Bisphenol A and Male Infertility: Role of Oxidative Stress

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Abstract

Bisphenol A (BPA) is an endocrine-disrupting chemical that is capable of mimicking, antagonizing, and interfering with the normal biological functioning of the endocrine system. BPA is used in diverse industries, hence its vast sources of exposure. Although the half-life of BPA is relatively short (<24 hours), studies have reported its detection in the urine of different populations. It, therefore, became important to investigate its effect on general health, including male reproductive health. The adverse effects of BPA on male fertility have been evaluated and reported from both in vivo and in vitro studies. Up to date, reports from randomized controlled trials remain controversial, as some revealed decreased sperm quality, sperm concentration, and total sperm count, while others reported that no adverse effect was seen after exposure. Findings from animal model studies and in vitro experiments have shown that exposure to BPA led to a reduction in sperm quality and increased sperm DNA fragmentation, and some even revealed altered expression of the gene that encodes gonadotropin-releasing hormone. This shows that BPA not only may adversely affect male fertility by acting as an endocrine disruptor but also can potentially impact male fertility via its possible contribution to oxidative stress. Therefore, this book chapter aims to identify and elucidate the effect of BPA exposure on male fertility, and to as well illustrate the mechanisms through which this occurs, while emphasizing the role of oxidative stress as a potential pathway.

Keywords

Bisphenol A · Oxidative stress · Male infertility · Hormone dysfunction · Endocrine-disrupting chemical

8.1 Introduction

The decline in male fertility has been attributed to diverse etiologies. This includes lifestyle choices (obesity), endocrinological abnormalities (Kallmann syndrome), congenital defects

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S. Roychoudhury, K. K. Kesari (eds.), *Oxidative Stress and Toxicity in Reproductive Biology and Medicine*, Advances in Experimental Medicine and Biology 1391,
https://doi.org/10.1007/978-3-031-12966-7_8

(cryptorchidism), idiopathic and genetic anomalies (Y-chromosome microdeletions), and acquired dysfunctions (varicocele) and could as well be caused by endocrine-disrupting chemicals (EDCs) and other environmental toxins (Vander Borght and Wyns 2018; Babakhanzadeh et al. 2020). Numerous types of EDCs have been shown to adversely affect male reproductive health. EDCs are a class of exogenous substances or chemical compounds that interfere with the function of the endocrine system, often exerting estrogen-like and/or anti-androgenic effects, which consequently cause adverse health effects in an intact organism, or its offspring, or subpopulation (Mima et al. 2018; Sharma et al. 2020). These substances, including bisphenol A (BPA), pesticides, and other environmental chemicals, may disrupt normal hormonal stimulatory effect, inhibitory action, or elimination of hormones. Exposure to organophosphates, for instance, a commonly used compound in pesticides, has been associated with abnormal sperm parameters including reductions in sperm counts, motility, viability, increased DNA damage, and abnormal morphology (Krzastek et al. 2021). Several studies have also reported its negative influence on serum reproductive hormones, as a reduction in total testosterone and an increased in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were observed following exposure to organophosphates (Mima et al. 2018; Melgarejo et al. 2015). Another compound is cadmium, a heavy metal, which is known to cause toxicity by impacting the hypothalamic-pituitary-gonadal (HPG) axis, testicular function, and spermatogenesis (Rana 2014). Exposure to cadmium can also induce endocrine disruption via interfering with the DNA zinc finger motif and substituting zinc for cadmium subsequently causing a decrease in steroidogenesis (Krzastek et al. 2021; Kumar and Sharma 2019). Another widely used chemical is BPA. BPA is a crystalline chemical compound used as a monomer or plasticizer in the production of epoxy resins and polycarbonate synthesis. It is also used in the production of medical equipment, aluminum cans, thermal papers, toys, food packaging, and jar caps, among others (Fig. 8.1). Due to the vast sources of BPA, more than 90% of people in Western countries have detectable BPA levels in the urine (Castellini et al. 2020). Exposure to BPA has been found to cause reduced sperm count, motility, and normal morphology (Chiang et al. 2017). After exposure to, and absorption of, BPA via inhalation, ingestion or skin contact, and distribution in the body (Fig. 8.1), it directly disrupts the HPG axis by lowering circulating levels of gonadotropins and reduces the expression of the gene that encodes gonadotropin-releasing hormone (GnRH) within cells in the preoptic area. This is caused due to BPA having an affinity to alpha and beta estrogenic receptors (ERα, ERβ), thereby inducing an estrogenic receptor-dependent gene expression which leads to endocrine disruption in the HPG feedback mechanism and, thus, causing hypostimulation and decreased spermatogenesis (Castellini et al. 2020). Additionally, BPA may also impair male fertility by causing imbalance between the generation of reactive oxygen species (ROS) and the antioxidant activities. When this trend persists, oxidative stress ensues. This shows that BPA not only may adversely affect male fertility by acting as an endocrine disruptor but also can potentially impact male fertility via its possible contribution to oxidative stress. Although environmental EDCs generally exist at low concentrations that may cause a negligible impact on general health, daily exposure to these toxins could potentially pose a threat to male reproductive health. Therefore, the purpose of this study is to identify and elucidate the effect of BPA exposure on male fertility and to as well illustrate the mechanisms through which this occurs, emphasizing the role of oxidative stress.

Key Statement

"Several environmental factors are associated with the global decline of male fertility. These compounds are abundant in our modern society, and the daily exposure to these compounds adversely affects male fertility."

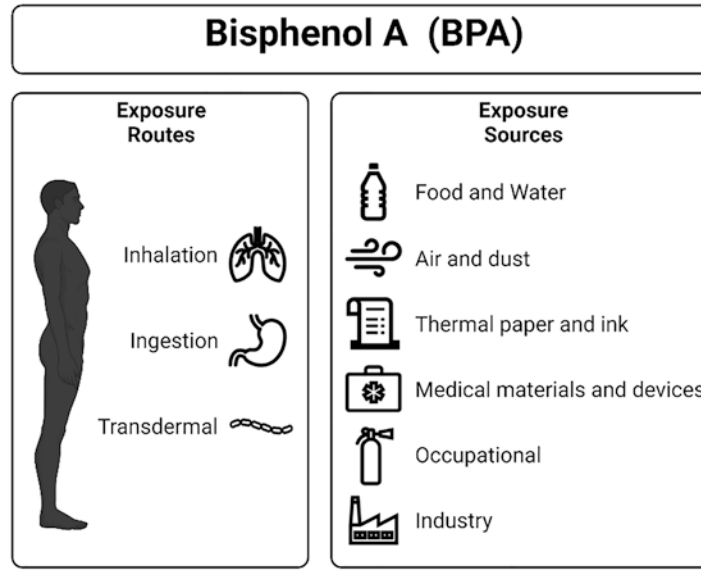

Fig. 8.1 The routes of exposure and sources of bisphenol A (BPA)

8.2 Bisphenol

8.2.1 Overview of Bisphenol

Bisphenols are chemical compounds that acquire two specific hydroxyphenyl capacities. Many of the derivatives of bisphenol are diphenylmethane-based except bisphenols M, P, and S (Liang et al. 2020). Other derivatives of bisphenol are BPA, bisphenol AP, bisphenol AF, bisphenol B, bisphenol BP, bisphenol C, bisphenol C2, bisphenol E, bisphenol F, bisphenol G, bisphenol M, bisphenol S, bisphenol P, bisphenol PH, bisphenol TMC, bisphenol Z, dinitrobisphenol A, and tetrabromobisphenol A. The derivatives are classified based on their reactants. For instance, acetone is the reactant of BPA, the most common derivative of bisphenol (Liang et al. 2020). This chapter will predominantly focus on BPA.

Key Statement
"The classification of the different types of bisphenol compounds depends on their reactant. In comparison to BPA, very limited studies are available on the effect of exposure to other substrates of bisphenol."

8.2.2 Bisphenol A

Approximately nine million tons of BPA are produced worldwide per year (Plasticstoday 2019). BPA is now widely considered as a structural component present in epoxy resin and polycarbonate materials used to manufacture medical devices, water supply pipes, safety equipment, beverage bottles, and food packaging, all of which are items and products that the average individual is exposed to daily (Fig. 8.1) (Castellini et al. 2020). The varied sources containing BPA allow for several different modes of BPA consumption, for instance, oral ingestion from canned foods, inhalation of dust from the air, and transdermal through physical contact (Fig. 8.1) (Loganathan and Kannan 2011). BPA is exceptionally prevalent in consumer merchandise production with approximately 90% of the people in the Western countries having a detectable amount of BPA in the urine, serum, seminal plasma, amniotic fluid, follicular fluid, placental tissue, and umbilical cord blood (Vandenberg et al. 2007). This is partly due to the act of consuming foods that are stored in BPA-containing packaging. The free monomers transfer from the packaging into the food

due to BPA's solubility which is then orally ingested, altering the cellular functionalities and development of the body, which is the leading cause of reproductive damage (Castellini et al. 2020). In recent years, the health risks following exposure to BPA have been examined. The data obtained through standardized toxicological tests displayed evidence that high exposure to BPA affects fertility and fetal development, hormonal levels, neurological and cardiac functionalities, and other physiological aspects of the human body. BPA is a known xenoestrogen, as it mimics estrogen effects due to its characteristic polycyclic phenolic chemical structure, which is similar to estradiol. BPA also affects redox homeostasis by altering the standard equilibrium of oxidative mediators, such as ROS and antioxidant enzymes. This causes the direct induction of cellular dysfunction due to the alteration of cell signaling pathways and activation of apoptosis (Castellini et al. 2020). Although studies have shown that low levels of BPA have no effect on human health, nonetheless, the increased exposure to high levels of BPA through direct and indirect contact causes potential adverse health effects (Gassman 2017).

Key Statement
"BPA has a chemical structure that is similar to estrogen, hence its affinity to bind to the estrogen receptors. Additionally, BPA damages redox homeostasis by altering the standard equilibrium of oxidative mediators, such as ROS and antioxidant enzymes."

8.3 Toxicokinetics of Bisphenol A

Data obtained through toxicological and epidemiological studies presented evidence of high concentrations of BPA in the body causing negative effects on physiological health and developmental capacity. It has been determined that BPA levels from 20 to 400 μg/kg/day and above interfere with normal human physiology (Acconcia et al. 2015). After ingestion, with the aid of uridine diphosphate glucuronosyltransferase, BPA binds to glucuronic acid to form BPA glucuronide (BPA-G) (Fig. 8.2). The same process occurs when BPA binds to sulfonic acid via sulfotransferases, such as phenol sulfotransferase, to form BPA sulfate (BPA-S). The glucuronidation or sulfation of BPA is a rapid process that makes it more soluble in water with a half-life of <24 hours. The toxicokinetic process of BPA can however be influenced by physiological changes (Castellini et al. 2020). The toxic accumulation of BPA is evident as the substance concentration diverts from the regular pharmaceutical range. There is no standard dose that causes detrimental effects in humans, as females and males react differently to specific concentrations of the toxin. Overall, the singular effect of BPA is weak. However, reports indicate that the excessive consumption of the toxin is the leading cause of adverse effects (Acconcia et al. 2015).

Key Statement
"The toxicokinetic process of BPA is rapid. The swiftness of the process makes it more soluble in water, especially when heat is applied. It has a half-life of 5.4–6.4 hours."

8.4 Bisphenol A, Sex Hormones, and Male Fertility

Testosterone is a crucial sex hormone in males as it regulates libido, the production of red blood cells and spermatozoa. Studies have emphasized the importance of preserving a specific level of estrogen in the male body in addition to testosterone to sustain the reproductive capacity. Exposure to BPA decreases the biosynthesis and secretion of testosterone, causing a decline in steroidogenic enzyme expression which affects testosterone production and spermatozoa concentration and quality. Studies have reported the concurrent decrease in testosterone production and an increased levels of FSH and LH following BPA toxicity (Meli et al. 2020).

Estradiol is the predominant hormone derivative of estrogen that plays an important role in maintaining male sexual maturation. During BPA toxicity, the transcription of target genes that are mediated by the estrogen receptor β is affected, as BPA inhibits receptor degradation and ubiquitina-

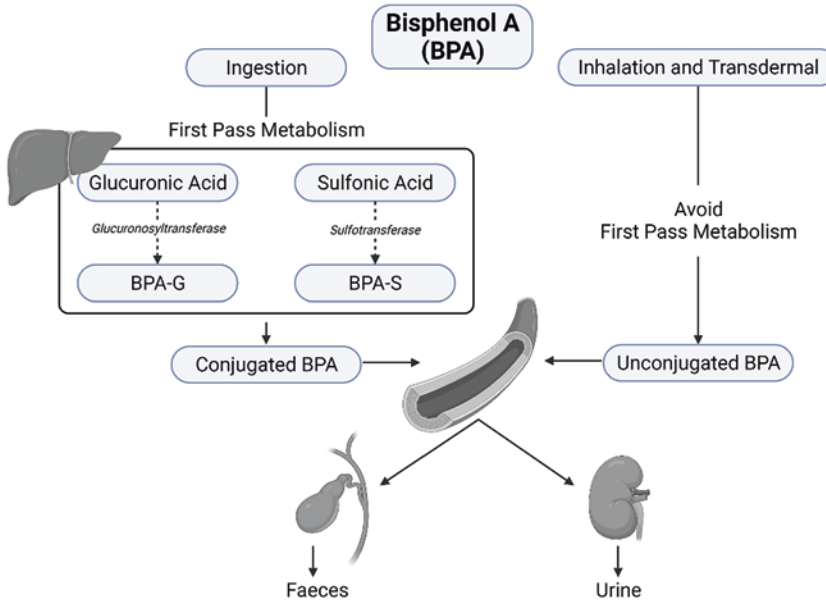

Fig. 8.2 Toxicokinetics of bisphenol A (BPA). Following oral ingestion, BPA undergoes first-pass metabolism in the liver (conjugation phase). Briefly, BPA binds to glucuronic acid with the aid of uridine diphosphate glucuronosyltransferase to form BPA glucuronide (BPA-G). This process is called glucuronidation. The same process occurs when BPA binds to sulfonic acid via sulfotransferases, such as phenol sulfotransferase, to form BPA sulfate (BPA-S), a process termed sulfonation. After glucuronidation or sulfonation, BPA metabolites are excreted either into the bile/GUT and then urine or into the blood and then passed into the kidney to be excreted as urine. The first-pass metabolic phase is avoided when BPA is absorbed through inhalation or transdermal route. This enhances the production of unconjugated BPA in the blood

tion (Masuyama and Hiramatsu 2004). This occurs as a result of BPA affecting the estrogen receptors (α and β) in target cells. The BPA molecule contains specific phenolic structural qualities that allow BPA to bind to the estrogen receptor subtypes α and β by imitating the estrogen receptor features, promoting alterations in cell migration, proliferation, and viability. BPA also stimulates cell growth which has a similar effect to estrogen on the human body, therefore presenting an association between the increased exposure of BPA and the reduced production of sperm and testosterone while prompting male reproductive diseases. Specific levels of the estrogen hormone are required in human males to regulate standard sexual development and fertility. The normal range of estradiol in human males to promote fertility is 10–40 pg/ml (Schulster et al. 2016).

Abnormal levels of the testosterone-to-estrogen ratio provoke damage to important processes such as spermatogenesis and steroidogenesis. The testicular compartments of the two cellular processes are vulnerable to BPA-induced damage due to their functionally similar state. The testosterone hormone is produced during steroidogenesis. BPA-induced oxidative stress alters the synthesis and distribution of the steroid receptors required for the mediation of hormonal activity by binding to the receptors and destroying the steroidogenic enzymes. As a result, the influence of BPA profoundly alters the quality of sperm produced and decreases the likelihood of successful male fertility (Acconcia et al. 2015).

Key Statement

"BPA is a known xenoestrogen as it mimics estrogen effects due to its characteristic polycyclic phenolic chemical structure, similar to estradiol."

8.5 Evidence of Bisphenol A-Induced Male Infertility: The Role of Oxidative Stress

Since the use of BPA in diverse "works of life" keeps increasing, it remains important to continually investigate the effect of its exposure on male reproduction. At present, several studies have pinpointed its adverse effects on male fertility (Li et al. 2021; Liu et al. 2021; Rahman et al. 2021; Mínguez-Alarcón et al. 2021), while some, however, showed no association (Benson et al. 2021). The controversy may be due to differences in methodological approaches, such as in vivo versus in vitro versus ex vivo. To reduce the disparity in findings, some authors have conducted meta-analyses and systematic reviews, establishing a conclusion from a holistic perspective (Castellini et al. 2020; Santiago et al. 2021). This section of the chapter will discuss some studies that have reported the effect of BPA exposure on male fertility (Fig. 8.3).

It has been previously established that BPA has estrogenic and anti-androgenic activity affecting the hypothalamus, which in turn disrupts the hypothalamic-pituitary-gonadal (HPG) axis. The disruption occurs by altering the gonadotropin-releasing hormone (GnRH) pulsatile release, resulting in impairment of adequate secretion of FSH and LH (Santiago et al. 2021). These two hormones play an important role in the male reproductive system, where LH stimulates the Leydig cells and FSH stimulates the Sertoli cells. Leydig cells produce testosterone, and Sertoli cells are responsible for testicular growth and promoting the production of androgen-binding protein. Both of these cells are found in the seminiferous tubules, which are responsible for hosting spermatogenesis and sustaining the maturing spermatozoa (Emedicine.medscape.com 2021). In summation, there is a reduction and/or inhibition of androgen production, as well as a decrease in the number and function of Sertoli cells leading to the degeneration and decline of spermatocytes. In other words, it disrupts the rather complex process of spermatogenesis (Santiago et al. 2021).

Prenatal maternal exposure of BPA when carrying male fetuses has been studied and was found to have an increased risk of urogenital developmental abnormalities. These abnormalities include cryptorchidism, hypospadias, and structural alterations of the testis of male fetuses (Pallotti et al. 2020). Similar to BPA exposure in adults, the main effect of this exposure is related to disruption of the preexisting and well-established hormonal homeostasis. Consequentially, this may hinder the appropriate development of the male genital tract as well as the induction of chronic structural modifications which is partly mediated by oxidative stress through ROS and inflammatory mechanisms (Pallotti et al. 2020). For instance, cryptorchidism may occur due to Leydig cell dysfunction, and it has been suggested that a history of cryptorchidism in boys could potentially increase the risk of fertility issues in the future (Komarowska et al. 2015). It may also be relevant to state that couples with a history of BPA exposure have reported difficulties in conceiving (Komarowska et al. 2015), and this may be attributed to the alteration in the mechanisms involving hormonal homeostasis and histopathological changes in the testicular structure.

Additionally, extensive in vivo and in vitro studies have been conducted to further elucidate the effect of BPA on male fertility. After exposure to BPA, the levels of circulating hormones (testosterone, estrogen) in the animal samples and histopathological changes in the testes were analyzed. It was identified that the circulating hormone levels were altered where testosterone levels decreased and estrogen levels had increased (Jia et al. 2020). Furthermore, the glandular cavity in the BPA group was slightly enlarged, with abnormal morphological changes in the spermatogenic cells and Leydig cells (Jia et al. 2020). It was identified that there is a reduction in the number of spermatozoa, the different phases of spermatogenesis were altered, and there were histopathological changes in the seminiferous tubule as vacuolation and shrinkage of the tubule occurred (Jia et al. 2020). It was also noted that there was a decline in the testicular mitochondrial

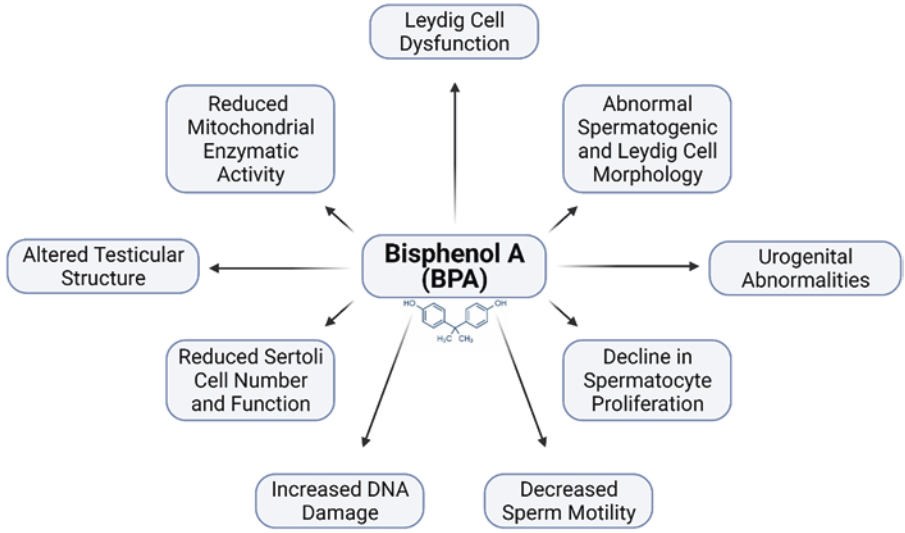

Fig. 8.3 Bisphenol A and male infertility. Exposure to BPA may lead to urogenital developmental abnormalities, decrease in testicular mitochondrial enzymatic activities, altered testicular structure, abnormal morphological changes in the spermatogenic and Leydig cells, increased DNA damage, decreased sperm motility, reduction in the number and function of the Sertoli cells, and decline in spermatocyte proliferation

enzymatic activities such as monoamine oxidase (MOA), NADH dehydrogenase (NDH), malate dehydrogenase (MDH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase (IDH) (Meli et al. 2020; Santiago et al. 2021). Unsparingly, it also reduces the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), and glutathione peroxidase (GSH-Px) (Meli et al. 2020; Santiago et al. 2021), thus promoting an imbalance that results in oxidative stress (Santiago et al. 2021).

In vitro studies using mice spermatozoa exposed to BPA demonstrated a significant decrease in the percentage of motile spermatozoa, decreased intracellular ATP levels, increased levels of ROS, and impaired epididymal sperm motility and viability potentially attributed to the oxidative stress (Rezaee-Tazangi et al. 2020). Findings from human studies reported mitochondrial dysfunction, reduced sperm motility, and increased oxidative DNA damage (Barbonetti et al. 2016). Furthermore, mitochondrial dysfunction in Sertoli cells may be a resultant of the increased ROS in the testes which propagate DNA damage and cellular apoptosis (Wang et al. 2017).

Exposure to BPA has been demonstrated to be dose-dependent (Rochester 2013), so, in theory, prolonged exposure to BPA and higher concentrations thereof have an increased risk of causing the mechanisms of change described above. Some studies have attempted to evaluate male adults with a known history of BPA exposure in their lifetime with known difficulties in conceiving and without. Nevertheless, there is a variation in these studies according to the dose-dependent exposure that could not be identified (Pallotti et al. 2020). Additionally, the measurements done to identify exposure included urine BPA which has been highlighted to be a suboptimal method of evaluating the exposure to BPA (Konieczna et al. 2015), serum BPA, and seminal BPA levels (Vitku et al. 2015). In comparison to healthy males, infertile males have significantly higher levels of seminal and serum BPA. It was identified that seminal BPA levels were associated with a reduction in the semen parameters (total sperm count and sperm concentration), while this was not true for serum BPA (Vitku et al. 2015). This finding provides an emphasis on the significance of seminal BPA levels which may aid and be a future focus in upcoming research attempting to approach this

issue. Furthermore, BPA exposure was found to be associated with increased serum prolactin levels in males (Liu et al. 2015), and this has a detrimental effect through inhibiting pulsatile GnRH secretion which as a result inhibits the release of FSH and LH (Dabbous and Atkin 2018). This will negatively affect and impact testosterone levels and the process of spermatogenesis. Furthermore, another study analyzed the urine samples of men undergoing IVF to investigate whether there is a link between IVF outcome and BPA concentration (Mínguez-Alarcón et al. 2021). The presence of BPA was identified in the urine samples, and the hazard ratio between cycle failure prior live birth and BPA concentration is greater than 1. This suggests that there is a probability that exposure to BPA may increase IVF failures before live births.

Key Statement
"Several adverse effects of BPA have been reported following its toxicity. This includes altered testicular structure, reduced mitochondrial enzymatic activities, and altered sperm quality, to mention a few. The hallmark mechanisms through which these adverse effects are exerted include (i) initiation of oxidative stress and (ii) disruption of the HPGA signaling."

8.6 Mechanisms Through Which BPA Impairs Male Fertility

Metabolically, BPA exposure will impair the homeostatic balance between the production of ROS and their neutralization. This occurs through an increase in the production of ROS and reduction of the antioxidant enzymes, which will lead to oxidative stress (Santiago et al. 2021). Oxidative stress is one of the main components of inflammatory reactions; these reactions will cause damage and changes to the male reproductive system mainly centered around testicular damage (Pallotti et al. 2020). The oxidative stress occurring in the epididymal and testicular sperm occurs through an increase in the levels of oxidants such as superoxide and hydrogen peroxide (H_2O_2) and a decrease in antioxidants, which consequently results in lipid peroxidation (LPO). Moreover, there are decreased levels and activity of glutathione reductase (GR), glutathione peroxidase (GPx), SOD, CAT, and glutathione (GSH). GSH is a known cofactor for multiple peroxidase enzymes that are involved in the detoxification process of ROS (Santiago et al. 2021). Additionally, BPA toxicity-induced oxidative stress may also cause mitochondrial dysfunction with resultant alteration of diverse cellular signaling and the concurrent initiation of apoptosis.

Hence, the main mechanisms of impairment fall under HPG axis dysfunction with hormonal imbalances and oxidative stress. Be that as it may, the induced oxidative stress and concurrent inflammation have a multitude of pathological and histopathological changes to the testicular structure. This is deemed to be significant and potentially more contributory to dysfunction of the male reproductive system and the risk of male infertility. In addition to altered hormone function and development of oxidative stress, BPA can also impair male fertility by promoting adipogenesis and lipid storage in adipocytes, thereby exhibiting obesity-related metabolic dysfunction. BPA also exerts anti-androgenic activity, as it interferes with androgen receptor signaling. That is, BPA acts as an antagonist of the androgen receptor and consequently results in decreased secretion of androgens. The different pathways through which BPA impairs male fertility are summarized in Fig. 8.4.

Key Statement
"Excessive accumulation of ROS and subsequent development of oxidative stress are key mechanisms through which BPA affects male fertility."

8.7 Summary of the Mechanisms Through Which BPA Impairs Male Infertility

All the available literature confirms that BPA is a potent endocrine disruptor affecting the HPG axis; this may occur during intrauterine and adult life. The two main mechanisms coexist and

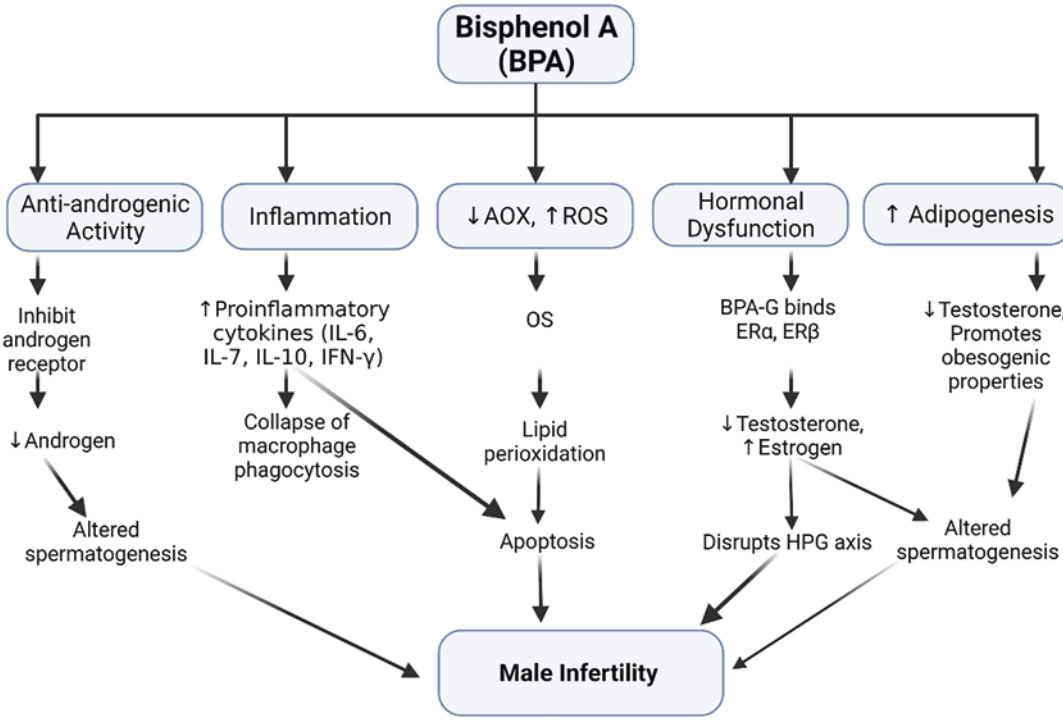

Fig. 8.4 Mechanisms through which bisphenol A (BPA) impairs male fertility. Briefly, BPA may impair male fertility by inducing oxidative stress and inflammation, cause hormonal imbalance via disruption of the HPG axis, and promote adipogenesis and lipid storage in adipocytes, thereby reducing the production of testosterone. It also acts as an antagonist of androgen receptor, which consequently results in decreased expression of androgen. The resultant outcomes of the diverse pathways are male subfertility or infertility

are collectively responsible for causing endocrine dysfunction and an imbalance in the cellular redox system as well as mitochondrial dysfunction, overall resulting in altered development of the testis in terms of structure and function, manifesting with abnormal sperm parameters. These parameters include concentration and motility, both of which are decreased with an increase in genetic abnormalities due to DNA damage. In general, there is a reduction in the semen quality and its parameters in exposed individuals.

While this dilemma has been ongoing for decades, the review and analysis of the existing literature do not provide a definitive answer to whether or not there is direct causation between BPA exposure and male infertility. It influences the HPG axis, estrogenic properties, anti-androgenic properties, oxidative stress, and overall impact on spermatogenesis. Consequently, these factors are known to have an impact on male fertility, yet we cannot conclude that they will ultimately lead to male infertility or that there is a direct causative effect. Hence, the current consensus is that BPA exposure and the sequel of events may increase the risk of male infertility or lead to difficulties in conception.

Key Statement

"Excessive exposure to BPA may not only have an effect on semen parameters such as sperm motility, concentration, or total count but may also cause genetic and epigenetic modifications."

8.8 Reactive Oxygen Species (ROS)

Having provided a background on BPA, the effects of BPA toxicity on male fertility, and the possible mechanisms through which these consequences are exerted, especially, the role of oxidative stress. This section and the succeeding sections of this article will briefly lay emphasis on ROS, the development of oxidative stress, and the management of oxidative stress-induced male infertility.

ROS is a collective term used to describe an array of oxygen-containing reactive species. Variations of ROS contain unpaired electrons and, therefore, are associated with free radicals (unstable atoms that sabotage cell integrity). ROS are predominantly valuable for ensuring appropriate functionality of cell development and proliferation for the maintenance of fundamental physiological processes, namely, immunological defenses to ultimately circumvent cell death. Nonetheless, overproduction of ROS within the human body can be detrimental to crucial cellular and biochemical functions as they are known toxic by-products of aerobic metabolism. The dual biological role of ROS exhibits the importance of a balance between ROS and antioxidants. Low levels of antioxidants in comparison to high levels of ROS obstruct the performance of neutralization activities. Thus, increased concentrations of ROS cause deliberate activation of a physiological cell death pathway and induce oxidative stress (Li and Trush 2016). Please refer to (Du Plessis et al. 2015) for a detailed review on ROS.

8.8.1 Pathophysiology of ROS in Human Semen

ROS play a fundamental role in the pathogenesis of various reproductive processes. An imbalance in ROS to antioxidant ratio due to the overproduction of ROS exhausts antioxidant defenses which directly affect male fertility. Hence, the regulation of ROS is vital. ROS present within human seminal plasma acquires a role in capacitation; ROS facilitates communication using the NADPH oxidase enzyme complex. ROS also modulates sperm chromatin condensation by altering the number of germ cells. Increased levels of ROS in seminal fluid induce apoptosis and proliferation of spermatozoa (Agarwal et al. 2003).

8.9 Oxidative Stress

Oxidative stress is a phenomenon that pertains to the disturbance between levels of production and elimination of ROS in the cells and tissues. The elevated levels of ROS within the human body modify the lipids, proteins, and DNA, thereby inhibiting the body's ability to detoxify the reactive products which activate an oxidative stress response. Cellular processes such as the activation of transcriptional factors, protein phosphorylation, immunity, and differentiation depend on adequate ROS production to commence proper functionality. Deviations made to the desired level of ROS pose harmful effects on crucial cellular structures (Tremellen 2012).

8.9.1 Origin of Oxidative Stress

Oxidative stress is associated with numerous intracellular and extracellular pathologies. ROS are the main cause of oxidative stress as they occur naturally in aerobic cells. There can be multiple sources of ROS, and the origins can include idiopathic and iatrogenic sources, as well as lifestyle and environmental factors such as smoking and pollution.

8.9.2 Idiopathic

The term idiopathic refers to a disease with an unknown cause and unspecified origin. Studies classify the idiopathic origin of oxidative stress-induced male infertility as multifactorial heterogeneous etiologies. Idiopathic male infertility is indicative of sperm abnormalities with no previous familial history of fertility problems,

medical information, or abnormal laboratory test results to corroborate the occurrence. The current consensus on idiopathic male infertility refers to an array of genetic disorders that could potentially affect fertility as a consequence (Alahmar 2019).

8.9.3 Iatrogenic

Iatrogenic causes of oxidative stress are consequences of certain medical treatments or examinations. In terms of oxidative stress-related male infertility, exposure to specific medications or medical equipments containing BPA could harm testicular function, spermatogenesis, and testosterone production due to hypothalamic-pituitary-testicular suppression; a dysfunctional reproductive axis causes irreparable consequences to developmental stages of fertility (Gandhi et al. 2017). Alteration at any stage of spermatogenesis may impair the overall sperm structure and quality, thereby affecting the vital fertilization functions. For instance, varicocele is one of the most common etiologies of male infertility. It is associated with elevated levels of oxidative stress-induced responses such as the impairment of sperm quality due to the overexpression of ROS, causing swollen veins in the scrotum. Moreover, an operative procedure such as varicocelectomy can be performed to remove the swollen varicoceles within the scrotum (Ni et al. 2016).

Several pharmaceutical medications have been approved to impair human spermatogenesis, including fluvoxamine maleate, cortisone acetate, and bosentan, danazol, among others (Ding et al. 2017). The mentioned medications are frequently prescribed to treat an array of psychological and physiological illnesses. Therapeutic drugs affect spermatogenesis function at varying degrees, causing temporary or persistent spermatogenesis impairment depending on their chemical properties and patients' immune response. Moreover, medications that alter the cellular function of either the testis or the epididymis will likely lead to adverse effects on fertility (Twigg et al. 1998).

Diet and lifestyle are among the prime contributory factors increasing the expression of free radicals within the human body. Medical conditions, treatment, and certain medications can also temporarily induce oxidative stress reactions due to mild inflammation. Also of importance is the exposure to BPA via oral ingestion, inhalation, or transdermal route. Oxidative stress occurs when the body is unable to facilitate the appropriate defense mechanisms against oxidative stress. Long-term exposure to oxidative stress leads to the development of chronic medical conditions, such as cardiac and neurological diseases (Pizzino et al. 2017).

8.9.4 Effect of Oxidative Stress on Male Fertility

The imbalance in the concentration of ROS and antioxidants alter DNA integrity, leading to the production of a lower quality of semen and inducing male infertility. ROS is required for sustaining regular cellular function. However, oxidative stress amplifies the production of ROS to a level of toxicity. Ooverproduction of ROS modifies sperm function by breaking DNA strands, altering bases and inducing chromatin cross-linking. The cellular characteristics are vulnerable to the influence of ROS, thereby leading to impaired defense mechanisms against ROS-induced oxidative stress damage (Agarwal et al., 2014).

8.10 Methods of Assessing Oxidative Stress-Related Male Infertility

Assessing the concentration of seminal ROS in infertile men is pivotal in determining therapeutic strategies that would offer the most effective

treatment. Numerous direct and indirect modes of detection have been developed to identify the ROS levels in seminal fluid (Alahmar 2019).

8.10.1 Direct Methods of Identification

Direct methods of identifying oxidative stress-related male infertility include the following:

- Chemiluminescence assay (CLIA) is a diagnostic tool that utilizes a variety of standard enzyme immunoassay methods with immunochemical reactions to detect oxidation or reduction through light generation (Alahmar 2019).
- Flow cytometry is an immunophenotypical mode of identification that is used to measure ROS concentration. A small sample of spermatozoa is required to facilitate the assay. Assessment of ROS concentration occurs by examining the visible light scatter and fluorescence parameters of single cells and particles that migrate past lasers in a buffered salt-based solution. The ability to simultaneously measure markers is a great advantage of the assay. However, it is a costly piece of equipment that is not sustainable for widespread clinical usage (Alahmar 2019).
- Electron spin resonance (ESR) is a spectroscopic method that allows for the detection and quantitative analysis of short-lived free radicals ESR-based methods have become widely used because the process can detect free radicals without interference from the sample properties, including its phase (solid, liquid, or gas). A limitation of ESR is the possibility of neutralization occurring by rapid reactions between a free radical and a molecule rather than a spin-trapping agent (Kohno 2010).
- The MiOXSYS System is used to measure oxidation-reduction potential (ORP). ORP measures the transfer of electrons from a reductant (or antioxidant) to an oxidant. ORP is measured in millivolts (mV). ORP is an overall measure of the oxidative stress to which a biological component is subjected. MiOXSYS System provides two measures of oxidative stress. Static ORP (sORP), measured in millivolts, is the integrated measure of the existing balance between total oxidants and reductants in a biological system. After this initial sORP reading is recorded, the analyzer automatically applies a small current sweep to the sample, resulting in the exhaustion of all antioxidant species, providing a measure of antioxidant capacity reserve (cORP), measured in microcoulombs (μC). Unlike other measures, sORP represents an integrated measure of all oxidants and reductants, making it a more clinically meaningful measure when diagnosing idiopathic cases associated with high levels of oxidative stress (Agarwal et al. 2016).
- Nitroblue tetrazolium assay is used to determine the ability of cells to produce ROS, giving insight into their oxidative metabolism. During this assay, NBT is reduced and precipitated, resulting in dark blue granules (formazan). Phorbol myristate acetate (PMA) in this assay acts as a stimulant, inducing the reduction of NBT to form formazan (Aitken 2018).

8.10.2 Indirect Methods of Identification

Indirect modes of identifying oxidative stress-related male infertility include the following:

- Lipid peroxidation levels are measured through colorimetric and thiobarbituric acid assays. MDA and toxic 4-HNE are detected by identifying the by-products of lipid peroxidation (Alahmar 2019).
- Myeloperoxidase identifies granulocytes in semen. Peroxidase charge (positivity) is measured through staining using benzidine. Myeloperoxidase is suitable for white blood cell differentiation from the immature germs present in semen. However, a disadvantage of the assay is its inability to identify ROS production in spermatozoa (Alahmar 2019).
- Cytochrome c reduction test quantifies oxidation by detecting the decrease in absorbance at 500 nm of ferricytochrome c caused by its oxidation, therefore displaying evidence whether an organism contains cytochrome c, an

enzyme derived from the electron transport chain. The assay measures oxygen released through the respiratory burst of neutrophils or isolated enzymes.

- Increased levels of sperm DNA damage have adverse effects on male reproductivity. Sperm chromatin structure assay (SCSA) is used to measure and identify sperm DNA damage. Sperm with an abnormal chromatin structure is more likely susceptible to acid and heat denaturation. SCSA measures the susceptibility of sperm DNA to acid-induced denaturation in situ.
- Chemokines are generated as a by-product of ROS-induced inflammation. Chemokines are measured using commercial ELISA. The prime disadvantage of using chemokines as a measurement of ROS in semen is that more than 0.5 L of biological material is required in order to facilitate proper ROS identification and measurement.
- Oxygen radical antioxidant capacity (ORAC) is a common assay used to determine antioxidant capacity. The assay measures antioxidant ability to reduce the degradation of fluorescent dye by ROS. Briefly, the assay measures the oxidative degradation of the fluorescent molecule (such as beta-phycoerythrin or fluorescein) after being mixed with free radical generators such as azo-initiator compounds. Azo-initiators are considered to produce peroxyl radical by heating, which damages the fluorescent molecule, resulting in the loss of fluorescence. Antioxidants are considered to protect the fluorescent molecule from the oxidative degeneration (Ou et al. 2001).

8.11 Male Infertility Treatments and Oxidative Stress Management

Oxidative stress-induced male infertility can be managed by combating the underlying cause of the pathogenesis, such as the use of antioxidants to reduce the excessive ROS production accrued due to BPA toxicity, and performing testicular sperm extraction for patients with low sperm count and/or azoospermia.

8.11.1 Antioxidants

Suboptimal fertility in men is associated with oxidative stress due to an increase in the levels of ROS production, which subsequently induces DNA damage resulting in lower rates of pregnancy. Occurrence of oxidative stress due to excessive production of ROS has been reported in BPA toxicity. This means that BPA toxicity can be ameliorated with the use of antioxidants. The role of antioxidants as a method to approach male infertility due to the increase in ROS has been explored. Favorable results such as improvement in sperm quality, mitigation of DNA damage, and combating lipid peroxidation have been elucidated following treatment with antioxidants (Martin-Hidalgo et al. 2019).

The nature and characteristics of antioxidants can be generally divided into enzymatic (SOD, CAT, etc.) and small organic molecules (ascorbate, urate, etc.). Organic molecules can be subsequently classified as lipid-soluble (vitamin E) and water-soluble molecules (glutathione, urate, and ascorbate). The main mechanisms by which antioxidants act are either by inducing a chain break via donating an electron to the free radical present in the system or by removing the ROS via quenching the chain-initiating catalyst. However, antioxidants may still act by other mechanisms such as metal-ion chelation and regulation of gene expression (Lobo et al. 2010; Ali et al. 2020; Stone and Pham 2021).

Antioxidants from a nutritional and synthesis point of view can be classified into endogenous and exogenous antioxidants. Endogenous antioxidants are made of smaller molecules, and it encompasses all enzymatic antioxidants and a few nonenzymatic antioxidants. Endogenous antioxidants depend heavily on the continuous synthesis of the reduced forms of reductants. On the other hand, exogenous antioxidants can only

be obtained via diet and cannot be naturally synthesized in eukaryotic cells due to their synthetic pathways being present only in plant and microbial cells. Common dietary sources for antioxidants include tomatoes, pineapples, watermelons, and all citrus fruits which contain high amounts of vitamin C, as well as vegetable oils, nuts, broccoli and, fish that are mainly abundant with vitamin E (Sharifi-Rad et al. 2020). The primary antioxidants found in the seminal plasma include SOD, CAT, GSH-px, vitamin C, vitamin E, and zinc (Pahune et al. 2013).

Extensive research exploring oral antioxidants and their possible role in terms of treating subfertility or infertility due to an increase in ROS has been performed throughout the years. However, only a few have demonstrated an improvement in terms of fertility rates and live births (Martin-Hidalgo et al. 2019). On the other hand, there is a possibility of causing more harm than good by using oral antioxidants, as demonstrated in the "Selenium and Vitamin E Cancer Prevention Study" (SELECT) where it showed that dietary supplementation with vitamin E significantly increased the risk of prostate cancer among men (Klein et al. 2011). In the interim, the overall evidence that is present in the literature is inconclusive due to a lack of proper methods and outcome reporting on live birth rates and pregnancy. This requires studies to include properly designed randomized placebo-controlled trials that would report the role of antioxidants on pregnancy and live births (Smits et al. 2019). In summary, the use of antioxidants remains essential when treating oxidative stress-induced male sub(in)fertility.

8.11.2 Testicular Sperm Extraction

Testicular sperm extraction (TESE) is the process of sperm retrieval from focal areas of spermatogenesis in the testis. It is usually used in men with nonobstructive azoospermia (NOA) caused by a multifarious array of etiology ranging from genetic disorders to gonadal toxins such as BPA, which impairs the process of spermatogenesis by causing hormonal imbalance. Men with NOA usually have focal areas of spermatogenesis on a background of germinal cell aplasia. There are several methods for sperm extraction, and they include fine-needle aspiration (FNA), percutaneous testicular biopsy, open testicular biopsy, and microdissection TESE. TESE, micro-TESE, and FNA carry the risk of vascular supply injury during the procedure which can lead to an intratesticular hematoma, and FNA could also cause epididymal injury (Janosek-Albright et al. 2015; Schlegel 1999). This method can be used to extract healthy sperm from the focal areas of spermatogenesis in men with BPA toxicity. Additionally, in cases where BPA toxicity resulted in oligospermia, asthenozoospermia, teratozoospermia, or combinations thereof, assisted reproduction such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) can be utilized.

8.11.3 Cryopreservation

Cryopreservation is the process by which biological structures are subjugated to extremely low temperatures. This is done mainly by first introducing the specimen to a cryoprotective agent such as dimethyl sulfoxide or polyvinylpyrrolidone and cooling the samples by using chemicals such as liquid nitrogen and then storing them until they are thawed again for usage (Martin-Hidalgo et al. 2019; Jang 2017). This procedure is useful when the source of oxidative stress cannot be immediately managed. For instance, if it is anticipated that workers would have a higher exposure to BPA, which may eventually lead to BPA toxicity, workers should be advised to cryopreserve their gametes to prevent the repercussions of BPA toxicity.

8.12 Conclusion

BPA is an ideal plasticizer because of its cross-linking characteristics. However, free monomers can be released in food content after polymerization, especially on exposure to high temperatures and with reuse of the containers. This chapter has

identified that BPA exerts both estrogenic and anti-androgenic properties, which interfere and impair testicular homeostasis. Although the half-life of BPA is relatively short, successive exposure to BPA poses a risk to male fertility. These threats are unraveled when the production of ROS begins to increase above the physiological level, thus leading to the induction of oxidative stress. The initiation of oxidative stress results in alteration of diverse signaling pathways which will adversely affect male fertility. Other ways through which BPA toxicity affects male fertility include endocrine dysfunction and the subsequent alteration in spermatogenesis, as well as the ability of BPA to promote adipogenesis and enhance obesogenic phenotype. Hence, caution must be taken, especially by men of reproductive age, to mitigate the exposure to BPA.

Acknowledgments This project was partially supported by the Al Jalila Foundation.

References

Acconcia F, Pallottini V, Marino M. Molecular mechanisms of action of BPA. Dose-Response. 2015; https://doi.org/10.1177/1559325815610582.

Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79:829–43.

Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014;32:1–17.

Agarwal A, Sharma R, Roychoudhury S, Du Plessis S, Sabanegh E. MiOXSYS: a novel method of measuring oxidation reduction potential in semen and seminal plasma. Fertil Steril. 2016; https://doi.org/10.1016/j.fertnstert.2016.05.013.

Aitken RJ. Nitroblue tetrazolium (NBT) assay. Reprod Biomed Online. 2018; https://doi.org/10.1016/j.rbmo.2017.09.005.

Alahmar A. Role of oxidative stress in male infertility: an updated review. J Hum Reprod Sci. 2019;12:4–18.

Ali SS, Ahsan H, Zia MK, Siddiqui T, Khan FH. Understanding oxidants and antioxidants: classical team with new players. J Food Biochem. 2020;44:e13145.

Babakhanzadeh E, Nazari M, Ghasemifar S, Khodadadian A. Some of the factors involved in male infertility: a prospective review. Int J Gen Med. 2020;13:29–41.

Barbonetti A, Castellini C, Di Giammarco N, Santilli G, Francavilla S, Francavilla F. In vitro exposure of human spermatozoa to bisphenol A induces pro-oxidative/apoptotic mitochondrial dysfunction. Reprod Toxicol. 2016;66:61–7.

Benson TE, Gaml-Sørensen A, Ernst A, et al. Urinary bisphenol a, f and s levels and semen quality in young adult danish men. Int J Environ Res Public Health. 2021;18:1–12.

Castellini C, Totaro M, Parisi A, D'Andrea S, Lucente L, Cordeschi G, Francavilla S, Francavilla F, Barbonetti A. Bisphenol a and male fertility: myths and realities. Front Endocrinol (Lausanne). 2020; https://doi.org/10.3389/fendo.2020.00353.

Chiang C, Mahalingam S, Flaws JA. Environmental contaminants affecting fertility and somatic health. Semin Reprod Med. 2017;35:241–9.

Dabbous Z, Atkin SL. Hyperprolactinaemia in male infertility: Clinical case scenarios. Arab J Urol. 2018;16:44–52.

Ding J, Shang X, Zhang Z, Jing H, Shao J, Fei Q, Rayburn ER, Li H. FDA-approved medications that impair human spermatogenesis. Oncotarget. 2017;8:10714–25.

Du Plessis SS, Agarwal A, Halabi J, Tvrda E. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. J Assist Reprod Genet. 2015;32:509–20.

Emedicine.medscape.com. Follicle-stimulating hormone abnormalities: practice essentials, pathophysiology, epidemiology. In: Online; 2021. https://emedicine.medscape.com/article/118810-overview#a1. Accessed 22 Oct 2021.

Gandhi J, Hernandez RJ, Chen A, Smith NL, Sheynkin YR, Joshi G, Khan SA. Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. Zygote. 2017;25:103–10.

Gassman NR. Induction of oxidative stress by bisphenol a and its pleiotropic effects. Environ Mol Mutagen. 2017;58:60–71.

Jang TH. Cryopreservation and its clinical applications | Elsevier Enhanced Reader. Integr Med Res. 2017;6:12–8.

Janosek-Albright KJC, Schlegel PN, Dabaja AA. Testis sperm extraction. Asian J Urol. 2015;2:79–84.

Jia J, Xu H, Chen C, Zhang X, Zhang X, Li W, Ma J. Quantitative proteomic analysis of mouse testis uncovers cellular pathways associated with bisphenol a (BPA)-induced male infertility. Gen Physiol Biophys. 2020;39:331–41.

Klein EA, Thompson IM, Tangen CM, et al. Vitamin E and the risk of prostate cancer: the selenium and vitamin E cancer prevention trial (SELECT). JAMA – J Am Med Assoc. 2011;306:1549–56.

Kohno M. Applications of electron spin resonance spectrometry for reactive oxygen species and reactive nitrogen species research. J Clin Biochem Nutr. 2010; https://doi.org/10.3164/jcbn.10-13R.

Komarowska MD, Hermanowicz A, Czyzewska U, Milewski R, Matuszczak E, Miltyk W, Debek W. Serum bisphenol a level in boys with cryptorchidism: a step to male infertility? Int J Endocrinol. 2015;2015:973154.

Konieczna A, Rutkowska A, Rachoń D. Health risk of exposure to Bisphenol A (BPA). Rocz Państwowego Zakładu Hig. 2015;66:5–11.
Krzastek SC, Farhi J, Gray M, Smith RP. Impact of environmental toxin exposure on male fertility potential. Transl Androl Urol. 2021;9:2797–813.
Kumar S, Sharma A. Cadmium toxicity: effects on human reproduction and fertility. Rev Environ Health. 2019; https://doi.org/10.1515/reveh-2019-0016.
Li YR, Trush M. Defining ROS in biology and medicine. React Oxyg Species. 2016;1:9–21.
Li C, Zhang L, Ma T, et al. Bisphenol a attenuates testosterone production in Leydig cells via the inhibition of NR1D1 signaling. Chemosphere. 2021; https://doi.org/10.1016/j.chemosphere.2020.128020.
Liang X, Yin N, Liang S, Yang R, Liu S, Lu Y, Jiang L, Zhou Q, Jiang G, Faiola F. Bisphenol a and several derivatives exert neural toxicity in human neuron-like cells by decreasing neurite length. Food Chem Toxicol. 2020; https://doi.org/10.1016/j.fct.2019.111015.
Liu X, Miao M, Zhou Z, Gao E, Chen J, Wang J, Sun F, Yuan W, Li DK. Exposure to bisphenol-A and reproductive hormones among male adults. Environ Toxicol Pharmacol. 2015;39:934–41.
Liu XX, Wang ZX, Liu FJ. Chronic exposure of BPA impairs male germ cell proliferation and induces lower sperm quality in male mice. Chemosphere. 2021;262:127880.
Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev. 2010;4:118–26.
Loganathan SN, Kannan K. Occurrence of bisphenol a in indoor dust from two locations in the Eastern United States and implications for human exposures. Arch Environ Contam Toxicol. 2011;61:68–73.
Martin-Hidalgo D, Bragado MJ, Batista AR, Oliveira PF, Alves MG. Antioxidants and male fertility: from molecular studies to clinical evidence. Antioxidants. 2019;8:89.
Masuyama H, Hiramatsu Y. Involvement of suppressor for Gal 1 in the ubiquitin/proteasome-mediated degradation of estrogen receptors. J Biol Chem. 2004; https://doi.org/10.1074/jbc.M312762200.
Melgarejo M, Mendiola J, Koch HM, Moñino-García M, Noguera-Velasco JA, Torres-Cantero AM. Associations between urinary organophosphate pesticide metabolite levels and reproductive parameters in men from an infertility clinic. Environ Res. 2015; https://doi.org/10.1016/j.envres.2015.01.004.
Meli R, Monnolo A, Annunziata C, Pirozzi C, Ferrante MC. Oxidative stress and BPA toxicity: an antioxidant approach for male and female reproductive dysfunction. Antioxidants. 2020;9:405.
Mima M, Greenwald D, Ohlander S. Environmental toxins and male fertility. Curr Urol Rep. 2018; https://doi.org/10.1007/s11934-018-0804-1.
Mínguez-Alarcón L, Bellavia A, Gaskins AJ, Chavarro JE, Ford JB, Souter I, Calafat AM, Hauser R, Williams PL. Paternal mixtures of urinary concentrations of phthalate metabolites, bisphenol A and parabens in relation to pregnancy outcomes among couples attending a fertility center. Environ Int. 2021; https://doi.org/10.1016/j.envint.2020.106171.
Ni K, Steger K, Yang H, Wang H, Hu K, Zhang T, Chen B. A comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic, and astheno/oligozoospermic clinical varicocoele. Andrology. 2016;4:816–24.
Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem. 2001; https://doi.org/10.1021/jf010586o.
Pahune PP, Choudhari AR, Muley PA. The total antioxidant power of semen and its correlation with the fertility potential of human male subjects. J Clin Diagnostic Res. 2013;7:991–5.
Pallotti F, Pelloni M, Gianfrilli D, Lenzi A, Lombardo F, Paoli D. Mechanisms of testicular disruption from exposure to bisphenol a and phtalates. J Clin Med. 2020;9:471.
Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. Oxidative Med Cell Longev. 2017;2017:8416763.
plasticstoday. BPA alternatives also pose health risks, study finds. In: Online; 2019. ps://www.plasticstoday.com/medical/bpa-alternatives-also-pose-health-risks-study-finds. Accessed 21 Oct 2021.
Rahman MS, Pang WK, Ryu DY, Park YJ, Ryu BY, Pang MG. Multigenerational impacts of gestational bisphenol a exposure on the sperm function and fertility of male mice. J Hazard Mater. 2021;416:125791.
Rana SVS. Perspectives in endocrine toxicity of heavy metals - a review. Biol Trace Elem Res. 2014; https://doi.org/10.1007/s12011-014-0023-7.
Rezaee-Tazangi F, Zeidooni L, Rafiee Z, Fakhredini F, Kalantari H, Alidadi H, Khorsandi L. Taurine effects on bisphenol a-induced oxidative stress in the mouse testicular mitochondria and sperm motility. J Bras Reprod Assist. 2020;24:428–35.
Rochester JR. Bisphenol a and human health: a review of the literature. Reprod Toxicol. 2013;42:132–55.
Santiago J, Silva JV, Santos MAS, Fardilha M. Fighting bisphenol a-induced male infertility: the power of antioxidants. Antioxidants. 2021;10:1–22.
Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod. 1999;14:131–5.
Schulster M, Bernie AM, Ramasamy R. The role of estradiol in male reproductive function. Asian J Androl. 2016;18:435–40.
Sharifi-Rad M, Anil Kumar NV, Zucca P, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694.
Sharma A, Mollier J, Brocklesby RWK, Caves C, Jayasena CN, Minhas S. Endocrine-disrupting chemi-

cals and male reproductive health. Reprod Med Biol. 2020;19:243–53.

Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. Cochrane Database Syst Rev. 2019; https://doi.org/10.1002/14651858.CD007411.pub4.

Stone WL, Pham TMS. Biochemistry, antioxidants. Online. StatPearls Publishing; 2021.

Tremellen K. Oxidative stress and male infertility: a clinical perspective. Stud Men Heal Fertil. 2012;14:325–53.

Twigg J, Irvine DS, Houston P, Fulton N, Michael L, Aitken RJ. Iatrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma. Mol Hum Reprod. 1998;4:439–45.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol a (BPA). Reprod Toxicol. 2007;24:139–77.

Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018; https://doi.org/10.1016/j.clinbiochem.2018.03.012.

Vitku J, Sosvorova L, Chlupacova T, Hampl R, Hill M, Sobotka V, Heracek J, Bicikova M, Starka L. Differences in bisphenol a and estrogen levels in the plasma and seminal plasma of men with different degrees of infertility. Physiol Res. 2015;64:S303–11.

Wang C, Qi S, Liu C, Yang A, Fu W, Quan C, Duan P, Yu T, Yang K. Mitochondrial dysfunction and Ca2+ overload in injured sertoli cells exposed to bisphenol a. Environ Toxicol. 2017;32:823–31.