2 The Role of Environmental Toxicant-Induced Oxidative Stress in Male Infertility

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Abstract

Infertility is a serious public health issue affecting around 15% of couples globally. Of the 60–80 million people of reproductive age affected by infertility, 40–50% are due to male factor while 30–40% of cases are still idiopathic. The recent global deterioration in sperm quality raises apprehensions regarding the toxic effects of environmental pollutants on reproductive health of males. Environmental toxicants have shown strong evidences for inducing oxidative stress affecting spermatogenesis severely, thereby leading to reduced sperm motility, count, and DNA damage. Reactive oxygen species (ROS) influences the spermatozoa development and transit process both internally and externally. Low level of ROS is indispensable for critical physiological sperm processes like sperm capacitation, motility, acrosome reaction, hyper-activation, sperm-oocyte interaction, etc., while excessive ROS disrupt antioxidant molecules which is detrimental to normal functioning of the sperm. Hence, identification of potential environmental toxicant may have clinical relevance for early screening and diagnosis of male infertility.

Keywords

Environmental pollutants · Pesticides · Heavy metals · Reactive oxygen species · Oxidative stress · Fertilization · Infertility

2.1 Introduction to Male Infertility

Infertility is defined as failure to conceive spontaneous pregnancy over more than a year after regular and unprotected sexual intercourse. The World Health Organization (WHO) has classified male infertility as a disease affecting 7–15% or 60–80 million couples worldwide (Datta et al. 2016). It is reported that 1 out of every 20 males in the reproductive age group experiences infertility (Rowe et al. 2000). Of the total infertility cases, 40–50% are contributed by male factors including genetic factors, varicocele, cryptorchidism, and hypogonadism, while 30–40% of cases are still idiopathic with normal hormonal, genetic, and biochemical parameters (Jarow 2007). In most couples, identifying a common reason of infertility is challenging since the

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pathology is often multifactorial and illusive. Reports suggest that environmental toxicant, genetics, epigenetics, age, lifestyle, sex, heat, stress, and obesity can affect male infertility (Agarwal et al. 2014). However, the exact pathophysiology is still poorly known. Global industrialization has amplified the risk of possible environmental toxin exposure to human beings. The exponential growth in automobiles and industrial sectors are held responsible for increased male infertility. Numerous environmental toxicants identified recently can affect male fertility severely.

2.2 Environmental Risk Factors and Male Infertility

The identification of high-risk environmental factors could contribute to exploration of disease etiopathology as well as the early detection and screening of high-risk patients and treatment strategies for male infertility. Environmental pollutants like organophosphate pesticides (OPs), persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), heavy metals, dioxin, bisphenol A (BPA), plastics, oils, solvents, explosives, disinfectants, radiation, etc. are severely affecting male fertility (Hauser et al. 2003; Geng et al. 2015; Babu et al. 2013; Ghafouri-Khosrowshahi et al. 2019). Humans in particular are exposed to environmental toxins by consuming contaminated food and water, use of consumable goods such as plastic ware, cosmetics, exposure to polluted air, etc. (Wong and Cheng 2011). Environmental toxicants can severely affect spermatogenesis process resulting in deprived semen quality, low sperm count, DNA damage, and irregular sperm morphology, primarily through the mechanism of oxidative stress (OS). OS and reactive oxygen species (ROS) have been identified as potential mechanisms for idiopathic infertility (Aitken et al. 2004; Sharpe 2010).

Sperm cells are surrounded by ROS both internally and externally during its formation and transit through the reproductive tracts of both male and female (Ribas-Maynou and Yeste 2020). The ROS are generated by both endogenous sources such as mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells, and exogenous sources such as environmental factors. Low levels of ROS are needed for regulation of essential normal sperm functions such as sperm capacitation, motility, acrosome reaction, hyper-activation, sperm-oocyte interaction etc., while excessive ROS disrupt the balance between antioxidant-oxidant molecules in seminal fluid which is detrimental to normal function of sperm (Ribas-Maynou and Yeste 2020). Environmental pollutants have been linked to an increase in ROS generation and a decrease in sperm quality. They can also affect male infertility by their endocrine-disrupting properties (Fig. 2.1). Environmental pollutants can affect male infertility by causing changes in hormone action, synthesis, transportation, and metabolism (Gore et al. 2015). In the last two decades, with the advancement in the area of environmental toxicology and developmental biology, research in the field of endocrine-disrupting chemicals (EDCs) has increased substantially, proving their hazardous impact on human life. Many countries in the world have banned the use of EDCs such as PCBs, BPA, and OCPs after experiencing their hazardous effects (Meeker 2012).

2.3 Pesticides Exposure and Risk of Male Infertility

Pesticides are chemicals or mixtures of chemicals commonly used to prevent, kill, repel, or mitigate pests. Pesticides have greatly helped in increasing agricultural yield to meet the food needs of fast-growing global population, while also lowering the risk of vector-borne diseases (Aktar et al. 2009). Pesticide exposure of humans and assessment of its hazards to human life needs attention regardless of the type of pesticide used. In the United States alone, approximately 750,000 new people are exposed to pesticides yearly (Perry et al. 2011). The fate of pesticide is determined by xenobiotic biotransformation enzymes, its metabolism, and degradation into

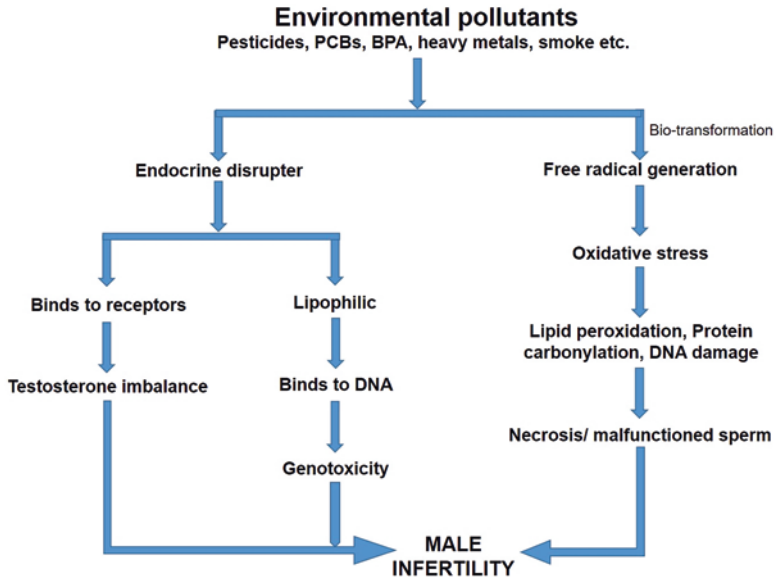

Fig. 2.1 Environmental toxicant induced toxicity and its role in male infertility

smaller weight metabolites that can stay longer in environment. Pesticides can affect the male reproductive system in many ways, including causing reproductive toxicity through direct cell structure damage or biotransformation into metabolites (Roy et al. 2017). The dose, frequency, duration, route of exposure, and genotoxic effect are the major determinants of pesticide hazard (Silva Pinto et al. 2020). The pesticides are categorized into organochlorine, organophosphate, carbamate, and pyrethroid insecticides.

2.3.1 Organophosphate Pesticides (OPs)

Pesticides such as OPs are widely used to control pests in agricultural crops, ornamentals, buildings, and homes (Jayaraj et al. 2016; Koureas et al. 2014). Humans are exposed to OPs either through their occupation or through their surroundings. The extent of OP exposure may be affected by the chemical type, the exposure route and period, age, gender, and genetic susceptibility. Similarly, occupational exposure can be affected by a variety of factors like nature of jobs, duration of exposure, precaution practice, personal protective equipment, and legislations (Mehrpour et al. 2014). OPs are potent neurotoxic chemicals that are characterized by their inhibitory action on acetylcholinesterase (AChE) enzyme activity in the cholinergic nerve terminal (Nili-Ahmadabadi et al. 2018). Acute exposures to OPs lead to the hyper-activation of the nicotinic and muscarinic receptors and often result in death if not managed properly (Peter et al. 2014). OPs are found to be associated with decreased sperm concentration and motility, resulting in infertility in men (Ghafouri-Khosrowshahi et al. 2019). The toxic effect of OPs on the spermatogenesis have also been evidenced in animal studies (Babazadeh and Najafi 2017). Chlorpyrifos, methyl parathion, and parathion are among the pesticides that have been shown to reduce sperm counts by disrupting the seminiferous epithelium by germ cell proliferation (Babazadeh and Najafi 2017; Perry et al. 2011). Furthermore, OPs have the ability to cross the epididymal epithelium and enter into stored spermatozoa due to their lipophilic nature, resulting in disruption of sperm structure and function (Adamkovicova et al. 2016). The positive correlation between OP exposure and total testosterone level was reported in Thai farmers (Panuwet et al. 2018). Increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in pesticide- exposed workers engaged in spraying insecticides has

also been reported (Jamal et al. 2016). An epidemiological study from Southern Iran has shown the incidence rate of primary infertility is substantially higher among farm workers as compared to normal population (Neghab et al. 2014).

OPs are bio-transformed by xenobiotic metabolizing enzymes, such as cytochrome P450 (CYPs) and the paraoxonase (PON). Therefore, OPs are not bioaccumulative in nature; the existence of secondary metabolites in biological matrices, especially dialkyl phosphates, confirms its exposure (Androutsopoulos et al. 2013; Sokoloff et al. 2016). The activity of plasma cholinesterase that affects testosterone levels is considered as biomarker for the chronic exposure to OPs (De Silva et al. 2006). OS induced by OPs may have negative impact on sperm production and gonadal function. Ghafouri-Khosrowshahi et al. (2019) reported higher OS in rural farmers accompanied by increase in serum lipid peroxidation (LPO) levels and decreased total antioxidant capacity (TAC).

2.3.2 Organochlorine Pesticides (OCPs)

Organochlorines, which include chlorinated pesticides and PCBs, are one of the forms of environmental pollutants. OCPs are absorbed through the skin, inhaled through the air, and ingested through food and water (Jayaraj et al. 2016). Of these, taking non-vegetarian food may cause greater exposure to organochlorines since they are lipid soluble and bioaccumulative in nature. Hence, their effects are more seen in the higher trophic organism such as human beings. In addition to this, OCPs are of great concern because they are chemically stable, endocrine disrupting in nature, strongly lipophilic, as well as have a very long half-life (Abhilash and Singh 2009) (Table 2.1).

Organochlorine hydrocarbons are classified into dichlorodiphenylethanes, cyclodiene compounds, hexachlorocyclohexane, endosulfans, and other related compounds. They may exert their toxicity through several mechanisms. Previous studies have reported environmental exposure to persistent OCPs results in their presence and accumulation in human follicular fluid (Younglai et al. 2002), seminal fluid (Toft et al. 2006), as well as in embryos and fetuses (Waliszewski et al. 2000). It results in abnormal sperm count, impaired sperm motility, and reduced fertilization capacity (Sengupta and Banerjee 2014). Many researchers have concluded that OCPs may increase OS and contribute to adverse reproductive outcomes (Pathak et al. 2010; Kumar et al. 2014). An in vitro study has also reported that lindane, the gamma-isomer of hexachlorocyclohexane (HCH) may cause OS and immunotoxicity (Mrema et al. 2013). Furthermore, Banerjee et al. (2001) have shown OCPs induce alteration in lipid peroxidation, protein oxidation, glutathione redox cycle, and excessive DNA damage.

Spermatozoa are extremely vulnerable to the deleterious effects of ROS because they are rich in polyunsaturated fatty acids (PUFA) in their plasma membrane and cytoplasm. ROS causes LPO leading to disintegration of membrane, increased permeability, decreased sperm motility, DNA damage, and apoptosis (Schuppe et al. 2008; Alahmar 2019). Moreover, OCPs are known to act as environmental xeno-estrogens and structurally resemble the sex hormones. Because estrogens contribute significantly in regulation of several reproductive processes, it is possible that exposure of humans to xeno-oestrogens in the environment could influence

Table 2.1 Environmental half-life of organochlorine pesticides (OCPs)

OCPs	Half-life in soil (years)	95% disappearance (years)
Aldrin	0.3–3.0	3.0
Lindane	1.2–6.5	6.5
Dieldrin	2.5–8.0	8.0
Dichlorodiphenyltrichloroethane (DDT)	2.8–10	10.0
β-Hexachlorocyclohexane (β-HCH)	7.2–7.6	7.6

male fertility. Several evidences have accumulated during the past decade to support the notion that environmental estrogens that may cause reproductive disorders (Bulayeva and Watson 2004).

Polymorphism in xenobiotic metabolizing gene may interfere with the proper metabolism of OCPs, and thus it induces OS causing excessive free radicals generation and alteration in antioxidant interference along with disruption in steroid hormonal synthesis (Mustafa et al. 2010, 2013). Dalvie et al. (2004) evaluated sperm parameters in regularly dichlorodiphenyltrichloroethane (DDT) exposed workers and observed that sperm morphology scores were lower than that of WHO or Tygerberg criteria in 84% of the workers, with the highest recording at 6%. In a large epidemiological study, a strong association between the percentage of DNA fragmentation index (% DFI) and p,p′-DDE levels has been reported. They have suggested that DDT/DDE can damage sperm DNA/chromatin if the extent of exposure is high (de Jager et al. 2009). In a study consisting a total number of 212 infertility cases in the United States, Hauser et al. (2003) registered that a mean serum level of DDE was 254 ng/g of lipid. Pant et al. (2004) reported higher levels of p,p′DDE and p,p′DDD in the sperm of infertile men as compared to normal population.

Lindane causes OS in adult rat testes, epididymis, and alters sperm dynamics (Joshi and Goyal 2004; Chitra et al. 2001). Several studies have shown that exogenous lindane therapy lowers serum testosterone levels, suggesting that lindane inhibits testicular steroidogenesis (Ronco et al. 2001; Saradha et al. 2008). It causes alterations in Leydig and Sertoli cells by impairing their functions (Suwalsky et al. 2000). In rats, lindane doses of 10 mg/kg/day for 15 and 45 days caused considerable loss in sperm production and mortality. Control rats had 100% positive fertility, while rats exposed to lindane for 15 and 45 days had 20% and 50% negative fertility (Dalsenter et al. 1997). Treating male mice with endosulfan dosage of 3 mg/kg body weight for 35 days caused defective sperm tail, morphed acrosome, coiled tail, decreased testosterone, and enhanced LH, confirming testicular dysfunction leading to infertility (Ali et al. 2012).

2.3.3 Bisphenol A (BPA)

BPA is a typical EDC affecting biosynthesis of steroid hormones. It has major public health concern due to its widespread use, toxicity, and persistent nature. It's a common chemical used in a variety of products used in our everyday lives, such as medical supplies, dental sealants, epoxy resins, can linings, and polycarbonate plastics. Its exposure has shown association with general male infertility (Manfo et al. 2014; Vitku et al. 2016), but the results are ambiguous (Yuan et al. 2015; Mínguez-Alarcón et al. 2016). Ingestion of contaminated food and wastewater are the major sources of BPA exposure whereas drinking water, air, and dust particles are other sources of exposure to humans and animals (Vom Saal and Welshons 2014). BPA is linked to affects on male reproductive function due to the breakdown of estrogen- or androgen-mediated pathways (Jambor et al. 2018). The hormone level analysis reflected that BPA may cause high FSH and lower inhibin B and estradiol:testosterone ratio (Meeker 2012). Its can decrease antioxidant capacity because of high free radical level causing its detrimental effect on sperm physiology and subsequent cause of infertility. Even at the lowest environmentally permissible dose, BPA can stimulate testicular seminoma cell proliferation through both putative membrane estrogen receptors (ER) and likely G-protein-coupled receptors (GPCR) (Bouskine et al. 2009).

BPA has shown its negative effects on spermatogenesis in both in vitro and in vivo studies. It has shown dose-dependent effect on sperm fertilizing ability, spermatozoa motility, and mitochondrial damage as a result of increased intracellular superoxide levels (Singh et al. 2015; Lukacova et al. 2015; Rahman et al. 2015). At high level, BPA can bind to the androgen receptor (AR) and act as antagonist. It may interfere with LH receptor-ligand binding and affect the expression of 17-hydroxylase/17,20 lyase and aromatase in Leydig cells, impairing steroidogenesis. However, BPA is considered as less potent than estradiol (Patel et al. 2015; Chen et al. 2013; Vitku et al. 2016). The Centers for Disease Control and Prevention has reported the biologically active levels of BPA in the urine

samples of >90% of American people (Calafat et al. 2008; Welshons et al. 2006). In China, strong association of polycyclic aromatic hydrocarbons (PAH) with male infertility was detected in 100% of test candidates reported (Xia et al. 2009). In another study, the average BPA levels in the urine have shown to be associated with poor sperm quality. It is worth noting that the average concentration of BPA in urine of infertile men is about 70 times lower, while in control group is 2000 times lower than the USEPA's recommended daily permissible amount. These numbers reveal that BPA can cause toxicity at far lower doses than the USEPA's daily recommended intake (Li et al. 2009).

2.4 Phthalates: Steroidogenesis and Spermatogenesis

Phthalates are one of the classes of EDCs which are ubiquitously distributed in the environment. They are used as plasticizers for polyvinyl chloride plastics. Global explosion of plastic use in daily life has increased its exposure to humans. Phthalates have the ability to damage male fertility by causing OS in the testes as well as disrupting endocrine synthesis pathway. The induced OS caused depletion of antioxidant capacity, especially in GPX (glutathione peroxidase) and GST (glutathione S-transferase) and increase LPO, CAT (catalase) and SOD (Cu/Zn superoxide dismutase) activity (Asghari et al. 2015). Reports suggested that di(2-ethylhexyl)phthalate (DEHP) affects the gene expression of antioxidant-related genes such as SOD1 and GPX expression at a dose of 100 μg/mL; however, decreased expression of anti-apoptotic factor (Bcl-2) and increased expression of pro-apoptotic factor (Bax) were noted at doses of 1, 10, and 100 μg/mL (Wang et al. 2012).

The ability of phthalates to bind and to activate peroxisome proliferator-activated receptors (PPARs) has long been known (Lapinskas et al. 2005). The phthalates binding to PPARs can cause increased intracellular OS by the mechanism of activating certain ROS generating enzymes (Mathur and Cruz 2011). Phthalates can also reduce Leydig cell activity by inducing ROS, which lowers the levels of steroidogenic enzymes. According to its toxicological profile, monoethylhexyl phthalate (MEHP) is ten times more toxic to Leydig and Sertoli cells than DEHP, indicating that DEHP is the pre-toxin action by metabolizing into MEHP (Koo et al. 2002).

2.4.1 Dioxins

Dioxins are lipophilic, persistent organic compounds that are highly resistant to degradation, allowing them to remain in the atmosphere for long duration. They are part of the "dirty dozen," a collection of hazardous chemicals known as POPs. These are the outcome of industrial processes including chlorine bleaching of pulp and paper, pesticide manufacturing, and medical waste and plastics incineration (Hewitt et al. 2006). The complex mixture of dioxins contains 75 dioxin congeners and 135 furan congeners; of the total, 7 and 10 congeners can bind to aryl hydrocarbon receptor (AhR) for its activation (Van den Berg et al. 2006). Dioxins and dioxin-like compounds such as polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and certain polychlorinated biphenyls have shown the similar structural and biological properties. Dioxin exposure has been considered as a risk factor for adverse male reproductive outcomes. The adverse effect of a single in utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure leads to reduction in sperm count in adult rats (Foster et al. 2010). TCDD has been reported to cause depletion in antioxidant enzymes may increase OS (Yoshida and Ogawa 2000).

2.4.2 Polychlorinated Biphenyls (PCBs)

PCBs are lipophilic, persistent organochlorines that were previously used in cutting oils and lubricants, as well as electrical insulators. Because of their toxicological effects on humans and laboratory animals, PCBs use and manufacture were banned in several countries in the late 1970s.

However, they are still present in food today, primarily in fish, poultry, and dairy products (Freels et al. 2007). PCBs are lipophilic, non-biodegradable, and bioaccumulative chemicals; hence, they tend to accumulate in higher tropic levels of the food chain (Gupta et al. 2018). The epidemiological studies reveal that prenatal exposure to PCBs may disrupt spermatogenesis and steroidogenesis processes resulting in reduced sperm count and male infertility (Guo et al. 2000). PCBs may induce OS by increasing ROS levels, which can damage membrane lipids by LPO and subsequently causes disruption of membrane (Halliwell and Gutteridge 1990). Both, in vivo and in vitro studies have reported deleterious effect of PCBs on LPO in Leydig cells (Aly et al. 2009; Venkataraman et al. 2008).

Petersen and colleagues included 266 fertile men for estimating exposure of PCBs by evaluating hormone concentration and semen quality. Their study showed that the ratio of testosterone to estradiol, as well as the levels of FSH and sex hormone binding globulin (SHBG), was related to serum PCB levels. In contrary to this, no association was reported between semen volume, sperm concentration, count and morphology, and abstinence duration by others (Petersen et al. 2015). Despite the fact that PCBs are not currently in use, their residues are continuously being reported in human beings. Hence, questions about no association of male infertility with PCBs or inverse correlation of PCBs with indicators of male reproductive function persist.

2.4.3 Heavy Metals and Male Infertility

Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), barium (Ba), mercury (Hg), and uranium (U) are dense elements with potential toxicity, especially in environmental context (Bánfalvi 2013) and are indicators of male infertility. Heavy metals are difficult to metabolize, hence toxic heavy metals can bioaccumulate in human body (Kamath and Bhattacharya 2012). Lower mean levels of zinc and higher level of heavy metals like Cu, Pb, and Cd in the serum and blood of infertile men indicates changes in their metabolism, which could be linked to the development of infertility among these men (Venkatesh et al. 2009; Fatma et al. 2012). Spermatogenesis and sperm function are depleted as a result of heavy metal-induced OS. Infertile men have higher levels of heavy metals and oxidant levels, as well as lower levels of enzymatic and non-enzymatic antioxidants and necessary antioxidant micronutrients (Venkatesh et al. 2009; Fatma et al. 2012). Cd, Pb, and selenium (Se) have been found in seminal plasma, which could affect semen parameters and DNA damage in human spermatozoa (Xu et al. 2003). The routine monitoring of heavy metals in humans could be helpful in improving the general health conditions including screening of high-risk male infertility.

2.5 Metabolism of Environmental Pollutants

Exposure of an organism to environmental toxins activates biological mechanisms in order to metabolize and detoxify xenobiotics by biotransformation. Biotransformation, also known as metabolic transformation, is a chemical process that converts organic compounds into relatively greater polarity compounds. It is essential for survival because it transforms ingested nutrients into substances necessary for normal body functions. Phase I and phase II reactions are the two forms of biotransformation reactions. In phase I reactions, adding a functional group to the parent compound activates xenobiotics, while in phase II reactions, a covalent linkage is built between the functional group and an endogenous water, resulting in the formation of a soluble conjugates like glucuronic acid to facilitate excretion. The cytochrome P450 (or CYPs) and glutathione S-transferases (GSTs) enzymes play an integral role in the biotransformation processes.

Cytochrome P450 enzyme family of proteins is involved in the synthesis and metabolism of both internal and external substances. The ferric

(Fe^{3+}) form of heme iron in CYP P450 is reduced to the ferrous (Fe2+) form during ligand binding. These enzymes catalyze mono-oxygenation reaction resulting in incorporation of one oxygen atom into the substrate (RH).

$$O_2 + 2e^- + R \quad H + 2H^+ \rightarrow H_2O + ROH$$

The polymorphism or mutations in CYP P450 genes may cause improper metabolism of xenobiotics leading to excess free radical generation. Phase II enzymes are involved in the hydrophilization of phase I molecules by conjugating them with glutathione, completing the detoxification step, and contributing to the excretion of conjugated compounds. GSTs catalyze this reaction as follows:

$$GSH + R \quad X \rightarrow GSR + H \quad X$$

GST enzyme brings electrophilic substrate close to GSH and allows the sulfhydryl group on GSH to be activated, resulting in completion of nucleophilic reaction and hydrophilic conjugate formation (R-X) (Eaton and Bammler 1999).

OCPs and PCBs can be activated by phase I and phase II metabolizing enzymes such as CYP1A1, CYP1B1, UGT1A6, and NQQ1 through the AhR pathway (Yan and Cheng 2006). Methoxychlor is primarily demethylated and hydroxylated by cytochrome P450 enzymes such as CYP3A4, CYP3A5, and CYP2B6. Phthalates are metabolized in a similar way to BPA, with glucuronidation reactions demonstrating a key mechanism in the detoxification process, while OPs are metabolized to the typical metabolite dialkyl phosphate (DAP) through the enzyme PON1. The functions of the parent compounds are retained in the metabolites of OPs.

2.6 Environmental Toxicant-Induced Oxidative Stress

The imbalance of oxidant molecules in cells causes OS. The sperm metabolism, fast cell division, and increased mitochondrial activity contribute significantly to the excessive free radical generation in sperm. The sperm is highly vulnerable to oxidative damage than any other cells because of the large amounts of unsaturated fatty acids in their cell membranes, scarcity of cytoplasm in a mature sperm, and limited quantity of antioxidants in the sperm cells (Saleh and Agarwal 2002). Moreover, sperm morphology also resists antioxidant enzymes to work against ROS and protect the membrane covering acrosome and tail of the sperm.

Free radicals can reduce sperm fertility potential by affecting various sperm parameters such as sperm count, motility, and genetic material. The OS plays a significant role in the production of abnormal sperm, as well as the reduction of sperm count, sperm transformation, and sperm DNA damage (Asadi et al. 2017). Thus, substantial amount of antioxidants in seminal plasma can protect fertility potential of sperm. Almonds, avocados, cabbage, and sweet potatoes are rich in vitamin E and should be included in our diet. They have strong antioxidant properties, neutralizing free radicals and inhibiting ROS, thus combating OS level in sperm (Alahmar 2019). In normal conditions, ROS produced in the sperm is constantly deactivated by seminal antioxidants (Showell et al. 2011) (Fig. 2.2).

Furthermore, ROS mediates sperm hyperactivation, which is necessary for fertilizing the oocyte (Barati et al. 2020; Griveau and Lannou 1997). Because of this partial consumption of ROS, sperm cells have low levels of ROS after spermatogenesis and epididymal maturation.

To investigate the relationship between exposure to OCPs and OS, Koner et al. (1998) used sub-acute doses of DDT and lindane in rats and reported high thiobarbituric acid reactive substance (TBARS) levels in serum after 8 weeks of treatment. Antioxidant status was also compromised in their study. According to Samanta and Chainy (2002), endosulfan causes LPO and OS in the testes of mice and rats. OCP-induced OS is the culmination of a multi-step pathway resulting in an imbalance between free radical and antioxidant levels. This imbalance causes increased peroxidation of membrane phospholipids, increased membrane permeability, loss of membrane integrity, enzyme inactivation, DNA damage, protein oxidation, and dysfunction of glutathione redox

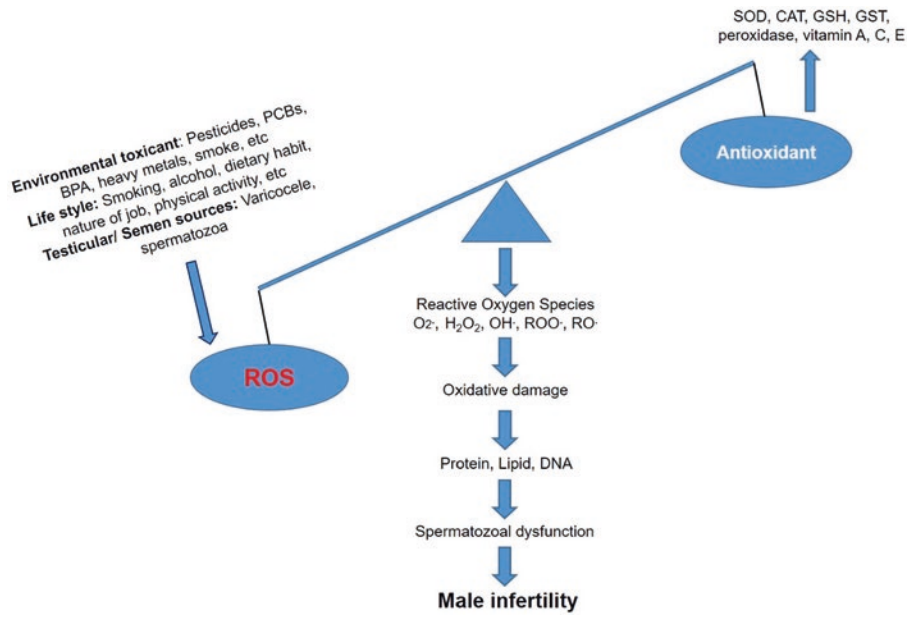

Fig. 2.2 Environmental toxicant-induced oxidative stress in male infertility

system (Banerjee et al. 2001; Koner et al. 1998; Pathak et al. 2011). HCH, DDT, and endosulfan have been shown to enhance OS in vivo and in vitro by producing a large number of free radicals (Srivastava and Shivanandappa 2005; Agarwal et al. 2005). Significant association of β-HCH, γ-HCH, α-endosulfan with content of malondialdehyde (MDA), and protein carbonyls suggests that OCPs play a role in the formation of ROS (Pathak et al. 2010). The study observed a negative association of GSH with OCPs which may be due to utilization of GSH to develop conjugates with electrophilic metabolites of OCPs or because of GSH being oxidized more efficiently by glutathione peroxidase.

2.7 Formation of Free Radicals

ROS or free radicals are oxygen derivate molecules consisting of free electrons or radicals that make them extremely reactive. Despite the fact that not all ROS are free radicals, the terms are often used interchangeably (Cheeseman and Slater 1993). Several forms of free radicals exist in biological system such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), proxyl radical (ROO), or hydroxyl (OH^-). The superoxide anion radical (O_2^-) is formed when one electron is added to dioxygen (O_2). It is the most common type of ROS. Further, this anion would then be converted either directly or indirectly to secondary ROS such as the hydroxyl radical (OH), peroxyl radical (ROO), or hydrogen peroxide (H_2O_2). Other than ROS, the reactive nitrogen species (RNS), consisting of nitric oxide (NO), dinitrogen trioxide (N_2O_3), and peroxynitrite ($ONOO^-$), are also present in sperm cells. Identification of the possible sources of ROS and RNS could be helpful to explore the underlying mechanism for the development and consequences of male infertility. It would also aid in the advancement of alternative therapeutic options as well as male reproductive health. ROS may be produced either endogenously or exogenously in the male germ line.

2.8 Sources of ROS

Reactive oxygen consists of at least one unpaired electron in the outer shell, which makes them extremely reactive molecules. It is worth to highlight that the spermatozoon produces ROS as a result of its metabolic activity.

In spermatozoa, ROS can be generated by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the plasma membrane level and/or the NAD-dependent redox reaction at the mitochondrial level, which is the most common mechanism (Dutta et al. 2019). The retention of excess cytoplasm caused by spermatogenesis arrest may trigger the NADPH system through the hexose-monophosphate shunt, which provides electrons for ROS generation and OS. Researchers have reported that environmental pollutants like BPA can affect the enzymatic H_2O_2/peroxidase and NADPH/CYP450 pathway and generate ROS (Babu et al. 2013).

Dysfunctional or immature spermatozoa in semen supply additional free radicals affecting mitochondrial function and motility of sperm (Agarwal et al. 2014). The most common ROS in human spermatozoa is superoxide ($O2^-$), which reacts with itself to create hydrogen peroxide through dismutation reactions. Further, transition metals such as iron and copper catalyze the reaction between H_2O_2 and O_2 resulting in formation of highly reactive hydroxyl radical (OH^-). At normal physiological conditions, low levels of ROS are essential for sperm maturation, hyper activation, capacitation, acrosome reaction, as well as fertilization. However, ROS imbalance causes lipid peroxidation, sperm DNA fragmentation, and apoptosis, all of which lead to infertility.

Capacitation, for example, necessitates NO and H_2O_2, which primes the spermatozoa to start the acrosome reaction that also needs ROS (Lamirande et al. 1998; Herrero et al. 1999). Furthermore, ROS mediate sperm hyperactivation or contact with the oocyte, making it necessary for fertilization to occur (Barati et al. 2020). Environmental toxins contribute significantly to the OS in testes of up to 80% of clinically reported infertile men (Tremellen 2008; Agarwal et al. 2005). On the other hand, the level of OS, may vary greatly based on chemical composition (Kabuto et al. 2004; Dhanabalan and Mathur 2009) (Fig. 2.3).

In addition to this, environment pollutant-induced OS may activate phosphatidylinositol 3-kinase (PI3K)/c-Src signaling pathway, which can increase the risk of male infertility. Sertoli cells are essential for normal spermatogenesis, owing to their role in nutrient supply, cell junction maintenance, and germ cell mitosis, and meiosis process. The possible mechanism behind this is the breaking of the cell junctions and adhesion between Sertoli-Sertoli cells and/or Sertoli-germ cells through the PI3K/c-Src/focal adhesion kinase (FAK) pathway. Also, tight junctions (TJ) and adherens junctions (AJ) are more susceptible to OS resulting in increased permeability (Lucas et al. 2009; Sandoval and Witt 2008; Rao et al. 2002). The environmental factor-induced OS may activate PI3K/c-Src signaling pathway which subsequently cause testicular damage. The incubation of spermatozoa under OS increases production of H_2O_2 which results in decreasing the rate and motility of sperm (Aitken and Clarkson 1987).

In living systems, the free radical components strike major cellular targets that include basic protein degradation resulting in decrease in sulfhydryls or thiols in the body and DNA destroyed by free radical-induced oxidative damage to chromatin structure. The structural damage to chromatin makes the individual susceptible to infertility. Because of this, Ca^{2+} membrane permeability increases causing damage to mitochondria, DNA, and proteins, which leads to cell swelling and apoptosis.

Exogenously ROS is produced by varicocele, infections and leukocytospermia, alcohol and tobacco consumption, physical exercise and heat stress, radiations and pollution, etc. which are responsible for exogenous ROS generation in humans.

2.9 Oxidative Damage to Sperm DNA

DNA integrity and accuracy are important factors for transfer of healthy genetic material from parents to offspring; otherwise, defective embryo could be formed. It could be a better diagnostic and prognostic marker to test the functional competence of the sperm. Hence, other than the con-

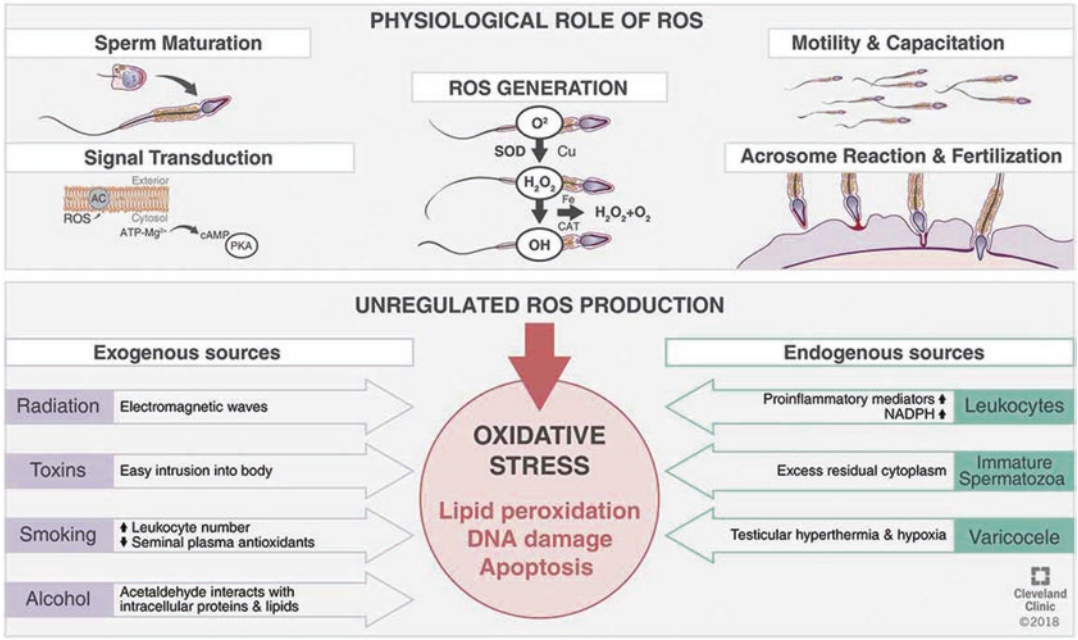

Fig. 2.3 Role of ROS in the sperm physiology and its toxic effects on spermatozoa (Dutta et al. 2019)

ventional semen analysis, usefulness of the molecular biomarkers should be explored (O'Brien and Zini 2005). Industrial explosion, environmental toxicant exposure, and life style changes of human being altogether may cause enhanced levels of ROS in abnormal spermatozoa or leukocytes conferring male infertility.

Both the exogenous and endogenous sources of ROS have deleterious effects on DNA integrity. The inheritance of abnormal genetic material to offsprings may cause autosomal dominant disorders, neuropsychiatric disorders, childhood cancers, etc. It has been reported that seminal leukocytes have the capacity to produce 1000-fold more ROS as compared to other cells with aerobic respiration (Plante et al. 1994). Therefore, excessive generation of ROS or decreased antioxidant capacity is recommended as potential threat and major cause for male infertility because it can increase DNA fragmentation, reduced sperm motility, and increased sperm death. High ROS concentration is reported in the sperm of the men whose partners have experienced abortion. The destruction of sperm membrane and DNA damage have been observed in these men, which may be the possible reasons for abortion.

2.10 Endocrine-Disrupting Chemicals (EDCs) and Male Infertility

EDCs are the substances that can disrupt male and female endocrine function through interacting with hormone receptors. Male infertility is more susceptible to EDCs because it can lead to reduction in fertility biomarkers, especially low sperm counts and testosterone level (Rehman et al. 2018; Slutsky et al. 1999; Jouannet et al. 2001). Humans are exposed to EDCs mainly through ingestion, dermal contact, inhalation, etc. (Joensen et al. 2009; Vilela et al. 2014; Zhu et al. 2016; Nordkap et al. 2012). Hence, the presence of more than the permissible limits of EDCs in our daily consumables such as foods, water, plastics, shampoos, clothing, toothpastes, soaps, fertilizers, paper, carpets, utensils, bedding, toys, cosmetics, etc. needs greater attention.

Environmental pollutants like OCPs, PCBs, BPAs, dioxin, etc. are known endocrine disrupters, which can disrupt hormone synthesis. OCPs including endosulfan and DDT as well as its

metabolites (DDE) are potential EDCs that can disrupt the hypothalamic pituitary testes axis and bind to sex steroid receptors affecting endocrine system in that specific tissue (Mehrpour et al. 2014). Pesticides may also affect neuroendocrine regulation at the testicular level, resulting in decreased testosterone secretion and increased free radical production (Petrelli and Figà-Talamanca 2001; Abdollahi et al. 2004). The exposure to OCPs raises the risk of morphological abnormalities in farm workers including fall in sperm count and a decreased level of viable sperm. Pesticides such as parathion and methyl parathion may cause genotoxicity in sperm, possibly through the OS mechanism. These OPs can also lower the concentration of the sperm by damaging the seminiferous epithelium (Perry et al. 2011). Lifeng et al. (2006) identified that sperm motility could be affected by fenvalerate pyrethroid pesticides. Male farmers in three different Malaysian communities exposed to malathion and/or paraquat showed significantly lower sperm concentration, pH, and mean volume motility of sperm cells compared to the non-exposed population (Hossain et al. 2010). Both malathion and parathion have been shown to reduce the body weights, weight of reproductive organ, and the sperm counts in rats (Narayana et al. 2006; Geng et al. 2015). The potential mechanism of action of OPs is that they alter the fold of Bax and Bcl-2 protein expression as well as reduce LH, FSH, and testosterone levels, resulting in sperm count depletion (Geng et al. 2015).

2.11 Conclusion

Available evidences suggest that the male reproductive system is highly vulnerable target of environmental toxicity. The metabolism of environmental toxicants in human body increases ROS generation, which at lower levels is required for critical physiological sperm processes like capacitation, motility, acrosome reaction, oocyte fusion, and fertilization. Higher ROS levels are detrimental to reproductive health of males and can cause a reduction in sperm count and motility, protein alterations, LPO, and DNA damage. The availability of antioxidants in seminal plasma improves the motility and fertilizing ability of spermatozoa by deactivating excessive ROS. Hence, for the normal functioning of spermatozoa, a balance between the benefits and risks of ROS and the antioxidants is essential. The identification of potential environmental toxicants in the body may have clinical relevance for early screening and diagnosis of male infertility.

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