Chapter

The Role of Endocrine-Disrupting Chemicals in Male Fertility Decline

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Abstract

Endocrine-disrupting chemicals (EDCs) are exogenous compounds with natural or anthropogenic origin omnipresent in the environment. These compounds disrupt endocrine function through interaction with hormone receptor or alteration of hormone synthesis. Humans are environmentally exposed to EDCs through the air, water, food and occupation. During the last decades, there has been a concern that exposure to EDCs may contribute to an impairment of human reproductive function. EDCs affect male fertility at multiple levels, from sperm production and quality to the morphology and histology of the male reproductive system. It has been proposed that exposure to EDCs may contribute to an impairment of sperm motility, concentration, volume and morphology and an increase in the sperm DNA damage. Moreover, EDCs exert reproductive toxicity inducing structural damage on the testis vasculature and blood-testis barrier and cytotoxicity on Sertoli and Leydig cells. This chapter will explore the effects of EDCs in male reproductive system and in the decline of male fertility.

Keywords: endocrine-disrupting chemicals, male infertility, lifestyle, environmental pollutants, body burden

1. Introduction

Endocrine-disrupting chemicals (EDCs) are exogenous substances or mixtures of chemicals that can disrupt male and female endocrine function through the interaction with hormone receptors. They lead to alterations in hormone action, synthesis, transport and metabolic processes [1]. Several compounds such as dioxins, plastic contaminants (e.g., bisphenols (BP)), triclosan (TCS), pesticides and herbicides (e.g., diphenyl-dichloro-trichloroethane (DDT)), metals and others are known EDCs [2].

Humans may be exposed to EDCs due to contamination of water and food chain, inhalation of contaminated house dust and through occupational exposure [2]. Although, in some westernized countries the use of certain EDCs has been banned, there are cases that human exposure to these chemicals is inevitable. Thus, during the past decades, human exposure to EDCs has received increased attention, and particular focus has been given to the harmful effects of EDCs to the male reproductive system. Evidences suggest that EDCs may have significant adverse

effects on human health and are contributing to the trends in occurrence of male reproductive health problems and the decline in male fertility [3]. According to the literature, male reproductive decline may result from a combination of morphological, functional and molecular alterations in the reproductive organs, often due to exposure to EDCs. Most studies are focused either on the evaluation of basic seminal parameters or reproductive outcomes, but there are evidences that EDCs may impact at the level of the reproductive and endocrine systems. For example, there are evidences that TCS has a tendency to bioaccumulate in the epididymis [4]. Bisphenol A (BPA) has been reported to have both estrogenic and antiandrogenic effects [5–7]. It has been also negatively associated with sperm quality [8–10]. Toxicological studies showed that BPA caused adverse reproductive outcomes, namely, decreased epididymal weight, daily sperm production and testosterone (T) levels in rodents [11–13]. Recently, our group performed a systematic review regarding the effect of exposure to mercury (Hg) on human fertility [14]. Results revealed that higher levels of Hg in blood and hair were associated with male subfertility or infertility status.

This chapter summarizes the effects of male exposure to EDCs on markers of male fertility. The agents discussed here, which include TCS, BPA, metals (such as cadmium (Cd) and Hg), polychlorinated biphenyls (PCBs) and others were chosen based on their human exposure prevalence and adverse effects on human reproductive health.

2. EDCs induce reproductive system toxicity: ultrastructural, cellular and molecular changes

The male reproductive system is composed by two testes, a system of genital ducts, the accessory glands (seminal vesicles, prostate, Cowper and Littre glands) and the penis [15]. Testes, the male sexual glands, are ovoid organs localized outside the abdominal cavity within the scrotum. This localization maintains the temperature at 2–4°C lower than the body temperature, optimal for the testes function. Testes are surrounded by two different layers of protective tissue, the *tunica albuginea* and the *tunica vaginalis*. The testicular parenchyma is composed of one to three seminiferous tubules, the functional unit of the testis, and of interstitial tissue surrounding the tubules that contain the Leydig cells (LC), which are responsible for the production of T in the presence of luteinizing hormone (LH) (**Figure 1**) [16]. The seminiferous tubules are composed of male germ cells (spermatogonia, spermatocytes and spermatids) and Sertoli cells (SC). SC are involved in the mechanical support and nutrition of germ cells, regulation of male germ cell proliferation and differentiation, phagocytosis, steroid hormone synthesis and metabolism and maintenance of the integrity of seminiferous epithelium. The male reproductive system is responsible for the production of spermatozoa, for the synthesis and secretion of male sex hormones and for the delivery of male gametes into the female reproductive tract. The process of spermatogenesis is highly regulated by the hypothalamic-pituitary-gonadal (HPG) axis.

Evidences suggest that the normal morphology and function of the male reproductive system are affected by several factors including environmental pollutants (**Figure 1**) (e.g., EDCs). In addition to altered testicular morphology and dysfunction, exposure to EDCs also increased the incidence of testicular pathologies. For instance, exposure to phthalates was associated with the development of testicular cancer, cryptorchidism and hypospadias [17]. This section discusses the current knowledge on reproductive system EDC toxicity in humans and other animals.

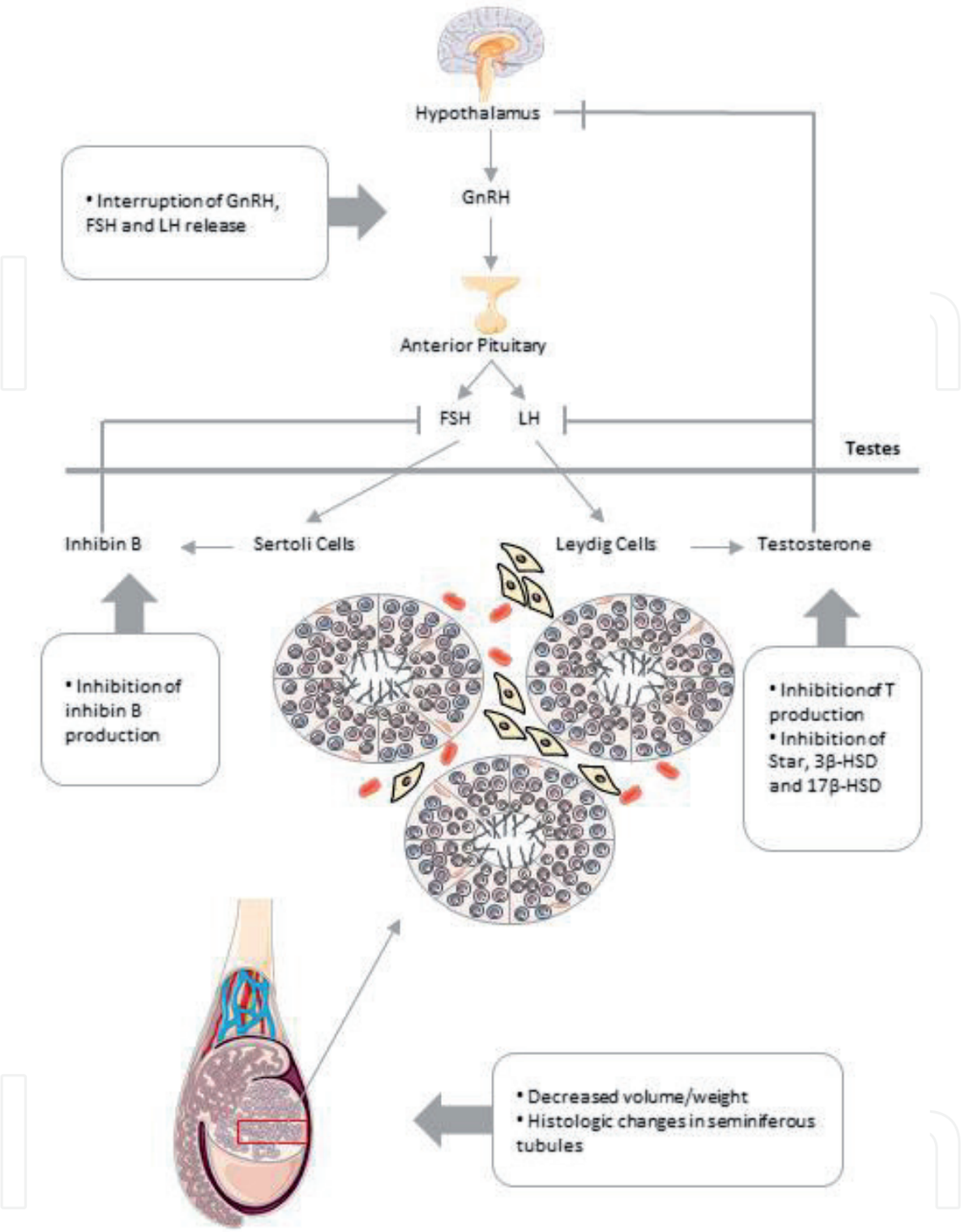

Figure 1.
Schematic representation of the effects of EDCs on HPG axis and testicular morphology.

2.1 Changes in volume/weight of reproductive organs

The volume/weight of the male reproductive organs is an important indicator of the integrity of this system. Several animal studies showed a significant decrease in the weight of the testes and sex accessory tissues in animals exposed to EDCs [4, 18–23]. For instance, male rats treated with 10 and 20 mg/(kg day) of TCS revealed a significant decrease in the weight of the testes, epididymis, ventral prostate, vas deferens and seminal vesicles [18]. However, an administration of 5 mg/(kg day) of TCS did not cause significant change in the testes and sex accessory tissues [18]. Recently, Lan et al. [4] showed that the absolute

weights of testes and epididymis of rats treated with 10, 50 or 200 mg/kg of TCS were not significantly affected.

Rodents were exposed to BPA by the oral route or subcutaneous injections [24, 25]. A dose of 2 ng/g body weight induced a decrease in epididymal weight and an increase in prostate weight. Bisphenol S (BPS), considered a safe substitute for BPA, has chemical similarities with BPA and may act as an EDC. Thus, a recent work compared the effects of BPA and BPS on the morphology and physiology of the ventral prostate of adult gerbils [26]. Animals treated with BPA and BPS showed no alterations in prostate weight. Regarding histopathology, BPS-treated animals showed intense prostatic hyperplasia; increased relative frequency of epithelium, muscular stroma and non-muscular stroma; and decreased luminal compartment, and BPA-treated animals showed increased occurrence of hyperplastic growth. But, in general the authors found that BPS promoted more structural and histopathological changes than BPA.

Exposure to metals also induced effects on testes size. A dose of 5 mg/kg body weight of cadmium chloride ($CdCl_2$) administered to rats by oral gavage caused a significant decrease in testes and epididymis weight [19]. Moreover, Hg and zinc (Zn) significantly decreased the absolute and relative testicular weights in murine, with Hg producing the highest reduction in weight [27]. Similar results were obtained by Narayana et al. [22] and Geng et al. [23] that showed a decrease in the weights of reproductive organs of rats exposed to pesticides.

Rats exposed to phthalates demonstrated reduced testicular weights and histologic changes in the seminiferous tubules [20, 21]. Moreover, rats exposed to phthalates during the prenatal period developed reproductive anomalies, namely, smaller testes and penis size [28].

Human studies related to the effects of exposure to EDCs on testicular volume/weight are limited but in accordance with animal studies. For instance, in a study in Croatian men, no occupational exposures were exposed to metals, and blood Cd was negatively correlated with testes size, suggesting that this metal exerts toxicity on human testes [29].

2.2 Alterations in testicular morphology

Experimental studies showed that exposure to EDCs had adverse effects on testes, resulting in testicular damage at structural and consequently functional level. Male rats treated with 20 mg/(kg day) of TCS exhibited several histopathological malformations in the testes and sex accessory tissues [18]. Lumen of vas deferens from the treated rats exhibited the presence of stereocilia detached from the epithelium and the presence of eosinophilic bodies. Moreover, the stereocilia were found to be thin, few or absent in the epithelium of TCS-treated rats. Rats treated with a high dose of TCS (200 mg/kg) showed changes in the cauda epididymis and in the testis compared with the control group [4]. In the cauda epididymis, the alterations included vacuolated and exfoliated epithelial cells. Moreover, these authors identified the absence of sperm tails in the seminiferous tubules in the TCS-treated groups.

Mice exposed to BPA showed the formation of morphologically multinucleated giant cells in testicular seminiferous tubules [30], disruption of the blood-testis barrier (BTB) and impaired spermatogenesis [31, 32]. Similar results were obtained by other study using pesticides that induced severe degenerative changes in seminiferous tubules [23]. Metals, such as Cd and Hg, also induced structural alterations in the testis structure, including damage in the vascular endothelium and in the BTB integrity and necrosis and disintegration of spermatocytes [27, 33]. In general, these animal studies showed that EDCs induced changes in testicular morphology, which may be a reason for the decline of male fertility. For instance, damage in epididymis

compromise the transport of testicular sperm out of the testis, the acquisition of progressive spermatozoa motility and the sperm storage. Moreover, damage at SC and LC levels compromise the structure of the BTB and seminiferous tubules.

2.3 Testicular dysfunction due to EDC exposure

The two main functions of the testes are spermatogenesis (exocrine function) and steroidogenesis (endocrine function). In normal conditions the gonadotrophin-releasing hormone (GnRH) is secreted by the hypothalamus, stimulating the synthesis of LH and the follicle-stimulating hormone (FSH) [34]. LH is recognized by LH receptors in LC stimulating T biosynthesis (steroidogenesis). FSH is recognized by FSH receptors in SC having an important role in spermatozoa production (spermatogenesis). Several studies showed that these functions are affected by exposure to EDCs (**Figure 1**) [10, 18, 35–39]. Prenatal exposure to EDCs was associated with testicular anomalies later in life, which includes reduced semen volume and quality, increased incidence of cryptorchidism and hypospadias and increased incidence of testicular cancer [40]. EDCs reduced SC number and impaired LC development, inducing testicular anomalies at morphological and functional level [39]. This section presents the studies that assessed the relationship between animal and human exposure to EDCs and testicular dysfunction, including alterations in reproductive hormone levels.

Evidences from animal studies suggest that TCS reduces the production of T in LC and disturbs the function of major steroidogenic enzymes [41, 42]. Male rats treated with TCS or pesticides showed a significant decrease in the levels of serum LH, FSH, cholesterol, pregnenolone and T compared to control [18, 23]. Regarding human studies, a case-control study showed that urinary levels of phthalates and TCS were negatively associated with inhibin B and positively with LH [39]. Additionally, an inverse association was found between urinary levels of phthalates or BPA and testosterone and estradiol (E_2) [38, 39]. Similar results were obtained by Meeker et al. [35] that showed an inverse association between BPA concentrations in urine and serum levels of inhibin B and E_2:T ratio in men recruited through an infertility clinic. Moreover, a positive association between BPA concentrations in urine and FSH and FSH:inhibin B ratio was found. Hanoaka et al. [36] did not found an association between exposure to BPA and free T and LH concentrations in men. However, a significant decrease in FSH concentrations was found in the BPA exposed men. Urinary levels of BPA were not associated with sperm quality in fertile men but were associated with markers of androgenic action [37]. A significant inverse association was found between urinary levels of BPA and free androgen index (FAI) levels and the FAI:LH ratio. Further, a significant positive association between BPA and sex hormone-binding globulin (SHBG) was found in fertile men. Recently, Lassen et al. [10] examined associations between urinary BPA concentration and reproductive hormones in young men from the general population. The authors found positive associations between urinary BPA concentrations and T, E_2, LH and free T levels. BPA and BPS induced significant changes in T and estradiol [26].

Meeker et al. [38] demonstrated that exposure to phthalates may be associated with altered male endocrine function. Urinary concentrations of some phthalates were inversely associated with T, E_2 and FAI.

Metals, namely, Cd, also affect the development of the male reproductive system and testis function. Mice prenatal exposed to Cd showed defects on the development of gonads, depletion of germ cells and impairment of spermatozoa maturation [43]. Cd also induces testicular dysfunction, which results of the functional impairment of SC and LC. Regarding human studies, the effect of Cd exposure to male endocrine function was assessed by several authors (as reviewed by de Angelis et al. [33]).

The results obtained are controversial; some authors found that Cd concentrations were positively correlated with FSH, T, E_2, LH and inhibin B and negatively correlated with prolactin [29, 44]. However, other authors did not find significant correlations between Cd concentrations and serum hormone levels [45, 46]. In general, these results suggest that exposure to EDCs may be associated with alterations in circulating hormone levels in men. Additionally, Yang et al. [47] showed that levels of GnRH and LH were significantly higher in occupationally manganese (Mn)-exposed group compared with the non-exposed men. The levels of T were lower in the exposed group. However, this study demonstrated that there was no association between exposure to Mn and E_2 and FSH and prolactin levels.

2.4 Molecular effects of EDCs

The effects of EDCs on the morphology and function of the male reproductive system may be attributed to the interactions of these chemicals with several molecules. Male rats treated with 20 mg/(kg day) of TCS showed a significant reduction in the testicular levels of mRNA for cholesterol side-chain cleavage enzyme (*Cyp11a1*), 25-hydroxyvitamin D-1 alpha hydroxylase (*Cyp27b1*), 3β-hydroxysteroid dehydrogenase (*Hsd3b1*), 17β-hydroxysteroid dehydrogenase (*Hsd17b6*), steroidogenic acute regulatory protein (*Star*) and androgen receptor (*Ar*) as compared to control [18]. Moreover, the authors found that there was a decreased localization of StAR protein in testicular LC as determined by immunolocalization indicating a reduced expression of this protein in animals treated with TCS as compared to control. These results could be correlated to the reduction in LC number.

In vitro studies investigated the effect of BPA on steroidogenesis [48, 49]. The authors found that BPA inhibited the production of testosterone in a concentration-dependent manner over the course of the 24 h incubation [48]. Moreover, the concentrations of E_2 were greater in the presence of BPA. The decrease in the concentrations of T is related with the inhibition of activities of some enzymes, such as 3β-hydroxysteroid dehydrogenase (*HSD3B1*) and 17α-hydroxylase (*CYP17A*). However, the activity of aromatase was not altered by BPA treatment. More recently, additional results in MA-10 Leydig cell line showed that BPA affects steroidogenic genes, for instance, induces the upregulation of *CYP11A1* and *CYP19* genes [49]. Moreover, the authors found that BPA treatment induced the phosphorylation levels of c-Jun and the levels of protein expression of SF-1, suggesting that the JNK/c-Jun pathway may be involved in BPA toxicity. Similar results were observed in an animal study [49].

The testes from male Sprague-Dawley rats treated with $CdCl_2$ showed a significant increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [19]. Geng et al. [23] found that pesticides altered the testicular protein expression of B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax). Moreover, these authors showed that the activities of testicular enzymes including acyl carrier protein (ACP), lactate dehydrogenase (LDH) and gamma-glutamyltransferase (γ-GT) were significantly altered by exposure to pesticides.

3. Spermatozoa

Sperm motility, together with concentration and morphology, is considered as one of the important predictors of male fertility in vivo. Declining human sperm quality has been demonstrated in several recent studies. Age, lifestyle, environmental pollutants and nutritional factors can affect semen quality [14, 50–52].

The present section focuses on studies of environmental exposure to EDCs and male reproductive function, as measured by declines in semen quality parameters or increased sperm DNA damage/fragmentation.

3.1 Effects of EDCs on sperm production, morphology, motility and velocity

Several studies have been published regarding the association of exposure to phenols and human semen quality [53–55]. A case–control study was conducted to evaluate the association between exposure to phenols and idiopathic male infertility [55]. For that, the authors recruited idiopathic infertile men and fertile controls and measured urinary levels of BPA, benzophenone-3, pentachlorophenol, TCS, 4-*tert*-octylphenol (4-*t*-OP), 4-*n*-octylphenol (4-*n*-OP) and 4-*n*-nonylphenol (4-*n*-NP) and semen parameters. The authors found that exposure to 4-*t*-OP, 4-*n*-OP and 4-*n*-NP was associated with idiopathic male infertility, and exposure to 4-*t*-OP and 4-*n*-NP was also associated with abnormal semen quality parameters. However, in this study the authors did not find more relationships between exposure to other phenols and idiopathic male infertility. In another study, urinary BPA concentrations were associated with declines in sperm concentration, motility and morphology [53]. An increasing urine BPA level was associated with lower semen concentration, lower total sperm count, lower sperm vitality and lower sperm motility [54]. Moreover, the authors demonstrated a dose–response relationship between increasing urine BPA level and reduction in semen quality. Lassen et al. [10] also found an inverse association between BPA concentrations and progressive motility, but in this study, BPA excretion was not associated with semen volume, sperm concentration, total sperm count or percentage morphologically normal forms. However, some authors did not find any association between urinary BPA concentrations and some semen parameters, such as semen volume or sperm morphology [8, 54].

TCS has been shown to decrease sperm density probably due to reduced testicular spermatogenesis [18]. A reduced sperm density was observed in the lumina of epididymal tubule from the treated rats. Rats treated with high doses of TCS (50 and 200 mg/kg) showed a significant decrease in the daily sperm production and an increase in the percentage of sperm abnormalities, which included elevated ratios of abnormal sperm head and tails [4]. Zhu et al. [56] performed a cross-sectional study to evaluate the association between exposure to TCS measured by urinary TCS concentration and semen quality in humans. The authors found an association between urinary TCS concentrations and poor semen quality parameters; namely, the authors found an inverse association between urinary TCS concentrations and percentage of sperm motility, sperm count, sperm concentration and percentage of normal morphology, suggesting that environmental exposure to TCS may have impact on semen quality.

Regarding exposure to PCBs, several studies showed an inverse association between exposure to PCB 153 and sperm motility, while relationships with sperm concentration or total sperm count were inconsistent [57–59]. Additionally, Hauser et al. [60] found an inverse dose–response relationship between PCB 138 and sperm concentration, motility and morphology.

The correlation between exposure to metals and adverse consequences for human and animal fertility is not completely established. Several studies determined the effects of exposure to metals on male gametes. In vitro studies, using bovine sperm, determined the effect of direct exposure to Hg on male gametes [61, 62]. Arabi et al. [61] showed that exposure to Hg (50, 100, 200, and 300 μmol/l) induced LPO (lipid peroxidation), decreased the glutathione (GSH) content and decreased the percentage of viable spermatozoa. Additionally, a more recent study showed that bovine sperm exposed to Hg at 8 nM and 8 μM have less motility and have impaired sperm

membrane integrity, increasing levels of reactive oxygen species (ROS) and LPO and decreasing the antioxidant activity and diminished fertility ability [62]. Regarding human fertility, in a cross-sectional study, participants with high blood Hg level had lower sperm with a normal morphology [63]. Cd is another male reproductive toxicant that exerts effects even at low levels of exposure by several mechanisms [64]. In vitro studies on human spermatozoa obtained through ejaculation allow to evaluate the effect of Cd treatment in semen parameters [65, 66]. Cd decreased sperm motility and sperm viability and induced detrimental effects on spermatozoa metabolism by inhibition of the activity of glycogen phosphorylase, glucose-6-phosphatase, fructose-1,6-diphosphatase, glucose-6-phosphate isomerase, amylase, Mg^{2+}–dependent ATPase and lactic and succinic acid dehydrogenases. As reviewed by de Angelis et al. [33], significant negative correlations were found between Cd levels and semen parameters, including total sperm count, concentration, motility and morphology. Results from a meta-analysis indicate that men with low fertility had higher semen Pb and Cd levels and lower semen Zn levels [67]. Sperm motility was significantly decreased in men occupationally exposed to Mn [47].

Occupational exposure to pesticides increased the risk of morphological abnormalities in sperm in addition with a decline in sperm count and a decreased percentage of viable spermatozoa. For instance, the exposure to pesticides reduced the seminal volume, sperm motility and concentration and increased the seminal pH and the abnormal sperm head morphology [68–70]. A study showed that young Swedish men exposed to phthalates presented a decrease in progressive sperm motility [71]. Additionally, levels of urinary phthalates and insecticides were also associated with lower sperm concentration, lower motility and increased percentage of sperm with abnormal morphology [72–75]. These results confirmed the results obtained by in vitro and in vivo studies [76, 77].

3.2 Sperm DNA damage

Sperm DNA integrity is essential for the correct transmission of genetic information [78]. Damage at sperm DNA level may result in male infertility. Sperm DNA damage is caused by oxidative stress that causes impairment in the sperm membrane [79]. It is well-known that some EDCs may induce oxidative stress and decrease the cellular levels of GSH and protein-sulfhydryl groups. Preclinical studies with male rats showed that exposure to BPA was associated with a significant increase in sperm DNA damage [80]. A statistically significant positive association between urinary concentrations of parabens and BPA and sperm DNA damage was found in male partners of subfertile couples [53, 81]. Contrary results were obtained by Goldstone et al. [8] that found a negative relationship between BPA and DNA fragmentations.

Additionally, other EDCs such as heavy metals (e.g., Hg), PCBs and insecticides induce sperm DNA damage [59, 61, 73, 75, 82–84]. Urinary levels of Hg and nickel in infertile men were associated with increasing trends for tail length, and the levels of Mn were associated with increasing trend for tail distributed moment [82]. The adverse effects of phthalates on sperm DNA were assessed by several studies among infertile men [75, 84]. Urinary concentrations of phthalate metabolites were associated with sperm DNA damage. These studies suggest that environmental and occupational exposure to EDCs may be associated with increased sperm DNA damage.

4. Conclusions

The results yielded in this chapter showed that both environmental and occupational exposures to EDCs affect male reproductive function at multiple levels.

In human populations, the majority of studies point toward an association between exposure to EDCs and male reproduction system disorders, such as infertility, testicular cancer, poor sperm quality and/or function. Exposure to EDCs was associated with declined semen quality, increased sperm DNA damage, alterations in testis morphology and endocrine function. However, there are studies exploring the effect of EDCs on male reproductive health including semen quality, reproductive hormones and male fertility that produced inconsistent results probably due to small-sized study populations and lack of control for potential confounding variables. These contrary results highlight the need to discuss and investigate the effect of environmental pollutants in the male reproductive health. Moreover, the identification of the sequence of events and mechanisms might be important to better understand the effect of exposure to EDCs on male reproductive system and their contribution to male fertility decline.

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Conflict of interest

The authors declare no conflicts of interest.

Abbreviations

ACP	acyl carrier protein
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
BP	bisphenols
BPA	bisphenol A
BPS	bisphenol S
BTB	blood-testis barrier
Cd	cadmium
$CdCl_2$	cadmium chloride
DDT	diphenyl-dichloro-trichloroethane
E_2	estradiol
EDCs	endocrine-disrupting chemicals
FAI	free androgen index
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
GSH	glutathione
Hg	mercury
LC	Leydig cells
LDH	lactate dehydrogenase

LH	luteinizing hormone
Mn	manganese
PCBs	polychlorinated biphenyls
ROS	reactive oxygen species
SC	Sertoli cells
SHBG	sex hormone-binding globulin
StAR	steroidogenic acute regulatory protein
T	testosterone
TCS	triclosan
Zn	zinc
γ-GT	gamma-glutamyltransferase

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