Effects of bisphenols on testicular steroidogenesis

Federica Barbagallo[1], Rosita A. Condorelli[1], Laura M. Mongioì[1], Rossella Cannarella[1], Antonio Aversa[2], Aldo E. Calogero[1], Sandro La Vignera[1*]

[1]University of Catania, Italy, [2]Department of Clinical and Experimental Medicina, Magna Graecia University of Catanzaro, Italy

Submitted to Journal:
Frontiers in Endocrinology

Specialty Section:
Reproduction

Article type:
Review Article

Manuscript ID:
523987

Received on:
01 Jan 2020

Revised on:
03 May 2020

Frontiers website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

FB, SLV, AEC, concept and design; RAC, LM and RC, articles research; FB, writing of the original draft; SLV, AEC and AA, final approval.

Keywords

Bisphenols, BPA (bisphenol A), Endocrine Disruptors, Testicular steroidogenesis, Testosteron

Abstract

Word count: 273

Over the last decades, the adverse effects of human exposure to the so-called “endocrine disruptors” have been the subject of scientific debate and media interest, with great concern for the toxicity on reproductive function. Bisphenols are synthetic chemicals, widely used in the manufacture of hard plastic products. Bisphenol A (BPA) is among the best known environmental toxicants proven to be related to the impairment of male reproductive function and other health problems. BPA is known to migrate from packaging materials into foodstuffs and liquids. Consumer concern resulted in “BPA free” products and in the gradual development of a number of bisphenol analogs (BPA-A) to replace BPA in several applications. However, these other bisphenols derivatives seem to have effects similar to those of BPA. BPA can exhibit weak estrogenic and antiandrogenic proprieties. It interferes with the hypothalamic-pituitary-testicular axis and modulates the gene expressions and enzyme activities involved in steroidogenesis. In addition, it also appears to be involved in DNA methylation. The antiandrogenic properties of BPA have been described in various experimental animal studies. However, the evidence on humans remains ambiguous. Contradictory outcomes may depend on several factors including experimental design, BPA dose, timing and route of exposure and other confounding factors. The effects of BPA appear to be most relevant during development. BPA has been proposed to influence fetal testis development and predispose to testicular dysgenesis syndrome. This includes anatomical abnormalities identified at birth, such as cryptorchidism and hypospadias, but also disorders that occur in adulthood, including testicular tumors, hypogonadism and/or infertility. This review aims to summarize the evidence on the relationship between BPA and testicular function, focusing on its effects on testicular steroidogenesis.

Contribution to the field

Bisphenols are synthetic chemicals, widely used in the manufacture of hard plastic products. Bisphenol A (BPA) is among the best known environmental toxicants proven to be related to the impairment of male reproductive function and other health problems. BPA can exhibit weak estrogenic and antiandrogenic proprieties. However, the evidence on humans remains ambiguous. Contradictory outcomes may depend on several factors including experimental design, BPA dose, timing and route of exposure and other confounding factors. This review aims to summarize the evidence on the relationship between BPA and testicular function, focusing on its effects on testicular steroidogenesis.

Funding statement

No funding has been received.

Effects of bisphenols on testicular steroidogenesis

Running title: Bisphenols and testicular steroidogenesis

Federica Barbagallo [1], Rosita A. Condorelli[1], Laura Mongioì[1], Rossella Cannarella[1], Antonio Aversa[2], Aldo E. Calogero[1], Sandro La Vignera[1]

[1]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

[2]Department of Experimental and Clinical Medicine, "Magna Graecia" University, Catanzaro, Italy

For submission to: Frontiers in Endocrinology

Keywords: Bisphenols, BPA, endocrine disruptors, testicular steroidogenesis

Corresponding author: Rosita A. Condorelli, Department of Clinical and Experimental Medicine, Via S. Sofia 78, 95123 Catania, Italy. Email: rosita.condorelli@unict.it

Abstract

Over the last decades, the adverse effects of human exposure to the so-called "endocrine disruptors" have been matter of scientific debate and public attention. Bisphenols are synthetic chemicals, widely used in the manufacture of hard plastic products. Bisphenol A (BPA) is one of the best known environmental toxicants proven to alter the reproductive function in men and to cause other health problems. Consumer concern resulted in "BPA free" products and in the development of bisphenol analogs (BPA-A) to replace BPA in many applications. However, these other bisphenol derivatives seem to have effects similar to those of BPA. Although a number of review have summarized the effects of BPA on human reproduction, the purpose of this article is to review the effects of bisphenols on testicular steroidogenesis and to explore their mechanisms of action. Testicular steroidogenesis is a fine-regulated process and its main product, testosterone (T), has a crucial role in fetal development and maturation and in adulthood for the maintenance of secondary sexual function and spermatogenesis. Contradictory outcomes of both human and animal studies on the effects of BPA on steroid hormone levels may related to various factors that include study design, dosage of BPA used in *in-vitro* studies, timing and route of exposure, and other confounding factors. We described the main possible molecular target of bisphenols on this complex pathway. We report that Leydig cells (LCs), the steroidogenic testicular component, are highly sensitivity to BPA and several mechanisms concur to the functional impairment of these cells.

1. Introduction

Over the last decades, the adverse effects of human exposure to the so-called "endocrine disruptors" have been matter of deep debate by the scientific community and the layman. Particular attention has been paid to their toxicity on the reproductive function. Bisphenol A (2,2-bis(4-hydroxyphenyl) propane) (BPA) is among the most well-known endocrine disruptors proven capable of impairing the male reproductive function and to cause other health problems. BPA is an organic synthetic compound, including to the group of dyphenylmenthane derivatives and bisphenols, widely used in the manufacture of hard plastic products. BPA has been used since the 1950s, in food packaging, industrial materials, dental sealants, personal hygiene products, and thermal receipts (Rochester et al., 2013; Huo et al., 2015). A significant exposure to BPA for children is given by toys, books, and feeding bottles (Brede et al., 2003; Sajiki et al., 2010). BPA penetrates the body through the skin, inhalation and the digestive system (Kang et al., 2006). Once adsorbed, BPA is then metabolized by the liver and excreted with the urine in 24 hours (Huo et al., 2015). Despite the rapid metabolism, BPA can accumulate in different tissues (Komarowska et al., 2015). Consumer concern for BPA effects on health, resulted in "BPA free" products and in the development of bisphenol analogs to replace BPA in many applications. However, these compounds seem to have endocrine disrupting capabilities similar to BPA and their impact on reproduction has been little investigated (Roelofs et al., 2015; Rochester & Bolden, 2015; Siracusa et al., 2018).

BPA seems to influence fetal testis development and predispose to the testicular dysgenesis syndrome (TDS). This syndrome may manifest itself not only at birth with cryptorchidism and hypospadias, but also in adulthood when it shows up with testicular tumors, hypogonadism and/or infertility (Matuszczak et al., 2019). Current evidence suggest that BPA can cause testicular histological abnormalities which encompass dysregulated proliferation and apoptosis of Leydig cells (LCs) and alteration of steroidogenesis (Williams et al., 2014). In mice, pubertal exposure to high doses of BPA causes LC and germ cells apoptosis, resulting in underdeveloped testis with histopathological changes including atrophied seminiferous tubules, decreased number of late spermatids and increased karyopyknotic cells (Li et al., 2009). The reduction of testicular weight and the alteration of spermatogenesis persist till adulthood, long after the period of BPA exposure (Li et al., 2009). The gestational period is a sensitive window of exposure to BPA. Male rats maternally exposed to BPA from gestation to the postnatal period have low testicular weight and testosterone (T) levels in the testicular

interstitial fluid in adulthood (Akingbemi et al., 2004). These effects may involve different molecular pathways discussed in the section 3b.

Many studies have investigated the effects of BPA on human reproduction and extensive reviews have addressed the strength of the evidence on BPA toxicity (Vom Saal et al., 2007; Perets et al., 2014; Siracusa et al., 2018; Matuszczak et al., 2019). Contradictory outcomes may depend on several factors including study design, BPA dose, timing and route of exposure and other confounding factors (Peretz et al., 2014). Several mechanisms of action have been described. First of all, BPA exhibits weak estrogenic and antiandrogenic proprieties. It binds to both estrogen receptors (ERs), ERα and ERβ (Rochester et al., 2013; Matuszczak et al., 2019) and. at high concentrations, BPA binds to the androgen receptor (AR) on which it acts as an antagonist (Hejmej et al., 2011). In addition to binding to the ARs, it disturbs the hypothalamic-pituitary-testicular axis and modulates gene expression and the enzymatic activity of testicular steroidogenesis (Hejmej et al., 2011). Furthermore, exposure to BPA is also associated with a decrease in the activity of the antioxidant system, resulting in increased oxidative stress, the most common cause of sperm damage (Wang et al., 2014; Lanzafame et al., 2009). Although several studies have supported the harmful effects of BPA on testicular function, its mechanism(s) remains not fully understood.

The purpose of this article is to review the evidence on the relationship between bisphenols and testicular steroidogenesis, focusing on their mechanism(s) of action on LCs function.

2. Testicular steroidogenesis

The testis is a complex endocrine organ regulated by intra- and extra-testicular pathways that interact synergistically (Tena-Sempere et al., 2002). Leydig cells (LCs) have a crucial role in the regulation of steroidogenesis and spermatogenesis. LCs produce testosterone (T), which has a main role in fetal development and maturation. During the masculinization programming window, the fetal testes begin to produce T, which allows male gonadal differentiation and development (Scott et al., 2009). Hence, T is necessary for the maintenance of secondary sexual function and spermatogenesis (Mathur & D'Cruz, 2011). Intratesticular T levels are approximately one hundred times higher than the levels found in systemic circulation (Ochsenkuhn and De Kretser, 2003). The high local production rate of T implies the need for its intratesticular transport from LCs to Sertoli cells which nourish and support the development of the germinal cells during the various stages of spermatogenesis (Dankers et al., 2013). LCs derive from mesenchymal cells located in the interstitial

compartment of the testis. Their development occurs through three different stages during which they are called progenitor, immature and adult LCs. Apoptosis seems to have a main role in maintaining a constant population of LCs, although other mechanisms may be involved (Siracusa et al., 2018).

LCs produce T in response to the luteinizing hormone (LH). LH binding to the LH receptors (LHR) on LCs activates Gs protein and adenylyl cyclase, increasing cAMP levels. cAMP acts as a key second messenger and up-regulates the expression of genes related to the steroidogenesis (Dufao et al., 1988). The steroidogenesis consists in a complex multi-enzyme process by which precursor cholesterol is converted to biologically active steroid hormones in a tissue specific manner (Figure 1). Cholesterol can be synthesized in the endoplasmic reticulum but the first source of this precursor for steroidogenesis is via uptake of cholesteryl esters from high-density lipoprotein by the scavenger receptor SR-B1 (Shen et al., 2016). Therefore, SR-B1 has a key role for the maintenance of cholesterol balance. The first step in steroidogenesis takes place within mitochondria. The steroidogenic acute regulatory protein (StAR) mediate the transport of cholesterol from the outer to the inner mitochondrial membrane (Devoto et al., 2002). The StAR-mediated transport of cholesterol is a crucial step for steroidogenesis (Stocco et al., 1996; Hasegawa et al., 2000) and appropriate concentrations of cAMP are necessary for the regulation of StAR expression (Stocco et al., 1997). However, cAMP/PKA is not the only pathway that regulates StAR expression. Other factors such as steroidogenic factor, activator protein and cAMP-response element-binding protein are also associated with StAR regulation (Stocco et al., 2005). Then, cholesterol is metabolized to pregnenolone into the smooth endoplasmic reticulum through a cascade of reactions that are catalyzed by the cytochrome P-450 proteins. Pregnenolone then is converted to T by 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α-hydroxylase/17,20 lyase (CYP17A1) and 17β-hydroxysteroid dehydrogenase (17β-HSD). This complex process of steroidogenesis itself can be responsible for the increase of reactive oxygen species (ROS) (Hanukoglu, 2006). Thus, the normal products of steroidogenesis can act as pseudosubstrates and interact with P-450 enzymes, resulting in a pseudosubstrate–P-450–O_2 complex, which is a source of dangerous free radicals (Quinn & Payne, 1985).

3. Bisphenols and testicular steroidogenesis

3.a. Effects of bisphenol A on steroid hormone levels

Experimental studies in male animals have shown that exposure to BPA is associated with altered hormone levels suggesting direct effects of BPA on LCs. However, these data are discordant. Low-dose BPA decreased

T levels in CD-1 mice exposed during perinatal and postnatal periods (Xi et al., 2011), but not in adult C57BL/6 mice exposed in utero (LaRocca et al., 2011). In addition, low-dose BPA lowered T levels in Holtzman rats exposed during gestation or in the neonatal age (Salian et al., 2009a; Salian et al., 2009b) and albino (El Beshbishy et al., 2012) and Wistar (D'Cruz et al., 2012a) rats exposed in adulthood. In contrast, by examining the gestational and neonatal exposure of low-dose BPA in Long Evans (Howdeshell et al., 2008) or Sprague-Dawley (SD) rats (Kobayashi et al., 2012, Qiu et al., 2013), the levels of T did not change. Treatment with increasing concentrations of BPA (1 to 1000 nM) did not significantly lower basal or hCG-stimulated T secretion by primary culture of LCs of young adult male rats (Muromo et al., 2001). However, although Sánchez and colleagues reported that low-dose BPA did not decrease T levels in Wistar rats, dihydrotestosterone levels decreased (Sánchez et al. 2013). Gamez and colleagues reported that exposure to low-doses BPA led to an increase in serum LH and FSH levels in young Wistar rats (Gamez et al., 2014). Nevertheless, another study in adult Wistar rats showed that exposure to BPA decreased serum T, LH and FSH levels, but increased the levels of 17ß-estradiol (E_2) (Wisniewski et al., 2015). In two studies in SD rats, postnatal exposure to low-dose BPA decreased serum T and E_2 levels (Guurmet et al., 2014). BPA exposure lowered T levels in Swiss albino and C57BL/6 mice, but at variable dosage between 0.5 µg/kg and 100 mg/kg (Chouhan et al., 2015; Zang et al., 2016). Sadowski and colleagues described a decrease in FSH concentrations in Long-Evans rats at weaning, after exposure to BPA at both 4 and 400 µg/kg/day (Sadowski et al., 2014). An *in-vitro* study conducted on fetal testes explanted from mice, rats and humans demonstrated that exposure to 10 nM of BPA was enough to decrease basal T secretion in human fetal testes, but higher concentrations were required in rats and mice (10 µM and 1 µM, respectively) (N'Tumba-Byn et al., 2012).

The epidemiological studies evaluating the effects of BPA exposure on serum hormone levels in men have also shown conflicting results. In the INChianti adult population study, Galloway and colleagues found a correlation between higher urinary BPA concentrations and higher serum T, but not E_2 levels in 307 Italian men living in Chianti, Italy (Galloway et al., 2010). Another study, conducted on 308 young men from Denmark's general population, reported that higher urinary BPA concentration was associated with a significant increase of LH, T and E_2 levels (Lassen et al., 2014). In contrast, in a cross-sectional study of 290 men, Zhou and colleagues found that increased serum BPA concentrations were statistically significantly associated with the reduction of androstenedione, free T and free androgen index (FAI) levels and with the increase of sex hormone-binding globulin (SHBG) levels (Zhou et al., 2013). Two cross-sectional studies,

respectively of 167 and 302 men, did not report any associations between BPA and T concentrations (Meeker et al., 2010; Mendiola et al., 2010). According to Meeker and colleagues, men with elevated urinary BPA concentrations had higher FSH and lower inhibin B levels with a higher FSH/inhibin B ratio and a lower E_2/T ratio (Meeker et al., 2010). Mendiola and colleagues found that higher urinary BPA levels were associated with lower FAI and FAI/LH and free T/LH ratios in fertile men (Mendiola et al., 2010). Two cross-sectional studies reported that urinary BPA levels were associated with higher SHBG in men occupationally exposed to BPA (Liu et al., 2015; Zhuangh et al., 2015). The NHANES 2011-2012 study showed an inverse correlation between urinary BPA levels and serum T concentrations in male adolescents (Scinicariello & Buser, 2016). However, a retrospective cohort study did not find any effects on hormone levels in boys aged 8 to 14 years after prenatal or childhood exposure to BPA (Ferguson et al., 2014).

Although these results are controversial, they suggest that BPA alters steroid hormones pathways in men.

3.b. Bisphenol A molecular mechanisms of action on testicular steroidogenesis

Although both animal and human studies support the harmful effects of BPA on steroid hormones, the mechanism of action of BPA in negatively interfering with testicular steroidogenesis still remains unclear. Since LCs are the site of testicular steroidogenesis, several studies have been conducted on these cells to investigate the effects of BPA. In Wistar/ST pubertal rats, continuous exposure to BPA at high doses reduced the number of LCs and the expression of steroidogenic enzymes in these cells (Nakamura et al. 2010). In contrast, Long-Evans rats exposed to low dose of BPA during gestation and at birth had an increase in the number of LCs in adulthood through the up-regulation of mitogen factors. However, although low-dose of BPA increased LC proliferation, the expression of steroidogenic enzymes and T biosynthesis decreased (Nanjappa et al. 2012). Chen and colleagues reported that BPA did not stimulate staminal LC proliferation but it induced the differentiation of stem LCs into more mature LCs. They used an *in-vivo* ethane dimethane sulfonate (EDS)-induced LC regeneration model to mimic the pubertal development of LCs. They treated rats with EDS to eliminate LCs and then, they injected BPA within the testis. The intratesticular injection of BPA avoided possible interference of hypothalamus and pituitary. The results of this study showed that BPA significantly increased the number of 11β-HSD1 positive cells, which is a biomarker for LCs at an advanced stage. Thus, BPA promoted the differentiation of staminal LCs, increasing T production and upregulating LC

specific genes (LHCGR, StAR, CYP11A1, HSD3B1, CYP17A1, HSD17B3 and HSD11B1). These findings suggest a possible role of BPA in sexual precocious puberty in males (Chen et al., 2018). Exposure to high-doses of BPA (480 and 960 mg/kg/day at postnatal days 31-44) has been reported to induce apoptosis in Leydig and germ cells *via* the upregulation of Fas, FasL and caspase-3 (Li et al., 2009). The apoptosis of LCs was associated with a decreased testicular testis weight and histopathological changes, which persisted into adulthood (Li et al., 2009). In another study, Thuillier and colleagues reported that SD rats exposed in-utero to BPA had an increase number of LCs but did not present significant change in serum T levels (Thuillier et al., 2009). Moreover, BPA can also induce *Nur77* gene expression, an orphan nuclear receptor which plays an important role in the regulation of LH-mediated steroidogenesis, altering LC steroidogenesis (Song et al., 2002). BPA induced Nur77 gene expression via PKA and MAPK signaling pathways in a time- and dose-dependent manner. BPA-mediated Nur77 expression resulted in the upregulation of steroidogenesis both *in-vitro* and *in-vivo*, with a significant increase of T synthesis (2-fold) (Song et al., 2002).

The inhibition of testicular steroidogenesis by BPA can be also associated with a decreased LH secretion. Akingbemi and colleagues reported that Long-Evans rats exposed to low doses of BPA (2.4 µg/kg*d) from postnatal days 21-35, decreased both serum LH and T levels, downregulating pituitary LHβ expression but increasing ERβ pituitary mRNA levels (Akingbemi et al., 2004).

The expression of LH and FSH receptors may also be altered by BPA. Li and colleagues showed that treatment of adult male zebrafish (Danio rerio) by 500 ng/L BPA for 7 weeks down-regulated the expressions of FSHr and LHCGr (Li et al., 2017). For the first time, Roelofs and colleagues demonstrated that BPA, BPF, and TBBPA showed clear glucocorticoid receptor antagonism, other than AR antagonism. They also found that bisphenol analogues up-regulated the 5αRed1 gene expression suggesting a redirection of steroidogenesis, which may have significant consequences for fetal testis development and function (Roelofs et al., 2015).

Within the steroid hormone biosynthetic pathway, steroidogenic enzymes are recognized as important targets for the actions of endocrine-disrupting chemicals. Several studies showed that BPA decreases the expression of steroidogenic enzymes (Nakamura et al. 2010; Xi et al. 2011; Horstman et al. 2012; Naijappa et al., 2012; Qiu et al. 2013; Samova et al., 2018). Moreover, some compounds, including BPA, seem to disturb steroidogenesis by inhibiting the cAMP pathway. Nikula and colleagues analyzed the effects of BPA at micromolar concentration in cultured mouse Leydig tumor cells (mLTC-1). BPA did not have any effects on hCG binding to LH receptors but it inhibited LH-receptor mediated signal transduction by decreasing hCG-

stimulated cAMP. Specifically, they found that after preincubation of mLTC-1 cells for 48 h with different doses of BPA, hCG-stimulated cAMP and progesterone production was inhibited. Whereas preincubation with 17ß-estradiol inhibited progesterone production but had no effect on cAMP. Thus, the effects of BPA did not seem to be estrogen-related (Nikula et al., 1999). Moreover, the inhibitory effect of BPA could not be seen when cAMP formation was directly stimulated by forskolin (Fk) or through Gs protein by cholera toxin (CT), and when steroidogenesis was directly activated by 8-Br-cAMP which can penetrate the plasma membranes and directly activate the protein kinase A. These results suggested that the negative effect of BPA is exerted between the LH receptor and the adenylate cyclase. Accordingly, Feng and colleagues found that BPA exposure inhibited progesterone secretion in hCG-stimulated mouse Leydig tumor cell line (mLTC-1) by decreasing SR-B1 and P450scc expression due to the adverse effects on cAMP. Moreover, lower SR-B1 levels cause a reduction in cholesterol levels within LCs that alters steroidogenesis (Feng et al., 2018). The role of StAR is instead controversial. According to Feng and colleagues (2018), StAR seem not be the molecular target of BPA. Similarly, male rats exposed to BPA showed decreased T levels but did not exhibit significant changes in StAR expression (Nanjiappa et al., 2012). However, other previous studies have reported that BPA decreased StAR expression in cell culture *in-vitro* (Peretz et al., 2011; Xi et al., 2011; Chouhan et al., 2014), but, in contrast, other studies have shown that StAR expression is upregulated (Qiu et al., 2013; Li et al., 2017). Takamiya and colleagues reported that StAR gene expression increased in presence of both hCG (10 µg/l) plus BPA (10^{-5} M) or by hCG alone, but was not influenced by BPA alone. They found that BPA had only a weak modulating effect on gene expression of hCG-stimulated mLTC-1 cells (Takamiya et al., 2007). Li and colleagues showed that the exposure of adult male zebrafish to low doses (0.22 nM-2.2 nM) of BPA for 7-weeks resulted in abnormal expression of genes involved in testicular steroidogenesis, specifically of 3β-HSD1, CYP17A1 and CYP11C1 (Li et al., 2017). Samova and colleagues found that BPA significantly and dose-dependently affected the functions of 3β-HSD and 17β-HSD in the testis of inbred Swiss strain male albino mice (Samova et al., 2018). Ye and colleagues reported that BPA significantly inhibited 3β-HSD, CYP17A1 and 17β-HSD3 activities in both human and rat testis. However, the inhibition of 17β-HSD3 activity was much weaker compared with that on the other two enzymes. They also found that human enzymes were more sensitive to BPA (Ye et al., 2011). Specifically, their results suggested that BPA did not exert a competitive inhibition of 3β-HSD against its substrate (pregnenolone), but it competed with the cofactor NAD+ in the cofactor binding site of the enzyme. Whereas BPA inhibition of CYP17A1 was mixed-type for

enzyme substrate progesterone, indicating a combination of two different types of reversible enzyme inhibition, both competitive and uncompetitive (Ye et al., 2011). Additionally, not only BPA, but also bisphenol S (BPS) and bisphenol F (BPF) exposure decreased T production in fetal mouse testis by inhibiting mRNA expression of StAR, 3β-HSD and cytochrome P45017A1 (CYP17A1), but not of P450scc (Eladak et al., 2015). Moreover, Dankers and colleagues suggested that the changes in T secretion after BPA or TBBPA exposure were only partly due to alterations of steroidogenic enzyme expression. These authors hypothesized that the inhibition of ATP-binding cassette (ABC) transporters, expressed in the blood-testis barrier (BTB), may play a role in this process. The BTB divides the seminiferous epithelium into a basal and an apical compartment and provides structural and protective support for the differentiation of spermatogonia into spermatocytes. It consists of tight junctions, testis-specific atypical adherent junctions, desmosomes and gap junctions. In the active part of BTB, ABC transporters are present to allow the passage of endogenous molecules involved in cellular signaling and to block the passage of dangerous compounds within the testes and to protect germ cells. The cellular membranes of LCs, Sertoli cells and capillary endothelial cells are provided of these transporters. For this reason, the association between endocrine disruptors and ABC transporters has a strong toxicological impact (Dankers et al., 2013). The breast cancer resistance protein (BCRP/*ABCG2*), the P-glycoprotein (P-gp/*ABCB1*) and the multidrug resistance proteins 1 and 4 (MRP1,4/*ABCC1,4*) are the major efflux transporters in the BTB with a differential expression in the various parts of the BTB (Dankers et al., 2013). LCs express P-gp, MRP1 and MRP4, but not BCRP in adult human testis (Bart et al., 2004; Morgan et al., 2012). Dankers and colleagues investigated the effects of BPA and of TBBPA (tetrabromobisphenol A) on BCRP, MRP1, MRP4 and P-gp. They found that TBBPA inhibited all these transporters; thus, it is considered a noncompetitive transporter inhibitor; whereas BPA inhibited only BCRP activity. They also showed that BPA, but not TBBPA, is transported by BCRP (Dankers et al., 2013). Interestingly, they found that, although exposure to BPA and TBBPA significantly increased T level in MA-10 cells, the effects on steroidogenic genes were not so significant. Thus, these authors hypothesized that the changes in T levels upon BPA or TBBPA exposure were associated to the inhibition of efflux of T precursors. Increased availability of these precursors, such as androstenedione or DHEA, could be responsible of the increased T levels found.

Moreover, many compounds increase the levels of ROS in the testis, altering steroidogenesis. Oxidative stress has also been found to induce apoptosis in LCs and germ cells (Song et al.,2008). Recent

studies have reported an inverse relationship between NOS activity and StAR expression (Srivastava et al., 2007). Srivastava and colleagues exposed Swiss albino mice to BPA at concentrations of 0.5, 50 and 100μg/kg body weight/day intraperitoneally for 60 days. They showed that BPA upregulated the expression of iNOS, downregulating the expression of StAR in mouse testis (Srivastava et al., 2007). It was also supposed that BPA impaired steroidogenesis by decreasing testicular glucose levels (D'Cruz et al., 2012). Glucose homeostasis is crucial for testicular spermatogenesis and steroidogenesis. D'Cruz and colleagues reported that low dose BPA exposure impaired insulin signaling interacting with GLUT-2 and GLUT-8 and inhibiting the uptake in the testis (D'Cruz et al., 2012).

Recently, a number of studies suggest epigenetic effects of BPA, including DNA methylation, histone modifications and non-coding RNAs. Epigenetic mechanisms can have long-term effects and may be transmitted across several generations (Kundakovic & Champagne, 2011). Specifically, Gao and colleagues (Gao et al., 2018) have recently investigated the epigenetic effects of BPA on the expression of non-coding RNAs (e.g. microRNAs) in the regulation of testicular steroidogenesis. They used both cell culture and *in-vivo* mouse models and showed that miR-146a-5p was expressed only in LCs and this expression was significantly induced by BPA. Consequently, the high miR-146a-5p expression intensifies the negative effects of BPA on testicular steroidogenesis by directly targeting the 3' UTR of Mta3 gene (Gao et al., 2018). Mta3 is a subunit of the Mi-2/nucleosome remodelling and deacetylase (NuRD) protein complex that is exclusively expressed in LCs (He et al., 2016). Specifically, Mta3 role in the control of testicular steroidogenic function is proven by its negative regulation by the high levels of circulated insulin (He et al., 2016). He and colleagues showed that a deficiency of Mta3 in LCs of diabetic mice was associated with low serum T level, indicating that Mta3 expression in LCs may be associated with androgen deficiency (He et al., 2016). Thus, the downregulation of mir-146a-5p/Mta3 cascade seems to be involved in steroidogenic alterations caused by BPA (Gao et al., 2018).

DNA methylation is one of the best characterized epigenetic mechanisms. Liu and colleagues investigated the effects of BPA on DNA methylation in rare minnow Gobiocypris rarus. DNA hypermethylation consists of an addition of a methyl group to the cytosine bases of DNA to form 5-methylcytosine and it may be associated with changes in gene expression. In their study, Liu and colleagues found that the global DNA methylation level was significantly increased in testis of male Gobiocypris rarus exposed to BPA for 7 days. Then, they specifically analyzed the change in DNA methylation in the 5' flanking region of the cytochrome P450 aromatase (CYP19A1A) gene. After 35-day exposure, the DNA methylation

levels of CYP19A1A did not have significant change in the testis, whereas they significantly increased in the ovary (Liu et al.,2014).

Conclusions

This review summarizes the current evidences on the association between BPA and testicular steroidogenesis. Altogether, these results show that LCs are very sensitive to BPA and that several mechanisms concur to the functional impairment of these cells. Testicular steroidogenesis is a complex and fine regulated process and each component of this pathway may be the molecular target of BPA. The main possible sites of BPA action are summarized in the Figure 2. The conflicting results of both human and animal studies may be related to various factors that include study design, dose of BPA, timing and route of exposure and other confounding factors. This review confirms that the widespread use of bisphenols is certainly dangerous for testicular development and function, and that a reduction of its use is necessary to preserve male sexual and reproductive health.

Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding Statement

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

1. Akingbemi Benson T, Sottas Chantal M, Koulova Anna I, Klinefelter Gary R, Hardy Matthew P. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells, Endocrinology, 145 (2), 592-603 Feb 2004.
2. Bart J, Hollema H, Groen HJ, de Vries EG, Hendrikse NH, Sleijfer DT, Wegman TD, Vaalburg W, van der Graaf WT. The distribution of drug-efflux pumps, P-gp, BCRP, MRP1 and MRP2, in the normal blood-testis barrier and in primary testicular tumours. Eur J Cancer. 2004 Sep;40(14):2064-70.
3. Brede C, Fjeldal P, Skjevrak I, Herikstad H. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Addit Contam. 2003 Jul;20(7):684-9.
4. Chen L, Zhao Y, Li L, Xie L, Chen X, Liu J, Li X, Jin L, Li X, Ge R. Bisphenol A Stimulates Differentiation of Rat Stem Leydig Cells in Vivo and in Vitro. Mol Cell Endocrinol, 474, 158-167 2018 Oct 15
5. Chouhan S, Yadav SK, Prakash J, Westfall S, Ghosh A, Agarwal NK, Singh SP, Increase in the expression of inducible nitric oxide synthase on exposure to bisphenol A: A possible cause for decline in steroidogenesis in male mice, Environ Toxicol Phar 39(1) (2015) 405–416.
6. Dankers AC, Roelofs MJ, Piersma AH, Sweep FC, Russel FG, van den Berg M, van Duursen MB, Masereeuw R. Endocrine disruptors differentially target ATP-binding cassette transporters in the blood-testis barrier and affect Leydig cell testosterone secretion in vitro. Toxicol Sci. 2013 Dec;136(2):382-91.
7. Delclos KB, Camacho L, Lewis SM, Vanlandingham MM, Latendresse JR, Olson GR, Davis KJ, Patton RE, da Costa GG, Woodling KA, Bryant MS, Chidambaram M, Trbojevich R, Juliar BE, Felton RP, Thorn BT, Toxicity Evaluation of Bisphenol A Administered by Gavage to Sprague Dawley Rats From Gestation Day 6 Through Postnatal Day 90, Toxicol Sci 139(1) (2014) 174–197.
8. D'Cruz SC, Jubendradass R, Jayakanthan M, Rani SJA, Mathur PP, Bisphenol A impairs insulin signaling and glucose homeostasis and decreases steroidogenesis in rat testis: An in vivo and in silico study, Food and Chemical Toxicology (3–4) (2012) 1124.

9. Devoto L, Kohen P, Vega M, Castro O, González RR, Retamales I, Carvallo P, Christenson LK, Strauss JF Control of human luteal steroidogenesis. Mol Cell Endocrinol. 2002 Jan 25;186(2):137-41.
10. Dufau ML. Endocrine regulation and communicating functions of the Leydig cell. Annu Rev Physiol. 1988;50:483-508.
11. El-Beshbishy HA, Aly HAA, El-Shafey M, Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats, Toxicology & Industrial Health 29(10) (2013) 875.
12. Ferguson KK, Peterson KE, Lee JM, Mercado-García A, Blank-Goldenberg C, Téllez-Rojo MM, Meeker JD. Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. Reprod Toxicol. 2014 Aug;47:70-6.
13. Hanukoglu I. Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. Drug Metab Rev 2006; 38: 171–96.
14. Hasegawa T, Zhao L, Caron KM, et al. Developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. Mol Endocrinol. 2000;14(9):1462–1471.
15. Hejmej A, Kotula-Balak M, and Bilinsk B. Antiandrogenic and estrogenic compounds: effect on development and function of male reproductive system," in *Steroids – Clinical Aspect*, 2011.
16. He Ke, Qu Hu, Wang Hui, Zhang Shun, Qian Xin-Hong, Li Wei. Regulated and Functional Expression of the Corepressor MTA3 in Rodent Testis, Endocrinology, 157 (11), 4400-4410 Nov 2016.
17. Horstman KA, Naciff JM, Overmann GJ, Foertsch LM, Richardson BD, Daston GP. Effects of transplacental 17-α-ethynyl estradiol or bisphenol A on the developmental profile of steroidogenic acute regulatory protein in the rat testis. Birth Defects Res B Dev Reprod Toxicol. 2012 Aug;95(4):318-25. doi: 10.1002/bdrb.21020. Epub 2012 Jul 2.
18. Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE, Gestational and Lactational Exposure to Ethinyl Estradiol, but not Bisphenol A, Decreases Androgen-Dependent Reproductive Organ Weights and Epididymal Sperm Abundance in the Male Long Evans Hooded Rat, Oxford University Press, Great Britain, 2008, p. 371.
19. Feng Y, Shi J, Jiao Z, Duan H, Shao B. Mechanism of bisphenol AF-induced progesterone inhibition in human chorionic gonadotrophin-stimulated mouse Leydig tumor cell line (mLTC-1) cells. Environ Toxicol. 2018 Jun;33(6):670-678.

20. Komarowska M, Hermanowicz A, Czyzewska U, Milewski R, Matuszczak E, Miltyk W and Debek W. Serum bisphenol A level in boys with cryptorchidism: a step to male infertility? International Journal of Endocrinology. Volume 2015, Article ID 973154.
21. Kundakovic Marija & Champagne Frances A. Epigenetic perspective on the developmental effects of bisphenol A. Brain Behav Immun, 25 (6), 1084-93, Aug 2011
22. Galloway T, Cipelli R, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Money C, McCormack P, Melzer D. Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environ Health Perspect. 2010 Nov;118(11):1603-8.
23. Gamez JM, Penalba R, Cardoso N, Ponzo O, Carbone S, Pandolfi M, Scacchi P, Reynoso R, Low dose of bisphenol A impairs the reproductive axis of prepuberal male rats, J Physiol Biochem 70(1) (2014) 239–246.
24. Gao GZ , Zhao Y, Li HX, Li W. Bisphenol A-elicited miR-146a-5p impairs murine testicular steroidogenesis through negative regulation of Mta3 signaling. Biochem Biophys Res Commun. 2018 Jun 22;501(2):478-485.
25. Gurmeet KSS, Rosnah I, Normadiah MK, Das S, Mustafa AM, Detrimental Effects of Bisphenol a on Development and Functions of the Male Reproductive System in Experimental Rats, Excli J 13 (2014) 151–160.
26. Huo X, Chen D, He Y, Zhu W, Zhou W, Zhang J. Bisphenol-A and Female Infertility: A Possible Role of Gene-Environment Interactions. Int J Environ Res Public Health. 2015 Sep 7;12(9):11101-16.
27. Kang JH, Kondo F, Katayama Y. Human exposure to bisphenol A. Toxicology. 2006 Sep 21;226(2-3):79-89. Epub 2006 Jun 16.
28. Kobayashi K, Kubota H, Ohtani K, Hojo R, Miyagawa M, Lack of effects for dietary exposure of bisphenol A during in utero and lactational periods on reproductive development in rat offspring, Journal of Toxicological Sciences 37(3) (2012) 565–573. [PubMed: 22687996]
29. La Rocca J, Boyajian A, Brown C, Smith SD, Hixon M, Effects of In Utero Exposure to Bisphenol A or Diethylstilbestrol on the Adult Male Reproductive System, Birth defects research. Part B, Developmental and reproductive toxicology 92(6) (2011) 526–533.
30. Lanzafame Francesco M, La Vignera Sandro, Vicari Enzo, Calogero Aldo E. Oxidative Stress and Medical Antioxidant Treatment in Male Infertility Reprod Biomed Online, 19 (5), 638-59 Nov 2009

31. Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, Skakkebaek NE, Juul A, Jorgensen N, Andersson AM, Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality, Environ Health Persp 122(5) (2014) 478–484.
32. Li X, Guo JY, Li X, Zhou HJ, Zhang SH, Liu XD, Chen DY, Fang YC, Feng XZ. Behavioural effect of low-dose BPA on male zebrafish: Tuning of male mating competition and female mating preference during courtship process. Chemosphere. 2017 Feb;169:40-52.
33. Li Y, Song T, Cai Y, Zhou J, Song X, Zhao X, Wu X. Bisphenol A exposure induces apoptosis and upregulation of Fas/FasL and caspase-3 expression in the testes of mice Toxicol Sci, 108 (2), 427-36 Apr 2009
34. Liu XQ, Miao MH, Zhou ZJ, Gao ES, Chen JP, Wang JT, Sun F, Yuan W, Li DK, Exposure to bisphenol-A and reproductive hormones among male adults, Environ Toxicol Phar 39(2) (2015) 934–941.
35. Liu Yan, Yuan Cong, Chen Shu, Zheng Yao, Zhang Yingying, Gao Jiancao, Wang Zaizhao. Global and cyp19a1a Gene Specific DNA Methylation in Gonads of Adult Rare Minnow Gobiocypris Rarus Under Bisphenol A Exposure. Aquat Toxicol, 156, 10-6 Nov 2014.
36. Mathur PP, D'Cruz SC. The effect of environmental contaminants on testicular function. Asian J Androl. 2011 Jul;13(4):585-91.
37. Matuszczak E, Komarowska MD, Debek W, Hermanowicz A. The Impact of Bisphenol A on Fertility, Reproductive System, and Development: A Review of the Literature. Int J Endocrinol. 2019 Apr 10;2019:4068717..
38. Meeker JD, Calafat AM, Hauser R, Urinary Bisphenol A Concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic, American Chemical Society, United States, 2010, p. 1458.
39. Mendiola J, Jørgensen N, Andersson AM, Calafat AM, Ye X, Redmon JB, Drobnis EZ, Wang C, Sparks A, Thurston SW, Liu F, Swan SH. Are environmental levels of bisphenol a associated with reproductive function in fertile men? Environ Health Perspect. 2010 Sep;118(9):1286-91.
40. Morgan JA, Cheepala SB, Wang Y, Neale G, Adachi M, Nachagari D, Leggas M, Zhao W, Boyd K, Venkataramanan R, Schuetz JD. Deregulated hepatic metabolism exacerbates impaired testosterone production in Mrp4-deficient mice. J Biol Chem. 2012 Apr 27;287(18):14456-66.

41. Murono EP, Derk RC, de León JH. Differential Effects of Octylphenol, 17beta-estradiol, Endosulfan, or Bisphenol A on the Steroidogenic Competence of Cultured Adult Rat Leydig Cells Reprod Toxicol, 15 (5), 551-60 Sep-Oct 2001
42. Nakamura D, Yanagiba Y, Duan Z, Ito Y, Okamura A, Asaeda N, Tagawa Y, Li C, Taya K, Zhang SY, Naito H, Ramdhan DH, Kamijima M, Nakajima T. Bisphenol A may cause testosterone reduction by adversely affecting both testis and pituitary systems similar to estradiol. Toxicol Lett. 2010 Apr 15;194(1-2):16-25.
43. Nanjappa MK, Simon L, Akingbemi BT, The Industrial Chemical Bisphenol A (BPA) Interferes with Proliferative Activity and Development of Steroidogenic Capacity in Rat Leydig Cells, Biology of Reproduction 86(5) (2012).
44. Nikula H , Talonpoika T, Kaleva M, Toppari J. Inhibition of hCG-stimulated Steroidogenesis in Cultured Mouse Leydig Tumor Cells by Bisphenol A and Octylphenols. Toxicol Appl Pharmacol, 157 (3), 166-73, 1999 Jun 15
45. N'Tumba-Byn T, Moison D, Lacroix M, Lecureuil C, Lesage L, Prud'homme SM, Pozzi-Gaudin S, Frydman R, Benachi A, Livera G, Rouiller-Fabre V, Habert R. Differential effects of bisphenol A and diethylstilbestrol on human, rat and mouse fetal leydig cell function. PLoS One. 2012;7(12):e51579.
46. Ochsenkühn R, De Kretser DM. The contributions of deficient androgen action in spermatogenic disorders. Int J Androl. 2003 Aug;26(4):195-201.
47. Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, Padmanabhan V, Taylor HS, Swan SH, VandeVoort CA, Flaws JA. Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013. Environ Health Perspect. 2014 Aug;122(8):775-86.
48. Qiu L-L, Wang X, Zhang X.-h., Zhang Z, Gu J, Liu L, Wang Y, Wang X, Wang S-L, Decreased androgen receptor expression may contribute to spermatogenesis failure in rats exposed to low concentration of bisphenol A, Toxicol Lett 219 (2013) 116–124.
49. Quinn PG, Payne AH. Steroid product-induced, oxygen-mediated damage of microsomal cytochrome P-450 enzymes in Leydig cell cultures. Relationship to desensitization. J Biol Chem 1985; 260: 2092–9.
50. Rochester JR. Bisphenol A and human health: a review of the literature. Reprod Toxicol. 2013 Dec;42:132-55.

51. Rochester JR1, Bolden AL. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. Environ Health Perspect. 2015 Jul;123(7):643-50.
52. Roelofs MJ, van den Berg M, Bovee TF, Piersma AH, van Duursen MB. Structural bisphenol analogues differentially target steroidogenesis in murine MA-10 Leydig cells as well as the glucocorticoid receptor. Toxicology. 2015 Mar 2;329:10-20.
53. Sadowski RN, Park P, Neese SL, Ferguson DC, Schantz SL, Juraska JM, Effects of perinatal bisphenol A exposure during early development on radial arm maze behavior in adult male and female rats, Neurotoxicol Teratol 42 (2014) 17–24.
54. Sajiki J., Yanagibori R. and Kobayashi Y. Study of experiment on leaching of bisphenol A from infant books to artificial saliva, Nippon Eiseigaku Zasshi, 2010, 65, 467–470.
55. Salian S, Doshi T, Vanage G, Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring, Life Sciences 85 (2009) 742–752.
56. Salian S, Doshi T, Vanage G, Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis, Toxicology 265 (2009) 56–67.
57. Samova S, Patel CN, Doctor H, Pandya HA, Verma RJ. The effect of bisphenol A on testicular steroidogenesis and its amelioration by quercetin: an in vivo and in silico approach. Toxicol Res (Camb). 2017 Sep 14;7(1):22-31.
58. Sánchez P, Castro B, Torres JM, Olmo A, del Moral RG, Ortega E. Bisphenol A modifies the regulation exerted by testosterone on 5 α -reductase isozymes in ventral prostate of adult rats. Biomed Res Int. 2013;2013:629235.
59. Scinicariello F, Buser MC. Serum Testosterone Concentrations and Urinary Bisphenol A, Benzophenone-3, Triclosan, and paraben levels in male and female children and adolescents: NHANES 2011-2012. Environ Health Perspect. 2016 Dec;124(12):1898-1904.
60. Scott HM, Mason JI, Sharpe RM. Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocr Rev. 2009 Dec;30(7):883-925.
61. Shen WJ, Azhar S, Kraemer FB. Lipid droplets and steroidogenic cells. Exp Cell Res. 2016;340(2):209–214.
62. Siracusa Jacob Steven, Yin Lei, Measel Emily, Liang Shenuxan, and Yu Xiaozhong. Effects of Bisphenol A and its Analogs on Reproductive Health: A Mini ReviewReprod Toxicol. Author

manuscript; available in PMC 2019 Aug 11Reprod Toxicol. 2018 Aug; 79: 96–123. Published online 2018 Jun 18.

63. Song KH, Lee K, Choi HS. Endocrine disrupter bisphenol a induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells.Endocrinology 2002; 143: 2208–15.
64. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev. 1996;17(3):221–244.
65. Stocco DM, Clark BJ. The role of the steroidogenic acute regulatory protein in steroidogenesis. Steroids. 1997;62(1):29–36.
66. Stocco DM, Wang XJ, Jo Y, Manna PR. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: More complicated than we thought. Mol Endocrinol. 2005;19(11):2647–2659.
67. Su L, Mruk DD, Cheng CY. Drug transporters, the blood-testis barrier, and spermatogenesis. J Endocrinol. 2011 Mar;208(3):207-23.
68. Takamiya Minako , Lambard Sophie, Huhtaniemi Ilpo T. Effect of Bisphenol A on Human Chorionic Gonadotrophin-Stimulated Gene Expression of Cultured Mouse Leydig Tumour Cells, Reprod Toxicol, 24 (2), 265-75, Aug-Sep 2007
69. Tena-Sempere M, Barreiro ML, González LC, Gaytán F, Zhang FP, Caminos JE, Pinilla L, Casanueva FF, Diéguez C, Aguilar E. Novel expression and functional role of ghrelin in rat testis. Endocrinology. 2002 Feb;143(2):717-25.
70. Thuillier R, Manku G, Wang Y, Culty M. Changes in MAPK pathway in neonatal and adult testis following fetal estrogen exposure and effects on rat testicular cells.Microsc Res Tech. 2009 Nov;72(11):773-86.
71. Vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette LJ Jr, Hauser R, Heindel JJ, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N, Prins GS, Richter CA, Rubin BS, Sonnenschein C, Soto AM, Talsness CE, Vandenbergh JG, Vandenberg LN, Walser-Kuntz DR, Watson CS, Welshons WV, Wetherill Y, Zoeller RT. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and

potential to impact human health at current levels of exposure. Reprod Toxicol. 2007 Aug-Sep;24(2):131-8.

72. Xi W, Lee CKF, Yeung WSB, Giesy JP, Wong MH, Zhang X, Hecker M, Wong CKC, Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypothalamus-pituitary-gonadal axis of CD-1 mice, Reprod Toxicol (4) (2011) 409.
73. Yuan SH, Xu SF. Leydig cell apoptosis and its regulation. Zhonghua Nan Ke Xue 2003;9: 218–20, 25.
74. Wang P, Luo C, Li Q, Chen S, Hu Y. Mitochondrion-mediated apoptosis is involved in reproductive damage caused by BPA in male rats. Environ Toxicol Pharmacol. 2014 Nov;38(3):1025-33.
75. Williams Cecilia, Bondesson Maria, Krementsov Dimitry N, Teuscher Cory.Gestational Bisphenol A Exposure and Testis Development Endocr Disruptors (Austin), 2 (1) 2014.
76. Wisniewski P, Romano RM, Kizys MML, Oliveira KC, Kasamatsu T, Giannocco G, Chiamolera MI, Dias-Da-Silva MR, Romano MA, Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic-pituitary-testicular axis, Toxicology 329 (2015) 1–9.
77. Ye Leping , Zhao Binghai, Hu Guoxin, Chu Yanhui, Ge Ren-Shan. Inhibition of Human and Rat Testicular Steroidogenic Enzyme Activities by Bisphenol A, Toxicol Lett, 207 (2), 137-42 2011 Nov 30
78. Zang Z, Ji S, Xia T, Huang S, Effects of Bisphenol A on Testosterone Levels and Sexual Behaviors of Male Mice, Advances in Sexual Medicine 6(04) (2016) 41.
79. Zhuang WL, Wu KS, Wang YK, Zhu HJ, Deng ZZ, Peng L, Zhu GH, Association of Serum Bisphenol-A Concentration and Male Reproductive Function Among Exposed Workers, Arch Environ Con Tox 68(1) (2015) 38–45.
80. Zhou Q, Miao M, Ran M, Ding L, Bai L, Wu T, Yuan W, Gao E, Wang J, Li G, Li DK. Serum bisphenol-A concentration and sex hormone levels in men. Fertil Steril. 2013 Aug;100(2):478-82.

Legends to figures

Figure 1. Leydig cell steroidogenesis. LH binds to its receptors (LHR) on the Leydig cell (LC) membrane. This results in activation of Gs protein and adenylyl cyclase and increased concentration of intracellular cAMP. cAMP stimulates the mobilization and transport of cholesterol within the mitochondria in part by activating PKA and MAPK signaling. The first source of cholesterol for steroidogenesis is via uptake of cholesteryl esters from high-density lipoprotein (HDL) by the scavenger receptor SR-B1. Steroidogenic acute regulatory enzymes (StARs) regulate cholesterol transport from the outer to the inner mitochondrial membrane. At the inner mitochondrial membrane cholesterol is converted into pregnenolone by CYP11A1 and pregnenolone is converted into testosterone by enzymes in the smooth endoplasmic reticulum (3β-HSD, CYP17A1 and 17β-HSD).

Figure 2. Mechanisms of action of bisphenol A on testicular steroidogenesis. Testicular steroidogenesis is a complex and fine-regulated process that bisphenol A (BPA) can perturb by acting with several mechanisms represented in this figure (circled in red).

Figure 1.JPEG

Barbagallo et al., Figure 1

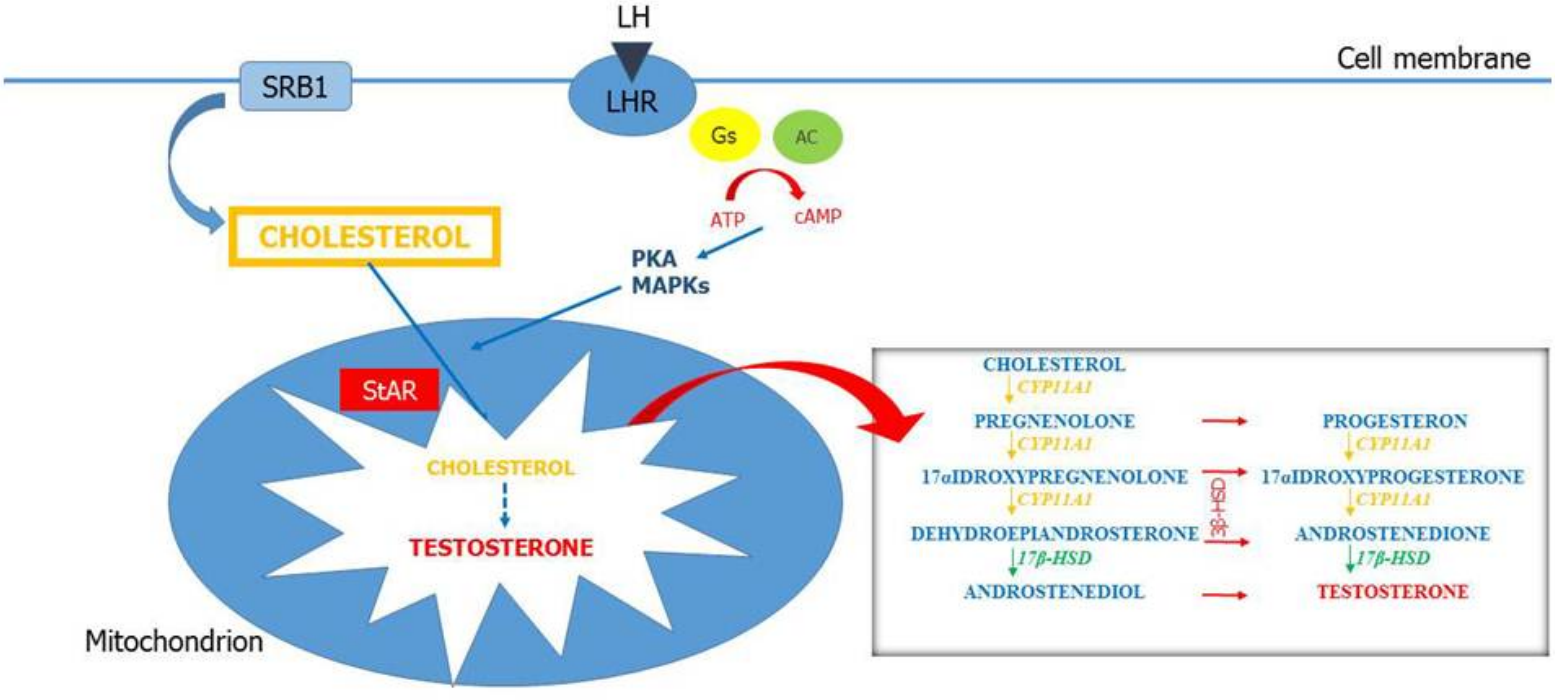

Figure 2.JPEG