Chapter 5
Endocrine-Distributing Chemicals and Reproductive Function

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Abstract Exposures to environmental chemicals affecting androgen action (endocrine-disrupting chemicals (EDCs)) are suspected to have a negative impact on male reproductive function by disrupting normal differentiation and development. In this chapter, the literature on the impact of exposure to endocrine-disrupting chemicals on male reproduction will be reviewed. We will specifically address the effects of exposure to organochlorine compounds, perfluorinated alkylate substances (PFAS), phthalates, and phenols on anogenital distance, reproductive hormones in childhood, puberty onset, and semen quality, focusing on prenatal or early exposures during vulnerable time points of development. Generally, anogenital distance (AGD) appears to be a promising, easily obtainable marker of male reproductive health. Maternal exposure to phthalates has consistently been associated with shorter AGD in male offspring, but no consistent associations between PFAS or bisphenol A exposure and AGD have been found. Prenatal exposure to organochlorine pesticides (OCPs) appears to lower children's testosterone concentrations and increase aromatase activity after birth. In addition, prenatal exposure to dioxins and OCPs may delay puberty, whereas exposure to polychlorinated biphenyls (PCBs) accelerates the onset of puberty in boys. Maternal, childhood, or adult phthalate exposure has been associated with lower reproductive hormone concentrations, changed onset of puberty and semen quality. No consistent associations between PFAS or phenol exposure and AGD, reproductive hormones, puberty onset, or semen quality have been found. We suggest that more research is urgently needed focusing on birth cohort studies addressing the adverse effects of exposures during vulnerable time windows during development, e.g., in utero, during early childhood, and puberty. The cohorts should have the necessary size, include biological

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material, focus on multiple exposures, and have long-term follow-up with repeated clinical examinations.

Keywords Anogenital distance · Reproductive hormones · Puberty onset · Testicular dysgenesis syndrome · Semen quality · Organochlorine compounds · Per- and polyfluoroalkyl substances · Phthalates · Bisphenols · Prenatal exposure

5.1 Introduction

In 2002, the World Health Organization (WHO), the International Programme on Chemical Safety (IPCS), United Nations Environment Programme (UNEP), and the International Labour Organization released the "Global Assessment of the State-of-the-Science of Endocrine Disruptors" report [1], which was updated as the "The State of the Science of Endocrine Disrupting Chemicals" in 2012 [2] to address concerns about the potential adverse effects of exposure to chemicals with endocrine-disrupting abilities (EDCs) on humans and wildlife. The Endocrine Society reported that exposure to environmental chemicals may contribute to the increasing incidences of obesity; diabetes mellitus; cardiovascular diseases; infertility; neurodevelopment and certain hormone-sensitive cancers such as the prostate gland, the thyroid, and the brain [3]. In the following we will focus on the adverse effects on male reproductive health.

Male reproductive health may be declining including increased incidence of testicular cancer, reduced semen quality, and increased birth prevalence of hypospadias and cryptorchidism [4]. The testicular dysgenesis syndrome (TDS) hypothesis suggests that these conditions are interlinked and signs of the same underlying unity founded in utero. Exposure to environmental chemicals affecting androgen action during the sensitive window of mascularization from week 8 to 14 of gestation is suspected to have a negative impact on male reproductive function by disrupting normal differentiation and development of the reproductive system. Consequences of such disruptions include compromising the development and function of the testicular Leydig and Sertoli cells [5] (Fig. 5.1 [6]) leading to cryptorchidism, hypospadias, testicular cancer, decreased testosterone production, and impaired spermatogenesis. More recently, decreased anogenital distance (AGD) has been suggested to be part of the TDC syndrome (see later) [7]. In humans, studying the effects of exposures in utero on adult male reproductive function, including semen quality, is challenging due to the long interval between fetal exposure and mature reproductive function.

In the following the literature of exposure to endocrine-disrupting chemicals (organochlorine compounds, perfluorinated alkylated substances (PFAS), phthalates, and phenols) on anogenital distance, reproductive hormones in childhood, puberty onset, and semen quality will be reviewed.

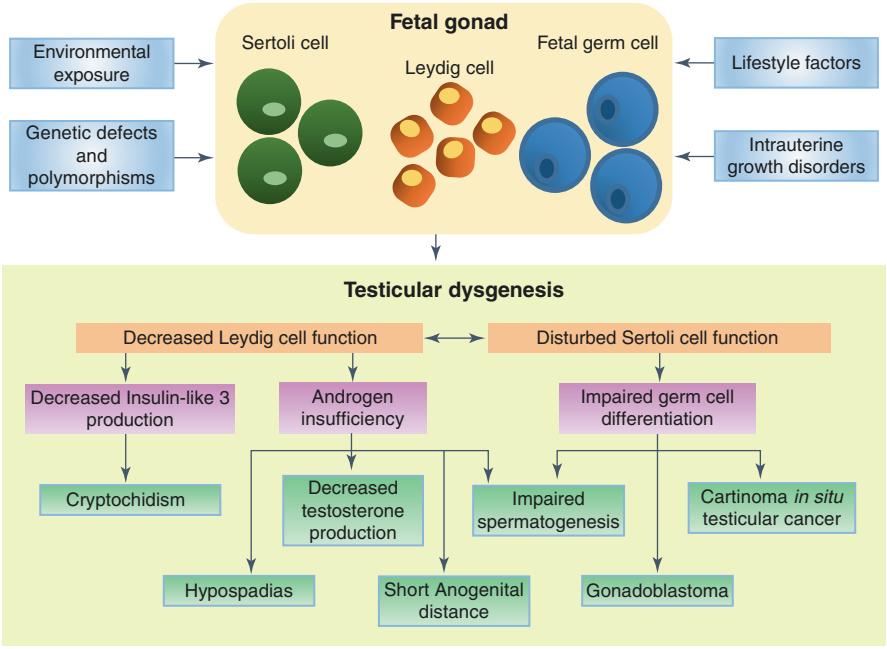

Fig. 5.1 Testicular dysgenesis syndrome. Multiple genetic and environmental factors ensure appropriate fetal testicular development, but adverse environmental exposures, genetic aberrations, lifestyle factors, and intrauterine growth disorders can all result in testicular dysgenesis. Testicular dysgenesis in early fetal life influences Leydig cells, Sertoli cells, and the niche supporting fetal germ cells. This influence can result in intermediate phenotypes (pink boxes), some of which can have late symptoms and consequences (green boxes). (Adapted from Juul et al. [6])

5.2 Evidence from Epidemiological Studies

5.2.1 Anogenital Distance as a Marker of Exposure

Anogenital distance (AGD; distance from anus to genitals) is routinely used in animal toxicology studies, and is sensitive to anti-androgenic exposure. In rodents, AGD has been shown to reflect the amount of androgen to which a male fetus is exposed in early development; higher in utero androgen exposure results in longer AGD. In male rodents, shortened AGD persists into adulthood [8] and predicts compromised reproductive function (reduced testis size) in the mature male [9].

5.2.1.1 AGD Definition

Interestingly, AGD has been measured in humans and may be a part of the TDS syndrome [5]. This is also in line with the theory that TDS outcomes result from a disturbance in the Sertoli cell and Leydig cell differentiation during fetal life,

leading to impaired testosterone production and decreased virilization. There is indirect evidence for a link between TDS conditions and AGD; AGD is shorter in patients with infertility [10], poor semen quality [11], lower testosterone concentrations [12], cryptorchidism and hypospadias [13]. This evidence suggests that shortened AGD is part of the TDS syndrome [7]. Because it can be measured in all boys, it is a more sensitive marker of genital development than the birth prevalence of cryptorchidism or hypospadias, which are found in less than 10% of newborns, thus requiring large study populations.

AGD can easily be measured by the use of a Vernier caliper; the shorter AGD measurement is measured from the center of anus to the posterior base of scrotum (AGDas) and the longer from the center of anus to the cephalad insertion of the penis (AGDap).

5.2.1.2 Organochlorine Compounds

Organochlorine compounds include organochlorine pesticides (OCP), polychlorinated biphenyl (PCB) compounds, and dioxins. Due to their highly persistent properties, the Stockholm Convention has regulated their use. OCP are chlorinated hydrocarbons, used extensively in the 1940s for agriculture and pesticide control. Most OCP were banned in the US, Europe, and many other countries in the early 1970s [14]. Although the Stockholm Convention has issued an exemption for the production and public health, use of dichlorodiphenyltrichloroethane (DDT) is still prominent to control vector-borne diseases. PCBs were used as a non-flammable insulate and heat stabilizer in industries until the 1970s. Dioxins and dioxin-like compounds are mostly the by-products of various industrial processes. Most OCP and PCBs have been prohibited for over 30 years; however, due to their long half-life, they are still detected in the blood of most individuals including pregnant women [15, 16].

In the U.S., a smaller study including 37 male offspring indicated reduced anogenital distance at higher *p*, *p*′-DDE exposure [17]. However, a larger study, among 781 mother–child pairs in Chiapas, Mexico, of which 29% reported living in DDT-sprayed homes, indicated no association between *p*, *p*′-DDE exposure and anogenital distance or penile length, suggesting that even high exposure to *p*, *p*′-DDE does not seem to have a significant impact on these outcomes in humans [18]. A smaller Mexican study with repeated AGD measurements found an association between maternal PCBs and AGD in 74 boys, whereas no associations were found for prenatal DDT exposure [19].

In a Spanish mother–child cohort, POP concentrations were measured in pregnant women, and AGDas was recorded in 43 offspring at 18 months of age. Anogenital index was calculated as AGD divided by weight and found to be inversely associated with lipid-adjusted concentrations of PBDE-99 and PBDE-153 but not with PCB congeners [20]. In a Danish mother–child cohort, no consistent association between prenatal exposure to the pesticide metabolites 3-phenoxybenzoic

acid (3-PBA), 3,5,6-trichloro-2-pyridinol (TCPY), 2,4-Dichlorophenoxyacetic acid (2,4-D), and dialkyl phosphates (DAPs) and AGD was found [21].

5.2.1.3 PFAS

PFASs compose a group of chemicals which are considered persistent organic pollutants because of their resistance to biodegeneration. They are mainly used as surfactants in a wide range of consumer products (e.g., paint and lacquers, carpets, impregnated outdoor clothing, food packaging) because of their water and soil-repellent properties. Compounds composed of chains of eight carbons, perfluorooctane sulfonic acid (PFOS), and perfluorooctanoic acid (PFOA) have been the most extensively produced. Due to their persistent properties, PFOS is listed in Annex B of the Stockholm Convention in 2009, and PFOA is listed in Annex A in 2019 [14].

To the best of our knowledge only one human study has addressed the association between maternal exposure to PFAS and AGD in the offspring. PFOS, PFHxS, PFNA, and PFDA were associated with a decreased AGD in *girls*, whereas no associations were reported in boys [22].

5.2.1.4 Phthalates

Phthalates are high volume production chemicals, and their applications in industrial production are determined by molecular weight. High molecular weight phthalates, such as di-(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP), are used as plastic softeners in numerous polyvinyl chloride (PVC) products, like rain wear and shoes, flooring, food packaging, toys, and medical devices. Low molecular weight phthalates, such as dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP), are primarily used as solvents in personal care products, lacquers, insecticides, and in coatings [23]. Humans are mainly exposed to environmental phthalates through ingestion (high molecular weight phthalates), indoor air, and dermal contact (low molecular weight phthalates) and inhalation [24]. The biological half-life of phthalates is in the range of hours to days, and they do not accumulate in the body; however, phthalate metabolites are present in urine in the majority of the human population indicating continuous exposure [25–27].

A U.S. study, which included 134 mother–son pairs, was the first investigation to explore AGD in humans. The study found a significant association between maternal exposure to several phthalates measured in urine and reduced AGD in the male offspring [28]. Later results found an inverse association between maternal urine DEHP exposure and AGD and penile size [29]. A recent review [30] and meta-analysis, which included 10 studies [31], reported that prenatal exposure to DEHP was significantly associated with shorter AGD. In addition, urinary monobutyl phthalate (MBP), monoethyl phthalate (MEP), and mono-*i*-butyl phthalate (MiBP) were found to be associated with short AGD (AGDas).

5.2.1.5 Bisphenol A (BPA)

Bisphenol A (BPA) is a high production volume chemical that is widely used in the manufacture of consumer products such as polycarbonate plastics, epoxy resin liners of canned foods, some dental sealants and composites, and thermal receipts. Due to its widespread use in consumer products, exposure to BPA is ubiquitous [32].

Many rodent studies have demonstrated that maternal BPA exposure decreased AGD in male offspring [33]; however, few human studies have been performed. Most studies have been conducted in China, where exposure concentrations are high. An occupational cohort study revealed that maternal exposure to BPA during pregnancy was associated with shortened AGD in male offspring [34]. However, few women were occupationally exposed to high doses of BPA (n = 18). In a Chinese cohort study, among 655 mother–son pairs, mothers with detectable BPA in urine in gestational week 12–16 gave birth to boys with shorter AGDap at 6 and 12 months of age [35]. These findings were not replicated in a Chinese study among 137 boys; i.e., no significant association between maternal BPA and AGD was found. However, data were not provided, and the measurement of AGD was not specified [36]. These findings are in accordance with findings from Canadian study among 198 boys, where no significant associations between maternal BPA exposure and AGD were reported [37]. A study conducted in Cypress measured BPA in cord blood, which may be difficult to compare to urine measurements, which is the gold standard. No significant correlations between cord blood BPA concentrations and AGD were found; however, a significant negative correlation between AGDas and cord blood BPA concentrations above the 90th percentile was found in 72 boys [38].

5.2.2 Environmental Chemical Exposure and Reproductive Hormones

5.2.2.1 Reproductive Hormone In Utero and Early Life

During early gestation, the reproductive organs differentiate into the testis and ovaries, and molecular stimuli may curtail these processes. In men, Sertoli cells secrete testosterone and cross-talk with Leydig cells, which produce virilization. Thus, during early development, exposure to proper concentrations of hormones is essential. Exposure to exogenous substances with endocrine-disrupting properties can mimic or antagonize these and other hormonal systems to change the developmental trajectory [3]. As a result, we looked at studies that examined the effect of exposure to EDCs on reproductive hormones at birth and during childhood. The results of birth cohort and longitudinal studies together with cross-sectional data at baseline are summarized in each section and Table 5.1.

Table 5.1 Prenatal exposures and reproductive hormones

Country, Year	Participants (number of samples)	Exposures	P4	T	E2	T/E2	SHBG	LH	FSH	Inhibin B	INSL3	Others	Ref.
Chlorinated chemicals													
Germany, 2008	Industrial area birth cohort (n = 104)	Prenatal dioxins		↓	↓								[41]
France, 2016	PELAGIE	Prenatal PCBs		↓		↓	↑						[16]
	(n = 282)	Prenatal α-endosulfan and HCE		→	↑	↓	↑						
Japan, 2018	Hokkaido Study: Sapporo Cohort (n = 257)	Prenatal dioxins (M)	→	→	→	→	→	→	→	↓	→		[112]
		Prenatal OCPs	→	↓	→	↑	↓	→	→	→	→	DHEA↑, T/androstenedione↓, prolactin↓	[42]
USA, 2017	CHAMACOS	Prenatal BDE-153		↑				↑	↑				[43]
	(males n = 234)	Prenatal PCBs		→				→	↑				
		Prenatal DDT		↓				↓	→				
		Prenatal DDE		→				↓	→				
Taiwan, 2005	Males with Yucheng mothers (cases n = 60, controls n = 61)	Cases compared to controls		→	→	↓		→	→			T/FSH↓, E2/FSH↑	[39]
Taiwan, 2005	Females with Yucheng mothers (cases n = 27, controls n = 21)	Cases compared to controls		→	→	→		→	↑				[40]

(continued)

Table 5.1 (continued)

Country, Year	Participants (number of samples)	Exposures	P4	T	E2	T/E2	SHBG	LH	FSH	Inhibin B	INSL3	Others	Ref.
Denmark, 2012	Faroe Islands birth cohort (males $n = 428$)	Prenatal PCBs		↓			↑	↓					[113]
		Postnatal (14 years of age) PCBs		→			↑	→					
PFAS													
Denmark, 2015	Cryptorchidism (cases $n = 270$), hypospadias (cases $n = 75$, controls $n = 300$)	Prenatal PFOS	→	↓							↓		[45]
Japan, 2016	Hokkaido Study: Sapporo Cohort ($n = 257$)	Prenatal PFOS (M)	↓	→	→	↓	→	→	→	↓	↓		[46, 47]
		Prenatal PFOA (M)	→	→	→	→	→	→	→	↑	→	DHEA↓	
		Prenatal PFOS (F)	↓	→	→	→	↓	→	→	→	→	Prolactin ↓	
		Prenatal PFOS	→	→	→	→	→	→	→	→	→	DHEA↑, cortisol↓, cortisone↓	
UK, 2015	ALSPAC (female $n = 75$)	Prenatal PFOS, PFOA, and PFHxS		↑			→						[48]
Denmark, 2013	Danish population-based cohort (males $n = 176$)	Prenatal PFOA		→	→	→	→	↑	↑	→	→		[106]

Phthalates												
Taiwan, 2011	Birth cohort (n = 155)	Prenatal 3rd trimester DEHP (F)		↓	→	↓						[49]
Japan, 2014, 2016	Hokkaido Birth Cohort Study: Sapporo cohort (n = 257)	Prenatal 2–3rd trimester DEHP (M)	↓	→	→	↓	→	→	→	↓	↓		[50, 51]
		Prenatal 2–3rd trimester DEHP	↓	→	→	→	→	→	→	→	→	Cortisol↓, cortisone↓, cortisol/cortisone↓, glucocorticoid/adrenal androgen↓, DHEA/androstenedione↑	
Denmark and Finland, 2006	Danish–Finnish prospective cohort (cryptorchidism cases n = 62, controls n = 68)	Postnatal (1–3 months) phthalate metabolites		↓			↑	↑				LH/T↑	[52]
Taiwan, 2017	TMICS	Prenatal 3rd trimester DEHP	↓ (F)	→	→								[53]
	(n = 193)	Postnatal 2, 5, 8, and 11 years of age DEHP	↓ (F)	↓ (M)	→								
Mexico, 2017	ELEMENT	Prenatal 3rd trimester phthalate metabolites (M)		→	↑		↑			→			[54, 55]
	(n = 229)	Prenatal 3rd trimester phthalate metabolites (F)		↑	→		→			→		DHEA-S↑	

(continued)

Table 5.1 (continued)

Country, Year	Participants (number of samples)	Exposures	P4	T	E2	T/E2	SHBG	LH	FSH	Inhibin B	INSL3	Others	Ref.
Denmark, 2013	Copenhagen Puberty Study	Postnatal (5–19 years of age) MBP (F)		→ (13 y)				→ (13 y)	→ (13 y)			DHEAS↓, Δ4-androstenedione↓	[81]
	Longitudinal study 168 children (males *n*= 84 and females *n* = 84)	Postnatal (5–19 years of age) MBP (M)		↑ (13 y)				→	→			DHEAS↓ (11 y)	
Bisphenols													
France, 2012	Prospective cohort (cryptorchidism cases *n* = 46, controls *n* = 106)	At birth BPA (M)		↑	→		→	→	→	↑			[56]
Japan, 2014	Hokkaido Study: Sapporo Cohort (*n* = 278)	At birth BPA (M)	↑	↑	→	→	→	→	→	→	→		[57]
China, 2017	Polluted area and control area (males *n* = 137)	At birth BPA		↓	→	↓							[36]
Czech Republic, 2018	Prospective birth cohort (*n* = 27)	At birth BPA, BPF, BPS, BPAF										No associations	[114]

BPA bisphenol A, *DDE* dichlorodiphenyldichloroethylene, *DDT* dichlorodiphenyltrichloroethane, *DHEA* dehydroepiandrosteone, *DHEA-S* dehydroepiandrosteone-sulfonate, *E2* Oestradiol, *F* Female, *FSH* follicle-stimulating hormone, *INSL3* insulin-like factor-3, *LH* luteinizing hormone, *M* male, *OCP* organochlorine pesticide, *PBDE* polybrominated diphenyl ether, *PCB* polychlorinated biphenyl, *PFHxS* perfluorohexanesulfonic acid, *PFNA, PFOA* perfluorooctanoic acid, *PFOS* perfluorooctanesulfonic acid, *SHBG* steroid hormone binding globulin, *T* testosterone, *T/E2* testosterone/oestradiol ratio, *y* year-old

↑, positive association, ↓, inverse association, →, no association

5.2.2.2 Organochlorine Compounds

A total of eight birth cohort studies were identified, as shown in Table 5.1. Two from Taiwan targeted Yucheng mothers, who were exposed to rice oil contaminated accidentally with PCBs and PCDFs in 1978–1979 [39, 40]. Children of Yucheng mothers aged more than 13 years showed significantly lower testosterone/oestradiol (T/E2) ratio and T/follicle-stimulating hormone (FSH) and higher E2/FSH ratio among boys of control group (non-Yucheng mothers) [39]. Girls of Yucheng mothers, significantly higher E2 and FSH concentrations were observed when compared control group of non-Yucheng mothers [39, 40]. Although these studies did not measure the blood concentrations of PCBs and dioxins, the lower T/E2 ratio among Yucheng children is in line with the results from other studies. In six studies chlorinated chemicals in either maternal or cord blood were measured and reproductive hormone concentrations in cord or child blood were assessed. Increased maternal blood or cord blood concentrations of PCBs and OCPs were consistently associated with lower testosterone concentrations, along with lower T/E2 ratio (or higher E2/T ratio), a marker of aromatase activity. These results were found at birth in Germany (dioxins) [41], France (PCBs and OCPs) [16], and Japan (OCPs) [42], at age 12 in the US (DDT) [43], and at age 14 in Denmark (PCBs) [44]. A U.S. study, however, reported that increased maternal BDE-153 exposure increased testosterone concentrations among boys at age 12 years [43].

5.2.2.3 PFAS

Few studies have examined the association between prenatal PFAS exposure and reproductive hormone concentrations among children. A Danish study compared cryptorchidism and hypospadias with matched controls and measured hormone concentrations in the amniotic fluid. PFOS concentrations in amniotic fluid were inversely correlated with testosterone and insulin-like factor 3 (INSL3) concentrations among all children [45]. Maternal PFOS concentrations and lower concentrations of progesterone T/E2, inhibin B, and dehydroepiandrosterone (DHEA) in cord blood was observed among boys in a birth cohort in Japan [46, 47]. The findings suggest that PFOS exposure resulted in undermatured testis. Whereas a positive association between PFOA and inhibin B and DHEA in cord blood among boys in a Japanese cohort suggested the opposite association between PFOS and PFOA [46, 47]. Moreover, prenatal PFOS exposure was positively associated with testosterone concentrations in child blood at 15 years of age among girls [48]. Thus, the effects of PFOS and PFOA on reproductive hormone concentrations may differ according to age and be modified by sex.

5.2.2.4 Phthalates

Two studies from Taiwan and Japan reported that concentrations of the most dominant phthalate, DEHP, or its metabolite mono(2-ethylhexyl) phthalate (MEHP) in mothers urine during pregnancy were inversely related to children's T/E2 ratio at

birth, which may reflect upregulation of aromatase cytochrome P450 (CYP19) [49, 50]. The Japanese study reported reduced concentrations of progesterone at birth but increased concentrations of DHEA/androstenedione, which may reflect the downregulation of hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (HSD3B1) [51]. Moreover, reduced concentrations of testosterone were also observed in the blood of boys aged 1–3 months [52]. Another Taiwan study measured DEHP metabolites both in mother and child urine and found an association between higher prenatal DEHP concentrations with reduced concentrations of female children's progesterone at a later age of 2, 5, 8, and 11 [53]. In the same cohort, child urinary metabolites of DEHP concentrations at 2, 5, 8, and 11 years of age were inversely correlated with testosterone among boys and progesterone among girls [53]. These results indicate that phthalate exposure may decrease concentrations of testosterone, progesterone, and T/E2 ratio, suggesting the upregulation of CYP19. However, a Mexican study found that higher exposure to phthalate metabolites during pregnancy was associated with increased concentrations of oestradiol among boys and testosterone and DHEA-sulfate among girls [54, 55] aged 8–13 years of age.

5.2.2.5 BPA

Three studies examined both BPA exposure concentrations and reproductive hormones at birth. A French and a Japanese study reported a positive association between maternal BPA and testosterone and progesterone [56, 57] in the male offspring, whereas a Chinese study reported an inverse association between prenatal BPA exposure and testosterone [36] in new-born boys.

5.2.3 Environmental Chemical Exposure and Onset of Puberty

5.2.3.1 Onset of Puberty

Hormones play an important part in defining the optimal conditions for human reproductive life to start [58]. Another critical period of development with a sensitive window of exposure to EDCs is puberty, during organs maturation [59]. To evaluate the onset of puberty most studies evaluate Tanner stage [60]. For girls, breast developmental and pubic hair stages, as well as menarche, are determined. For boys, genital and pubic hair stages and testicular volume were mainly used. The current evidence and longitudinal studies on exposure to environmental chemicals and their associations with the onset of puberty are collected and summarized in Table 5.2.

Table 5.2 Exposures to EDCs and onset of puberty

Country, Year	Design and participants	Exposure and outcome measurements	Breast development	Pubic hair	Axillary hair	Genital stage	Menarche	Testicular volume	Others	Ref.
Chlorinated chemicals										
The Netherlands, 2008	Longitudinal cohort ($n = 33$)	Prenatal PCDD/PCDF	Delay (F)	→	→	→	→			[61]
Denmark, 2013	Danish population-based cohort established in 1988–1989 (females 20 years of age $n = 436$)	Prenatal *p,p′*-DDE, HCB					→		Antral follicle number ↓	[62]
UK, 2016	ALSPAC females (menarche age <11.5 years as cases $n = 218$, controls $n = 230$)	Prenatal 9 OCPs					→			[63]
USA, 2015	Breast Cancer and Environment Research Centers (BCERC) project (females 6–8 years old, $n = 645$)	Postanal 8–6 years of age ΣPCB	Delay (cross-sectional)							[64]
		Postnatal 8–6 years of age ΣPCBs, PBDEs, OCPs	Delay (2, 5 years follow-up)	Delay (2, 5 years follow-up)						
Russia, 2011	Prospective cohort (males $n = 489$)	Prenatal PCB		→		Delay		→		[65]
		Prenatal TEQ		→		→		→		
Russia, 2011	Prospective cohort (males $n = 499$)	Postnatal 8–9 years of age TEQ		→		→		Delay		[66]
		Postnatal 8–9 years of age 2,3,7,8-TCDD (8–9 years)		→		→		Delay		

(continued)

Table 5.2 (continued)

Country, Year	Design and participants	Exposure and outcome measurements	Breast development	Pubic hair	Axillary hair	Genital stage	Menarche	Testicular volume	Others	Ref.
Russia, 2014	Prospective cohort (males 8–9 years old $n = 350$)	Postnatal 8–9 years of age HCB		Delay		→		Delay		[67]
		Postnatal 8–9 years of ageβ-HCH, *p,p′*-DDE		→		→		→		
Russia, 2016	Prospective cohort (males $n = 473$)	Postnatal 8–9 years of age TEQ	TEQ	Delay (17–18 years)						[68]
		Postnatal 8–9 years of age Non-DL PCB		Early (17–18 years)						
PFAS										
Denmark, 2013	Danish population-based cohort established in 1988–1989 (females $n = 343$)	Prenatal PFOA					Delay			[70]
UK, 2011	ALSPAC (females menarche age <11.5 years old as cases $n = 218$, control $n = 230$)	Prenatal PFAS					→			[71]
Phthalates										
Taiwan, 2015	TMICS birth cohort study	Prenatal 3rd trimester MEHP, total DEHP						→	Reduced uterus size (F)	[73]
	($n = 437$ at baseline)	Prenatal 3rd trimester MBzP	↑ (F)					→	Bone age/ chronological age ratio (F)	

Mexico, 2017	ELEMENT birth cohort (females 8–13 years old, $n = 120$)	Prenatal 3rd trimester MEHP	Delay	Early						[74, 75]
	(males 8–14 years old, $n = 109$)	Prenatal 3rd trimester MBzP		Delay						
USA, 2010	Breast Cancer and Environment Research Centers (BCERC) project	postnatal (6–8 years of age) high molecular weight phthalate	→	Delay						[76]
	Females aged 6–8 years $n = 1151$ (baseline of [77])	postnatal (6–8 years of age) low molecular weight phthalate	Early	→						
USA, 2010	BCERC project (females 6–8 years old, $n = 1151$)	Postnatal (6–8 years of age) high molecular weight phthalate	→	Delay						[77]
USA, 2017	BCERC project (females 6–8 years old, $n = 1051$)	Postnatal (6–8 years of age) MCPP					Delay			[78]
Denmark, 2012	Copenhagen Puberty Study (baseline of [81]) (males $n = 555$)	Postnatal 6.07–19.83 years of age phthalate metabolites		→		→				[79]
	(females $n = 725$)	Postnatal 5.6–19.1 years of age (phthalate metabolites except MEP)	→	Delay					No association with precautious puberty	[80]

(continued)

Table 5.2 (continued)

Country, Year	Design and participants	Exposure and outcome measurements	Breast development	Pubic hair	Axillary hair	Genital stage	Menarche	Testicular volume	Others	Ref.
Denmark, 2013	Copenhagen Puberty Study (longitudinal, $n = 168$)	Postnatal follow-up for 5 years per 6 months 14 urinary phthalate	→ (F)	→ (F)						[81]
		Postnatal follow-up for 5 years per 6 months MBP		Early (M)		→ (M)				
Taiwan, 2015	Prospective cohort at baseline 9.7 ± 2.2 years (males $n = 222$; females $n = 208$)	Postnatal 9.7 years of age MnBP		Delay (M)						[82]
	(females $n = 208$)	Postnatal 9.7 years of age MEHP, MEHHP, and MEOHP	Early (F)				Early (F)			
Phenol										
USA, 2017	BCERC project (females 6–8 years old, $n = 1051$)	Postnatal (6–8 years of age) 2,5-dichlorophenol					Early			[78]

B Tanner scale breast development stage, *DDE* dichlorodiphenyldichloroethylene, *DDT* dichlorodiphenyltrichloroethane, *DEHP* di(2-ethylhexyl) phthalate, *DL-PCB* dioxin like-polychlorinated biphenyl, *G* Tanner scale genitals, *HCB* hexachlorobenzene, *HCH* hexachlorocyclohexane, *MBzP* monobenzyl phthalate, *MCPP* mono-(3-carboxypropyl) phthalate, *MEHHP* mono(2-ethyl-5-hydroxyhexyl) phthalate, *MEP* monoethyl phthalate, *MEHP* mono(2-ethylhexyl) phthalate, *MEOHP* mono (2-ethyl-5-hydroxyhexyl) phthalate, *MnBP* mono-*n*-butyl phthalate, *MMP* monomethyl phthalate, *OCP* organochlorine pesticide, *PCDD* polychlorinated dibenzo-*p*-dioxins, *PCDF* polychlorinated dibenzofuran, *PBDE* polybrominated diphenyl ether, *PCB* polychlorinated biphenyl, *PFAS* perfluorinated alkylated substances, *PFOA* perfluorooctanoic acid, *PH* Tanner scale pubic hair, *TEQ* toxic equivalent

5.2.3.2 Organochlorine Compounds

The adverse effects of exposure to organochlorine compounds such as PCBs, dioxins, and chlorinated pesticides have been extensively studied. In eight prospective studies, three were birth cohorts from the Netherlands, Denmark, and the UK (see Table 5.2). The Dutch study examined prenatal and lactational dioxin exposure and found that higher exposure delayed initiation of breast development among girls, although the sample size was only 33 [61]. In Denmark and the UK concentrations of organochlorine pesticides in maternal blood were measured and no association with age of menarche [62, 63] was found. In an American study, blood concentrations of PCB and OCP in girls aged 7–8 years [64] were associated with delayed onset of breast and pubertal hair development (Tanner stage 2) [64]. Four studies from Russia reported on the same population of boys [65–68]. The blood concentrations of PCBs, dioxins and organochlorine pesticides in these boys at 8–9 years of age were measured. Relatively consistent results were obtained indicating that higher concentrations of dioxins, dioxin-toxicity equivalents (TEQ), and hexachlorobenzene (HCB) were associated with a delayed onset of puberty, while an earlier onset was observed for boys with higher concentrations of non-dioxin like PCB. Although not listed in Table 5.2, two cross-sectional studies from Belgium and Kazakhstan [69] also reported an association between higher concentrations of PCBs, dioxins, and OCPs and delayed pubertal development among both boys and girls, which is consistent with the findings from longitudinal studies.

5.2.3.3 PFAS

Only two prospective studies examined the association between prenatal exposure to PFAS and age of puberty, both studies focused on females [70, 71]. A study from Denmark found that higher concentrations of prenatal PFOA were associated with delayed onset of menarche [70], whereas a UK study found no association [71]. The results of a cross-sectional study conducted in the Mid-Ohio Valley in the US suggested that PFOA and PFOS were associated with a later age of puberty in both boys and girls [72].

5.2.3.4 Phthalates

In a Taiwanese study, higher concentrations of DEHP in maternal urine were associated with girls at 8 and 11 years of age of reduced uterus size, while higher concentrations of butyl benzyl phthalate (BBzP) were associated with increased bone age/chronological age ratio of girls at 8 and 11 years of age [73]. Among Mexican girls, higher exposure to DEHP in utero was associated with the earlier development of pubic hair and delayed breast development at 8–13 years of age [74], whereas a higher exposure to DEHP delayed pubic hair development among boys at 8–14 years

of age [75]. Higher urinary phthalate metabolites in girls at 6–8 years of age were associated with delayed onset of puberty both cross-sectional at baseline and after 2 or 5 years follow-up in American girls [76–78]. In contrast, in a cross-sectional setting, increased concentrations of phthalates delayed puberty among Danish girls (5–19 years of age), whereas no association was found in boys (6–19 years of age) [79, 80]. However, at 5 years follow-up, higher exposure to DBP was related to an early age of puberty among boys but not in girls [81]. Finally, a Taiwan study examined phthalates in urine among 9 year olds and found that DBP delayed pubic hair development in boys, whereas DBP, DMP, DEP, and DEHP exposures accelerated breast and menarche onset in girls after 1.5 years follow-up [82].

5.2.3.5 BPA and Phenols

A prospective U.S. cohort among girls aged 6–8 years urinary concentrations of 2,5-dichlorophenol at baseline was associated with earlier onset of menarche after 2 or 5 years of follow-up [78]. One review examined previous knowledge of the impact of BPA on puberty [83]. The authors concluded that currently available data do not allow the establishment of a clear role of BPA in pubertal development, as the results were conflicting. Moreover, most of the performed studies were cross-sectional or case–control studies comparing precocious puberty children to children without precocious puberty children. As a result, no conclusions on the effects of BPA exposure on puberty onset can be drawn.

5.2.4 Semen Quality

5.2.4.1 Definition and Trends

Semen quality is assessed by concentration, volume, total sperm count (concentration × volume), morphology and motility. In addition, more sophisticated measures of DNA fragmentation, sperm apoptosis and sex-ratio ect. can be performed. Semen quality is an important marker for couple fecundity (ability to conceive) [84], and some studies have suggested that semen quality may be a marker for subsequent morbidity and mortality [85, 86]. A possible decline in semen quality has prompted discussion after a meta-analysis in 1992 suggested a decline of 0.9 ml/ml/year during a 50-year period from 1940 to 1990 [87]. Interestingly, a newly published meta-analysis including studies from 1973 to 2011 reported a similar yearly decline of 0.7 mill/ml [88].

The literature in this field is large, and we have therefore included reviews and meta-analyses when available and focused on studies examining the effect of fetal exposure, as the TDS hypothesis suggests that these are considered most relevant.

5.2.4.2 Organochlorine Compounds

An extensive number of epidemiological studies have addressed the possible effects of exposure to POPs on male reproductive health, but the results are conflicting (reviewed by [89]). Overall, studies of exposure to PCBs during adulthood indicate some association between PCB and lower sperm motility and to some extent decreased sperm DNA chromatin integrity [89]. However, two Faroese studies among high exposed young men and fertile men found no association with semen quality [90, 91].

In high exposed South African and Mexican populations, an inverse association between *p*, *p*′-DDE exposure and semen volume, total sperm count, and computer-assisted sperm analysis mean motility were reported [92, 93]. However, the adverse effects of low exposure to *p*, *p*′-DDE on sperm motility have been contradictory [89]. Some studied have suggested a positive association between *p*, *p*′-DDE exposure and sperm concentration, whereas several studies have suggested that *p*, *p*′-DDE is not related to sperm morphology and sperm DNA integrity [89].

Most studies have been cross-sectional and investigated exposure during adulthood. Only a few studies have been able to evaluate, whether intrauterine exposure to POPs has long-term consequences for male reproductive health with measurable effects on semen quality in adulthood. A Danish study included 176 male offspring from a Danish cohort of pregnant women, who participated in a study in 1988–1989. Results suggested that in utero exposure to PCB and DDE was not significantly associated with semen quality measures [44]. Among 39 sons and mothers exposed to dioxin after the devastating, industrial accident in Seveso, Italy in 1976 and 58 unexposed [94] average sperm counts were almost halved in the exposed group and the effect was most pronounced among breastfed men.

5.2.4.3 Phthalates

Many Chinese studies have addressed the adverse effect of phthalate exposure on semen quality. In 1040 Chinese men from an infertility clinic, higher MBP was associated with low sperm concentration and total sperm count [95]. Higher exposure to MEHP increased the percentage of abnormal heads. A subset of the men had phthalates measured in semen, and higher concentrations and MEHP and monobenzyl phthalate (MBzP) reduced sperm motility parameters and semen volume [96]. In older Chinese men, higher urinary MEP was significantly associated with a decreasing percentage of normal morphology [97].

Two meta-analyses of the impact of phthalate exposure on semen quality have been performed [30, 98]. The first from 2015 included 14 studies and found that urinary MBP and MBzP were associated with reduced sperm concentration. MBP and MEHP were inversely associated with motility [30]. No associations were observed between MEP and any semen parameters. A meta-analysis from 2017 [98]

included 15 studies. Many of the individual study results were not significant, which may be due to small sample size and large both inter- and intra-individual variation in semen quality. Overall, the association between increased DBP exposure and decreased semen quality, specifically sperm concentration, was robust, whereas moderate evidence of an association between increased DEHP and decreased semen quality, particularly for sperm concentration was suggested. Given the consistency across studies for morphology, the relationship between DiNP exposure and sperm parameters was considered moderate. The relationship between DiBP exposure and semen parameters was considered slight, whereas moderate evidence of an adverse effect of BBzP exposure specifically for motility was suggested [98].

Two studies have assessed maternal phthalate exposure and subsequent semen quality in her son [99, 100]. These are of special interest, as this is the relevant exposure window. DiNP metabolites in maternal serum from 12 weeks of pregnancy were analyzed and semen quality assessed among 112 adolescent Swedish sons. Higher prenatal exposure to DEHP and DiNP was associated with lover testicular size and semen volume, whereas no association with semen concentration was found. Among 185 young Australian men, maternal serum phthalate metabolite concentrations of mono-isononyl phthalate (MiNP) and DEHP and DiNP metabolites were negatively correlated with testis volume. It is difficult to draw conclusions from these two studies, as phthalates have a short half-life and were measured in serum and not the golden standard urine, but they suggest that prenatal exposure to DEHP and DiNP may affect testis development and thereby adult testis size.

5.2.4.4 PFAS

In a review [101], nine studies were identified investigating the association between PFAS exposure and semen characteristics. The findings for semen volume, sperm concentration, and total sperm count were inconsistent. Two studies found serum concentrations of PFAS to be associated with sperm morphology [102, 103]. However, in an American study PFAS concentrations were not consistently associated with overall sperm morphology [104, 105] but with makers of immature sperms with tail deficiencies. A few studies reported on the possible associations between PFAS exposure and sperm DNA integrity and apoptotic markers. No strict conclusions can be drawn from these studies [101].

Among 169 young Danish men, whose pregnant mothers were recruited in 1988–1989, prenatal PFOA exposure was associated with lower adjusted sperm concentration and total sperm count, while no associations were found for PFOS [106]. This is interesting, as these men are unselected and exposed during the vulnerable period in utero. In addition, PFAS have long half-lives and therefore maternal concentrations of PFAS represent the exposure during pregnancy.

5.2.4.5 BPA

In a review from 2016, five studies were identified [107]. Li and colleagues explored the association between urinary BPA concentrations and semen parameters among 218 factory workers from four regions in China [108]. Results indicated a negative association between urinary BPA concentrations and sperm concentration, total sperm count, sperm vitality, and sperm motility. However, results only remained significant for sperm concentration for non-occupationally exposed men [108]. In a cross-sectional Danish study, among young men from the general population, urinary BPA was inversely associated with progressive sperm motility [109]. One study among infertile men found that urinary BPA concentrations were negatively associated with sperm concentration, normal morphology, and sperm DNA damage and lower percentage progressively motile sperm, whereas two other studies, of fertile men or men trying to conceive, found no association between urinary BPA and semen quality [107].

To the best of our knowledge, no studies have examined the effect of prenatal BPA exposure on semen quality.

5.3 Challenges for Future Studies

Most studies of the association between endocrine-disrupting chemicals (EDC) and male reproductive health have been observational, as interventions or randomization is not possible. Thus, only association not causation may be drawn. Also, for cross-sectional studies (which constitute the majority) reverse causation is a possibility, as the outcome may proceed the exposure. Participants with poor semen quality, earlier onset of puberty, or lower testosterone may have an unhealthier lifestyle in addition to the EDC exposure, and despite taking confounders into account in the data analysis, it is difficult to disentangle the adverse effect of one single exposure.

While it is challenging to study semen quality, therefore, many studies include men undergoing infertility treatment, as they are easier to recruit. Infertile men constitute a very heterogeneous population consisting of men with both impaired and normal fertility potential because of infertility due to female factors. Other investigations therefore include young healthy men or donors, it is, however, difficult to obtain a participation rate above 30% in such studies, and the participants may be healthier or have a greater concern about their fertility than non-participants. Many of the semen quality studies included few participants, and as both intra- and inter-individual variation in semen quality is large, this may explain the lack of associations found in these studies. Also, most studies are cross-sectional, and as semen takes approximately 90 days to mature it is difficult to assess causality. Often

questionnaires focus on EDC exposure, behavior, and lifestyle of the recent 4–6 weeks, and thus do not cover the entire timespan of the spermatogenesis process. In addition, due to the lack of intervention, the question whether a change of EDC exposure can actually restore or improve a reduced reproduction remains to be elucidated.

When studying timing of puberty onset, it is essential to perform a clinical examination. Alteration of hormone concentrations may be a biological responsiveness to protect the body system from EDC exposure. Follow-up is necessary to find if these alterations of hormone concentrations and disruption of puberty onset proceed to infertility or cause of other diseases, such as cancer in reproductive organs.

Many chemicals behave as endocrine disruptors. However, it is important to bear in mind that many chemicals have different exposure pathways, and endocrine disrupting may not be the most prominent. In addition, we are all exposed to these chemicals, and it is difficult to identify an unexposed group. Also, large spatial and temporal trends in exposure concentrations occurs, and it is not within the limits of this review to address these variations. Mixed exposure to various chemicals is also a considerable challenge in terms of delineating the possible health effects of chemical exposure. Humans are exposed to a complex mixture of EDCs, and when investigating the effect of one chemical, it may be another correlating co-exposure that actually does the harm, or the mixture of a number of chemicals, the so-called cocktail effect.

Lastly, genetic susceptibility may also modify the effects of EDCs on reproductive functions. The results from a Japanese cohort study suggest that maternal dioxin concentrations may differ due to variations in the aryl hydrocarbon receptor and polymorphisms in families of metabolizing enzymes such as cytochrome P450 [110]. A Russian cohort suggested that single nucleotide polymorphisms (SNPs) in the glucocorticoid receptor (GR/NR3C1) and estrogen receptor-α (ESR1) genes were significant modifiers of the association between peripubertal dioxin concentration and male pubertal onset [111]. Thus, it is also important to consider genetic factors in the relationship between environmental chemical exposure and reproductive health.

5.4 Conclusions

In this chapter, we review the literature on exposure to endocrine-disrupting chemicals, e.g., organochlorine compounds, perfluorinated alkylated substances, phthalates, and phenols on anogenital distance, reproductive hormones in childhood, onset of puberty, and semen quality focusing on prenatal or early exposures during vulnerable time points of development. Prenatal exposure to OCPs appears to lower children's testosterone concentrations and increase aromatase activity after birth. Prenatal exposure to dioxins and OCPs is consistently associated with delayed onset of puberty in both sexes, whereas PCBs accelerate the onset of puberty in boys. Generally, AGD appears to be a promising, easily obtainable marker of male repro-

ductive health. Maternal exposure to phthalates has consistently been associated with shorter AGD in male offspring, whereas childhood or adult phthalate exposure has been associated with lower reproductive hormone concentrations, changed the onset of puberty and semen quality. No consistent associations between PFAS or phenol exposure and AGD, reproductive hormones, puberty onset, or semen quality have been found; however, the number of studies is limited and further studies are urgently warranted. Future studies are suggested to be birth cohort studies focusing on the effect of exposures during vulnerable time windows during development, e.g., in utero, during early childhood and puberty. They should have the necessary size, include biological material, focus on multiple exposures, and have long-term follow-up with repeated clinical examinations.

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