Chapter

Endocrine Disruptors: Very Low Doses with Genuinely High Impacts on Male Reproduction

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Abstract

Endocrine disruptors (EDs) are chemical substances that affect physiological processes in the body via hormonal regulation. They are often detected in food, plastic water bottles, cosmetics, and many other daily need items. Thereafter, EDs are detected in many bodily fluids, pointing out the real exposure to even very low doses. Permanent and long-term utilization of EDs has harmful effects on male reproductive health mainly due to interference with sex hormone synthesis and mechanism of action. However, with decreasing dosage of EDs, the possibilities of unpredictable modes of action arise. In addition to various molecular actions of individual EDs, the interference of individual ones represents another dimension of the ED issue. This review provides an overview of the EDs and their possible impact on reproductive health in males, with focus on sperm quality with the mighty potential of epigenetic transmission to further generations. The "posttranslational" effect of EDs in really low doses in real exposure routes is stigmatized in this review, being strongly considered as creeping molecular action of individual EDs as well as amplifications of their copresence in the environment.

Keywords: endocrine disruptor, bisphenol, regretable substitution, posttranslational modification

1. Introduction

Maturation of sperm cells (spermatogenesis) is a continuous process starting in puberty. The process is stimulated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Until the onset of puberty, spermatogonia are quiescent and their quantity does not change significantly. After sexual maturity is reached, an expressive activation of mitochondrial activity and the process of spermatogenesis begin, leading to the formation of spermatids. They are then transformed into spermatozoa by the spermiohistogenesis process, when a round spermatid changes into a sperm cell with a tail, middle section, and head. However, whole spermatogenesis, including gonadal ridge colonization and differentiation of primordial germ cells (PGCs), followed by further development, begins during early embryogenesis. In light of this fact, there are several exposure windows when environmental noxi can hit spermatogenesis along the entire process.

Considering the transmission of extraneous agents, the hemotesticular barrier (HTB) represents the morphological division of the seminiferous tubulus into two

compartments: basal and adluminal. The barrier is crucial for full functionality of germinal epithelium, as well as for the elimination of negative impacts of environmental pollutants. Physiologically, this strict division ensures free paracellular movement of substances among the compartments, such as water, nutrients, electrolytes, hormones, and paracrine factors. HTB provides protection of the emerging sperm cells from autoimmune damage by antibodies to sperm cells produced if the barrier was impaired, and the immune system would be in contact with spermatozoa during puberty when the body has already reached immunocompetence. Therefore, the cross talk of the immune system and HTB is potentially another sensitive target to a pollutant impact.

There is a basal compartment in close proximity to the basal membrane. This segment contains vessels and nerves, and spermatogenesis is initiated here. Spermatogonia and spermatocytes up to the proleptotene stage are present here. This segment is necessary for nutrition. The spermatogonia are subsequently transported through tight intercellular junctions to the adluminal compartment which is a place of spermatogenesis completion and subsequent metamorphosis of spermatids to spermatozoa. Both compartments are demarcated by the blood-testis barrier (BTB). Vessels and nerves are no longer present in this segment, and the nutrition of the germinal epithelium cells is covered by the Sertoli cells. The impact of various doses and concentrations of EDs on the male reproductive system can affect the functions of this barrier. The differentiation and development of the male reproductive system depends on elementary estrogen/androgen ratio, and the antagonistic and agonistic effects of EDs often disrupt their balance. The development of testicular tissue is crucial for further development of the entire reproductive system, as the endocrine activity of testicles determines overall masculinization of the body. Any disruption in the development of the testicles can therefore impair the overall masculinization process and sperm production.

Sperm concentration in men decreases worldwide, and spermiogram parameters deteriorate mainly in the Western world population [1]. Among others, huge amounts of endocrine disruptors (EDs) in our environment can cause this state. This final manifestation of the noxious effect of EDs has an unknown background, such as dose, kinds of EDs, interactions, and crosstalk of individual EDs and/or the timing of the exposure. Therefore, biomonitoring data represents significant input for experimental designing, leading to the description of molecular action in simulated conditions. Based on the newest findings, the record of the biological impact of individual EDs is an ongoing research issue leading to indicating the found compounds as endocrine disruptors.

Many cases of impaired sexual development due to the effects of EDs are also known from the animal kingdom. For example, reduction of penile length was observed in crocodiles living in waters contaminated with EDs [2]. EDs can significantly influence not only the process of spermatogenesis but also the development of testicular tissue. It has been documented that increased exposition of pregnant mice to BPA caused alterations of organelles, that is, mitochondria and lysosomes, in Sertoli and Leydig cells, respectively. These alterations led to maturation disorders in spermatocytes and androgen synthesis inhibition [3].

2. Spermatogenesis, epigenetics, biochemical status of spermatozoa, and implications for male reproduction

The creation of the spermatozoon leads to the terminally differentiated cell with an extremely high level of chromatin methylation and silencing. The final shape of the spermatozoon, often species-specific, requires many morphological and biochemical changes, in particular, dynamic remodelation of the chromatin [4].

Protamination, histone-protamine exchange in elongating spermatids, represents a drastic, expressible change of sperm chromatin [5]. A tight protamine-derived DNA package protects sperm chromatin against damage and, interestingly, even the ratio of protamines PRM1 (sperm protamine P1) and PRM2 (sperm protamine P2) is decisive about sperm quality [6]. In accordance with the tight chromatin package, DNA is strongly methylated, and, therefore, general chromatin silencing is required for sperm stability [7, 8]. Protamination represents a tool for the protection of paternal gene imprinting [9]. Temporal protamine-packaged sperm DNA undergoes a second exchange of chromatin proteins after fertilization, and then maternal histones are incorporated into the paternal pronucleus. Both protamine-histone transition events, first and second in testicular seminiferous tubuli and fertilized oocyte, respectively, are obviously sensitive to environmental influences and represent susceptible exposure windows [10, 11].

Although most core histone is substituted by protamines, a residual species-specific amount of histones resists in the sperm head. In addition to DNA methylome, epigenetic hallmarks of mature spermatozoa include the epigenetic code of residual histones, based on many posttranslational modifications (PTMs) of individual amino acids [12, 13]. These chromatin-repressive histone marks positively correlate with DNA methylome and accompany imprinted genes. Moreover, the sperm histone code shows an exact physiological role in fertilization and early embryonic development [14]. The histone code establishment is highly orchestrated [15] and, therefore, enforces spermatid sensitivity to exposure to environmental pollutants.

Following comprehensive demethylation of parental chromatin after fertilization, the total erasure of the methylation pattern, including gene imprinting on paternal and maternal alleles, is needed for the re-establishment of gene imprinting adequate to the paternal pattern in the sperm cell. This erasure comes early after gonadal ridge colonization, and primordial germ cells (PGCs) occur, at human embryonic days E32 and E10.5 in mice [16]. The recurrent "writing" of the epigenetic pattern into imprinted loci occurs in the late prenatal period when the spermatogonia are formed. This period between erased PGCs and remethylated spermatozoa represents a highly sensitive and quite extensive exposure window, when the epigenetic status can be changed by environmental factors during embryonic development in utero. There is another dynamic chromatin demethylation, many years later, when sperm chromatin remodeling occurs when paternal and maternal pronuclei are developed in the early zygote. This methylation erasure is not complete and excludes parent-of-origin methylation, that is, erasure-resistant loci, such as IAPs, LINEs, and transposon-related loci. Taken together, the transgenerational and intergenerational inheritances of epigenetic shifts (i.e., non-genomic or non-Mendelian inheritance) are based on these two exposure windows, when epigenetic erasure, including gene imprinting in PGCs and imprinted gene-excluding erasure, occur, respectively [17]. The renewal of gene imprinting between PGCs and mature gamete is another power of transgenerational epigenetic inheritance [18]. The dynamics of the epigenetic code is subjected to a well-tuned orchestra of "erasures" (TET oxygenases, histone deacetylases, and demethylases) and "writers" (DNA methyltransferases, histone methyl transferases, and acetyl transferases) (reviewed in [19]). It is assumed that, via EDs, they change the epigenetic code through these upstream factors (the possible methods of exposure are summarized in **Figure 1**).

Doubtless, a properly established epigenetic code plays an extremely important role, in particular in imprinted genes in epimutation-prone gametes. The epigenetic code of the spermatozoon is highly protected by the protamination, determining the stability of the genome and gene imprinting. Otherwise, epigenetic disorders arise: Prader-Willi syndrome, Angelman syndrome, or Silver-Russell syndrome. Moreover, residual histones bring the epigenetic information via histone PTMs.

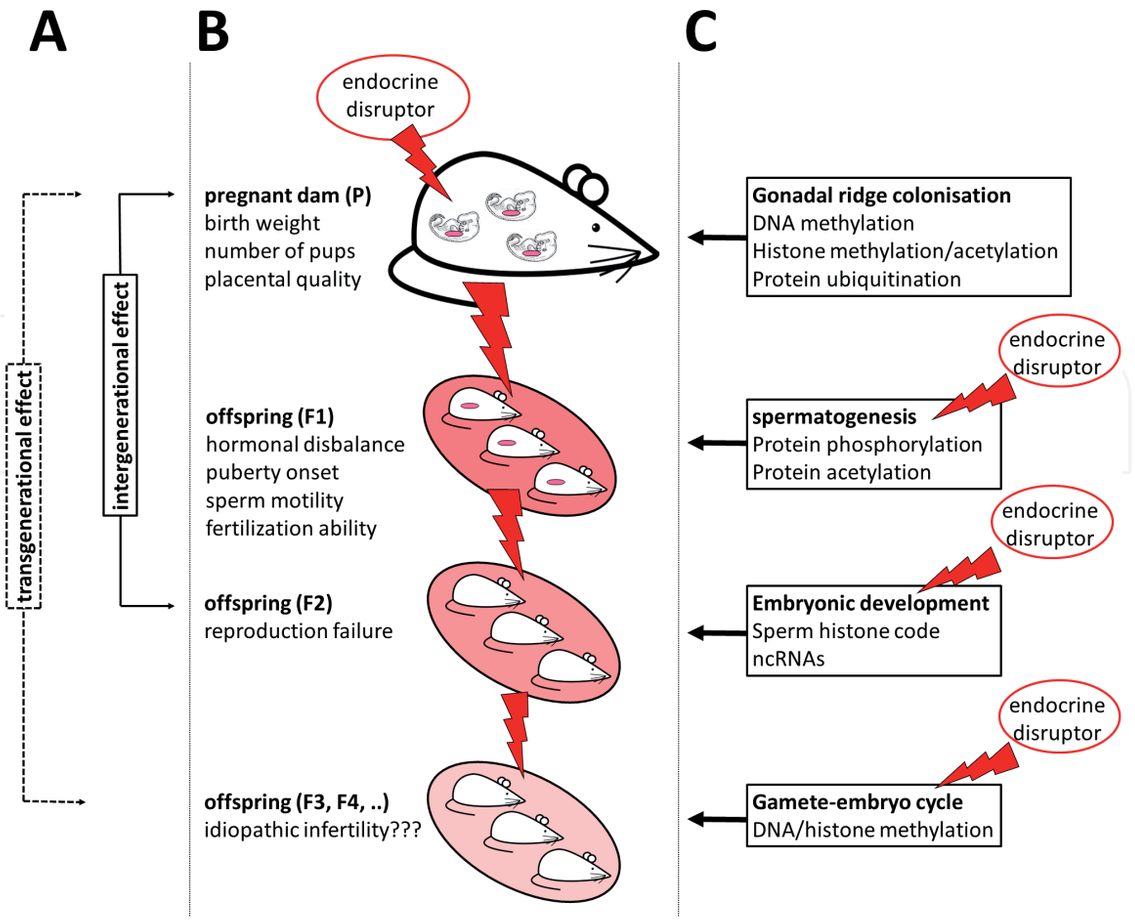

Figure 1.
Endocrine disruptors induce non-genomic inheritance through posttranslational modifications (PTMs) of various epigenetic factors. (A) Environmentally impacted posttranslational modifications of proteins incoming into intergenerational and transgenerational effects. (B) Endocrine disruptors are able to affect developing gonads through transplacental transmission in utero. Gonad activity is changed and hormonal levels, puberty onset, and sperm quality are affected. Sperm quality contributes to embryonic development and can influence the health of an offspring, and, therefore, the intergenerational transmission of the ED effect to F2 generation is obvious. Gene imprinting and epigenetic erasure are assumed to be the tools of this effect. The epigenetic code of erasure-resistant loci is possibly affected by ED, and the transgenerational effect appears. Nonspecific symptoms accompany these epigenetic faults, and many disorders are classified as idiopathic. (C) From the molecular point of view, inadequate changes in DNA and chromatin proteins, including PTMs of core histones and/or RNA polymerases, are responsible for the epigenetic record and gene manifestation, and ED becomes potentially dangerous for these protein modifications through "posttranslational" effect. Obviously, male reproduction is endangered through several exposure windows during gamete formation, including epigenetic code erasure and re-establishment. Therefore, in addition to direct modification of chromatin, responsible "erasures" and "writers" (responsible for de-differentiation and gene imprinting, respectively) undergo regulation via PTMs when the EDs' effect is considered.

Obviously, in addition to the genetic information, the sperm head carries a package of epigenetic notice, very sensitive to the disruption through its establishment throughout the spermatogenesis.

In addition to the establishment of epigenetic code of sperm histones, achievement of other PTMs of regulating proteins is required. Frequently, the loss of a PTM leads to protein activity lacking, sometimes leading to fatal clinical manifestations, for example, the inability of PARKIN1 S-sulfhydration of cysteine followed by sporadic Parkinson's disease [20]. During post-ejaculation the sperm changes, such as capacitation and acrosomal reaction; there are many PTMs of key proteins necessary for the achievement of fertilization ability. Therefore, protein kinase A (PKA)-driven phosphorylation of Arg-X-X-(Ser/Thr) motifs is required, as the result of upstream regulation by soluble adenylyl cyclase and cAMP production [21]. However, acetylation of ε-amino group of lysine residues arises as regulatory tool for

PKA, and, accordingly, the hyperacetylation of sperm proteins is needed for sperm capacitation [22], essential for sperm hyperactivation in female reproductive tract. Versatile role of protein acetylation is obvious, including aforementioned residual histones as well as protein kinases. Taken together, the impact of endocrine disruptors on histone PTMs [23, 24] as well as sperm phosphorylation [25–27] has been described, and, therefore, the modifications of proteins (protein PTMs) become the likely manner in which disruptors (EDs) work in their real doses.

3. Endocrine disruptors: mode of action and nonlinear effect

There are many shared features of EDs, such as spatiotemporal omnipresence, exposure to very low doses, and, therefore, often a nontoxic effect [28]. Nevertheless, the affection of hormonal balance represents a major sign of them, giving the name to endocrine disruptors [29]. Indeed, there is an increasing number of observations of exposure to EDs, across all age, race, profession, lifestyle, and health status categories [30]. These findings are in accordance with the ubiquity of EDs through the presence in daily need items.

Compound	Phenotype of filial generation	Species	Reference
Antibiotics (Geneticin)	Up-/downregulation of genes responsible for basic metabolism, cell cycle, stress response, and development	*Drosophila melanogaster*	[108]
Atrazine	Reproduction, altered transcriptome responsible for steroidogenesis, and DNA methylation	Medaka (*Oryzias latipes*)	[109]
Benzylisoquinoline alkaloids	Reduction of lipid accumulation	*Caenorhabditis elegans*	[110]
BPA	Affected neurogenesis and damaged social interactions	Mouse (C57BL/6 J)	[111]
DDT	Pathology of gonads, obesity	Rat (Sprague Dawley)	[112]
Dioxin	Testicular tissue abnormalities	Zebrafish (*Danio rerio*)	[113]
Di(2-ethylhexyl)phthalate (DEHP)	Reproduction failure	Mouse (CD-1)	[114]
Glyphosate	Obesity, prostate, and ovary diseases; kidney failure; birth abnormalities	Rat (Sprague Dawley)	[115]
Methoxychlor	Obesity, ovary, and kidney diseases	Rat (Sprague Dawley)	[116]
Vinclozolin	Alterations transcriptome with disease susceptibility of gonads, ancestry glands, *mamma*, and kidney	Rat (Sprague Dawley)	[117]

Representative studies are included, testing different compounds (in toxic and sub-toxic doses) on various biomodels, mostly exposed during the establishment of germ cells and gonad maturation. These exposures lead to changed phenotype of filial generation through the epimutation of germ cells. In addition to pregnant exposure, PTMs of epigenetic factors and/or histone code represent a molecular tool of endocrine disruptor-inherited impact along generations, even though the exposure is during adulthood. Although direct human evidence is lacking, there are several indications of the effect of transmission of endocrine disruptors in very low doses, on further generations due to PTM-driven epimutations [106, 107].

Table 1.
Overview of recent knowledge of environmental inheritance of endocrine disruptor effects.

The "family" of EDs is wide and still growing, as is our awareness of their biological impact. Therefore, EDs include polybrominated diphenyl ethers, phthalates, polyethylene terephthalate (PET), bisphenols, and others (**Table 1**). Hence, flame retardants in electronic devices, perfumes, plastic bottles, and polycarbonates, respectively, are the most usual source of EDs. Surprisingly, some daily need items, such as paper bags, cans, receipts, and dental sealants, include bisphenols, although they seem to be free of any endocrine disruptors. Even strict elimination and usage control of pesticides are not able to exclude the endocrine-disrupting effect through contamination of food with residua of some of them, for example, glyphosate [31], atrazine [32], and imidacloprid [33]. Because EDs are so widespread, humans are exposed to them via different routes: oral intake with food and beverages and transdermal exposure and/or inhalation. Some specific routes of exposure are derived from the uniqueness of the stage of ontogenesis, such as transplacental in utero exposure during pregnancy, followed by translactational exposure when a baby is nursed.

Most EDs are released into the environment in a very low amount, and, therefore, the human intake is appropriately much smaller. This is the result of the legacy action of responsible authorities (European Food Safety Authority, EFSA; Food and Drug Administration, FDA), which has established the limits of intakes (tolerable daily intake, TDI) for many ED compounds. However, extremely low doses have been recognized as having a biological effect. In the light of this fact, the earlier

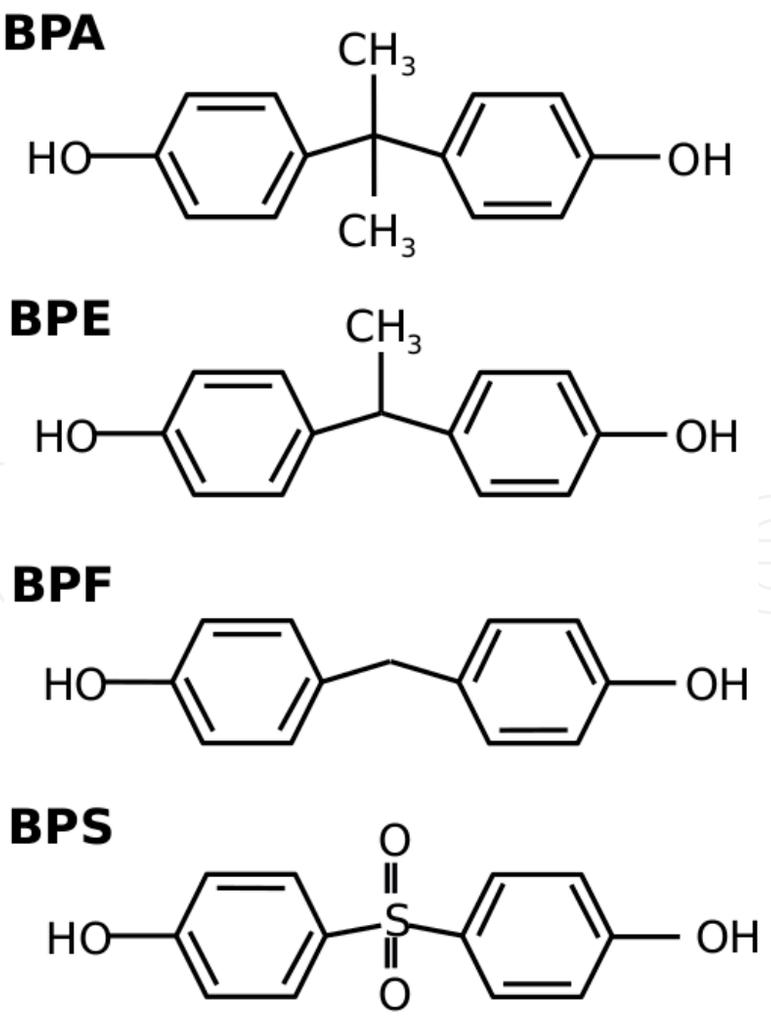

Figure 2.
Molecular structure of BPA and its alternatives. BPA, bisphenol A (2,2-Bis(4-hydroxyphenyl)propane); BPE, bisphenol E (4-(1-(4-hydroxyphenyl)ethyl)phenol); BPF, bisphenol S (4,4'-dihydroxydiphenylmethane); BPS, bisphenol S (4,4'-sulfonylbisphenol).

accepted quantities of no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) lose importance. Interestingly, lower doses show often more deleterious effect than the higher ones, pointing out the nonlinear effect [29]. The response of the cell, tissue, or an organism on the dose is in a non-monotonic (i.e., U-shape) curve [34]. It was difficult to accept this phenomenon, but recently we consider it to be one of the features of EDs [35–37].

After many substances were described to be an ED, elimination or total restriction followed. Therefore, several compounds have been introduced as a substitution. *n*-Hexane and alternative bisphenols (BPS, BPF, and BPAF) have become widespread, such as alternatives to dichloromethane and bisphenol A (BPA), respectively, although the unambiguous safety of these substitutions has not been proved (chemical structure of selected bisphenols is presented in **Figure 2**). For instance, BPA usage has been banned in children's toys and baby bottles, and, in addition to these, BPA-free products were introduced based on the consumer preferences [30]. However, BPS to BPA exchange has taken place, although "endocrine" safety has not been elucidated. In this point of view, we can denote it to be a regrettable substitution, and comprehensive testing of these alternative compounds is required.

4. The impact of EDs on male reproductive health

According to a range of studies, the effect of EDs is significant mainly during the development of the male reproductive system. The cocktail effect of multiple substances in low concentrations with similar action target has been described many times. Particularly trans-uterine exposition during embryonic development is critical, when testicular dysgenesis syndrome can develop [38]. There is a presumption that it is caused by impaired function of Leydig cells and testosterone production [39]. For initiation of prostate development and masculinization of the sex ducts, the presence and correct ratio of steroid hormones is necessary. Nevertheless, EDs often act as inhibitors of 5-alpha reductase enzyme that is necessary for the conversion of androgens to testosterone and inhibitors of aromatase necessary for androgens aromatization to estrogens [40].

4.1 Effect on gonads and accessory glands

Cryptorchidism: This is a serious developmental disorder which may be also caused by exposition of the fetus to EDs in utero and subsequent feeding with breast milk with a high concentration of EDs [41].

Hypospadias: It has been documented that utilization of EDs in the form of medication for pregnant women led to various disorders of testicular development [42].

Testicular cancer: The half-life of the EDs with lipophilic character is up to 30 years. It has been observed that mothers of adult men with testicular cancer had high levels of polychlorinated biphenyls (PCBs) in the blood, which led to the conclusions that the ability of PCBs to accumulate in the body makes their presence one of the factors contributing to development of this type of cancer [43]. Considering the half-life of many toxins, for example, PCBs, we can assume that these toxins will achieve their endocrine-disrupting effect as their real amount in the environment decreases, while their toxic effects are not taken into account anymore.

Prostate hyperplasia: It has been described in rats that exposition of males to low doses of estrogens and xenoestrogens led to prostate hyperplasia. These results support concerns that, in today's plastic era, this phenomenon will also manifest

in adult men [44]. Moreover, in 2017, it was documented that doses of BPA equivalent to doses potentially present in the environment caused increased growth of prostate cells [45].

4.2 Effect on spermatogenesis

Sperm concentration in ejaculates of men has been decreasing for a long time, mainly in Western world populations (the USA, Europe, Australia, and New Zealand) [1]. This long-term process has been observed since the 1970s. The situation might be caused by environmental changes, primarily by the increased occurrence of various EDs [46]. It is generally acknowledged that the process of sperm production is significantly reduced by FSH and LH, while alterations on this level may cause impairment of spermatogenesis to infertility [47].

4.3 Effect on cells of germinal epithelium

It is known that EDs are capable of influencing the offspring in utero through transplacental transmission and via breast milk and that they cause disorders that can be transferred epigenetically to further generations. In certain periods of fetal development, testicles are estrogen-sensitive, and their excessive exposure to this hormone can result in complete arrest of steroidogenesis. EDs with an estrogenic character can interfere with the correct functioning of the reproductive system.

During spermatogenesis, spermatogonia are transformed to spermatozoa when a round spermatid changes into a sperm cell with tail, middle piece, and sperm head. For this process, Sertoli cells play a key role as they form the functional blood-testis barrier (BTB) with very tight junctions. This barrier is dynamic and demarcates the basal compartment and adluminal compartment of seminiferous tubules. The barrier is necessary to prevent damaging of sperm cells by the immune system, since contact of blood and mature sperm cells leads to the production of antibodies to spermatozoa. These antibodies can then enter the seminal plasma and damage sperm cells. The principle of the hemotesticular barrier are very tight junctions between the Sertoli cells which divide the structure of seminiferous tubules into basal and adluminal compartments.

4.4 Disruption of the blood-testis barrier (BTB)

Effects on the hemotesticular barrier can significantly affect spermatogenesis and can have an impact on embryonic development of testicular tissue. The division contributes to unlimited capillary supply of nutrients, hormones, and other biomolecules which are needed for mitotic renewal of spermatogonia, their proliferation, and differentiation. However, the other developmental stages must not come into contact with blood. If this barrier did not exist or was damaged, antibodies to sperm cells would be produced, which could ultimately result in male infertility [48].

BTB and effect of EDs: Detachment of both compartments is ensured by tight intercellular junctions of adjacent Sertoli cells. These are very tight connections represented mainly by tight junction, adherens junction, desmosome, and gap junction types. The riskiest period is when spermatocyte at the proleptotene stage passes through the barrier, which needs to undergo structural changes. It is this particular period when the effect of substances such as the endocrine disruptors is most significant.

It is known that the level of free BPA in blood plasma decreases the concentrations of occludin, N-cadherin, and connexin 43, which are proteins that

significantly contribute to the production and regeneration of tight junctions. Decreased levels of these proteins affect the function of BTB [49].

Taken together, a very low dose of EDs seems to have the most deleterious impact. There are obviously different modes of action of EDs, and, all the more so, the molecular targets of EDs are the center of interest of the current studies describing disruptors.

5. Molecular mechanism of EDs in extremely low doses

The toxic effects of many compounds are well-known and described, and the amount of published findings is still growing by thousands of papers each year. In general, genotoxicity and carcinogenesis [50], oxidative stress induction [51], and DNA damage and cell senescence [52–54] are known impacts of several toxic compounds. However, sub-toxic effects of toxic compounds (pesticides and drugs) described earlier as well as seemingly safe compounds (alternative bisphenols) represent a serious risk for human public health. For this reason, there are many biomonitoring initiatives, followed by legislation and the development of next-generation plastics.

In accordance with toxin elimination during the last decades, people in developed countries have been recently exposed to rather sub-toxic doses in trace amounts. This effect is known as endocrine disrupting, affecting the body in other ways than toxins, that is, genomic, non-genomic, and epigenetic modes of action. While the genomic effect is similar to toxin action, the non-genomic effect is the closest to endocrine disruption; the mimicking of the presence of a hormone, targeting of hormonal signaling, and/or misregulation of hormone production and expression of receptors are known mechanisms of endocrine-disrupting effects [55–57]. Hormonal disbalance impacts the hypothalamus-pituitary-gonadal axis [58], with possible clinical manifestations: changed anogenital distance, morphological changes of sex determinations, and earlier puberty onset [59]. However, the tested doses are very high, whereas, on the contrary, very low doses correspond to the real exposure, often leading to small differences on the level of tissue and cell, without any demonstration of clinical aspects. Although changes in hormonal balance are well-known [60, 61], EDs are even capable of affecting hormonal action directly in a cell without a shift in hormonal profile. Therefore, the estrogen-like and estrogenic effects of BPA have been described in germ [62], ovarian [63], and testicular cells [64, 65]. Frequently, the G-protein-coupled estrogen receptor is a target of the estrogen-like effect of BPA [65, 66], as well as alternative bisphenols [67]. Transcription and subcellular distribution of estrogen receptors ERα and ERβ and aromatase, an enzyme converting adro- to estrogens, are changed in bisphenol S-exposed oocytes [68]. These non-genomic alterations are accompanied by cytoskeleton abnormalities. In particular, the meiotic spindle is extremely sensitive [69] and, indeed, affected in mammalian oocytes exposed to bisphenols [68, 70, 71], leading to increased incidence of aneuploidy [72].

The comparable effect of EDs is known during spermatogenesis: BPA is capable of affecting meiotic division and chromosome segregation, increasing the incidence of aneuploidy-derived disorders [73]. In addition, the molecular mechanism of BPA consists in impacting several signal pathways and results in the change of protein kinase A activity and protein tyrosine phosphorylation, ATP generation, and oxidative stress-related enzymes (i.e., peroxiredoxin-5, glutathione peroxidase 4, succinate dehydrogenase), crucial for sperm motility and ability of oocyte fertilization [26, 27, 74]. Dose-response association of BPA and motility parameters of human sperm has been observed [75]. Interestingly, some EDs have shown a

stronger negative impact on Y-chromosome-bearing spermatozoa, and the sex ratio of offsprings can be changed [76, 77].

Many non-genomic methods of ED action lead to inappropriate epigenetic changes of DNA and core histones. Although the sequence of nucleotide remains unaffected, the changes of genome-wide methylation status, as well as silencing or enhancing the individual loci, follow the exposure of EDs. These epimutations result in changed transcriptional activity of the genome with many negative impacts, such as failure of scavenging of reactive oxygen species, DNA damage repair, and/or inadequate mitochondrial biogenesis. These cellular changes lead to clinical manifestations, most of which are diagnosed as "idiopathic." Obviously, exposure to EDs causes obesity [78], type 2 diabetes [79], metabolic disorders, and infertility [80].

While the exposure of somatic cells creates health problems for exposed individuals, influence on gametes leads to an intergenerational effect when the burden is transduced to the next generation of daughters and sons [81]. Indeed, the exposure to bisphenols impairs genome-wide DNA methylation, as well as histone code in oocytes [71, 82], followed by changes in the imprinting of genes in the embryo and placenta [83]. In spermatozoa, DNA methylation [84] is potentially affected by environmental pollutants, leading to aberrant gene imprinting [85, 86]. It can be assumed that the sperm histone code is sensitive to endocrine disruptors, with effect similar to estrogens, as well as to the involvement of estrogen receptors in histone code establishment [15]. Moreover, the negative role of environmental pollutants in the influence on noncoding RNAs in spermatozoa, another tool of epigenetic regulation [87] with ability to drive epigenetic inheritance [88], is well-known [89, 90].

The exposure in utero and transplacental transmission of an ED affect DNA demethylation in developing PGCs and result in transgenerational inheritance of this burden. Accordingly, the exposure of pregnant rat females to fungicide vinclozolin [91] or DDT [92] leads to modified epigenome, that is, DNA methylome, histone retention in sperm, and ncRNAs. Translactational exposure, another way of indirect influence with environmental agents, is a reason of changes of male reproduction after lactating female mice were exposed to BPA [93]. Moreover, this type of exposure to bisphenols creates a risk of changed nursing behavior and also affects the mammary glands of mothers [94].

Whereas endocrine-disrupting hypothesis is assumed for very low doses of EDs, there is a relevant phenomenon of interactions of individual EDs. The comprehensive work of T. Pollock and his colleagues produced valuable results, describing cross talk of common EDs. The combined presence of bisphenols is considered to be deleterious [95] as well as the simultaneous presence of triclosan, a soap compound [96, 97]. Degradation of bisphenol is inhibited under other ED exposure, and, obviously, the co-exposure achieves various modes on how to affect the body [98]. In addition to human and mammalian models, there is evidence of interaction of xenobiotics and pesticide residua [99], as well as synergistic interactions of organophosphates and pyrethroids [100], potentially leading to the collapse of honey bee colonies [101]. In contrast to synergic effects leading to the increase of the deleterious impact, competition of some pollutants is known, and, surprisingly, a reverse effect of the synergic activity of pollutants has been described, where one pollutant protects cells against damage caused by another pollutant [102]. The molecular action of interacting pollutants remains to be unexplained in mammalian models, and there is obvious need for further study. Also the results of these studies will influence public health protection.

6. Perspectives

The aforementioned routes of exposure to EDs, including their interactions, obviously lead to different systemic response as the result of molecular action in tissues and cells. The molecular mode of action seems to be the key for the elimination of EDs' negative effect on the body. Based on already described manifestations of EDs in higher and lower doses, two dose-dependent modes of action are recognized: toxic effect and endocrine disruption. It seems that the current issue of EDs is in extremely low doses without clinical manifestations, leading to "idiopathic" infertility, metabolic syndrome, and other failures with nonspecific symptoms. Moreover, intergenerational and transgenerational inheritances occur because of the change of the epigenetic code of germ cells. The posttranslational modifications of crucial proteins, particularly regulating epigenetic factors, seem to be a common feature of these very low doses. In accordance with this, we can mark this effect to be "posttranslational." The possible contribution of posttranslational modifications of key proteins is indicated in **Figure 1**.

There is an obvious direct impact of EDs on male reproduction due to oral, respiratory, and/or transdermal exposure. Thereafter, both the gonads and accessory glands are affected, leading to the failure of male reproduction, often diagnosed as idiopathic. On the spermatozoon level, direct protein targeting is assumed, including cytosolic proteins as well as sperm histone code. Even protamine PTMs are considered to have a biological role, and, in accordance with the abovementioned importance of acetylated lysines, protamine acetylation seems to be most potent for sperm quality. The impact on DNA and chromatin proteins (i.e., histones and protamines) represent hazardous mode of inter- and transgenerational transmission of ED-driven epigenome.

In addition to the direct impact of EDs, indirect impact is also observed. The exposure of EDs during pregnancy and prenatal life represents the most dangerous exposure method when the germline is affected during gene imprinting erasure and re-imprinting in developing spermatozoa [85] and oocytes [103]. This exposure window allows an ED to affect the health of a generation of grandchildren through transgenerational inheritance [104, 105]. Epigenetic transmission to further generations involves various modifications, such as DNA and histone methylation, histone acetylation, and other PTMs of core histones, as well as epigenetic writers and erasures, translational factors, and others. Obviously, PTMs actually drive the phenomenon of the epigenetic inheritance, and the molecular impact of individual EDs is still unknown, as is their interaction (**Table 1**).

There is a strong need for further study focused on the ED-modulated epigenetic code and its manifestation in the body. In accordance with our "posttranslational" hypothesis of ED action, comprehensive screening of the most crucial PTMs should be taken into account in an assessment of individual EDs. Taken together, biomonitoring has an extremely significant role in the fight against EDs, as does the subsequent testing of EDs in the ascertained doses. Simulation of real exposures to individual EDs and their interactions are appropriate, using both in vitro and in vivo experimental assessments. Finally, advanced screening methods capable of identifying PTMs are needed for qualified recognition of an ED as harmful/harmless.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, et al. Temporal trends in sperm count: A systematic review and meta-regression analysis. Human Reproduction Update. 2017;**23**:646-659. DOI: 10.1093/humupd/dmx022

[2] McLachlan JA, Simpson E, Martin M. Endocrine disrupters and female reproductive health. Best Practice & Research. Clinical Endocrinology & Metabolism. 2006;**20**:63-75. DOI: 10.1016/j.beem.2005.09.009

[3] Liu X-L, Chen X-Y, Wang Z-C, Shen T, Zhao H. Effects of exposure to bisphenol A during pregnancy and lactation on the testicular morphology and caspase-3 protein expression of ICR pups. Biomed Reports. 2013;**1**:420-424. DOI: 10.3892/br.2013.79

[4] Rathke C, Baarends WM, Awe S, Renkawitz-Pohl R. Chromatin dynamics during spermiogenesis. Biochimica et Biophysica Acta, Gene Regulatory Mechanisms. 2014;**1839**:155-168. DOI: 10.1016/j.bbagrm.2013.08.004

[5] Oliva R. Protamines and male infertility. Human Reproduction Update. 2006;**12**:417-435. DOI: 10.1093/humupd/dml009

[6] Carrell DT, Emery BR, Hammoud S. Altered protamine expression and diminished spermatogenesis: What is the link? Human Reproduction Update. 2007;**13**:313-327. DOI: 10.1093/humupd/dml057

[7] Nanassy L, Carrell DT. Analysis of the methylation pattern of six gene promoters in sperm of men with abnormal protamination. Asian Journal of Andrology. 2011;**13**:342-346. DOI: 10.1038/aja.2010.160

[8] Rahiminia T, Yazd EF, Fesahat F, Moein MR, Mirjalili AM, Talebi AR. Sperm chromatin and DNA integrity, methyltransferase mRNA levels, and global DNA methylation in oligoasthenoteratozoospermia. Clinical and Experimental Reproductive Medicine. 2018;**45**:17-24. DOI: 10.5653/cerm.2018.45.1.17

[9] Carrell DT, Emery BR, Hammoud S. The aetiology of sperm protamine abnormalities and their potential impact on the sperm epigenome. International Journal of Andrology. 2008;**31**:537-545. DOI: 10.1111/j.1365-2605.2008.00872.x

[10] Zatecka E, Castillo J, Elzeinova F, Kubatova A, Ded L, Peknicova J, et al. The effect of tetrabromobisphenol A on protamine content and DNA integrity in mouse spermatozoa. Andrology. 2014;**2**:910-917. DOI: 10.1111/j.2047-2927.2014.00257.x

[11] Ankolkar M, Deshpande SS, Balasinor NH. Systemic hormonal modulation induces sperm nucleosomal imbalance in rat spermatozoa. Andrologia. 2018;**50**:e13060. DOI: 10.1111/and.13060

[12] Brunner AM, Nanni P, Mansuy IM. Epigenetic marking of sperm by post-translational modification of histones and protamines. Epigenetics & Chromatin. 2014;**7**:2. DOI: 10.1186/1756-8935-7-2

[13] Luense LJ, Wang X, Schon SB, Weller AH, Lin Shiao E, Bryant JM, et al. Comprehensive analysis of histone post-translational modifications in mouse and human male germ cells. Epigenetics & Chromatin. 2016;**9**:24. DOI: 10.1186/s13072-016-0072-6

[14] Schon SB, Luense LJ, Wang X, Bartolomei MS, Coutifaris C, Garcia BA, et al. Histone modification signatures in human sperm distinguish clinical abnormalities. Journal of Assisted Reproduction and Genetics.

2019;**36**:267-275. DOI: 10.1007/s10815-018-1354-7

[15] Dumasia K, Kumar A, Deshpande S, Balasinor NH. Estrogen, through estrogen receptor 1, regulates histone modifications and chromatin remodeling during spermatogenesis in adult rats. Epigenetics. 2017;**12**:953-963. DOI: 10.1080/15592294.2017.1382786

[16] Lucas-Herald AK, Bashamboo A. Gonadal Development. Endocrine Development. 2014;**27**:1-16. DOI: 10.1159/000363608

[17] Sales VM, Ferguson-Smith AC, Patti M-E. Epigenetic mechanisms of transmission of metabolic disease across generations. Cell Metabolism. 2017;**25**:559-571. DOI: 10.1016/j.cmet.2017.02.016

[18] Iqbal K, Tran DA, Li AX, Warden C, Bai AY, Singh P, et al. Deleterious effects of endocrine disruptors are corrected in the mammalian germline by epigenome reprogramming. Genome Biology. 2015;**16**:59. DOI: 10.1186/s13059-015-0619-z

[19] Zeng Y, Chen T. DNA methylation reprogramming during mammalian development. Genes (Basel). 2019;**10**:257. DOI: 10.3390/genes10040257

[20] Vandiver MS, Paul BD, Xu R, Karuppagounder S, Rao F, Snowman AM, et al. Sulfhydration mediates neuroprotective actions of parkin. Nature Communications. 2013;**4**:1626. DOI: 10.1038/ncomms2623

[21] O'Flaherty C. Phosphorylation of the arginine-X-X-(serine/threonine) motif in human sperm proteins during capacitation: Modulation and protein kinase a dependency. Molecular Human Reproduction. 2004;**10**:355-363. DOI: 10.1093/molehr/gah046

[22] Ritagliati C, Luque GM, Stival C, Baro Graf C, Buffone MG, Krapf D. Lysine acetylation modulates mouse sperm capacitation. Scientific Reports. 2018;**8**:13334. DOI: 10.1038/s41598-018-31557-5

[23] Men Y, Zhao Y, Zhang P, Zhang H, Gao Y, Liu J, et al. Gestational exposure to low dose Zearalenone disrupting offspring spermatogenesis might Be through epigenetic modifications. Basic & Clinical Pharmacology & Toxicology. 2019:1-12. DOI: 10.1111/bcpt.13243

[24] González-Rojo S, Lombó M, Fernández-Díez C, Herráez MP. Male exposure to bisphenol A impairs spermatogenesis and triggers histone hyperacetylation in zebrafish testes. Environmental Pollution. 2019;**248**:368-379. DOI: 10.1016/j.envpol.2019.01.127

[25] Xie F, Chen X, Weng S, Xia T, Sun X, Luo T, et al. Effects of two environmental endocrine disruptors di-n-butyl phthalate (DBP) and mono-n-butyl phthalate (MBP) on human sperm functions in vitro. Reproductive Toxicology. 2019;**83**:1-7. DOI: 10.1016/j.reprotox.2018.10.011

[26] Rahman MS, Kwon WS, Karmakar PC, Yoon SJ, Ryu BY, Pang MG. Gestational exposure to bisphenol A affects the function and proteome profile of F1 spermatozoa in adult mice. Environmental Health Perspectives. 2017;**125**:238-245. DOI: 10.1289/EHP378

[27] Rahman MS, Kwon W-S, Lee J-S, Yoon S-J, Ryu B-Y, Pang M-G. Bisphenol-a affects male fertility via fertility-related proteins in spermatozoa. Scientific Reports. 2015;**5**:9169. DOI: 10.1038/srep09169

[28] Žalmanová T, Hošková K, Nevoral J, Prokešová Š, Zámostná K, Kott T, et al. Bisphenol S instead of bisphenol A: A story of reproductive disruption by regretable substitution–A review. Czech

Journal of Animal Science. 2016;**61**. DOI: 10.17221/81/2015-CJAS

[29] Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee D-H, et al. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocrine Reviews. 2012;**33**:378-455. DOI: 10.1210/er.2011-1050

[30] Scherer LD, Maynard A, Dolinoy DC, Fagerlin A, Zikmund-Fisher BJ. The psychology of "regrettable substitutions": Examining consumer judgements of bisphenol A and its alternatives. Health, Risk & Society. 2014;**16**:649-666. DOI: 10.1080/13698575.2014.969687

[31] Nardi J, Moras PB, Koeppe C, Dallegrave E, Leal MB, Rossato-Grando LG. Prepubertal subchronic exposure to soy milk and glyphosate leads to endocrine disruption. Food and Chemical Toxicology. 2017;**100**:247-252. DOI: 10.1016/j.fct.2016.12.030

[32] Fang Y, Ni C, Dong Y, Li H, Wu S, Li X, et al. In utero exposure to atrazine disrupts rat fetal testis development. Frontiers in Pharmacology. 2018;**9**:1391. DOI: 10.3389/fphar.2018.01391

[33] Mesnage R, Biserni M, Genkova D, Wesolowski L, Antoniou MN. Evaluation of neonicotinoid insecticides for oestrogenic, thyroidogenic and adipogenic activity reveals imidacloprid causes lipid accumulation. Journal of Applied Toxicology. 2018;**38**:1483-1491. DOI: 10.1002/jat.3651

[34] Lagarde F, Beausoleil C, Belcher SM, Belzunces LP, Emond C, Guerbet M, et al. Non-monotonic dose-response relationships and endocrine disruptors: A qualitative method of assessment. Environmental Health. 2015;**14**:13. DOI: 10.1186/1476-069X-14-13

[35] Molina A, Abril N, Morales-Prieto N, Monterde J, Ayala N, Lora A, et al. Hypothalamic-pituitary-ovarian axis perturbation in the basis of bisphenol A (BPA) reproductive toxicity in female zebrafish (*Danio rerio*). Ecotoxicology and Environmental Safety. 2018;**156**:116-124. DOI: 10.1016/j.ecoenv.2018.03.029

[36] Oudir M, Chader H, Bouzid B, Bendisari K, Latreche B, Boudalia S, et al. Male rat exposure to low dose of di(2-ethylhexyl) phthalate during pre-pubertal, pubertal and post-pubertal periods: Impact on sperm count, gonad histology and testosterone secretion. Reproductive Toxicology. 2018;**75**:33-39. DOI: 10.1016/j.reprotox.2017.11.004

[37] Ramos C, Ladeira C, Zeferino S, Dias A, Faria I, Cristovam E, et al. Cytotoxic and genotoxic effects of environmental relevant concentrations of bisphenol A and interactions with doxorubicin. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2019;**838**:28-36. DOI: 10.1016/j.mrgentox.2018.11.009

[38] Lymperi S, Giwercman A. Endocrine disruptors and testicular function. Metabolism. 2018;**86**:79-90. DOI: 10.1016/j.metabol.2018.03.022

[39] Eladak S, Grisin T, Moison D, Guerquin M-J, N'Tumba-Byn T, Pozzi-Gaudin S, et al. A new chapter in the bisphenol A story: Bisphenol S and bisphenol F are not safe alternatives to this compound. Fertility and Sterility. 2015;**103**:11-21. DOI: 10.1016/j.fertnstert.2014.11.005

[40] Sweeney MF, Hasan N, Soto AM, Sonnenschein C. Environmental endocrine disruptors: Effects on the human male reproductive system. Reviews in Endocrine & Metabolic Disorders. 2015;**16**:341-357. DOI: 10.1007/s11154-016-9337-4

[41] Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, Bongain A, et al. Cryptorchidism at birth in Nice

area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. Human Reproduction. 2008;**23**:1708-1718. DOI: 10.1093/humrep/den186

[42] Toppari J, Virtanen HE, Main KM, Skakkebaek NE. Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): Environmental connection. Birth Defects Research. Part A, Clinical and Molecular Teratology. 2010;**88**:910-919. DOI: 10.1002/bdra.20707

[43] Hardell L, van Bavel B, Lindström G, Carlberg M, Dreifaldt AC, Wijkström H, et al. Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. Environmental Health Perspectives. 2003;**111**:930-934. DOI: 10.1289/ehp.5816

[44] Taylor JA, Richter CA, Ruhlen RL, vom Saal FS. Estrogenic environmental chemicals and drugs: Mechanisms for effects on the developing male urogenital system. The Journal of Steroid Biochemistry and Molecular Biology. 2011;**127**:83-95. DOI: 10.1016/j.jsbmb.2011.07.005

[45] Huang D, Wu J, Su X, Yan H, Sun Z. Effects of low dose of bisphenol A on the proliferation and mechanism of primary cultured prostate epithelial cells in rodents. Oncology Letters. 2017;**14**:2635-2642. DOI: 10.3892/ol.2017.6469

[46] Le Moal J, Rolland M, Goria S, Wagner V, De Crouy-Chanel P, Rigou A, et al. Semen quality trends in French regions are consistent with a global change in environmental exposure. Reproduction. 2014;**147**:567-574. DOI: 10.1530/REP-13-0499

[47] Bretveld R, Brouwers M, Ebisch I, Roeleveld N. Influence of pesticides on male fertility. Scandinavian Journal of Work, Environment & Health. 2007;**33**:13-28

[48] Cheng CY, Mruk DD. The blood-testis barrier and its implications for male contraception. Pharmacological Reviews. 2012;**64**:16-64. DOI: 10.1124/pr.110.002790

[49] Li MWM, Mruk DD, Lee WM, Cheng CY. Disruption of the blood-testis barrier integrity by bisphenol A in vitro: Is this a suitable model for studying blood-testis barrier dynamics? The International Journal of Biochemistry & Cell Biology. 2009;**41**:2302-2314. DOI: 10.1016/j.biocel.2009.05.016

[50] Harada T, Takeda M, Kojima S, Tomiyama N. Toxicity and carcinogenicity of dichlorodiphenyltrichloroethane (DDT). Toxicology Research. 2016;**32**:21-33. DOI: 10.5487/TR.2016.32.1.021

[51] Buha A, Antonijević B, Milovanović V, Janković S, Bulat Z, Matović V. Polychlorinated biphenyls as oxidative stress inducers in liver of subacutely exposed rats: Implication for dose-dependence toxicity and benchmark dose concept. Environmental Research. 2015;**136**:309-317. DOI: 10.1016/j.envres.2014.11.005

[52] Angelé-Martínez C, Goodman C, Brumaghim J. Metal-mediated DNA damage and cell death: Mechanisms, detection methods, and cellular consequences. Metallomics. 2014;**6**:1358-1381. DOI: 10.1039/c4mt00057a

[53] Kašuba V, Milić M, Rozgaj R, Kopjar N, Mladinić M, Žunec S, et al. Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line. Environmental Science and Pollution Research. 2017;**24**:19267-19281. DOI: 10.1007/s11356-017-9438-y

[54] Møller P, Wils RS, Jensen DM, Andersen MHG, Roursgaard M. Telomere dynamics and cellular senescence: An emerging field in environmental and occupational toxicology. Critical Reviews in Toxicology. 2018;**48**:761-788. DOI: 10.1080/10408444.2018.1538201

[55] Goyal HO, Braden TD, Williams CS, Williams JW. Estrogen-induced developmental disorders of the rat penis involve both Estrogen receptor (ESR)- and androgen receptor (AR)-mediated Pathways1. Biology of Reproduction. 2009;**81**:507-516. DOI: 10.1095/biolreprod.108.071951

[56] Cotter KA, Yershov A, Novillo A, Callard GV. Multiple structurally distinct ERα mRNA variants in zebrafish are differentially expressed by tissue type, stage of development and estrogen exposure. General and Comparative Endocrinology. 2013;**194**:217-229. DOI: 10.1016/j.ygcen.2013.09.014

[57] Sheng Z, Wang C, Ren F, Liu Y, Zhu B. Molecular mechanism of endocrine-disruptive effects induced by bisphenol A: The role of transmembrane G-protein estrogen receptor 1 and integrin αvβ3. Journal of Environmental Sciences. 2019;**75**:1-13. DOI: 10.1016/j.jes.2018.05.002

[58] Bellingham M, Fowler PA, Amezaga MR, Rhind SM, Cotinot C, Mandon-Pepin B, et al. Exposure to a complex cocktail of environmental endocrine-disrupting compounds disturbs the Kisspeptin/GPR54 system in ovine hypothalamus and pituitary gland. Environmental Health Perspectives. 2009;**117**:1556-1562. DOI: 10.1289/ehp.0900699

[59] Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. Exposure to bisphenol A advances puberty. Nature. 1999;**401**:763-764. DOI: 10.1038/44517

[60] Pollock T, Greville LJ, Tang B, deCatanzaro D. Triclosan elevates estradiol levels in serum and tissues of cycling and peri-implantation female mice. Reproductive Toxicology. 2016;**65**:394-401. DOI: 10.1016/j.reprotox.2016.09.004

[61] Pollock T, Weaver RE, Ghasemi R, deCatanzaro D. Butyl paraben and propyl paraben modulate bisphenol A and estradiol concentrations in female and male mice. Toxicology and Applied Pharmacology. 2017;**325**:18-24. DOI: 10.1016/j.taap.2017.04.001

[62] Karmakar PC, Kang H-G, Kim Y-H, Jung S-E, Rahman MS, Lee H-S, et al. Bisphenol A affects on the functional properties and proteome of testicular germ cells and spermatogonial stem cells in vitro culture model. Scientific Reports. 2017;**7**:11858. DOI: 10.1038/s41598-017-12195-9

[63] Shi X-Y, Wang Z, Liu L, Feng L-M, Li N, Liu S, et al. Low concentrations of bisphenol A promote human ovarian cancer cell proliferation and glycolysis-based metabolism through the estrogen receptor-α pathway. Chemosphere. 2017;**185**:361-367. DOI: 10.1016/j.chemosphere.2017.07.027

[64] Wang H, Ding Z, Shi Q-M, Ge X, Wang H-X, Li M-X, et al. Anti-androgenic mechanisms of Bisphenol A involve androgen receptor signaling pathway. Toxicology. 2017;**387**:10-16. DOI: 10.1016/j.tox.2017.06.007

[65] Ge L-C, Chen Z-J, Liu H-Y, Zhang K-S, Liu H, Huang H-B, et al. Involvement of activating ERK1/2 through G protein coupled receptor 30 and estrogen receptor α/β in low doses of bisphenol A promoting growth of Sertoli TM4 cells. Toxicology Letters. 2014;**226**:81-89. DOI: 10.1016/j.toxlet.2014.01.035

[66] Fitzgerald AC, Peyton C, Dong J, Thomas P. Bisphenol A and related

alkylphenols exert nongenomic estrogenic actions through a G protein-coupled Estrogen receptor 1 (Gper)/epidermal growth factor receptor (Egfr) pathway to inhibit meiotic maturation of Zebrafish Oocytes1. Biology of Reproduction. 2015;**93**:135. DOI: 10.1095/biolreprod.115.132316

[67] Cao L-Y, Ren X-M, Li C-H, Zhang J, Qin W-P, Yang Y, et al. Bisphenol AF and bisphenol B exert higher estrogenic effects than bisphenol A via G protein-coupled estrogen receptor pathway. Environmental Science & Technology. 2017;**51**:11423-11430. DOI: 10.1021/acs.est.7b03336.

[68] Žalmanová T, Hošková K, Nevoral J, Adámková K, Kott T, Šulc M, et al. Bisphenol S negatively affects the meotic maturation of pig oocytes. Scientific Reports. 2017;**7**:485. DOI: 10.1038/s41598-017-00570-5

[69] Holubcová Z, Blayney M, Elder K, Schuh M. Human oocytes. Error-prone chromosome-mediated spindle assembly favors chromosome segregation defects in human oocytes. Science. 2015;**348**:1143-1147. DOI: 10.1126/science.aaa9529

[70] Campen KA, Kucharczyk KM, Bogin B, Ehrlich JM, Combelles CMH. Spindle abnormalities and chromosome misalignment in bovine oocytes after exposure to low doses of bisphenol A or bisphenol S. Human Reproduction. 2018;**33**:895-904. DOI: 10.1093/humrep/dey050

[71] Nevoral J, Kolinko Y, Moravec J, Žalmanová T, Hošková K, Prokešová Š, et al. Long-term exposure to very low doses of bisphenol S affects female reproduction. Reproduction. 2018;**156**:47-57. DOI: 10.1530/REP-18-0092

[72] Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, et al. Bisphenol A exposure causes meiotic aneuploidy in the female mouse. Current Biology. 2003;**13**:546-553

[73] Mandrioli D, Belpoggi F, Silbergeld EK, Perry MJ. Aneuploidy: A common and early evidence-based biomarker for carcinogens and reproductive toxicants. Environmental Health. 2016;**15**:97. DOI: 10.1186/s12940-016-0180-6

[74] Rahman MS, Kwon W-S, Ryu D-Y, Khatun A, Karmakar PC, Ryu B-Y, et al. Functional and proteomic alterations of F1 capacitated spermatozoa of adult mice following gestational exposure to bisphenol A. Journal of Proteome Research. 2018;**17**:524-535. DOI: 10.1021/acs.jproteome.7b00668

[75] Ji H, Miao M, Liang H, Shi H, Ruan D, Li Y, et al. Exposure of environmental Bisphenol A in relation to routine sperm parameters and sperm movement characteristics among fertile men. Scientific Reports. 2018;**8**:17548. DOI: 10.1038/s41598-018-35787-5

[76] You Y-A, Mohamed EA, Rahman MS, Kwon W-S, Song W-H, Ryu B-Y, et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin can alter the sex ratio of embryos with decreased viability of Y spermatozoa in mice. Reproductive Toxicology. 2018;**77**:130-136. DOI: 10.1016/j.reprotox.2018.02.011

[77] Song W-H, Mohamed EA, Pang W-K, Kang K-H, Ryu D-Y, Rahman MS, et al. Effect of endocrine disruptors on the ratio of X and Y chromosome-bearing live spermatozoa. Reproductive Toxicology. 2018;**82**:10-17. DOI: 10.1016/j.reprotox.2018.09.002

[78] Darbre PD. Endocrine disruptors and obesity. Current Obesity Reports. 2017;**6**:18-27. DOI: 10.1007/s13679-017-0240-4

[79] Song Y, Chou EL, Baecker A, You N-CY, Song Y, Sun Q, et al. Endocrine-disrupting chemicals, risk of type 2 diabetes, and diabetes-related

metabolic traits: A systematic review and meta-analysis. Journal of Diabetes. 2016;**8**:516-532. DOI: 10.1111/1753-0407.12325

[80] Rutkowska AZ, Diamanti-Kandarakis E. Polycystic ovary syndrome and environmental toxins. Fertility and Sterility. 2016;**106**:948-958. DOI: 10.1016/j.fertnstert.2016.08.031

[81] Pietryk EW, Clement K, Elnagheeb M, Kuster R, Kilpatrick K, Love MI, et al. Intergenerational response to the endocrine disruptor vinclozolin is influenced by maternal genotype and crossing scheme. Reproductive Toxicology. 2018;**78**:9-19. DOI: 10.1016/j.reprotox.2018.03.005

[82] Wang T, Han J, Duan X, Xiong B, Cui X-S, Kim N-H, et al. The toxic effects and possible mechanisms of Bisphenol A on oocyte maturation of porcine in vitro. Oncotarget. 2016;**7**:32554-32565. DOI: 10.18632/oncotarget.8689

[83] Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol A exposure disrupts genomic imprinting in the mouse. PLoS Genetics. 2013;**9**:e1003401. DOI: 10.1371/journal.pgen.1003401

[84] Lu Z, Ma Y, Gao L, Li Y, Li Q, Qiang M. Urine mercury levels correlate with DNA methylation of imprinting gene H19 in the sperm of reproductive-aged men. PLoS One. 2018;**13**:e0196314. DOI: 10.1371/journal.pone.0196314

[85] Doshi T, D'souza C, Vanage G. Aberrant DNA methylation at Igf2–H19 imprinting control region in spermatozoa upon neonatal exposure to bisphenol A and its association with post implantation loss. Molecular Biology Reports. 2013;**40**:4747-4757. DOI: 10.1007/s11033-013-2571-x

[86] Zhang X-F, Zhang L-J, Feng Y-N, Chen B, Feng Y-M, Liang G-J, et al. Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Molecular Biology Reports. 2012;**39**:8621-8628. DOI: 10.1007/s11033-012-1716-7

[87] Reza AMMT, Choi Y-J, Han SG, Song H, Park C, Hong K, et al. Roles of microRNAs in mammalian reproduction: From the commitment of germ cells to peri-implantation embryos. Biological Reviews of the Cambridge Philosophical Society. 2019;**94**:415-438. DOI: 10.1111/brv.12459

[88] Rodgers AB, Morgan CP, Leu NA, Bale TL. Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. Proceedings of the National Academy of Sciences. 2015;**112**:13699-13704. DOI: 10.1073/pnas.1508347112

[89] Brieño-Enríquez MA, García-López J, Cárdenas DB, Guibert S, Cleroux E, Děd L, et al. Exposure to endocrine disruptor induces Transgenerational epigenetic deregulation of MicroRNAs in primordial germ cells. PLoS One. 2015;**10**:e0124296. DOI: 10.1371/journal.pone.0124296

[90] Horan TS, Marre A, Hassold T, Lawson C, Hunt PA. Germline and reproductive tract effects intensify in male mice with successive generations of estrogenic exposure. PLoS Genetics. 2017;**13**:e1006885. DOI: 10.1371/journal.pgen.1006885

[91] Ben Maamar M, Sadler-Riggleman I, Beck D, Skinner MK. Epigenetic Transgenerational inheritance of altered sperm histone retention sites. Scientific Reports. 2018;**8**. DOI: 10.1038/s41598-018-23612-y

[92] Skinner MK, Ben Maamar M, Sadler-Riggleman I, Beck D, Nilsson E, McBirney M, et al. Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational

inheritance of disease. Epigenetics & Chromatin. 2018;**11**:8. DOI: 10.1186/s13072-018-0178-0

[93] Kalb AC, Kalb AL, Cardoso TF, Fernandes CG, Corcini CD, Junior ASV, et al. Maternal transfer of bisphenol A during nursing causes sperm impairment in male offspring. Archives of Environmental Contamination and Toxicology. 2016;**70**:793-801. DOI: 10.1007/s00244-015-0199-7

[94] LaPlante CD, Catanese MC, Bansal R, Vandenberg LN. Bisphenol S alters the lactating mammary gland and nursing Behaviors in mice exposed during pregnancy and lactation. Endocrinology. 2017;**158**:3448-3461. DOI: 10.1210/en.2017-00437

[95] Pollock T, Greville LJ, Weaver RE, Radenovic M, deCatanzaro D. Bisphenol S modulates concentrations of bisphenol A and oestradiol in female and male mice. Xenobiotica. 2019;**49**:540-548. DOI: 10.1080/00498254.2018.1480818

[96] Pollock T, Mantella L, Reali V, deCatanzaro D. Influence of Tetrabromobisphenol A, with or without concurrent triclosan, upon bisphenol A and estradiol concentrations in mice. Environmental Health Perspectives. 2017;**125**:087014. DOI: 10.1289/EHP1329

[97] Pollock T, Tang B, deCatanzaro D. Triclosan exacerbates the presence of 14C-bisphenol A in tissues of female and male mice. Toxicology and Applied Pharmacology. 2014;**278**:116-123. DOI: 10.1016/j.taap.2014.04.017

[98] Pollock T, Weaver RE, Ghasemi R, deCatanzaro D. A mixture of five endocrine-disrupting chemicals modulates concentrations of bisphenol A and estradiol in mice. Chemosphere. 2018;**193**:321-328. DOI: 10.1016/j.chemosphere.2017.11.030

[99] Hawthorne DJ, Dively GP. Killing them with kindness? In-hive medications may inhibit xenobiotic efflux transporters and endanger honey bees. PLoS One. 2011;**6**:e26796. DOI: 10.1371/journal.pone.0026796

[100] Johnson RM, Pollock HS, Berenbaum MR. Synergistic interactions between in-hive miticides in Apis mellifera. Journal of Economic Entomology. 2009;**102**:474-479. DOI: 10.1603/029.102.0202

[101] vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, et al. Colony collapse disorder: A descriptive study. PLoS One. 2009;**4**:e6481. DOI: 10.1371/journal.pone.0006481

[102] Lee G-A, Choi K-C, Hwang K-A. Treatment with phytoestrogens reversed triclosan and bisphenol A-induced anti-apoptosis in breast cancer cells. Biomolecules & Therapeutics (Seoul). 2018;**26**:503-511. DOI: 10.4062/biomolther.2017.160

[103] Trapphoff T, Heiligentag M, El Hajj N, Haaf T, Eichenlaub-Ritter U. Chronic exposure to a low concentration of bisphenol A during follicle culture affects the epigenetic status of germinal vesicles and metaphase II oocytes. Fertility and Sterility. 2013;**100**:1758-1767.e1. DOI: 10.1016/j.fertnstert.2013.08.021

[104] Skinner MK. Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. Molecular and Cellular Endocrinology. 2014;**398**:4-12. DOI: 10.1016/j.mce.2014.07.019

[105] Brehm E, Flaws JA. Transgenerational effects of endocrine disrupting chemicals on male and female reproduction. Endocrinology. 2019. DOI: 10.1210/en.2019-00034

[106] Latchney SE, Fields AM, Susiarjo M. Linking inter-individual variability

to endocrine disruptors: Insights for epigenetic inheritance. Mammalian Genome. 2018;**29**(1-2):141-152. DOI: 10.1007/s00335-017-9729-0

[107] Wei Y, Schatten H, Sun Q-Y. Environmental epigenetic inheritance through gametes and implications for human reproduction. Human Reproduction Update. 2015;**21**:194-208. DOI: 10.1093/humupd/dmu061

[108] Stern S, Snir O, Mizrachi E, Galili M, Zaltsman I, Soen Y. Reduction in maternal *Polycomb* levels contributes to transgenerational inheritance of a response to toxic stress in flies. The Journal of Physiology. 2014;**592**:2343-2355. DOI: 10.1113/jphysiol.2014.271445

[109] Cleary JA, Tillitt DE, vom Saal FS, Nicks DK, Claunch RA, Bhandari RK. Atrazine induced transgenerational reproductive effects in medaka (*Oryzias latipes*). Environmental Pollution. 2019;**251**:639-650. DOI: 10.1016/j.envpol.2019.05.013

[110] Chow Y-L, Sato F. Transgenerational lipid-reducing activity of benzylisoquinoline alkaloids in *Caenorhabditis elegans*. Genes to Cells. 2019;**24**:70-81. DOI: 10.1111/gtc.12657

[111] Wolstenholme JT, Drobná Z, Henriksen AD, Goldsby JA, Stevenson R, Irvin JW, et al. Transgenerational bisphenol A causes deficits in social recognition and alters post-synaptic density genes in mice. Endocrinology. 2019. DOI: 10.1210/en.2019-00196

[112] King SE, McBirney M, Beck D, Sadler-Riggleman I, Nilsson E, Skinner MK. Sperm epimutation biomarkers of obesity and pathologies following DDT induced epigenetic transgenerational inheritance of disease. Environmental Epigenetics. 2019;**5**:dvz008. DOI: 10.1093/eep/dvz008

[113] Baker BB, Yee JS, Meyer DN, Yang D, Baker TR. Histological and Transcriptomic changes in male Zebrafish testes due to early life exposure to low level 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. Zebrafish. 2016;**13**:413-423. DOI: 10.1089/zeb.2016.1275

[114] Pocar P, Fiandanese N, Berrini A, Secchi C, Borromeo V. Maternal exposure to di(2-ethylhexyl)phthalate (DEHP) promotes the transgenerational inheritance of adult-onset reproductive dysfunctions through the female germline in mice. Toxicology and Applied Pharmacology. 2017;**322**:113-121. DOI: 10.1016/j.taap.2017.03.008

[115] Kubsad D, Nilsson EE, King SE, Sadler-Riggleman I, Beck D, Skinner MK. Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: generational toxicology. Scientific Reports. 2019;**9**:6372. DOI: 10.1038/s41598-019-42860-0

[116] Manikkam M, Haque MM, Guerrero-Bosagna C, Nilsson EE, Skinner MK. Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult-onset disease through the female germline. PLoS One. 2014;**9**:e102091. DOI: 10.1371/journal.pone.0102091

[117] Skinner MK, Nilsson E, Sadler-Riggleman I, Beck D, Ben Maamar M, McCarrey JR. Transgenerational sperm DNA methylation epimutation developmental origins following ancestral vinclozolin exposure. Epigenetics. 2019;**14**:721-739. DOI: 10.1080/15592294.2019.1614417