Chapter 7
Endocrine Disruption in the Male

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7.1 Introduction

Reproductive health has emerged as an important healthcare need involving many clinical and public health issues, including sexually transmitted infections (STIs), declining fertility and rising rates of testicular cancer [1–4]. Importantly, it is now recognized that many causes and risk factors for testicular dysfunction and infertility indeed act early during life [5]. Many andrological pathologies that we see in adults actually arose in younger age, due to the strong susceptibility and vulnerability of male gonads to external insults, starting from gestational age and during all growth phases.

Of particular scientific and public interest is the possible contribution of endocrine disruptors to increased incidence of male sexual and reproductive problems, such as infertility, hypogonadism, cryptorchidism, hypospadias and testicular cancer. An endocrine-disrupting chemical (EDC) is defined as "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action" [6]. Contamination from EDCs is almost inevitable, when such chemicals are used in occupational activities or are widely dispersed across the environment. The daily used products like pesticides, plastic items containing bisphenol A and phthalates, flame retardants, personal care products containing antimicrobials, heavy metals and perfluoroalkyls are regularly being manufactured in industries. These are some of the most potential candidates as testicular disruptors among EDCs. Although the biological effects of many EDCs are well known at the molecular and cellular levels in in vitro studies, their mechanism of action is not readily and easily assessed in vivo, as their effects can appear after prolonged and/or continuous exposure to a

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low dose. Importantly, the effects can be transgenerational and therefore two or three generations are necessary to highlight some modest effects, making epidemiological studies in humans very challenging. Furthermore, these effects are often the result of the simultaneous interaction of several substances (mixture effect) at low doses. On the contrary, in vitro and animal studies often use single compounds at a high dose. Human EDC-related diseases are more likely to be the result of long-term exposure to low concentrations of EDCs mixtures, rather than of acute exposure to single compound at high concentration. Anyway, many EDCs that have been linked to impaired male sexual and reproductive development and function seem to act as antiandrogenic and/or estrogenic compounds, after binding or mimicking the actions of either the androgen receptor (AR) or the oestrogen receptor (ER). EDCs are highly heterogeneous and can be classified according to their origins in: (1) natural and artificial hormones (e.g. fitoestrogens, 3-omegafatty acids, contraceptive pills and thyroid medicines); (2) drugs with hormonal side effects (e.g. naproxen, metoprolol and clofibrate); (3) industrial and household chemicals (e.g. phthalates, alkylphenoletoxilate detergents, plasticizers and solvents) and (4) side products of industrial and household processes (e.g. polycyclic aromatic hydrocarbons, dioxins and pentachlorobenzene). As a consequence, many pathways might be disrupted, depending on the period of life when they act (ranging from impairment of sexual differentiation, organogenesis, spermatogenesis and steroidogenesis) and the cocktail of contaminants involved.

In general, three main phases of a man life are particularly susceptible for subsequent normal testis development and function (Fig. 7.1): the intrauterine phase, the

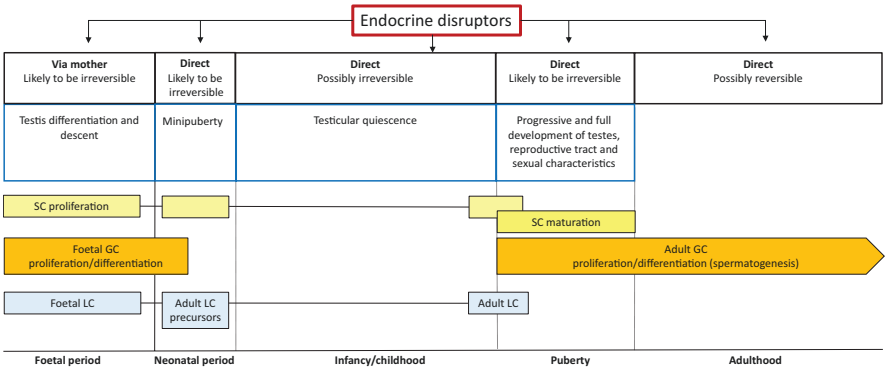

Fig. 7.1 Windows of susceptibility for testicular development and function from the foetal period to adulthood and effects of EDCs. *SC* sertoli cells, *GC* germ cells, *LC* leydig cells. Reproduced with permission from: Ferlin A, Di Nisio A, De Toni L, Foresta C. Impact of Endocrine Disruptors on Male Sexual Development. In: Foresta C, Gianfrilli D (eds), Pediatric and Adolescent Andrology. Trends in Andrology and Sexual Medicine, 2021. Springer, Cham. https://doi.org/10.1007/978-3-030-80015-4_2

neonatal phase comprising the so called “minipuberty” in the first months of life and puberty. However, even during infancy, when the testes are apparently “sleeping”, damaging causes with permanent effects on testicular function can occur. This is, for example, the case of the iatrogenic, devastating effect of chemotherapy in this period of life. Risk factors acting via the mother during pregnancy might compromise definitively testicular function later in life, by disrupting foetal germ cell proliferation and differentiation, Sertoli cell proliferation and establishing of the Leydig cell population (Fig. 7.1). Similarly, risk factors acting directly on minipuberty might compromise germ, Sertoli and Leydig cell differentiation and proliferation. Iatrogenic, environmental and life style risk factors during childhood might interfere above all with the germ cell compartment and those acting during puberty might disrupt Sertoli cell maturation, the establishment of adult Leydig cell population and spermatogenesis (Fig. 7.1) [5, 7]. These fundamental phases of vulnerability are also important when dealing with EDCs, even if the intrauterine, transplacental phase seems to be the most important for future and transgenerational effects. Also adolescence is a vulnerable window for the development and maturation of the genitourinary tract [8]. Risk factors, lifestyles and EDCs effects in adolescence may negatively affect adult health as well as that of future generations, through epigenetics.

A large body of evidence has been published dealing with various molecular and cellular aspects of the action of EDCs and their association with urogenital diseases. However, most studies focused on a single or single class of EDCs. Evidence from epidemiological and clinical studies is less robust for the intrinsic difficulties highlighted above. Indeed, a systematic review and meta-analysis [9] of epidemiological studies reporting association between male reproductive disorders and exposures (documented by biochemical analyses of biospecimens) to chemicals that have been included in the European Commission’s list of Category I EDCs showed that there is evidence for a small increased risk following prenatal and postnatal exposure to some persistent environmental chemicals, but the evidence is low, with an overall odds ratio across all exposures and outcomes of 1.11 (95% CI 0.91–1.35). Most studies are focused on bisphenol A and phthalates [10], and more recently on perfluoroalkyl compounds (PFC) (Table 7.1) [11].

Table 7.1 Main endocrine-disrupting chemicals (EDCs) and related mechanisms and effects during the three main phases of male sexual development

	Pre-natal period		Neo-natal period/Infancy		Childhood/Adolescence	
EDCs	Mechanisms	Effects	Mechanisms	Effects	Mechanisms	Effects
BPA	Foetal Leydig cell dysfunction; germ cell toxicity	Reduced T and/or INSL3; impaired germ cells maturation; impaired HPG maturation	GnRH interference; impaired germ cells maturation	Impaired HPG maturation; congenital malformations (crtyptorchidism, hypospadias)	HPG disruption; reduced steroidogenesis; testicular toxicity on germ cells and Leydig cells	Delayed puberty; reduced spermatogenesis; developmental disorders; delayed sexual maturation; reduced testicular volume
Phthalates	Histological alterations; Leydig cell dysfunction	Reduced T and/or INSL3; impaired germ cells maturation	Impaired germ cells maturation	Congenital malformations (crtyptorchidism, hypospadias)	Reduced steroidogenesis; testicular toxicity	Impaired spermatogenesis
PFAS	Impairment of foetal Leydig cells, germ cells and Sertoli cells	Reduced T and/or INSL3; congenital malformations; impaired germ cells maturation; impaired HPG maturation	GnRH interference; impaired germ cells maturation	Impaired HPG maturation; congenital malformations (crtyptorchidism, hypospadias); reduced AGD	HPG dysruption; reduced steroidogenesis	Delayed puberty; reduced spermatogenesis; developmental disorders; delayed sexual maturation; reduced testicular volume

Abbreviations: *BPA* bisphenol A, *PFAS* perlfuoroalkyl substances, *T* testosterone, *INSL3* insulin-like 3 hormone, *HPG* Hypothalamic–pituitary gland, *GnRH* gonadotropin-releasing hormone, *AGD* anogenital distance

7.2 Mechanism of Action of EDCs on Hypothalamic–Pituitary–Gonadal Axis

As reported above, the interactions involved in gonadal function and hormonal communication are various and complex. Different targets could be impaired by EDCs, and different time points are involved, even at different generations. Various mechanisms can lead to the impairment of endocrine regulation, but mainly involves a reduction in steroid hormones biosynthesis, storage or release and transport, or could involve an antagonistic effect on binding of sex hormones to their receptors, and/or post receptor signal transduction [reviewed in 12].

There is evidence of altered steroid biosynthesis of different hormones from a wide range of toxicological studies on different chemicals [6]. The first reported mechanism of reduced steroidogenesis mainly involves the inhibition of specific enzymatic steps. Another mechanism has been observed by direct inhibition of aromatase activity, therefore blocking the conversion of testosterone to estrogen in the testis. Altogether these chemicals are defined as environmental estrogens and antiandrogens, as they interfere with hormones biosynthesis regulated by gonadal or extragonadal steroids through a series of signals at transcriptional and translational levels [5]. Pituitary hormone synthesis is affected by both estrogen and testosterone, directly or indirectly through changes in the glycosylation of LH and FSH. Therefore, any factor that interferes with the glycosylation has a negative impact on the biological activity of these hormones. As a consequence, any EDCs that mimics or antagonizes the action of these steroid hormones could presumably alter glycosylation.

Another mechanism is the alteration of hormone storage and/or release. For example, after LH stimulation in Leydig cells, the testis synthesizes testosterone. Therefore, any EDC that antagonizes the binding of LH to its receptor on one hand, or that inhibits the activation of the 3′,5′-cyclic AMP (cAMP)-dependent pathway involved in steroidogenesis on the other hand, has the potential to impair testosterone biosynthesis. As for cAMP, second messenger pathways are one of the main routes involved in the release of various hormones. Therefore, any compound that interferes with these processes has the potential to reduce the bioavailability of hormones. For example, disruption of pituitary hormone release has been reported for heavy metals, mainly by interfering with Ca^{2+} flux [12].

Another possible mechanism of hormonal interference on the hypothalamic–pituitary–gonadal (HPG) axis is the perturbation of hormonal transport and clearance. Hormones typically circulate in blood in the free or bound state. Steroids, and androgens in particular, are transported by specific transporters, named steroid hormone-binding globulin (SHBG) or testosterone-estrogen-binding globulin (TEBG). Any modification in the concentration of these carrier proteins in the circulation can lead to an increase or decrease in steroid hormone bioavailability. For example, DDT analogs have been shown to induce an enhancement in the degradation of transport proteins, leading to reduced release of androgen from the testis to the circulation, then limiting its biological systemic activity [10].

Probably the most frequent mechanism of EDCs interference on sex hormones is the altered hormone receptor recognition and/or binding. Since hormones represent a complex system of signal transduction and communication across various body cell types, the correct recognition of hormones with their receptors is fundamental in order to elicit correct responses in target cells. The binding of the physiological ligand to its receptor, which can be either cytoplasmic, nuclear or membrane-bound, is therefore highly specific and represents a crucial step in hormonal signaling. Intracellular receptors, there including steroid receptors, adrenal receptors, thyroid hormones receptors, vitamin D receptor and retinoic acid receptor, normally act by regulating gene transcription upon ligand binding and subsequent nuclear translocation, where they interact with specific DNA target sequences, known as responsive elements that ultimately activate the transcription of target genes. A huge variety of EDCs have been proven to interfere with this process, either by resembling the physiological agonist or acting as an agonist, or even by inhibiting the hormone binding and acting as an antagonist [10–12]. At first, the most studied EDCs were shown to inhibit estrogen receptor activity, such as DDT, some PCBs and BPA. Nonetheless, this interference on steroid hormones is not exclusive of only one compound or towards only one receptor, indeed many EDCs classified as estrogen-like or anti-androgenic compounds have the ability to reduce receptor binding and/or affinity on more than one type of hormone receptor. Classical hormonal receptors are located on and in the cell membrane, upon binding, transduction of a signal across the membrane requires the activation of second messenger signal transduction pathways. Among these, the most frequent involve alterations in G-protein/cAMP-dependent protein kinase A (e.g. after LH stimulation of the Leydig cell), phosphatidylinositol regulation of protein kinase C and inositol triphosphate (e.g. after GnRH stimulation of gonadotrophs; thyrotropin releasing hormone stimulation of thyrotrophs), (c) tyrosine kinase (e.g. after insulin binding to the membrane receptor) and (d) calcium ion flux. Xenobiotics are an example of interference on signaling pathways involving second messengers regulated by peptide hormones. EDCs can also target the cascade of events that follows the hormone binding to its receptor and fundamental to fulfil the physiological response of target cells to hormonal stimulation. Various mechanisms can interfere with activation of steroid hormone receptors. Among these, the most frequent one involves the reduction of receptor sensitivity to its ligand, as observe, for example, after tetrachlorodibenzo-p-dioxin (TCDD) exposure (including the estrogen, progesterone and glucocorticoid receptors). As a result, a wide range of pathways can be altered by EDCs; therefore, any evaluation of their effects on human health should include the possibly largest set of influences on hormonal signaling, receptor function or regulation of feedback [9].

Finally, another possible mechanism relies in the stimulation of oxidative stress, which frequently results in increased apoptosis due to cellular damage as a consequence of oxygen and oxygen-derived formation of free radicals, which is reactive oxygen species (ROS). The generation of ROS has been proven to induce testicular damage after exposure to various chemicals that are associated with hormonal impairment, ultimately leading to infertility. Another target of ROS is the

endothelium, where highly reactive radicals can induce cell damage, leading to a reduction of blood flow to the testis with consequent impairment of testicular function. Finally, ROS can also directly damage DNA, by oxidation of DNA bases or by covalent binding that induces strand breaks or cross-linking [12].

7.3 Bisphenol A

Bisphenols, and in particular the phenol compound 2,2 Bis (4-hydroxylphenyl)–propane, universally known as Bisphenol-A (BPA), are widely used as additives for the production of plastic materials, such as polycarbonate, phenol and epoxy resins, polyesters and polyacrylates, as well as antioxidant in foodstuffs and cosmetics [6, 13]. Specifically, nearly 75% of the industrial production of BPA is intended for the manufacture of polycarbonate-based products, which find wide application in food industry such as containers for food and beverages, in plastic dishes, in kitchen utensils, in containers for microwave cooking and, until 2011, in bottles [14]. Of note, BPA is also used in epoxy-resins films used as binary patina: the internal coatings in the cans for canned food [15].

As a result, there is a significant risk of human exposure to BPA through ingestion, skin contact or inhalation [16, 17]. Epidemiological data from the United States have reported detectable levels of BPA in urine samples from more than 90% of general population, resulting in a major problem of exposure to chemical substance [18].

Concerns about BPA issues on the human health date back to 1930s, when severe impact on male sexual development had been suggested. From a mechanistic point of view, the most relevant risks associated with the exposure to BPA are mainly due to its action as an EDC. Available reports in late 1990s first documented a stimulating activity of BPA on ERα [19, 20] confirmed later [21–23]. In addition, unconjugated BPA showed a binding activity to other two receptors: the G protein-coupled oestrogen receptor 30 (GPR30), also known as membrane estrogen receptor alpha (mERα) [24, 25], and the orphan nuclear oestrogen-related receptor gamma (ERR-gamma) [25]. Finally, experimental animal studies demonstrated that BPA binds also to AR, to the peroxisome proliferator-activated receptor gamma (PPAR-gamma) and the thyroid hormone receptor [22].

A wide amount of data from animal studies shows a clear effect of BPA on male reproductive system, even at very low doses. In rodent models, BPA exposure has been associated with reduced sperm count and significant reductions of the absolute weights of the testes and seminal vesicles [26–33]. Furthermore, the exposure to BPA has been associated with the alteration of other non-conventional markers of sperm quality such as the index of DNA fragmentation, suggesting a possible role as mutagen [29, 32, 34–42]. Also, acrosomal integrity, an overall marker of the fertilization potential, was significantly reduced by PBA exposure in murine models [27].

Several studies have been performed to disclose the possible disruption of the hypothalamus–pituitary–testis (HPT) axis associated with BPA exposure in animal models, with the result of a fairly complex picture that invariably leads to the impaired production of testosterone [28, 43], both by direct effects on steroidogenesis of the Leydig cells [39, 44, 45] and indirect effects on HPT. This latter is mediated by indirect suppression of the pituitary LH release through the massive aromatase upregulation in the testes [46]. Importantly, because of its high lipid solubility, BPA undergoes to trans-placental transfer in animal models with a consequent detection in cord blood, an evidence reported also in humans [47–49]. Accordingly, BPA exposure during the prenatal period was associated with the impairment of both foetal development and the endocrine function of the testis, with reduced Leydig cell proliferation and foetal testosterone production [50–52]. Maternal exposure to BPA was associated with reduced sperm count and motility in male offspring and, in turn, with post implantation loss and decreased litter size [53]. Of note, very recent studies disclosed some transgenerational effects associated with BPA exposure [54].

Despite the large availability of data in animal models, fewer studies assessed the possible relationship between BPA exposure and semen quality in humans and a negative association between urinary BPA and sperm concentration [55], motility, morphology and sperm DNA damage [56]. However, two independent studies on male partners from infertile couples attending infertility clinics were not able to retrieve any significant association between BPA urinary concentration and altered semen parameters [57, 58].

Another field of investigation pursued was the possible correlation between exposure to BPA and alteration of the endocrine pattern, but widely varying scenarios can be observed. Lower serum levels of follicle-stimulating hormone (FSH) in exposed workers compared to those non-exposed was found [59], but also a positive and significantly association with serum testosterone levels was observed [60]. Another study found increased serum testosterone, free testosterone, LH and oestradiol in subjects pertaining to higher urinary BPA concentrations quartile, compared with the lowest quartile. Subjects in the highest urinary BPA quartile also showed reduced progressive sperm motility compared with the lowest quartile [61]. On the contrary, urinary BPA concentrations were found positively associated with serum SHBG levels and inversely correlated with free androgen index (FAI) [58].

Finally, few studies aimed to assess the possible impact of BPA exposure on the overall fertility potential in males through the overall evaluation of the relationship between BPA levels and the reproductive outcome in the setting of assisted reproduction facilities. Minimal association between paternal urinary propyl paraben levels and reduced live birth rate in a correlation model corrected by possible confounders has been reported [62]. However, no significant association emerged between paternal urinary BPA and reproductive outcomes after fertility treatments. On the other hand, urinary BPA concentration in either males or females was not associated with increased time to pregnancy [63].

Overall, available data are supportive of detrimental role of BPA on semen parameters, but this is not accompanied by clear data on sex hormones and on fertility outcomes. As suggested by other authors [64], within the limits of the availability of data in humans, a possible reconciling explanation could rely on a greater direct toxicity of BPA on germ line cells, rather than in an albeit important endocrine disruption of the HPT axis.

In conclusion, BPA represents one of the most controversial chemical pollutants, with the typical features of an EDC. Early toxicological evidence on BPA date back to nearly 30 years ago, when major interference with estrogen signaling pathway was claimed. Since that time, a wide range of cell mechanisms of both endocrine and metabolic disruption have been claimed by the use of experimental models. In particular, major impairment of the HPT axis has been recognized as associated with the exposure to BPA during both the foetal and the adult life, resulting in altered testis development, impaired endocrine function and infertility. To this regard, direct disruption of sperm characteristics, such as reduced motility performances and development genetic abnormalities, has been identified. On the other hand, data obtained in humans are actually limited and poorly conclusive to identify a strict causal role of BPA in reduced male fertility potential.

Methodological differences and different study populations are factors that can explain some discrepancies. Moreover, available clinical outcomes, such as semen parameters and time to pregnancy, are likely susceptible of variation related to many different confounding factors. It should be noted that, as for most of chemical pollutants, the identification of a reliable marker of exposure remains a major issue. Specifically, for BPA, urinary concentrations are surely reliable data from an analytical point of view but may not be representative of the real exposure to BPA due to its short half-life. To this regard, Vitku et al. reported that BPA levels in blood plasma were positively correlated with BPA levels in semen, but only seminal BPA was negatively associated with seminal quality [65]. Finally, the cross-sectional design of the available studies surely provides proof of association but limited evidence of causality.

One of the main problems associated with exposure to endocrine disruptors in general, and to BPA in particular, is represented by the potential activity at low concentrations. This represents a critical issue during the development phases, such as embryo/foetal life, newborn or peri-pubertal age, since the effects in these time windows may result irreversible and are generally detected only at adulthood. Accordingly, populations at higher risk include pregnant women, infants and adolescents (Fig. 7.1). On these bases, the current European law restricted the use of BPA in the production of packaging and materials in direct contact with food by limiting migration rate to 0.05 mg/kg of food and prescribing the total absence in products for newborns, from food to food containers and clothes [66]. In addition, based on new toxicological data and methodologies, the European Authorities adjusted the tolerable daily intake from 50 to 4 μg/kg body weight/day with an overall lowering rate of 12 times, highlighting the increasing level of attention for these health concerns.

7.4 Phthalates

Phthalates are employed in virtually all industrial applications and consumer products as additives, used as plasticizers in a broad range of industrial and commercial products [67, 68]. The most commonly used phthalates are di-(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diethyl phthalate (DEP) and benzylbutyl phthalate (BzBP). More than 75% of DEHP produced worldwide is used in plastic products. The other phthalates are largely used in personal care products like foams, shampoos, dyes, lubricants and food packaging materials [69]. Since these compounds are not covalently bound polymers, their exposure to heat over time has the potential to favour their migration into food [70]. Indeed, plasticizers such as phthalate esters, because of their anti-androgen and oestrogen-like activity, are indicated as major EDCs. Both in vitro and in vivo toxicology studies have demonstrated their endocrine-disrupting potential in model organisms, with endpoints such as antiandrogen effects, reproductive abnormalities, testicular lesions and reduced sperm production [71]. However, as for other EDCs, dose ranges used for traditional reproductive toxicological studies were much higher than those observed in human epidemiological studies. Therefore, it is not surprising that these studies do not entirely align with the human studies. Nevertheless, in vitro and in vivo toxicology studies with low exposures to phthalates were linked to decreased semen quality and male infertility in animals, as well as to decreased androgen production and steroidogenesis [64, 72–80]. Phthalates have mostly shown the antiandrogen effect on testicular function during steroid formation [81–83]. Furthermore, phthalates as well as their metabolites (e.g. DEHP/MEHP, DBP/MBP) have stimulatory effects at low doses through inducing the production of progesterone, testosterone, steroidogenesis-related proteins and gene expression [64, 74, 75, 77, 78, 80]. The adverse effects of phthalates on sperm quality were confirmed by ex vivo studies, where spermatozoa were exposed to high concentrations of phthalates, showing that sperm motility was affected and that cytotoxicity was caused at long-term exposures (>3 days) to the metabolite DEHP [84]. In parallel, DHEP has been shown to inhibit testosterone production, when cultured in vitro with explants derived from human testes [85].

Epidemiological studies reported an association between phthalates exposure and altered seminal parameters [86]. It is important to note that exposure of infants to phthalates is due to both maternal exposure and breastfeeding. In fact, breast milk levels of the phthalate metabolites are positively associated with maternal diet and water consumption.

Studies in humans corroborated the in vitro findings and suggested that exposure to phthalate metabolites is correlated with lower motility of spermatozoa in men from subfertile couples [87]. The DNA damage induced in spermatozoa, the motility and morphology of the spermatozoa were weakly associated with the exposure to phthalates [88–91], whereas an inverse association between MEHP exposure and testosterone and oestradiol levels was reported [92].

Apart from infertility, data available on the effect of phthalates on male reproductive health are limited [93]. Phthalates are rapidly metabolized and excreted in

urine and feces and therefore the assessment of exposure to phthalates in humans relies on the measurement of urinary concentrations of phthalate metabolites. However, little or even no attention is given to the possible accumulation of unmetabolized phthalates in different tissues [94]. This evidence raises some concerns about the appropriateness of parameters employed as index of exposure to contaminants, in particular for those substances like phthalates that, showing specific tissue accumulation, may exert risk associated to long-term exposures [82]. To this regard, quantification of both parent compound and corresponding metabolites in specific body fluids may represent an informative parameter with better correlation with clinical parameters [83].

7.5 Perfluoroalkyl Compounds

Perfluoroalkyl compounds (PFCs) or substances (PFAS) are a class of organic molecules characterized by fluorinated hydrocarbon chains extensively used in industry and consumer products including oil and water repellents, coatings for cookware, carpets and textiles. PFCs possess unique physical chemical properties due to their amphiphilic structures and their strong carbonfluorine bonds. Therefore, long-chain PFCs are non-biodegradable and bioaccumulate in the environment [95, 96]. PFCs have been found in humans and in the global environment and their toxicity, environmental fate and sources of human exposure have been a major subject of research. Currently, 23 PFCs are distinguished, including perfluorooctanoic acid (PFOA) and perfluooctane sulfonate (PFOS), which are the predominant forms in human and environmental samples. Both in vitro and animal studies on PFCs toxicity have shown a detrimental effect of PFOA and PFOS on testicular function, through alteration of steroidogenic machinery and subsequent defect of spermatogenesis [97–101]. Among the endocrine effects of PFOS in particular, it should be emphasized that this compound can affect the HPT axis activity [102, 103]. It is also able to exert its toxicity at testicular level [104], as reported in rats [102, 105] and in testis models [106]. According to a recent study on male rats [107], high doses of PFOS orally administered for 28 days seem to modify the relative gene and protein receptor expressions of several hormones of the reproductive axis (GnRH, LH, FSH and testosterone). Recently, exposure to PFOA was associated to reduction in sperm motility through alteration of sperm membrane fluidity [108].

Various PFCs compounds have been found in human serum [109], seminal fluid [110], breast milk [111] and even umbilical cord [112], suggesting a life-long exposure to PFCs in humans, from foetal stages until the adult life. Indeed, PFCs act as endocrine disruptors on the foetus and newborns, leading to developmental defects [113]. This has led to strict regulation of PFOA and PFOS use in industrial processes, as the compounds were added to the Annex B of the Stockholm Convention on Persistent Organic Pollutants. In addition to the health concerns related to foetal development, epidemiological studies have focused also on the relationship between PFCs and human fertility. In utero exposure to PFOA was

associated later in adult life with lower sperm concentration and total sperm count and with higher levels of LH and FSH [114].

Besides the impact of PFCs on the professionally exposed populations, recent evidence of pollution from chemical industries producing PFCs has emerged also in the general population from at least four different area worldwide: Mid-Ohio valley in the USA, Dordrecht area in Netherlands, Shandong district in China and Veneto region in Italy [115]. Despite strong evidence pointing towards a negative role of PFCs on male reproductive function, to date few evidence is available on the actual effect of these substances on seminal parameters in men, with conflicting results [110, 116, 117]. Two cross-sectional studies reported negative associations of PFOS, or high PFOA and PFOS combined, with the proportion of morphologically normal spermatozoa in adult men [116, 118]. Furthermore, in a study of men attending an in vitro fertilization clinic, Raymer et al. [110] reported that LH and free testosterone significantly and positively correlated with plasma levels of PFOA, although PFOA was not associated with semen quality. Conflicting results are also reported for the association between PFCs and sperm DNA quality, although a significant trend is evident for increased DNA fragmentation in exposed men [117, 119, 120]. In infertile males, PFOS levels were higher than fertile counterparts, together with a higher gene expression of ERα, ERβ and AR [121, 122], suggesting that PFCs activity might be linked also to the genetic expression of sex hormones nuclear receptors. With respect to AR, PFOS and PFOA induce a decrease of the protein expression of this receptor in the hypothalamus and pituitary gland as well as in the testis [123]. These findings clearly suggest an antiandrogenic potential of PFCs. More recently, in a cross-sectional study on 212 exposed males from the Veneto region in Italy, and 171 nonexposed controls, increased levels of PFCs in plasma and seminal fluid positively correlated with circulating testosterone and with a reduction of semen quality, testicular volume, penile length and anogenital distance [124]. Furthermore, the anti-androgenic property of PFOA was related to antagonism on the binding of testosterone to AR [124].

In conclusion, in men, there is little evidence for an association between PFCs exposure and semen quality or levels of reproductive hormones. As is the case for many epidemiological studies, causality cannot be definitively established in these studies, largely because of their cross-sectional design. However, the consistency of findings in preclinical studies strongly suggests a causal relationship for some endpoints.

7.6 Conclusions

EDCs can potentially cause harmful effects to the male reproductive system. In addition to the classical action of EDCs that includes the agonism and/or antagonism with hormone and nuclear receptors, the last decade of scientific research has given significant advances in the field of molecular biology that identified several compounds as endocrine disruptors, by interfering with the cell cycle, the apoptotic machinery and the epigenetic regulation of the target cells [125]. However, action

mechanisms should not be generally extrapolated since each chemical has different routes to interfere with endocrine activity. Among the tens of known EDCs, BPA, phthalates and PFCs are particularly intriguing for male sexual and reproductive consequences given the strong experimental evidence of effects on hormone nuclear receptors (AR and/or ER), HPT axis and direct action on spermatogenesis and steroidogenesis [126, 127]. However, epidemiological studies in humans have shown controversial and inconsistent results. This discrepancy can be attributed to several factors that could affect the outcome of the studies, notably to the complexity of the clinical protocols used, the degree of occupational or environmental exposure, the selection of the target group under investigation, the determination of the variables measured and the sample size of the subjects examined. Despite the lack of consistency in the results of the human studies, the overall conclusion points toward a positive association between exposure to EDCs and alteration of the reproductive system.

EDCs and environmental factors can lead to male reproductive alterations at different stages of sexual development and maturation. In addition to the classical mechanism of endocrine disruption by chemicals, there including agonistic and/or antagonistic interference on hormonal and intracellular receptors, the last decades of scientific research have provided new evidence at different experimental levels, from in vitro studies to animal and human studies. Altogether these results have recognized a wide range of chemicals with endocrine-disrupting features that interfere with various biological processes in target cells, such as cell proliferation or apoptosis, and their epigenetic regulation. However, a common mechanism of action cannot be identified, given the very wide range of chemical structures, exposure routes, environmental levels and so on among different chemicals with endocrine activities. Among the tens of known EDCs, BPA, heavy metals, phthalates, organophosphates and PFAS are particularly intriguing for male HPT axis function, given the agreement in experimental studies showing a consistent effect on steroid receptors (AR and/or ER), hormonal metabolism and related enzymes and direct action on steroidogenesis. Although the observed effects may be subtle on an individual level, the biological link between them (i.e. TDS: decreased androgen levels contributing to cryptorchidism, reduced penile length and reduced testicular volume) should raise concerns about the effects of EDCs at population levels in young men. More longitudinal studies performed on a wide number of subjects are clearly needed in order to identify other putative damaging compounds, to clarify new routes of exposure and to replace legacy EDCs with harmless substances. However, epidemiological studies in humans have shown controversial and inconsistent results for different EDCs classes. The lack of consistency across studies and between human and animal studies can be explained by different factors possibly affecting the outcomes of these studies, such as the differences in investigation protocols and study designs, the crude levels of contaminations at different degrees, from occupational to environmental exposure, the different analytical approaches for the quantification of exposure, the selection of different variables of interest as outcomes indicative of endocrine-disrupting features and finally even the wide range of sample sizes of subjects included in the studies. Despite the lack of consistency in the results of the human studies, the overall

conclusion points toward a positive association between exposure to EDCs and alteration of the hypothalamus–pituitary–testis axis. Nonetheless, it should be pointed out that humans are not exposed to a single compound during their life, from foetal period to adulthood. Therefore, it is crucial to stress out the importance of toxicological and clinical studies that take into account a cocktail of chemicals rather than the single compound. Although this clearly represent a complicate step, it is fundamental in order to have a more comprehensive view of exposure risk to EDCs in well-define groups, and in particular in those developmental windows more sensitive to hormonal alterations, such as foetus, newborns and adolescents. The collection of new evidence on the cumulative effect of different chemicals with different properties and mechanisms would therefore provide a new avenue for the treatment and prevention of male reproductive alterations.

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