Journal of Steroid Biochemistry & Molecular Biology 155 (2016) 217–223

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Steroids and endocrine disruptors—History, recent state of art and open questions

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ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 14 April 2014
Accepted 20 April 2014
Available online 9 May 2014

Keywords:
Steroids
Endocrine disruptors
Sites of action
Overreview

ABSTRACT

This introductory chapter provides an overview of the levels and sites at which endocrine disruptors (EDs) affect steroid actions. In contrast to the special issue of Journal of Steroid Biochemistry and Molecular Biology published three years ago and devoted to EDs as such, this paper focuses on steroids. We tried to point to more recent findings and opened questions.

EDs interfere with steroid biosynthesis and metabolism either as inhibitors of relevant enzymes, or at the level of their expression. Particular attention was paid to enzymes metabolizing steroid hormones to biologically active products in target cells, such as aromatase, 5α-reductase and 3β-, 11β- and 17β-hydroxysteroid dehydrogenases. An important target for EDs is also steroid acute regulatory protein (StAR), responsible for steroid precursor trafficking to mitochondria.

EDs influence receptor-mediated steroid actions at both genomic and non-genomic levels. The remarkable differences in response to various steroid-receptor ligands led to a more detailed investigation of events following steroid/disruptor binding to the receptors and to the mapping of the signaling cascades and nuclear factors involved. A virtual screening of a large array of EDs with steroid receptors, known as in silico methods (=computer simulation), is another promising approach for studying quantitative structure activity relationships and docking.

New data may be expected on the effect of EDs on steroid hormone binding to selective plasma transport proteins, namely transcortin and sex hormone-binding globulin.

Little information is available so far on the effects of EDs on the major hypothalamo–pituitary–adrenal/gonadal axes, of which the kisspeptin/GPR54 system is of particular importance. Kisspeptins act as stimulators for hormone-induced gonadotropin secretion and their expression is regulated by sex steroids via a feed-back mechanism. Kisspeptin is now believed to be one of the key factors triggering puberty in mammals, and various EDs affect its expression and function.

Finally, advances in analytics of EDs, especially those persisting in the environment, in various body fluids (plasma, urine, seminal fluid, and follicular fluid) are mentioned. Surprisingly, relatively scarce information is available on the simultaneous determination of EDs and steroids in the same biological material.

This article is part of a Special Issue entitled 'Endocrine disruptors & steroids'.

Contents

Abbreviations: BPA, bisphenol A; DDE, 2,2′-bis-(4-chlorophenyl)-1,1′-dichloroethene; DDT, dichlorodiphenyltrichloroethane; FSH, folitropin; LH, lutropin; PCB(s), polychlorinated biphenyl(s); PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; SHBG, sex hormone-binding globulin.

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http://dx.doi.org/10.1016/j.jsbmb.2014.04.013
0960-0760/

1. Introduction

1.1. History

The term endocrine disruptors (EDs) as substances which can interfere with the endocrine or hormone system in mammals dates back to the early nineties [1], but it was well known much earlier that many chemicals present in the environment may affect, mostly adversely, human and animal life and health. This includes many civilization diseases across the life cycle, such as cancer, genetic modification, metabolic diseases, the malfunction of various organs and, last but not least, reproduction. Concerning their effect on reproduction and, in broader terms the endocrine system, it is not surprising that a great part of this matter deals with steroids. This has been the subject of numerous reviews and monographs, such as that of Gore [2]. Some of these topics were addressed and discussed in this journal two years ago [3].

1.2. Classification

EDs may be divided into natural compounds such as soy phytoestrogens, extracts and formulas from various plants or fungi and a broad spectrum of human and industrial products. The latter comprise chemicals used in fighting undesired wildlife and agricultural threats (pesticides, fungicides, insecticides, and rodenticides) or various synthetic compounds as substances used in the production of plastics and plasticizers, packaging materials, including also non-intentionally added substances (bisphenol(s), phthalates) or a broad spectrum of industrial chemicals used as building materials, paints, isolation materials (PCBs, metals). Endocrine disruptors also include many drugs derived from natural hormones, particularly all contraceptives. Given their duration in the environment, EDs are usually divided into persistent and short-life compounds. The use of many of these chemicals (see e.g. DDT) was banned after their effects on wild life were discovered, but they still persist in the environment.

1.3. Effects

Generally, EDs may intervene in the hormonal function at various sites: they can directly affect hormone biosynthesis, the metabolism, transport, and mechanism of action on both receptor and post-receptor levels. They may act at a genome level by influencing gene expression and even via epigenetic mechanisms, including effects on genomic imprinting. Many EDs are known to affect fetal (prenatal) development. Other characteristic features of some EDs are their transgenerational effects and their often non-linear or non-monotonic dose–response curves. Some EDs act in an additive way with natural hormones and act complexly upon multiple targets. Finally, EDs can interfere with feed-back mechanisms typical for the endocrine system.

In the following text we will not repeat known facts about EDs, but focus on their effect on steroids, namely their biosynthesis, metabolism, mechanism of action, their co-existence with steroids in body fluids as potential biomarkers, and also discuss their participation in feed-back mechanisms in an attempt to point to open questions.

2. EDs and steroid biosynthesis and metabolism

EDs may influence steroid biosynthesis and metabolism either as inhibitors or rarely as activators of key enzymes, or on the level of the respective enzyme expression. Many excellent reviews have appeared since the beginning of this century demonstrating *in vitro* as well as in vivo inhibitory effects of an array of EDs, covering most of their classes – pesticides, plasticizers, dioxins, PCBs and polycyclic aromatic hydrocarbons, and their impact on ovarian or testicular functions [4–9]. The reviews covered all steroidogenic enzyme issues, most of which belong to the cytochrome P450 family [4,5,7]. Some of them dealt preferably with gonadal, ovarian [7,8] or Leydig cell [6,9] steroid biosynthesis.

Particular attention was devoted to aromatase activity [8], not only in humans or rodents, but also in fish regarding the strong impact of EDs pollutants in water on fish reproduction [10]. In rodents, it was shown that bisphenol A from plasticizers may even increase aromatase activity in rat prostate. At the same time, this chemical affects the expression of another important steroid metabolizing enzyme – 5α-reductase – existing in form of three isoenzymes (5α-R1, 5α-R2 and 5α-R3). While the expression of the first two isoenzymes is inhibited by bisphenol A, the third, 5α-R3, known as a biomarker for malignancy, is increased. It is an example of the synergic effect of the disruptor at various sites, each leading to an increased risk of cancer [11]. 5α-Reductase isoenzymes as targets for EDs are important enzymes not only due to metabolizing testosterone and its precursor to peripherally active androgens [12], but also in biosynthesis of 5α-saturated C21 neuroactive steroids as allopregnanolone [13]. So far little is known about EDs action on this enzyme in brain.

3β-Hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) are key enzymes involved in androgen biosynthesis in Leydig cells. Various phthalates were tested as potential inhibitors of these enzymes in human and rat testicular preparations. Their inhibitory activities differed according to the length of carbon chains in the ethanol moieties [14]. Both enzymes were also inhibited by perfluorinated chemicals [15]. For a review of the effect of a broad spectrum of EDs covering industrial materials (perfluoroalkyl compounds, phthalates, bisphenol A and benzophenone) and pesticides/biocides (methoxychlor, organotins, 1,2-dibromo-3-chloropropane and prochloraz) and plant constituents (genistein and gossypol) see e.g. [9].

17β-HSD and 11β-hydroxysteroid dehydrogenase (11β-HSD) are also enzymes regulating the actual concentration of biologically active hormonal steroids in peripheral tissues. While 17β-HSD acts on sex steroids (testosterone and estradiol and its 17-oxo precursors), 11β-HSD isoenzymes convert 11-oxo corticoids into their hormonally active 11β-hydroxy-derivatives and vice versa. While the first type of 11β-HSD acting as reductase is ubiquitous, the especial role of Type 2 11β-HSD acting as oxidase, protects kidney and also testicular Leydig cells from an excess of glucocorti-

coid. Both hydroxysteroids dehydrogenases are targets for various endocrine disrupting chemicals, which may affect their activity as well as expression [16–18].

Another target for EDs are isoenzymes responsible for the sulfation of estrogen, androgens and their precursors in biosynthetic pathway. Sulfation plays an important role both in detoxification and in the control of steroid activity and, moreover, in the case of some so-called neurosteroids as e.g. dehydroepiandrosterone, it changes its activity the opposite way. Sulfotransferases were inhibited by phthalates, chlorinated phenols and also by some phytoestrogens [19–21].

An important step in the steroid hormone biosynthesis is the transport of cholesterol substrate across the inner mitochondrial membrane, mediated by Steroidogenic Active Regulatory protein (StAR) identified ten years ago [22]. StAR is present in mitochondria of typical steroidogenic cells as in adrenocortex and in gonads. Its gene belongs to those, the expression of which may also be inhibited by various EDs [23–25]. Besides "typical" EDs its expression at least in fish was also inhibited by heavy metal, namely cadmium [26].

The selection of the reviews and original papers should reflect the large publication activity in this field and is far from complete: for instance, as this chapter was being prepared there were as many as 150 references alone under the key words EDs and estrogen biosynthesis and review.

2.1. EDs and receptor-mediated steroid action

Steroid hormones exert their actions in the target cell through intracellular or membrane receptors. The classical genomic mechanism employing intracellular receptors consists of regulation – stimulation or inhibition – of the expression of particular genes. The individual steps started by hormone binding to the intracellular receptor, followed by the translocation of the steroid–receptor complex to the nuclei, its binding to steroid regulated elements and transactivation making accessible the initiation sites for mRNA transferase(s) and the initiation of transcription, actually includes a number of protein–protein interactions with nuclear factors (often called co-activators or co-repressors), many of which have only recently been revealed [27,28]. EDs may interfere with all these steps, leading to incredible diversity and complexity of EDs action. The investigation of EDs interaction with these transcription factors opens an interesting new field in the mosaic of their actions.

Regulatory mechanisms mediated by membrane receptors are not so frequent and, in most instances reported to date, deal with reproductive functions mediated by estrogen, progestine and androgen receptors, but examples concerning other steroid action have been reported as well; for the review see e.g. [29–31]. These mechanisms involve the binding of the steroid to a membrane receptor coupled with a G-protein, the activation of an effector (usually adenylate cyclase) and the initiation of a signaling cascade (usually phosphorylation), resulting in a variety of activation/inhibition effects.

Of interest are newly discovered "mixed mechanisms", beginning with steroid binding to a membrane receptor and initiating a signaling cascade, the final step of which is the activation of a so-called cAMP activated regulatory protein (CREB), which binds to the gene-regulatory elements and thus promotes transcription as in the "classical" mechanism; for examples see [32,33]. EDs may interfere with such a mechanism, too [34].

The methods of investigating how EDs interfere with receptor-mediated steroid action underwent remarkable progress) over the past few decades. For a review of these approaches see e.g. Adler in the cited monograph [35]. The first experiments in the early nineties dealt with the binding of EDs to steroid receptors as ligands and their competition with native steroid hormones. Various sources of steroid receptors from animal or human tissues or cell cultures were used. The great deal of reports concerned estrogen receptors (ERs), since most of the effects of EDs consisted of their estrogenic/antiestrogenic properties. The experimental data were compared with observed in vivo effects. It was clear that the binding of EDs to the respective steroid receptors differs in their affinity and kinetics, and that the binding characteristics need not correspond to the final disrupting effect.

Another way of investigating EDs actions consisted of studying the effects of EDs on gene expression. Steroid hormones regulate thousands of genes and a great advance brought the methods enabling the screening of a large array of expressed genes after the isolation and multiplication of cDNA from various hormone-responsive tissue preparations. The more detailed follow-up of gene expression in time and dose–response studies enabled methods employing gene reporters (e.g. luciferase) co-transfected with hormone response elements (HRE) of selected steroid-responsive gene. It was enabled by advances in molecular biology in the eighties, crowned by the characterization of human and animal genome. The remarkable differences in response to various steroid-receptor ligands led to a more detailed investigation of the events that followed steroid/disruptor binding to the respective receptors and the mapping of the signaling cascades and individual components involved. As mentioned above, these are very current topics and an interesting finding may be expected.

The differences in the effects of disruptors on various targets and more detailed knowledge of steroid receptor structures led to computer modeling of suitable ligand structures. This approach known as in silico methods (≡computer simulation) [36,37], in fact a virtual screening, is an inexpensive tool for studying quantitative structure activity relationships and docking, which undoubtedly belongs to perspective approaches in investigating EDs actions.

3. EDs and steroid hormone binding in circulation

Reversible binding to plasma transport proteins considerably influences the availability of free, biologically active, steroid hormones. Of particular importance are corticosteroid-binding globulin (CBG, transcortin) and sex hormone binding-globulin (SHBG). The structure and genes coding for these proteins have been well characterized. The main source of both proteins is the liver, but their expression is not limited to this tissue. Though fulfilling similar function, there is no homology between them. For the review covering the main information about their properties and functions see e.g. [38–41]. The SHBG function is broader: it possesses its own receptors on the membrane of cells from some reproductive tissues and triggers a signaling cascade by way of cAMP [41]. The binding of various non-steroidal ligands to SHBG could considerably modulate steroid hormone action; SHBG and CBG thus represent possible targets for various endocrine disruptors [42]. Surprisingly, the only reports on the interaction of EDs with the SHBG analog concerned fish [43], but analogous interaction of EDs was described for the thyroid binding protein transthyretin [44]. The reports on the effects of EDs on steroid-binding transport proteins have been scarce to date and new data may be expected.

3.1. How EDs may influence hypothalamic–pituitary hormonal axes on central levels

Steroid hormones, in concert with other hormones, genes, neurotransmitters, growth and other factors play an important role in the development and function of the brain and neural system in general. Since embryonic life, any of these well coordinated and programmed sequence of events may be affected or even dis-

rupted by environmental agents. Especially vulnerable are prenatal or early postnatal periods and puberty. Changes in brain development, including the central nervous system, caused by exposure to toxic and endocrine-disrupting agents may have serious consequences for the entire life, especially for reproductive functions, behavior, motor activity and cognition; for the review see e.g. [45]. In the following text we will focus only on the mechanisms by which EDs influence the central–peripheral regulations of steroid actions through major hypothalamic–pituitary–peripheral axes.

Gonadal and adrenal steroid hormone biosynthesis and secretion is controlled by feed-back mechanisms known as hypothalamic–pituitary–adrenal- or gonadal axes (HPA, HPG). Steroids interact with hypothalamic as well as pituitary receptors of the trophic cells. Both genomic and non-genomic mechanisms may take part there. This results in the secretion or biosynthesis of hypothalamic liberines or pituitary hormones. Various EDs can compete as antagonists or agonists with natural steroid hormones for the receptors thanks to their structural similarity or, in a genomic way by affecting their expression in various neuronal or pituitary cells.

The mechanisms are the same as those that occur in other target cells for steroid hormones. Some recent examples of ways how EDs intervene with hypothalamic–pituitary regulations are shown here. The cited papers demonstrate on animal models how EDs affect further development of hypothalamic–pituitary regulation system in prenatal or neonatal period(s): even low doses of bisphenol A administration in early life altered sex-specific ER expression in rat hypothalamus and amygdala [46]. Besides ER, bisphenol A also disrupted hypothalamic gonadoliberin (LHRH) mRNA processing in hypothalamic nuclei, both leading to changes in estrous cyclicity in adult rats [47]. Similarly acted PCBs, which altered the expression of a set of genes including estrogen receptors alpha in rat hypothalamus, also resulting in a change in the trajectory of postnatal development [48].

Bisphenol A also disrupted non-genomic estrogen induced signaling via ERs coupled with G-protein [49].

Among the protein actors expressed in the hypothalamus and pituitary, of particular importance is kisspeptin/GPR54 system. Kisspeptins, encoded by the *KISS1* gene, act as stimulators for hormone-induced gonadotropin secretion and their expression is regulated by sex steroids via a feed-back mechanism. Kisspeptin is now believed to be one of the key factors triggering puberty in mammals [50]. It is not surprising that it is a potential target for EDs and first reports brought evidence that various EDs affect its expression and function; for examples see [51,52]. Organochlorine EDs as DDT and its metabolites may even increase gonadoliberin secretion through its effects on steroid and glutamate receptors [53].

Compared with the HPG axis, less attention was paid to the effect of EDs on the HPA axis, for the review see e.g. [54]. Interestingly, the effect of atrazine, a widely used herbicide on LH release was in the first line mediated by stimulation of HPA axis and the alteration of adrenal hormone secretion. Among other things, it demonstrates a close linkage between both hypothalamic–pituitary–adrenal- and gonadal axes [55].

The examples shown here, performed mostly on animal models, demonstrate the diverse actions of EDs on hypothalamic and pituitary levels; further studies, especially in humans, are needed to complete the mosaic of EDs actions on the autonomous nervous system.

3.2. Steroids and endocrine disruptors in body fluids

Since EDs influence steroid biosynthesis and its actions at various levels, it logically raises the question of how steroid levels correlate with actual concentration of EDs. Advanced analytical techniques [56–58] enable the assessment of a large array of EDs and their metabolites (some of which may be more active than parental compounds) in various body fluids. The latter concerns not only blood serum (plasma) or urine, but also follicular fluid, semen, maternal milk, saliva and even tissues obtained by biopsy. The EDs content in male semen (seminal plasma, fluid) is of particular interest with respect to the observed decline of sperm quality within the last decades [59,60].

Two ways of investigation may be traced: how steroid levels in the body associate with concentration of EDs and how exposure to EDs influences actual steroid levels. Both studies of the general population and special cohorts of humans exposed to EDs risks were reported.

In Table 1 we attempted to summarize chronologically major reports on EDs from the last decade and, if measured too, steroids, in serum, urine and also maternal milk [61–63] and follicular fluid [64]. Besides general population from various geographic regions and including also pregnant women, the studies focused on various common disorders associated with hormonal imbalance as ovarian dysfunction and PCOS [65–67]. The most frequent analytes were bisphenol A and PCBs. A consensus was reached on the positive association of BPA levels with biomarkers of PCOS including insulinoresistance [67] and hyperandrogenemia [65]. Surprisingly, as may be seen, there are relatively few reports on the simultaneous determination of EDs and hormonal steroids in the same biological material, above all in serum. In females it concerned pregnant women [68,69] and the already mentioned PCOS [66,67]. In men, two large studies provided evidence of the association of decreased testosterone levels with PCB and perfluorooctanesulfonic acid (PFOS) concentration, respectively [57,70]. More studies are needed to address the issue regarding the association of other EDs concentrations, especially persistent and steroid levels in the same material.

From the point of view of male reproduction, more attention was devoted to determining various EDs in seminal plasma and its association with the impairment of semen quality. This issue has been reviewed in the previously cited chapter of Hauser et al. in Gore's monograph [60]. Only a few reports, however, have dealt with the simultaneous determination of EDs and steroids in seminal plasma, though there was a clear association between actual steroid- and EDs concentration in this fluid. We have reviewed this matter recently [71].

4. Conclusive remarks and further perspectives

Three years ago a special issue of Journal of Steroid Biochemistry was devoted to endocrine disruptors as such, but many themes more or less dealt with steroids. To avoid overlapping, we tried in this issue to focus more on steroids and, at the same time, to point to news of this very vital topic.

As concerns steroid metabolizing enzymes, a promising topic is the effects of EDs on brain 5α-reductase with respect to its key role in metabolizing of progesterone to saturated metabolites, known to act as modulators of $GABA_A$ receptors. More information is needed about EDs effects on steroid transport to mitochondria and their influence on StAR protein.

Hand in hand in learning of steroid-triggered signaling cascades the effects of EDs on individual steps may be expected. Interesting would be the impact of EDs on pluripotent glucocorticoid actions in the light of their immunomodulatory, anti-inflammatory and apoptotic properties. The virtual screening of large sets of genes regulated by steroids and their affection by EDs could be a promising approach.

Very little is known about the effects of EDs on steroid binding to plasma proteins.

Table 1
Endocrine disruptors and steroids in body fluids.

Author, year	Ref.	Subjects	Analyzed material	EDs	Steroid(s) and hormones	Main findings
Takeuchi et al., 2004	[65]	47 normal and women with various ovarian dysfunctions	Serum (both hormones and EDs)	BPA	Testosterone, androstenedione	Strong relationship between serum BPA and androgen concentrations
Wang et al., 2006	[68]	50 pregnant women aged 25–34 years, 3rd trimester	Serum and placental tissue (both hormones and EDs)	17 dioxin congeners, 12 dioxin-like PCBs, and 6 indicator PCBs	4- and 2-hydroxylated E2 metabolites	The ratio of 4- to 2-hydroxylated estradiol decreased with increasing exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, while levels of 4-OH-estradiol increased with increasing concentrations of high-chlorinated dibenzofurans
Mocarelli et al., 2008	[72]	135 males (3 age groups) exposed in the past to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin	Serum (both hormones and EDs)	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	Estradiol, FSH	Exposure in either period leads to permanent reduction of estradiol and increased FSH
Brucker-Davis et al., 2008	[61]	Nursing mothers of 6246 boys screened for cryptorchidism (1.6% cryptorchic)	Colostrum, 125 samples (56 for cryptorchid and 69 for controls)	15 antiandrogenic and/or antiestrogenic EDs including DDE, PCBs and others		All maternal milk available was contaminated with EDs, insignificantly higher concentrations were found in milk from mother of cryptorchid boys
Goncharov et al., 2009	[57]	Native Americans (Mohawks), 257 adult men and 436 women	Serum (both hormones and EDs)	PCBs – 101 congeners	Testosterone	Elevation in serum PCB levels is associated with a lower concentration of serum testosterone in men only
Bushnik et al., 2010	[73]	5319 general population	Serum, urine	BPA, lead		Blood lead was found in 100% samples, urinary BPA in 91%
Galloway et al., 2010	[74]	715 adults between 20 and 74 years of age	Urinary excretion and serum (steroids only)	BPA	Testosterone, estradiol, SHBG	No associations with the serum hormone levels and urinary BPA excretion, with exception of association between BPA and SHBG concentrations in premenopausal women
Weldon et al., 2010	[75]	366 low-income, Mexican-American pregnant women	Serum	PCB congeners, DDT, DDE		Persistent organic pollutants were not associated with shortened lactation duration, but may be associated with longer lactation duration
Wan et al., 2010	[69]	26 pregnant women and 28 matching fetuses	Serum (both hormones and EDs)	10 polybrominatd diphenyl ethers plus BPA	Estradiol	Concentrations of 6-hydroxylated polybrominated diphenyl ethers in maternal and cord serum were positively correlated, being significantly greater in cord blood serum
Kandaraki et al., 2011	[66]	71 women with PCOS and 100 healthy women	Serum (both hormones and EDs)	BPA	Testosterone, androstenedione	Higher BPA levels in PCOS women than controls and a statistically significant positive association between androgens and BPA
Kadar et al., 2011	[62]	30 breast milk samples from French women	Breast milk	Perfluorinated compounds, PFOS, PFOA and other		Advanced analytics
Ye et al., 2012	[76]	936 children 3–11 years, general population	Serum, urine	BPA + 7 other phenols	Urine, not serum is the preferred matrix for EDs monitoring	
Gyllenhammar et al., 2012	[77]	100 young women, general population	Serum	Nonylphenol, BPA		Association with nutritional habits
Petro et al., 2012	[64]	40 women undergoing IVF	Folicullar fluid, serum	PCB 153 and other PCBs, p,p′DDE	Estradiol	Higher EDC contamination in the follicular fluid was associated with a decreased fertilization rate
Kim et al., 2012	[63]	Healthy women at delivery and their newborns, 21 pairs	Umbilical cord and maternal blood, breast milk	Polybrominated EDs		A strong correlation was found for studied EDs between breast milk and cord blood or maternal blood and cord blood samples
Meijer et al., 2012	[78]	53 pregnant women and their male newborns	Maternal serum at 35 weeks of pregnancy	8 neutral and 4 phenolic polychlorinated EDs		Organohalogen compounds correlated with markers of sexual development in boys up to 18 months of age
Joensen et al., 2012	[79]	881 healthy men, who provided serum, urine and semen samples	Serum (hormones), urine (EDs)	14 phthalate metabolites, including di(2-ethylhexyl)- and diisononyl-phthalate metabolites	Serum testosterone, estradiol, SHBG, LH, FSH, inhibin-B	Negative association of EDs with total and free testosterone and testosterone/estradiol ratio
Toft et al., 2012	[80]	588 partners of pregnant women who provided semen samples	Serum	4 perfluorinated chemicals		Negative associations between PFOS exposure and sperm morphology
Joensen et al., 2013	[70]	247 men, general population	Serum (both hormones and EDs)	Serfluorooctanesulfonate (PFOS)	Testosterone (total and free), LH, FSH	Negative association between serum PFOS and testosterone
Tarantino et al., 2013	[67]	40 women with PCOS and 20 healthy women	Serum (both hormones and EDs)	BPA	Testosterone, SHBG	Association of BPA with PCOS markers incl. insulinoresistance

Further studies are needed for a more complex understanding of the impact of EDs on major hypothalamic–pituitary–gonadal or adrenal axes and on the role of kisspeptin systems.

Finally, advanced analytical methods would enable the simultaneous assessment of EDs and a broad steroid spectrum in biological fluids and their association with various endocrine diseases. There is still a scarcity of data on the concentrations of EDs and steroids in the same body fluids. EDs and steroids could be measured not only in blood or urine, but also in seminal plasma, follicular fluid, cerebrospinal fluid and saliva, and new data may be expected.

Acknowledgment

The work was supported by the Grant no. 13369-4 from the Internal Grant Agency of the Czech Ministry of Health.

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