in human male reproductive function disorder over the past 50 years, with the suggestion of a relation with the increase in the amounts of endocrine disruptors in the environment. Testicular cancer, which is the most prevalent cancer in young men, has steadily increased in all countries studied, rising from 3.4% in 1973 to 5.5% in 1997 in North America. Hypospadias and cryptorchism also increased from 0.2 and 2% respectively in 1970 to 0.38 and 3.5% respectively in 1991. Finally, the sperm count decline have been controversial but large-scale prospective studies using standardized methodologies have shown a decline from 170 to 70million spermatozoa per milliliter between 1940 and 1990 in Europe.

There are grounds for linking these four disorders. For example, a comparative study in European countries showed that the incidence of each of these four abnormalities (sperm count decline, testicular cancer, hypospadias, and cryptorchism) was maximal in Denmark and minimal in Finland. Moreover, having a history of cryptorchism increases the risk of other three disorders by a factor of 3 in the case of testicular cancer. Similarly, hypospadias increases the chances of developing testicular cancer and oligospermia is frequently observed in men, who go on to develop testicular cancer. Therefore, it has been suggested that these four alterations are symptoms of a single syndrome, the testicular dysgenesis syndrome TDS.¹¹

¹¹ DELBES et al: Estrogen effects on fetal and neonatal testicular development, *Reproduction*, 2006, issue 132, p. 527-528.

2.1 Fertility and semen quality

Long-term trends of decrease in fertility and semen quality are difficult to confirm and are still being debated. The debate has focused mainly on sperm concentration. A much-cited paper published in 1992 reviewed the world literature. Its claim of a 50% decline in mean concentration over 50 years, from 113 to 66 million/ml, should be treated with great caution. Another similar analysis along the same lines found the decline in sperm density to be much steeper in Europe than in America.

In 1992 several centers analyzed their semen quality data, which had been continuously collected for some two decades. Those data are less likely to have been distorted by possible changes in the method of semen examination or in selection processes affecting the populations studied. The principal conclusions that emerge are these:

1. declines in semen quality have occurred in some places (*e.g.* Paris, Edinburgh, Gent) but not in others (Toulouse, Finland and the five US cities with published data);
2. at most, the available data go back to the early 1970s;
3. where concentration has deteriorated, there usually have been also alterations in sperm motility and morphology.

Where a decline has occurred, the findings are compatible not only with a period effect but also with a birth cohort effect, men born in the 1940s having better quality semen than those born in the 1960s. It is not possible to locate the year when the decline started or what the pre-decline values were. As semen quality is inferior in humans compared with other mammalian species, it is possible that deterioration from a 'natural' level has a much longer history than we have the data to substantiate.

This deterioration involved not only sperm concentration, but also morphology and motility. No evidence is available on earlier periods, so that a decline may possibly have begun earlier. Substantial spatial variation in sperm concentration has been demonstrated, within both Europe and America. Based on the available evidence, concentrations appear to be relatively high in New York and Finland and low in California and north-western Europe including Denmark and Britain.

Couple fertility is high in parts of southern Europe compared with the north, with the exception that it is also high in Finland. The congruence of the findings for Finland suggests that the higher levels of sperm concentration observed there are not the result of differences in methodology or to longer abstinence (less frequent intercourse).

2.2 Testicular cancer

There has been an increasing trend in incidence shown for this disease in recent decades throughout the developed world, typically with rates trebled or more. An important and often overlooked question is when this began. In England and Wales, mortality started rising around 1920, having been stable before World War I. In Denmark, a continuous rise in age-standardized incidence is observable since cancer registration began in 1943.

Clinical research strongly suggests that the predisposition to testicular cancer is present from an early age, probably *in utero*, so that the possible influence of environmental agents needs to be evaluated in relation to time of birth rather than of diagnosis or death. Accordingly, if these trends are examined in terms of birth cohorts, mortality started rising among men born before 1900 in England and Wales, and incidence in Denmark, Norway

and Sweden started rising among men born around 1905. In these latter three countries, rates stabilized or fell for men born during 1935–45, whereas the rise was rapid and inexorable among men born from 1920 until at least 1960 in East Germany, Finland and Poland. Recent data indicate that the rates may be stabilizing for Danish men born since about 1960, but the 1965 birth cohort shows a continuing rise in other countries.

There is considerable spatial and ethnic variation. Denmark has the highest incidence in the world, the lifetime risk now being almost 1%.

However, the Nordic countries do not have a uniformly high risk, as Finnish men have comparatively low rates, with Norway and Sweden in intermediate positions. The spatial pattern for testicular cancer in the Nordic countries does not resemble that of other hormone-sensitive carcinomas such as those of the prostate or female breast, but is similar to that of colo-rectal cancer in both sexes.

Other high-risk populations include Switzerland and New Zealand, whereas the Baltic states and African-Americans have comparatively low rates. The tumor is rare among Chinese and Japanese men.

2.3 Anomalies of the male genitalia

Hypospadias and cryptorchism have been grouped with male infertility and cancer of the testis into the 'testicular dysgenesis syndrome', on the grounds that they occur together more often than expected by chance, and that they all probably originate early in life. Therefore, it is argued, they probably share at least some risk factors.

Both anomalies are likely to be unreliably ascertained at birth, particularly in mild cases, and the study of cryptorchism is further complicated by the difficulty of distinguishing testes that have not descended from those that readily but reversibly retract back into the abdominal cavity in early infancy. The consequence is that published data from congenital malformation registries cannot be relied on to reflect real variations: reported time trends and differences between registries may both merely reflect differences in ascertainment and reporting. Self-reported data (by mothers) are similarly unreliable.

For hypospadias, the apparent increase in many countries may well be because of variations in the registry system rather than a real change, apart from a step increase between 1982 and 1985 in the severe form in Atlanta, Georgia. Recent studies in Denmark and Finland using strict criteria have shown a higher rate in Denmark.

In the case of cryptorchism, a study was carried out in Oxford during the 1950s, using strict diagnostic criteria and examination of the baby boys at 3 months when the diagnosis is more reliable. A subsequent study in southern England using the same criteria found almost double the proportion of boys having cryptorchism. Recent studies in New York and in Finland using the same criteria found a similar proportion to the original (lower) Oxford estimate, whereas in Denmark it was close to the later English value. Unlike for testicular cancer, African-Americans do not appear to have a lower risk.¹²

¹² JOFFE Michael: Infertility and environmental pollutants, *British Medical Bulletin*, 2003, vol.68, p. 51-56.

3. Effects of xenoestrogens on male reproductive system

As the descriptive epidemiology cannot help us understand the underlying biological processes and human tests are out of question the animal tests are the only feasible alternative. Here is a summary of some of the findings and how they are interpreted.

Male mice exposed to high doses of bisphenol A (BPA), a low potency estrogen-like effector, *in utero* during gestational days 16–18 showed increased anogenital distance, increased prostatic size and decreased epididymal weight. These changes persisted during adulthood. Male fetuses also exhibited an increase in the number of prostatic glandular buds. In adulthood, these animals exhibited decreased daily sperm production and enlarged prostates. Exposure of fetal prostates to BPA *in vitro* also resulted in prostate enlargement, which was shown to be mediated through the ERs present in the stroma, and this effect was blocked by antiestrogens. BPA has been shown to increase the expression of androgen receptor in the prostate stroma of mice, while fetal exposure of rats to BPA induced alterations in the differentiation pattern of the peritubular stroma in this same organ. A recent study also revealed that the increase in the number and size of dorsolateral prostate ducts and overall increase in prostate duct volume observed in male mouse fetuses is due to an increase in the proliferation of basal epithelial cells. Malformations in the urethra were also observed; its connection to the bladder was significantly constricted. Taken together, these results indicate that prenatal BPA exposure results in permanent alterations of the morphology,

histoarchitecture, and cell proliferation control in the prostate and other androgen-target tissues predisposing the affected individual to disease in adult life.¹³

Results of another study on rats provided evidence that exposure of neonatal male pups to estrogen induced permanent dysmorphogenesis of the penis characterized by replacement of cavernous spaces and smooth muscle cells by fat cells in the corpora cavernosa penis. Similar novel effects have not been reported in the rat or any other species except the rabbit, where bisphenol A or tetrachlorodibenzodioxin treatment at puberty resulted in deposition of fat, a reduction in cavernous spaces. These data point to smooth muscle cells and cavernous spaces in the body of the penis as primary targets for estrogen action. Studies aimed at elucidation of mechanisms through which aberrant estrogen exposure affects restructuring of the corpora cavernosa will provide important insights into mechanisms governing early development of the penis. The study showed that rats treated prior to 12 days of age, but not after, developed permanent penile abnormalities and loss of fertility, implying postnatal days 1–12 as the period when penis was sensitive to estrogen exposure.

It is noteworthy here that the rat penis during this estrogen sensitive period contains only stromal cells in the corpora cavernosa (lacks smooth muscle cells and cavernous spaces) and thus is similar developmentally to the human penis in the first and second trimesters of pregnancy.

Whether the observed effects of estrogen on penile dysmorphogenesis result from an ER-mediated pathway or an AR-mediated pathway, or both ways remains unclear. Support

¹³ MAFFINI Maricel V. et. al.: Endocrine disruptors and reproductive health: The case of bisphenol-A, *Molecular and Cellular Endocrinology*, 2006, issue 254/255, p. 184.

for an ER-mediated pathway comes from observations that both ER α and β are present in stromal cells in 1-day-old rat penis, estrogens inhibit proliferation of smooth muscle of injured blood vessels, and the estrogen metabolite, 2-methoxyestradiol, inhibits angiogenesis. Additionally, ER α up-regulation is associated with abnormal development in rodents of male reproductive tract, prostate gland, and seminal vesicles.

As far as isolated effects of estrogens on humans are concerned it has been reported that diethylstilbestrol, a medication that had been used from 1940s until the late 1970s as estrogen-replacement therapy for estrogen deficiency states, caused increased hypospadias and cryptorchism in the born boys.¹⁴

There are however some authors (Joffe et al.) who disagreed with this conclusion. They state that while it is superficially plausible that estrogens 'demasculinize' the developing male, this is biologically naive because mammals are adapted to starting life inside their mothers, whose internal environment is estrogen-rich (even before the early pregnancy surge). The risk of testicular cancer for the born males may be raised, but by less than the trend observed throughout the developed world, and the position for sperm concentration is similar; hypospadias has only been implicated because of a propagated error in the literature. Therefore the explanation of the symptoms by estrogen exposure only is not satisfactory and we have to look for other chemicals, such as anti-androgenic pesticides and other mutagens (dioxins, furans etc.).¹⁵

¹⁴ FABER, K. A. and HUGHES, C. L.: The effect of neonatal exposure to diethylstilbestrol, genistein and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. *Biol. Reprod.*, 1991, issue 45, p. 649-653.

¹⁵ JOFFE Michael: Infertility and environmental pollutants, *British Medical Bulletin*, 2003, vol.68, p. 59-60.

Another concern are phytoestrogens which are a natural content of soya beans and other types of food. Despite the numerous reports of phytoestrogen-rich diets causing adverse effects in animals, there are relatively few reported cases of phytoestrogens having adverse effects in humans (most probably because the dosage tested in animals was many times higher than amounts generally consumed in human diet). Results of yet another study led to the conclusion that the isoflavone dosage (40 mg) in the supplement, which is similar to the amounts of phytoestrogens consumed in many Asian nations, had no effect on gonadotropin or sex hormone levels or on semen quality. The authors of the study however caution against consumption of high amounts of phytoestrogen-rich food by infants.¹⁶

¹⁶ MITCHELL Julie H.: Effect of a phytoestrogen food supplement on reproductive health in normal males, *Clinical Science*, 2001, issue 100, p. 613–618.

Conclusion

As studies on rodents and clinical observations on humans have shown the male reproductive system responds to estrogen exposure and thus estrogen indeed is capable of affecting male reproductive system in several loci. The question is however whether the current levels of estrogens in the environment are high enough to have any effect. Yet another question one must ask is how do we measure those levels to which human body is exposed. As we are on the very top of the food chain we are exposed not only directly (e.g. through water we use) but also indirectly (accumulation in plants and animals we eat). Furthermore, the substances can be consumed not only orally but also transcutaneously, in which case they aren't processed by liver and therefore their effects multiply.

The fact is that there are only rough estimates of what the amounts of xenoestrogens in the environment might be. I agree with the conclusion of those authors who argue that the levels are still too low to have any substantial effects. As Joffe states in his article (Infertility and environmental pollutants, British Medical Bulletin 2003) the recent deterioration of male fertility and dysmorphogenesis can be predominantly attributed to other chemicals such as phthalates, dioxins, pesticides etc.

Nonetheless, as we know estrogens are quite stable molecules and if we keep dumping them to our back yard they will eventually accumulate in the levels high enough to influence us. There are documented cases of professional exposure that can provide an insight of the health issues which could arise when the levels in the environment reach dangerous limits . Extensive research still needs to be done in this area to determine what the safe limits of xenoestrogen levels for humans are.

Souhrn

Tato diplomová práce shrnuje základní fyziologii a histologii spermatogenezy s ohledem na funkci estrogenů a vliv xenoestrogenů na mužskou potenci. Dále zmiňuje také epidemiologii mužské fertility za posledních několik dekad a klade si otázku, do jaké míry jsou trendy v poklesu mužské potence a zvýšené incidence dysmorfogenezí a karcinomu varlat ovlivněny akumulací xenoestrogenů v životním prostředí. Na základě analýzy relevantních článků dochází autor k závěru, že přestože studie dokazují vliv xenoestrogenů na mužský pohlavní systém, není zatím akumulace hormonů dostatečná na to, aby měla prokazatelný nepříznivý dopad.

Summary

This thesis is a review of basic physiology and histology of spermatogenesis with respect to estrogen function and effect of xenoestrogens on male fertility. Furthermore it mentions also epidemiology of male fertility within the last few decades and it poses a question to which extent are the trends of male fertility deterioration, increased incidence of dysmorphogenesis and carcinoma of testes affected by accumulation of xenoestrogens in our habitat. Based on an analysis of relevant articles, the author reaches a conclusion that even though studies show an effect of xenoestrogens on male reproductive system the accumulation of hormones is not high enough to have any evident adverse impact.

List of tables and graphs

Table 1: Distribution of ERs in testes	14
Table 2: Endocrine disruptors	15
Graph 1: Estrogen in rete testis fluid	11

List of literature

- DELBES** et. al: Estrogen effects on fetal and neonatal testicular development, *Reproduction*, 2006, issue 132, p. 527-528.
- FABER**, K. A. and **HUGHES**, C. L.: The effect of neonatal exposure to diethylstilbestrol, genistein and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. *Biol. Reprod.*, 1991, issue 45, p. 649–653.
- FRANCA** LR et. al.: Germ cell genotype controls cell cycle during spermatogenesis in the rat. *Biol Reprod*, 1998, issue 59, p. 1371–1377.
- GOYAL**, H.O. et. al.: Role of estrogen in induction of penile dysmorphogenesis: a review, *Reproduction*, 2007, issue 134, p. 199-208.
- JOFFE**, Michael: Infertility and environmental pollutants, *British Medical Bulletin*, 2003, vol.68, p. 47-70.
- MAFFINI**, Maricel V. et. al.: Endocrine disruptors and reproductive health: The case of bisphenol-A, *Molecular and Cellular Endocrinology*, 2006, issue 254/255, p. 179-186.
- MITCHELL**, Julie H.: Effect of a phytoestrogen food supplement on reproductive health in normal males, *Clinical Science*, 2001, issue 100, p. 613–618.
- O'DONELL**, Liza et. al.: Estrogen and Spermatogenesis, *Endocrine Reviews*, 2001, vol.22, no.3, p. 289-318.
- SIERENS**, Jayne E. et. al.: Estrogens in Testis Biology, *Ann. N.Y. Acad. Sci.*, 2005, issue 1061, p. 65–76.
- SIKKA**, Suresh C. et. al.: Endocrine disruptors and estrogenic effects on male reproductive axis, *Asian Journal of Andrology*, 2008, vol.10, no.1, p. 134-145.