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Review Article

Effects of Endocrine Disrupting Chemicals on Spermatogenesis and Reproductive Hormones in Bony Fishes

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Abstract

Endocrine disrupting chemicals (EDCs) are naturally occurring or synthetic molecules present in an ecosystem that have the potential to alter the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body. They act by mimicking endogenous hormones, antagonizing normal hormones, altering the natural pattern of hormone synthesis or metabolism, or modifying hormone receptors. The chemicals, particularly disrupting the reproductive system, can be androgenic, anti-androgenic, estrogenic or anti-estrogenic. This review provides a brief overview on spermatogenesis, disruption of spermatogenesis and effects of various EDCs on hormones involved in spermatogenesis. EDCs affect spermatogenesis in two ways, by affecting the development and function of male gonads or by disrupting the synthesis and action of hormones involved in spermatogenesis, such as, GnRH, gonadotropin, dihydroandrogen, 11-ketotestosterone, estrogen, and progesterone.

Keywords: Endocrine Disrupting Chemicals; Spermatogenesis; Hormones; Gonadotropin; 11-Ketotestosterone

Introduction

The book 'Our Stolen Future' was published by Theo Colburn and colleagues (1996), and this book highlighted group of natural and synthetic chemicals to the forefront to which we are exposed in our day-to-day life directly or indirectly. These chemicals induce hormonal imbalances in the victim's body and are called as endocrine disrupting chemicals (EDCs) [1]. Endocrine disrupters are exogenous substances which interfere with the endocrine homeostasis, beginning from the synthesis of hormones to their binding with receptors, mode of action, and elimination in the body. These compounds mimic natural steroid hormones and are fat-soluble. They exert their effects on the endocrine system in several ways by imitating or antagonizing endogenous hormones altering the natural pattern of hormone synthesis or metabolism or modifying the expression of hormone receptors [2]. The EDCs drained to aquatic bodies affect the aquatic life and those chemicals upon entering the food chain, affect land animals, including humans. Among aquatic animals, fish are consumed worldwide. EDCs deposited in the fish body come into contact with humans by consuming the fish and exert the same effect as that of fish. In order to have sustainable aquaculture, we need to look into the reproduction of fishes. EDCs

affect the reproductive system by interfering with the production, transport, and binding of hormone to the receptor (Figure 1). Different groups of EDCs affect fish reproduction in different ways by altering plasma concentration of gonadotropins, development of gonads, gametogenesis, spawning, and survival of the larvae (Table 1).

Chemical group	Name of the EDC	Exposed fish	Concentration of EDC and duration of exposure	Effects	Reference
Organochlorine	p,p′-DDE	Juveniles of *Danio rerio*	0.01 to 20 µg/ Lfor 14 days and kept in control water for 4 months	Enhanced VTG production Reduced number of mature oocytes	[43]
Organophosphate	Dimethoate	Juvenile of *Cyprinuscarpio*	0.96 mg/L for 96h and 0.48 mg/L for 36 days	Necrotic spermatogonia and spermatocytes and clumping of spermatids and spermatozoa.	[44]
	Glyphosate	*Anabas*	2.6, 3.9 and 7.8ppm for 45 days	Decreased GSI, HSI, and spawning performance, vacuolation in tissue, dilution of sinusoid.	[45]
	Monocrotophos	Carassius auratus	0.01, 0.10 and 1.00 mg/ L for 21 days	Induction of VTG production, increased level of 17β-estradiol, decreased level of testosterone, increased LH β subunit mRNA expression, increased expression of aromatase gene.	[32]
		Anabas	3.5, 5.3 and 10.6mg for 45 days	Decreased GSI, Fertilization and hatching rates and vitellogenesis, ruptured follicular wall and oocyte atresia in female, ruptured seminiferous tubule in males	[46]
Pyrethroids	Deltamethrin	Clarias gariepinus	0.22, 0.44, 0.88 and 1.76 µg/L for 7, 14, 21 and 28 days	Increase of plasma E2 and decrease of testosterone. Development of ovotestis in male. Enlargement and degradation of testicular seminiferous tubules	[47]
	Cypermethrin	*Esomus danricus*	0.02, 0.2and 2ppb for 45 days	Decreased Vitellogenin and Estrodiol-17β	[48]

Other	Microcystin-LR	*Danio rerio*	1 or 50 µg/L for 21 days	Reduced spawning , reduction of fertilization and hatching rates, increase in concentration of 17β-estradiol (E2), testosterone (T) and vitellogenin (VTG) in female zebrafish, protein levels of 17βhsd and cyp19a remarkably	[49]
	Bisphenol A	*Alburnoides bipunctatus*	0, 0.5, 1.25, 2.5 and 5 µg/L	Decreased activity of superoxide dismutase and glutathione peroxidase, increased activity of catalase and lipid peroxidation in sperms. Decreased percentage of motile sperms	[50]

Table 1: Classification of EDCs based on chemical property and their impact on fish reproduction.

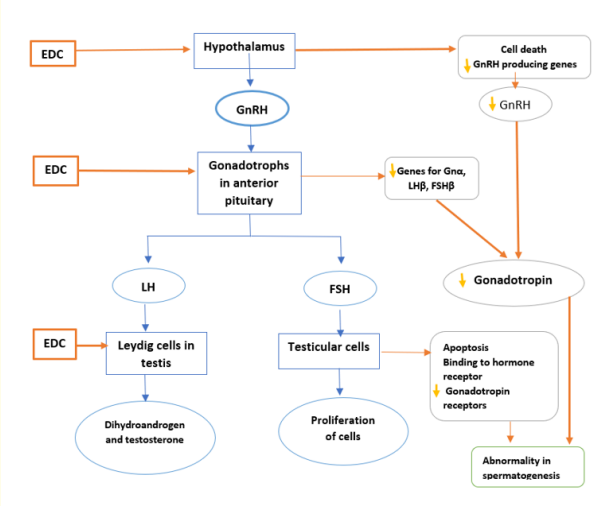

Figure 1: Effects of EDCs on spermatogenesis. Downward arrows (inside the box) indicate decreased concentration of hormones or downregulation of gene.

Spermatogenesis is a type of gametogenesis in which the diploid spermatogonia undergoes mitotic and meiotic cycles with structural modifications to produce the male gametes, sperm [3]. In animals, spermatogenesis takes place inside the seminiferous tubules of the testis [4], but in fishes, it takes place inside a cyst formed by the Sertoli cells. In fish, three different types of testes are present. The tubular testis type occurs in lower teleosts such as-salmonids, cyprinids, and lepisosteids. The unrestricted spermatogonial testis type is present in neoteleosts except Atherinomorph and the restricted spermatogonial testis type is found in all Atherinomorpha. But the process of spermatogenesis is the same in all three types of testes [5]. The fish testis is made up of two compartments: a germinal compartment containing cyst, and an interstitial compartment consisting of compressed stromal elements [6]. The two compartments are separated by a basement membrane. The cyst structure is formed by Sertoli cells surrounding the germinal epithelium and germ cells. The cystic structure, within which spermatogenesis takes place, is formed of developing spermatozoa surrounded by Sertoli cells. Proliferation of those Sertoli cells occurs by mitotic division, which doesn't have a blood testis barrier. Follicular stimulating hormone (FSH), the insulin family of growth factors, activin, and cytokines regulate the process of proliferation of the Sertoli cells. The signaling pathways involved are protein kinase (PKA), extracellular protein kinase 1/2 (ERK1/2), phosphatidylinositol-3 kinase (PI3K)/Akt, and mammalian target of rapamy-

cin C1 (mTORC1)/p70S6K pathways [7]. Spermatogenesis begins with the mitotic proliferation of type A spermatogonia, which is the largest germ cell measuring 12-16µm in diameter. Spermatogonia B are of 9-12 µm and present within the cyst. The spermatogonia undergoes 11 mitotic divisions before differentiation into primary spermatocytes [8]. Two secondary spermatocytes are produced by the meiotic division of the primary spermatocyte. Four spermatids are produced from two secondary spermatocytes. With two consecutive divisions primary and secondary spermatocytes are produced. The spermatids undergo the process of spermiogenesis and develop into sperms (Figure 2). The sperm in fishes are of two types, isodiametric aqua sperm having a globular head and anisodiametric, elongated introsperm [5]. The testis undergoes morphological changes during the annual reproductive cycle. It passes through 5 phases, such as: regression, early maturation, mid maturation, late maturation, and regression. The regressed phase is characterized by diploid cells consisting of primary germ cells and spermatogonia. In the early maturation stage, the testes contain spermatogonia and spermatocytes and a few spermatids. Intensive spermatogenesis is a characteristic of the mid maturation stage. Primary germ cells, spermatogonia, spermatocytes, and spermatids are found to be abundant in the testis. In the late maturation stage, the testes are filled with lobules containing spermatozoa. The regressed stage is the period after discharge of most of the milt. The empty lobules contain unrepelled sperm only, which undergo phagocytosis later [9]. There are several genes and hormones regulating spermatogenesis. Various processes in spermatogenesis, such as DNA synthesis, renewal and/or proliferation of spermatogonia, are induced by several steroid hormones, like 11-ketotestosterone (11-KT), 17α,20β-dihydroxy-4-pregnen-3-one, and 17β-estradiol. 11-KT is required to initiate spermatogenesis and spermiation. The 17α and 20β-DP play a significant role in the mitosis and meiosis phases of the final maturation stage. Estrogen is essential during the mitotic phase of testicular function [10].

The dmrt1, AMH, or sox9, sox8, and fgf9 are expressed during male fish development and are associated with the male testis-determining pathway [11]. In harsh environmental conditions, such as gamma rays, hypoxia, high density, high temperature, altered thermocycles, and poor nutrition, sex ratio tends to shift towards males. It is evident from literature that the sex associated loci are present both on Chr3 and Chr4. This study aims to discuss the changes to spermatogenesis caused by interference of EDCs with sex determination, production of hormone and expression of hormone receptors, development of male gonads, and male gametes in fishes.

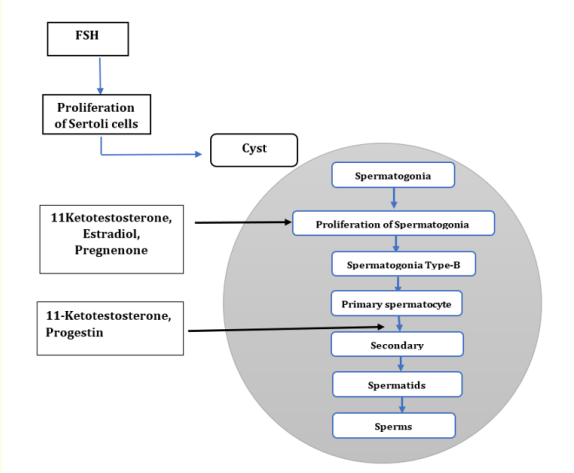

Figure 2: Spermatogenesis in fish and hormones involved in the process.

Effects of EDCs on spermatogenesis

The endocrine disruptors affect spermatogenesis by interfering with sex determination, gonadal development, hormones involved in spermatogenesis and by disrupting the morphology and production of sperms.

Effect of EDCs on gonadal development

Endocrine disruptors affect gonadal development by mediating alteration of the migration of primordial germ cells, and expression of genes involved in testicular differentiation and steroidogenesis. Proliferation and migration of primordial germ cells to the genital ridge is affected by bisphenol-A (BPA) since this environmental estrogen interacts with the estrogen receptor α [12,13]. The transcripts of this gene appear in the lateral mesoderm. The migration of germ cells during the critical period requires interaction between PGCs and somatic cells. The chemokine sdf1a, its receptor cxcr 4b, and the docoy receptor Cxcr 7b are all involved in this interaction [14]. Expression of cxcr 4b is regulated by the estrogen receptor [15]. BPA affects the sf1a gene, which is essen-

tial for ACKR3. Both these genes are essential for normal testicle development. In sperm, histone-3 lysine-9 acetylation(H_3K_9ac) is found to be decreased [16]. BPA exposure decreased levels of double sex mab-3 related transcription (dmrt1) and Mullerian inhibiting substance (MIS) mRNA in both hermaphrodite and secondary males, while increasing levels of female-specific gene and factor in the germline (Figure 1) in both genders' gonads. The expression of dosage sensitive sex reversal adrenal hypoplasia critical region chromosome X, gene1(*dax1)* and steroidogenic factor 1 (*sf1*) was inhibited in the brain and gonads of both genders. Hence, BPA modulates transcription and steroidogenesis towards feminization. Pharmaceutical ethynyl estradiol (EE2) leads to undifferentiated or intersex gonads in zebrafish [17].

Effects of EDCs on maturation

The endocrine disruptors affect the maturation of fishes in following ways: The sewage effluent affects the development of the gonopodium and hemal spine *Gambusiaholbrooki.* in 14th 15th and 16th vertebra [18]. This development is under the regulation of androgen hormone and it requires an increased concentration of androgen. The 17α-ethylene estradiol leads to development of the juvenile ovary in male zebrafish and fadrozole induces masculinization when the zebrafish are exposed from 2hour post fertilization to 60days post fertilization to 17α-ethylene estradiol and fadrozolealone and in a mixture with fadrozole [19].

Effect of EDCs on vitellogenesis

Vitellogenesis is the process of yolk synthesis under the stimulation of ovarian estradiol. The receptors for estrogen are present in the hepatic cells of males, females, and immature juvenile fishes. Hence, vitellogenin (VTG) is produced in liver of mature female fish only and is absent in male fish and juveniles. The VTG in circulation shows a sharp increase between May and June, reaches its peak in the month of July, drops in August and September and becomes negligible in December [20]. 17α-ethynyl estradiol (EE2) induces expression of vitellogenin in male *Sparusaurata* [21]. Exposure of male zebrafish to 2000µg/L of BPA can induce expression of the VTG gene. Pyrithroids such as bifenthrin can induce upregulation of the vitellogenin gene in fathead minnow [22]. Estrogenic compounds affect not only the juvenile and adult but also early life stages which have been demonstrated in Atlantic salmon (*Salmo salar*) taking the concentration of plasma vitellogenin into consideration. The early life stages, such as late-stage embryos, newly hatched larvae, feeding fry and smolts were exposed to three potent estrogenic compounds, 17 α-ethinylestradiol (EE2), 17 β-estradiol (E2), and nonylphenol (NP) for 96 hours. An upregulation of VTG mRNA was observed in all treatments and in all four stages. The expression level of mRNA was highest in feeding fry [23].

Effects of EDCson gonadotropin releasing hormone (GnRH)

GnRH is a family of neuropeptides that play a vital role during development and maintenance of reproductive system. At present, there are 16 GnRHs which have been isolated and sequenced. This hormone stimulates the gonadotrophs of the anterior pituitary to synthesize and release gonadotropins [24]. The synthesis and action of GnRH is affected by EDC since they mediate apoptosis in the hypothalamus and down-regulation of genes that synthesize GnRH receptors. During early development, exposure of zebrafish to 0.02 nm., 0.1 nm., and 0.5 nM concentrations of ethinylestradiol, resulted in disruption of the ontogeny of the GnRH system. The number of GnRH-ir neurons were increased, whereas GnRH fibers and the size of GnRH-ir soma were decreased [25]. The EE2 induces the expression of the brain aromatase gene. Overexpression of this gene aromatizes the testosterone into estrogen. This results in a higher concentration of estrogen in the brain. The GnRH neurons do not contain any receptors for estrogen [26]. The estrogen acts indirectly by the kiss peptin system. The receptors for kiss peptin are found on GnRH neurons [27]. Bisphenol A affects both the synthesis and action of GnRH by regulating gene expression for synthesis of hormone and expression of GnRH receptors on gonadotrophs. Exposure of adult rare minnow *Gobiocyprisrarus* to 15µg/Lconcentration of BPA for 35 days resulted in a significant down regulation of GnRH2, GnRH3, GnRHR1A and GnRHR1B genes. A significant upregulation of GnRH3 andGnRHR1A was found in female [28]. The polychlorinated bisphenols suppress the synthesis of GnRH and also mediate necrosis as well as apoptosis of hypothalamic cells. It has been proved from the experiment by Dickerson., *et al*. 2009 [29], in which, they exposed an immortalized hypothalamic GT1-7 cell line, which synthesizes GnRH to different forms and concentrations of PCBs for 1,4,8 and 24 hours. GnRH concentration and viability of cells were reduced with increased concentration and duration of exposure to the ED, whereas apoptosis and necrosis were increased. In the extrinsic pathway of apoptosis, caspase- 8 is activated with stimulation of membrane death receptors, whereas in the intrinsic pathway, caspase- 9 is activated with release of mitochondrial signaling factors and their cleavage. In both the extrin-

sic and intrinsic pathways caspase-3 is activated. It is considered as the effector caspase and its activation denotes the "point of no return" in apoptosis [30]. In this study, a twofold increase in the activity of caspase 3/7 was observed in intermediate and low concentrations of PCBs.

Effects on gonadotropin hormones

The luteinizing hormone (LH) and follicular stimulating hormone (FSH) are two hormones released from the anterior pituitary. The stimulus for secretion of these two gonadotropins is from binding of GnRH with the GnRH receptors on gonadotroph cells, which regulates spermatogenesis and the secretion of male secondary sexual hormones (Figure 3). The β subunit of these two hormones binds to a common α subunit. The expression of these three subunits (Gonadotropin α, βLH and βFSH) is a potential biomarker of exposure to EDC. 4-nonylphenol also affects the gonadotropin subunits. When the juveniles of female FSH subunit mRNA and protein levels were increased in goldfish exposed to 0. *Oncorhynchusmasouwere injected with 10 or 50 mg/Kg body weight of 4-NP, an over expression of gonadotropin subunit was observed [31].* 01, 0.10, and 1.00 mg/L of a 40% MCP for 21 days in semi-static conditions, while LH subunit mRNA and protein levels were decreased [32]. The juveniles of masu salmon (*Oncorhynchus masou*) were treated with10 or 50 mg/ kg body weight of 4-nonyl phenol. The low dose treatment resulted in increased expression of GTHα and FSHβ, whereas the higher dose reduced FSHβ mRNA and increased VTG mRNA [31].

Effects of EDCs on progesterone

Any change in synthesis, release or action of progesterone can affect spermatogenesis since it regulates the meiotic division. In zebrafish, a significant decrease in body weight, inhibition of the Cyp19a1 gene and an increased rate of sperm maturation were observed when the males were treated with7, 84 and 810 ng/L of synthetic progestin norethindrone (NET) for 90 days [33]. 17α,20β-dihydroxy-4-pregnen-3-one (DHP) induces the mitotic proliferation of cells, including spermatogonia.

Effect of EDCson estradiol

Estradiol at higher concentration inhibits spermatogenesis and mediates sex reversal. But this hormone is essential for mitotic division and the proliferation of spermatogonia. In vertebrates, it is essential for development and sex determination process. Hua., *et*

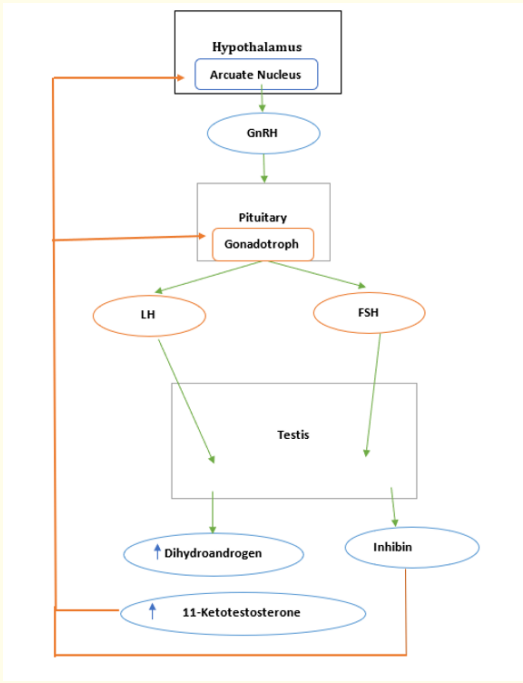

Figure 3: Hypothalamic-pituitary-gonadal axis. Green arrows indicate stimulation and red arrows indicate inhibition. Upward arrow notifies.

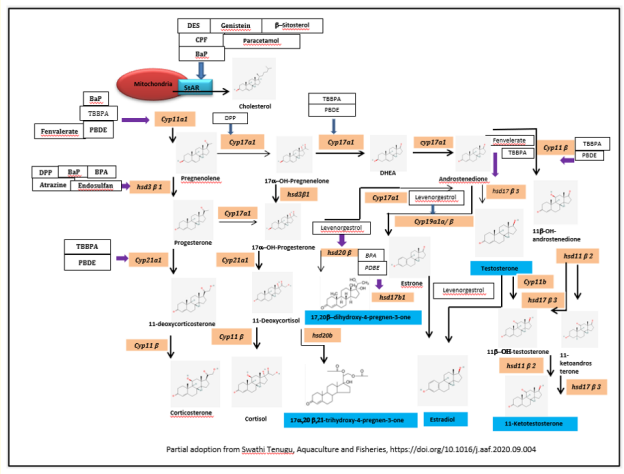

Figure 4: Effect on endocrine disruptor on steroidogenesis.

al. [34] found that when the Chinese rare minnow (*Gobiocyprisrarus*) was exposed to 1ng/L and 10ng/L concentrations of megestrol acetate, there was a decrease in the plasma concentration of estradiol. But there is no evidence whether the decreased plasma concentration of estradiol affects spermatogenesis or not.

Effects of EDCs on androgens

Androgens are a group of hormones responsible for the differentiation of the reproductive tracts into male. Testosterone and 5α-dihydrotestosterone are the major androgens found in all tetrapods. In the case of fishes, 11-ketotestosterone(11KT) plays a major role in stimulating male characteristics [35]. EDCs affect spermatogenesis by interfering with the synthesis of this hormone (Fig. 04). Treatment of goldfish with 0.01, 0.10 and 1.00mgL^{-1} of 40% MCP for 21 days in a semi-static condition caused a higher level of aromatase in gonads, which converts androgen to estrogen. By this, the plasma concentration of testosterone was decreased and 17β-estradiol was increased. This resulted in the expression of VTG mRNA in the liver [32]. The synthetic progestin megestrol acetate (MTA) reduces the plasma concentration of 11-KT. Hua., *et al.* [34] brought the above findings to notice with the larvae of Chinese rare minnow (*G.rarus*) exposed to MTA at concentrations of 1ng/L and 10ng/L for 6 months.

Effect of EDCson progesterone receptors

The predominant progestins in the case of fishes are 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S). The mRNA for nuclear progesterone receptor (nPR) is expressed in all tissues of the adult fathead minnow, but the highest level of expression was exhibited in brain, pituitary, and gonads [36]. It has been observed in goldfish that the diethylstibestrol (DES) and its analogues, such as dipropionate-DES and hexestrol can bind to the membrane progestin receptor-α. The binding sites of DES on PRα are different from 17, 20- β DHP, but it can induce the same effects as that of 17, 20 β DHP binding [37].

Effect of EDCson androgen and estrogen receptors

Unlike proteinaceous and amine hormone receptors, the receptors for steroid hormones are located inside the cytoplasm. Binding of a steroid hormone to its specific receptor brings about conformational changes to the receptor and the hormone-receptor complex is formed. This complex binds to specific response elements inside the nucleus and regulates gene expression. Any changes to these receptors or the synthesis of these hormones can alter the expression of some particular genes that are regulated by that particular hormone-receptor complex. Estrogen receptor α and G protein-coupled estrogen receptor were expressed in gonad of adult malegiltheadseabream (*Sparusaurata* L.) exposed to 17α-Ethynyl estradiol (EE2) and tamoxifen (Tmx) at a concentration of 5 µg EE2/g food, 100 µgTmx/g food or 5 µg EE2 + 100 µgTmx/g food [38].

Effects of EDCson sperm

EDCs affect spermatogenesis by altering sperm structure and other parameters. Asifa and Chitra [39] conducted an experiment where cichlid fish, *Pseudetroplus maculates* was exposed to 3.5 and 7µg/L concentrations of chlordane for 4, 7, 15, and 30 days. As a result of this exposure, sperm count, viability and motility were decreased. The viability of sperm is less due to the damage to testis caused by oxidative stress from chlordane [40].

Impact of EDCson sex determining genes with respect to male

In the case of fishes, a 373-amino-acid protein is coded by Dmrt-1which is homologous to the sex-development double sex gene of *Drosophila* and the Mab3 gene of *Caenorhabditis elegans.* This gene suppresses the aromatase pathway and estrogen production gene expressed in the female development pathway. The antimullerian hormone, expressed in differentiated Sertoli cells, prevents the development of Mullerian duct. Sf-1 is another sex determining gene that is expressed in steroidogenic cells. The homologous gene of Sf-1, ff1d is found in zebrafish. Sry-related HMG box-9 (Sox9) and sf1 interact together and mediate upregulation of amh in presertoli cells. It is evident from the literature that bisphenol F supresses the expression of all these genes. When zebrafish were exposed to 1,10,100, 1000 µg/L of BPF from fertilization to 60 days post fertilization (dpf), downregulation of dmrt1, ff1d, sox9a, AMH genes was observed. BPA at concentrations ranging from 0.1 to 10 nM/L resulted in the downregulation of the Cyp19a1b gene in juveniles of the rare minnow (G. rarus) after 6 months of exposure [41].,17 hsd, and cyp19a were upregulated in marine medaka (*O.melastigma*) larvae when they were exposed to 0.1 and 0.5 mg/L concentrations of both di-(2-ethylhexyl)-phthalate (DEHP) and mono-(2-ethylhexyl)-phthalate (MEHP) for a period of 6 months [42].

Conclusion

Endocrine disrupting chemicals affect spermatogenesis either by affecting the gonads and gametes or by disrupting the synthe-

sis and action of hormones involved, such as, GnRH, LH, FSH, Dihydroandrogen, 11-Ketotestosterone, Estradiol and Progesterone. During sex determination, the concentration of estradiol and the enzyme aromatase play a crucial role. Increased level of estradiol and activity of aromatase leads to sex reversal in fishes. A lower level of aromatase gene expression and a low concentration of 17-estradiol lead to the development of male gonads. Xenoestrogens mimic the estradiol and induce feminization in fishes. As a result, a decrease in the population of male fishes is seen in waterbodies polluted with xenoestrogens. Further investigation is required to establish the co-relation between estradiol and progesterone disruption, and male gametogenesis in fish and environmental factors inducing maturation of fish and spermatogenesis in male fish.

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Authors' Credit

Wati Sucharita Panda and Jitendra Kumar Sundaray conceptualized the manuscript. Sujata Mohapatra, Swati Sucharita Panda and Rajesh Kumar drafted the manuscript. Structural editing, proof reading and language editing were done by IpsitaIswari Das, Jitendra Kumar Sundaray and Lakshman Sahoo.

Conflict of Interest

All the authors declare no conflict of interest.

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