Bisphenol A and Reproductive Health: Update of Experimental and Human Evidence, 2007–2013


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Abstract

**Background:** In 2007, an expert panel reviewed associations between bisphenol A (BPA) exposure and reproductive health outcomes. Since then, new studies have been conducted on the impact of BPA on reproduction.

**Objective:** This review summarizes the data obtained since 2007, focusing on: 1) findings from human and animal studies, 2) the effects of BPA on a variety of reproductive endpoints, and 3) mechanisms of BPA action.

**Methods:** We reviewed the literature using a PubMed search from 2007-2013 based on keywords related to BPA and male and female reproduction.

**Discussion:** BPA is an ovarian toxicant because it affects the onset of meiosis in both animal and *in vitro* models, interferes with germ cell nest breakdown in animal models, accelerates follicle transition in several animal species, alters steroidogenesis in multiple animal models and women, and reduces oocyte quality in animal models and women undergoing IVF. BPA is a uterine toxicant because it impairs uterine endometrial proliferation, decreases uterine receptivity, and increases implantation failure in animal models. BPA exposure may be associated with adverse birth outcomes, hyperandrogenism, sexual dysfunction, and impaired implantation in humans, but additional studies are required to confirm whether this is the case. BPA is a testicular toxicant in animal models, but the data in humans are equivocal. Finally, insufficient evidence exists regarding effects of BPA on the oviduct, placenta, and pubertal development.

**Conclusion:** BPA is a reproductive toxicant because it impacts female reproduction, and has the potential to affect male reproductive systems in humans and animals.
Early oogenesis and ovarian follicle formation

All studies on BPA exposure and early oogenesis and follicle formation have been conducted using animal models or in vitro systems. These studies indicate that developmental BPA exposure has the potential to affect two stages of oogenesis: 1) the onset of meiosis in the fetal ovary and 2) germ cell nest breakdown and follicle formation (Supplemental Material, Table S1). Several reports have confirmed the original findings from studies in mice (reviewed in Richter et al. 2007) that gestational exposure affects the onset of meiosis and induces nondisjunction in meiosis in the fetal ovary, but does not induce aneuploidy. In a macaque study designed to mimic serum levels of unconjugated BPA reported in human biomonitoring studies (Vandenberg et al. 2010), daily low dose BPA exposure (measured <1 ng/ml in maternal serum) significantly disrupted synapsis and recombination between homologous chromosomes at the onset of meiosis (Hunt et al. 2012), consistent with previous findings (Susiarjo et al. 2007). In three studies, gestational low dose BPA exposure of mice induced changes in gene expression in germ cells and early meiocytes. BPA exposure increased expression of Stra8 (stimulated by retinoic acid 8 homolog) and a variety of meiotic genes in C57BL/6 mice (Lawson et al. 2011). However, longer gestational BPA exposure down-regulated the expression of Stra8, Dazl (deleted in azoospermia-like), and Nobox (newborn ovary homeobox) in CD-1 mice (Zhang X et al. 2012). Lastly, BPA exposure commencing after the onset of meiosis induced a meiotic delay at gestational day (GD) 17.5 in CD-1 females (Zhang H et al. 2012). Collectively, these studies provide strong evidence that BPA exposure disrupts meiosis in mice and macaques, as well as alters gene expression in germ cells and early meiocytes in two different strains of mice.

In vitro studies provide further evidence that BPA impacts the onset of meiosis. BPA (1 to 30 µM) increased oocyte degeneration by impairing meiotic progression in cultured human fetal
oocytes and, similar to mouse studies, human fetal oocytes that progressed to prophase exhibited increased levels of recombination (MLH1 foci) and gene expression changes (Brieño-Enriquez et al. 2011a, 2011b). BPA also increased methylation errors in differentially methylated regions of maternally imprinted genes of oocytes in cultured preantral follicles of C57/BL6xCBA/Ca mice (Trapphoff et al. 2013). Thus, the data from different mouse strains, macaques, and in vitro studies consistently provide strong evidence to conclude that BPA exposure has detrimental effects on the meiotic process both at the gene expression and phenotypic level.

Studies also suggest that BPA interferes with germ cell nest breakdown in animal models. In neonatally exposed lambs, low dose BPA increased the incidence of multi-oocyte follicles (Rivera et al. 2011). Similarly, in gestationally exposed macaques, dietary low dose BPA exposure increased the number of oocytes present in secondary and antral follicles at birth and continuous BPA exposure (measured <1 ng/ml in maternal serum) increased the incidence of unenclosed oocytes (Hunt et al. 2012). Further, in gestationally exposed CD-1 mice, low dose BPA increased the number of unenclosed oocytes, while it decreased the number of primordial follicles in a dose-dependent manner (Zhang H et al. 2012). In another study, prenatal BPA exposure altered the fetal ovarian steroidogenic gene and microRNA expression that mediate gonadal differentiation and folliculogenesis in sheep (Veiga-Lopez et al. 2013). Collectively, these studies provide strong evidence that gestational BPA exposure across multiple exposure routes, doses, and species, impairs proper germ cell nest breakdown, leading to the formation of multi-oocyte follicles. The presence of multi-oocyte follicles is of concern because they are considered a pathologic condition that may lead to ovulatory problems (Iguchi et al. 1990).

BPA exposure also appears to accelerate follicle transition and growth in several species. Neonatal BPA exposure accelerated follicle transition in lambs, decreasing primordial and
increasing primary follicle numbers, without affecting total follicle numbers (Rivera et al. 2011).
A similar enhanced activation of follicular recruitment was observed in neonatally exposed
Wistar rats (Rodríguez et al. 2010). BPA exposure also increased cell proliferation, indicative of
follicular growth, in small antral follicles in neonatally exposed lambs and Wistar rats (Rivera et
al. 2011; Rodríguez et al. 2010). Taken together, the data suggest that BPA enhances the
recruitment and growth of primordial and primary follicles across species. Combined with the
effects on germ cell nest breakdown, there is strong evidence that BPA induces ovotoxicity by
acting on developing and immature follicle stages in animals models. However, the
consequences of these effects on reproductive potential/longevity are unclear. In one study, low
dose neonatal BPA exposure decreased numbers of all follicle types and increased atretic
follicles in rats during adulthood (Li Y et al., 2013). These effects of BPA on follicles could lead
to premature reproductive senescence, but this needs to be confirmed in future studies.

*In vitro* studies on the effects of BPA have focused on mature ovarian follicles. In murine
preantral follicles, BPA (3nM) accelerated development to antral follicles (Trapphoff et al.
2013). In murine antral follicles, exposure to BPA (440μM) aberrantly up-regulated expression
of cell cycle regulators and the pro- and anti-atretic factors *Bax* (BCL2-associated X protein),
*Trp53* (tumor protein 53), and *Bcl2* (B-cell lymphoma 2), inhibiting follicle growth and inducing
apoptosis (Peretz et al. 2012). Further, BPA (110-438μM) inhibited antral follicle growth in mice
(Ziv-Gal et al. 2013). These studies suggest that low dose BPA exposure may alter follicle
formation, but high dose BPA may directly inhibit growth, cause atresia, and induce changes in
gene expression in rodent antral follicles. In future studies, it will be important to validate
findings from these *in vitro* studies at the *in vivo* level and determine the consequences of BPA-
induced follicle toxicity on reproduction function.
Steroidogenesis in females

Multiple studies have investigated the association between BPA exposure and ovarian steroid hormone production in women (Supplemental Material, Table S2). In three publications based on women undergoing in vitro fertilization (IVF), BPA exposure was associated with a decrease in peak serum estradiol levels prior to oocyte retrieval (Ehrlich et al. 2012b; Mok-Lin et al. 2010; Bloom et al. 2011a). Additionally, in a case-control study, BPA was associated with increased testosterone and androstenedione levels in women with PCOS (Kandaraki et al. 2011). In a study of 60 women undergoing IVF, urinary BPA concentrations were not associated with a negative linear dose-response association with the expression of the steroidogenic enzyme Cyp19 in granulosa cells collected at the time of oocyte retrieval, but instead a suggested non-monotonic dose-response association (Ehrlich et al. 2013). Conversely, BPA was not associated with estradiol or testosterone levels in women in the INChianti study, a prospective, population based study of adults living in Chianti, Italy (Galloway et al. 2010). Given the limited information on BPA exposure and ovarian steroidogenesis in women and the discrepant study results, additional studies utilizing sensitive and reliable steroid hormone and BPA assays are required to delineate whether BPA levels negatively impact reproductive hormonal patterns in women. Further, given that most existing studies on BPA exposure and steroid levels were conducted in IVF populations, it is critical to examine the association between BPA exposure and steroidogenesis in women from the general population.

Several experimental studies have examined the effect of BPA exposure on ovarian steroidogenesis in laboratory animals (Supplemental Material, Table S1). In three rodent studies, perinatal (Xi et al. 2011) and postnatal (Fernández et al. 2010; Tan, et al. 2013) low dose BPA exposure increased serum estradiol levels. Further, in one Sprague-Dawley rat and one pregnant
ICR mouse study, low dose BPA increased testosterone and progesterone levels (Fernández et al. 2010; Tan et al. 2013). Interestingly, in the Xi et al. study (2011), postnatal BPA exposure alone did not affect serum hormone levels in the mice. Similarly, in other studies using rats, mice, and lambs, gestational and/or gestational and neonatal BPA exposure had no effect on steroidogenesis (Kobayashi et al. 2012; Mendoza-Rodríguez et al. 2011; Rivera et al. 2011; Varayoud et al. 2011). In these studies, the doses used were lower than those in the affected studies, indicating that BPA doses less than 20mg/kg may not increase hormone production in animal models. In contrast, though BPA did not alter estradiol levels, low dose BPA decreased progesterone levels in adult mice during early pregnancy (Berger et al. 2008). Additionally, in adult rats, low dose BPA (below 0.1 mg/kg/day) decreased estradiol, testosterone, Cyp19 (aromatase), and Star (steroidogenic acute regulatory protein) (Lee SG et al. 2013). Further, in adult mice, low dose BPA decreased expression of estrogen and progesterone receptors, though BPA did not alter hormone levels (Berger et al. 2010). The differences in study results may be a function of exposure times, internal doses of BPA, or species.

The results of in vitro studies on the effects of BPA on steroidogenesis are also equivocal. BPA exposure (44 and 440μM) inhibited estradiol, testosterone, androstenedione, estrone, dehydroepiandrosterone, and progesterone production, and decreased StAR and Cyp11a1 (cytochrome P450 side-chain cleavage) expression in cultured intact murine antral follicles (Peretz et al. 2011; Ziv-Gal et al. 2013). However, in isolated rat theca-interstitial cells, BPA (100 nM to 100 μM) had opposite effects, increasing testosterone synthesis and Cyp17a (cytochrome P450 17alpha hydroxylase/lyase), Cyp11a1, and StAR expression (Zhou et al. 2008). In isolated rat granulosa cells, BPA (100 μM) decreased progesterone synthesis and increased StAR expression (Zhou et al. 2008). In a separate study using porcine granulosa cells,
0.1 μM BPA increased estradiol levels, while higher doses (1 and 10 μM) decreased estradiol levels. All three concentrations of BPA decreased progesterone levels (Grasselli et al. 2010). Collectively, these studies indicate that BPA adversely affects steroidogenesis in vitro, but the effects depend on BPA concentration, and that different levels of BPA have different effects on steroidogenesis depending on whether intact follicles or isolated cells are used in the cultures.

**Oocytes: quantity, quality, and fertilization**

A few human studies have analyzed the association between urinary BPA levels and oocyte yield, maturation, and fertilization (Supplemental Material, Table S2). A small prospective study of 58 infertile women and 37 male partners undergoing intracytoplasmic sperm injection (ICSI) or conventional IVF found an association between serum BPA concentrations and oocyte maturation only among Asian women, but an overall correlation between increasing serum concentrations and the developmental potential of human oocytes (Fujimoto et al. 2011). In two publications from the same prospective cohort of 84 women (Mok-Lin et al. 2010) and 174 women (Ehrlich et al. 2012b) undergoing IVF, increasing urinary BPA concentration was associated with decreased numbers of retrieved oocytes, mature oocytes (MII), and normally fertilized oocytes (2PN). These results suggest that BPA is associated with impaired oocyte yield, maturation, and fertilization, adversely affecting the success of IVF treatment.

Several recent experimental results support the findings from earlier studies that suggested BPA exposure affects the resumption of meiosis in the periovulatory oocyte (reviewed in vom Saal et al. 2007; Supplemental Material, Table S1). Neonatal low dose BPA exposure inhibited germinal vesicle breakdown (GVBD) in CD-1 F1 hybrid female mice (Chao et al. 2012), confirming the previous work of Hunt et al (2003). Conversely, low dose BPA administered to MF-1 (C57BL/6 x CBA/Ca F1 hybrid) mice from PND22 to PND28 did not reduce GVBD and polar body
extrusion or increase spindle aberrations (Eichenlaub-Ritter et al. 2008). Similarly, low dose BPA given orally to 4 or 9 week old superovulated female C57Bl/6 mice did not affect oocyte retrieval, meiotic maturation, or induce aneuploidy (Pacchierotti et al. 2008). While the exact reasons for the subtle discrepancies among studies are unknown, there are numerous possible reasons for the variation (reviewed in Hunt et al. 2009), including the window of neonatal exposure and potential co-exposure to phytoestrogens that may modulate the effects of BPA on the periovulatory oocyte (Muhlhauser et al. 2009).

In *in vitro* studies, BPA (43.8 μM) significantly altered spindle formation, distribution of pericentriolar material at spindle poles, and induced congression failure in MF-1 mouse oocytes isolated from antral follicles (Eichenlaub-Ritter et al. 2008). Additionally, BPA (30 μM) impaired spindle alignment and caused meiotic arrest after GVBD, but prior to polar body extrusion in follicle-enclosed oocytes from adult mice (Lenie et al. 2008). Importantly, a recent study of human oocytes exposed during *in vitro* maturation, with doses of BPA within the range measured in human follicular fluid (20, 200 ng/ml, and 20 μg/ml), found a dose-dependent increase in the incidence of meiotic arrest, disturbances in spindle formation and chromosome alignment, and spontaneous oocyte activation (Machtinger et al. 2013). Because the doses used were in the range measured in human follicular fluid, these data together with the results of experimental studies, provide compelling evidence that BPA adversely affects the maturing oocyte.

**Polycystic ovarian syndrome (PCOS)**

Studies on the effects of BPA on polycystic ovarian syndrome (PCOS) are limited (Supplemental Material, Table S2). Women with PCOS are characterized by oligo-anovulation, functional hyper-androgenism, and multi-follicular ovaries (accumulation of several small sized antral
follicles), with the majority of women showing insulin resistance and luteinizing hormone excess (Hampton 2013). One case control study (71 women with PCOS and 100 women without PCOS) reported an association between serum BPA levels and increased testosterone, androstenedione levels and insulin resistance in PCOS (Supplemental Material, Table S2; Kandaraki et al. 2011). Given the limited number of studies assessing the association between BPA exposure and PCOS symptoms, more studies are needed before firm conclusions can be made about the impact of BPA exposure on PCOS or PCOS symptoms.

The ovarian phenotype in BPA-treated rodents (cystic appearing follicles) differs from the ovarian phenotype of women with PCOS (accumulation of small antral follicles). In rodents, pre- and neonatal low dose BPA exposure lead to disruption of estrous cyclicity (Adewale et al. 2009), increased testosterone production (Fernández et al. 2010), and ovarian cysts (Newbold et al. 2009) (Supplemental Material, Table S1). High dose BPA exposure also leads to ovarian cysts (Fernández et al. 2010) and an accumulation of large antral follicles (Adewale et al. 2009). Additionally, BPA exposure decreased GnRH levels measured from hypothalamic explants in vitro (Fernández et al. 2010). Additional studies in other animal models are needed to fully understand whether BPA exposure causes PCOS or PCOS-like conditions and to determine why outcomes differ in animal models and women.

Oviduct

Only one study investigated the effects of BPA on the oviduct (Supplemental Material, Table S1). This study demonstrated that prenatal low dose BPA exposure causes progressive proliferative lesions in the oviducts of the offspring of CD-1 mice (Newbold et al. 2009). Because alterations in oviduct morphology would be expected to adversely affect both
fertilization and embryo transport, future studies are clearly warranted to examine the impact of BPA not only on the oviduct, but also on fertilization and embryo transport.

**Uterine morphology**

While no studies have reported the impact of gestational BPA exposure on uterine morphology in women due to the extreme difficulty of monitoring adult outcomes of prenatal exposure to BPA, animal studies suggest that gestational BPA exposure perturbs uterine gross morphology in the adult (Supplemental Material, Table S3). Specifically, low dose BPA exposure induces benign and malignant lesions (Newbold et al. 2009; Signorile et al. 2010) and endometrial polyps in the uterus as well as perturbs Wölfian duct regression in gestationally exposed, adult mice (Newbold et al. 2009). Further, adult hens exposed in ovo on day 4 of incubation to BPA (134 ng/kg) had decreased thickness of their tunica mucosa and density of uterine glandular structures compared to unexposed hens (Yigit and Daglioglu 2010). Together, these studies indicate that gestational BPA exposure may be potentially deleterious to uterine morphology in adult females. Importantly, since abnormalities have been identified in middle age that were not observed in young adults (Newbold et al. 2009), future studies should assess effects in exposed animals at different life stages.

**Uterine endometrium**

Limited data exist on BPA exposure and uterine endometrium in women. One case control study of 69 women suggests that serum BPA concentrations may be associated with the occurrence of endometriosis (Supplemental Material, Table S2) (Cobellis et al. 2009). Another study of 495 individuals in an operative cohort and 131 women in population cohort found no association between BPA exposure and endometriosis (Buck Louis et al. 2013). However, this study was not originally intended to investigate BPA exposure, was not appropriately powered to assess
endometriosis, and included a lapse between sample collection and endometriosis evaluation, further confounding these data. Given the lack of studies on BPA and endometrial disorders in humans, more studies are required before making conclusions about whether BPA adversely impacts the uterine endometrium in women.

Experimental studies support the findings from the aforementioned human study (Supplemental Material, Table S3). Adult female Balb-c mice exposed gestationally and neonatally to low dose BPA developed endometrial like structures with glands and stroma in adipose tissue surrounding the genital tract. These structures expressed \textit{Hoxa10} (homeobox A10), a transcription factor that mediates proliferation of stromal tissue prior to implantation (Signorile et al. 2010). Both low and high dose BPA increased expression of \textit{Hoxa10} in gestationally exposed adult CD-1 and ICR mice as well (Bromer et al. 2010; Hiyama et al. 2011).

Other \textit{in vivo} studies provide evidence that BPA impairs proliferation in the uterus. BPA exposure over a wide range of doses, times, and routes decreased expression of uterine \textit{Esr1} (estrogen receptor alpha) in the rodent, which may lead to inhibited endometrial proliferation in the uterine epithelium and stroma (Berger et al. 2010; Bromer et al. 2010; Varayoud et al. 2008; Bosquiazzo et al. 2010). In two separate studies of adult rats, gestational and neonatal low dose BPA exposure decreased uterine epithelium proliferation in response to hormone treatments (Varayoud et al. 2008; Mendoza-Rodríguez et al. 2011). While low dose BPA did not affect progesterone receptor expression, it dampened glandular and stromal progesterone receptor expression in response to estradiol stimulus in another (Aldad et al. 2011). Low dose BPA also impaired apoptosis of the uterine epithelium during estrus in neonatally exposed, adult rats (Mendoza-Rodríguez et al. 2011).
*In vitro* studies also support the hypothesis that BPA exposure adversely impacts the uterus. BPA (50 and 100 µM) significantly decreased proliferation of human endometrial stromal fibroblasts cultured for 48h (Aghajanova et al. 2011). BPA (50 µM) was also found to decrease the proliferation of cultured human endometrial endothelial cells (Bredhult et al. 2009). Further, in cultured, primary heterogeneous populations of uterine cells, BPA (10 µM) significantly inhibited uterine cell contractions, increased oxytocin-related pathways, and decreased prostaglandin-related signaling after 48h (An et al. 2013). Taken together, the existing animal and *in vitro* studies provide strong support that BPA impairs uterine cell proliferation.

**Uterine receptivity and implantation**

Only one study has reported on the association between BPA exposure and uterine receptivity/implantation in women (Supplemental Material, Table S2). In 137 women undergoing IVF, higher quartiles of urinary BPA concentrations were associated with increased odds of implantation failure (Ehrlich et al. 2012a). Given the limited information on this topic, future studies need to be conducted to determine whether BPA exposure is associated with adverse uterine receptivity/implantation outcomes in women.

Several experimental studies indicate that BPA exposure impairs uterine receptivity and implantation (Supplemental Material, Table S3). Repeated exposure of pregnant mice to low dose BPA during early gestation completely ablated embryo implantation (Berger et al. 2008; Xiao et al. 2011). A single exposure to high dose BPA on GD0 or GD1, but not on GD2 was found to decrease implantation in mice (Berger et al. 2008, 2010). Neonatal low dose BPA exposure also decreased implantation sites in pregnant rats (Varayoud et al. 2011). Interestingly, low dose BPA increased pre-implantation loss in unexposed females mated to neonatally exposed male rats (Salian et al. 2009a) and decreased implantation sites in females mated to
adult exposed male rats (Tiwari and Vanage 2013). Further, when untreated and healthy embryos were transplanted into the uteri of low dose BPA exposed mice, BPA prevented implantation (Xiao et al. 2011). Together, these studies provide strong evidence that BPA exposure, in both males and females, affects uterine receptivity in females. However, a need still exists to explore whether BPA-exposed embryos attach to the uterine epithelium and initiate implantation.

Neonatal low dose BPA exposure also decreased pregnancy maintenance in experimental studies (Varayoud et al. 2011). Low dose BPA increased resorption rates in uteri of unexposed females mated to either neonatally exposed male rats (Salian et al. 2009a), gestationally and neonatally exposed male rats (Salian et al. 2009b), and adult exposed rats (Tiwari and Vanage 2013), suggesting that male exposure can significantly contribute to pregnancy loss. These effects were also evident in F1 and F2 rat offspring (Salian et al. 2009a, 2009b), suggesting transgenerational effects of BPA. The potential ability of BPA to cause transgenerational effects in animal models is further supported by Hiyama et al (2011) who showed that developmental high dose BPA exposure reduced uterine weight, expanded the uterine lumen, and induced demethylation of the $Hoxa10$ gene in the F2 generation in mice. Collectively, these experiments indicate that BPA exposure causes adverse effects on implantation and pregnancy maintenance in animal models, which may be transgenerational in nature.

**Embryo development**

Few studies have examined the effects of parental BPA levels on subsequent *in vitro* embryo development in humans (Supplemental Material, Table S2). In one prospective preconception cohort study of 174 women, total urinary BPA concentration was associated with a decreased rate of blastocyst formation (Ehrlich et al. 2012b). Further, a prospective cohort study involving 27 couples undergoing IVF treatment found that increasing urinary BPA concentrations in the
male, but not female partner, decreased the odds of a high embryo fragmentation score, suggesting the embryos were low quality for IVF treatments (Bloom et al. 2011b). Collectively, these studies indicate adverse associations of parental BPA levels on the development of early embryos *in vitro*, but further studies are required to determine whether the observed effects occur *in vivo*.

Although only two experimental studies have investigated the effects of BPA exposure on early embryo development *in vivo*, the results are intriguing. One study showed that high dose BPA given to pregnant C57BL/6 mice from GD0.5 to 3.5 delayed embryo development (Supplemental Material, Table S3) (Xiao et al. 2011). A more recent study of F1 female C57BL/6 Cast7 mice showed that low dose BPA exposure initiated prior to breeding and continuing through pregnancy disrupted the expression of imprinted genes in mid-gestation embryos and placentae (Susiarjo et al. 2013). Given the limited number of studies on the effects of BPA on embryo development *in vivo*, additional studies are needed prior to making firm conclusions about the effects of BPA on early embryos.

**Placenta**

Although epidemiological studies on BPA and the placenta have not been published, one experimental study suggests that both low and high doses of BPA increase plasma estradiol, testosterone, and corticotropin releasing hormone levels due to an increase in mRNA expression of corticotrophin releasing hormone and activation of protein kinase C ζ/λ and δ in the placenta (Supplemental Material, Table S3; Tan et al. 2013). A few *in vitro* studies indicate that BPA affects placental cell proliferation. After 24h or 48h of culture, BPA (1 to 10 µM) increased apoptosis and decreased proliferation of cultured human trophoblast cells from first trimester placentas, while BPA at 10 µM decreased cell viability (Morice at al. 2011). Similarly, after 24h,
BPA (87.7 nM to 8.77 μM) increased apoptosis in cultured human cytotrophoblasts (Benachour and Aris 2009). In addition, recent data from mouse studies suggest that BPA alters gene expression in the placenta (Susiarjo et al. 2013). Taken together, these in vitro data suggest that BPA may affect placental function, but additional animal and human studies are required to substantiate these data.

**Pregnancy outcomes**

Only one study has examined the association between BPA exposure and pregnancy outcome in humans. Specifically, a nested case control study of 60 pregnant women found a positive association between urinary BPA concentration and pre-term birth (Supplemental Material, Table S4; Cantonwine et al. 2010). Given the limited information on BPA and pregnancy outcomes in humans, more studies are needed before making firm conclusions about whether BPA exposure is association with adverse pregnancy outcomes in humans.

In contrast, several experimental studies have investigated the effects of BPA exposure on pregnancy outcomes in animal models such as mice and rats that are altricial species in which pups are born at a stage of development equivalent to mid-gestation in humans. Low dose BPA did not alter gestation length in gestationally and neonatally exposed mice (Supplemental Material, Table S5; Cabaton et al. 2011; Kobayashi et al. 2010), adult exposed mice (Tyl et al. 2008), or gestationally and neonatally exposed SD rats (Kobayashi et al. 2012). In other studies, low dose BPA exposure decreased the number of pregnancies and successful deliveries in gestationally exposed mice (Cabaton et al. 2011). BPA also decreased the percent of hatchings of chickens exposed in ovo to BPA (134 ng/kg) on day 4 on incubation (Yigit and Dagliogu 2010). Conversely, neither low nor high dose BPA affected the number of litters born to unexposed
female SD rats mated to gestationally exposed male rats (Thuillier et al. 2009), suggesting that maternal, but not paternal, BPA exposure may influence successful delivery of offspring.

Many experimental studies have reported that low dose BPA does not affect the number of live pups (Howdeshell et al. 2008; Kobayashi et al. 2010, 2012; Thuillier et al. 2009; Xi et al. 2011) or total number of delivered pups (Kobayashi et al. 2010, 2012; Nanjappa et al. 2012; Ryan et al. 2010; Tyl et al. 2008; Xi et al. 2011) in mice and rats. In a few studies, however, low dose BPA exposure decreased the number of live pups born to gestationally and neonatally exposed CD-1 mice (Cabaton et al. 2011) and Holtzman rats (Salian et al. 2009b). Low dose BPA also decreased the total number of pups born to gestationally and neonatally exposed CD-1 mice (Cabaton et al. 2011) Interestingly, in these studies, BPA acted differently from the positive control used (diethylstilbestrol: DES), suggesting the effects of BPA may differ from those of DES in CD-1 mice. Low dose BPA also decreased the total number of pups born to unexposed female Holtzman rats mated to neonatally and gestationally exposed male Holtzman rats (Salian et al. 2009a, 2009b). In these studies, BPA exposure decreased pup numbers after multiple litters, similar to DES used in the study. Collectively, these studies suggest that BPA exposure affects pregnancy outcomes in many, but not all studies depending on experimental protocol. Clearly, more studies are required to determine why results differ between studies and to determine if BPA exposure does indeed affect pregnancy outcomes in animal models.

**Birth weight**

Human studies of BPA and birth weight are equivocal (Supplemental Material, Table S4). In a cross-sectional study of 97 pregnant women, those women with serum BPA concentrations greater than 2.51 ng/mL had a higher risk for having low birth weight male neonates that were small for gestational age, compared to women with lower serum BPA concentrations. No
with induced prostatic hyperplasia, high dose BPA increased prostate gland mass and increased relative weight of the dorsolateral prostate lobe (Wu et al. 2011). High dose BPA exposure also increased epithelial cell heights of the ventral prostate and dorsolateral prostate lobes (Wu et al. 2011). Lastly, neonatal exposure to low dose BPA led to transient and permanent hypomethylations in the rat epigenome implicated in the manifestation of prostatic carcinogenesis (Tang et al. 2012). Collectively, these studies support the concept that both low and high doses of BPA promote changes in the steroidogenic pathways and morphology of the prostate, which may in turn affect homeostasis and pathogenesis.

Currently, no information is available about whether BPA exposure causes benign urologic disease. However, studies have shown that estrogens and estrogen receptor pathways negatively affect the lower urinary tract (Nicholson et al. 2012; Ricke et al. 2008; Wang et al. 2007; Willingham and Baskin 2007), supporting a potential role for estrogen-like molecules, including BPA, in the manifestation of urological diseases. Future experimental studies evaluating the effects of BPA on the lower urinary tract as well as epidemiological studies in humans are to gain insight into the role BPA plays in disease processes in the lower male urinary tract.

**Puberty and sexual receptivity**

Two epidemiological studies investigated the effect of BPA on puberty and measured BPA levels in girls at similar ages (Supplemental Material, Table S2). In these studies, one of 1151 girls aged 6-8 years and another of 192 girls aged 9 years, BPA exposure was not associated with accelerated breast or pubic hair development (Wolff et al. 2008a, 2010). Additionally, BPA exposure was not associated with precocious puberty in a study of 82 patients with precocious puberty and 32 patients without precocious puberty (Lee SH et al. 2013). However, in a study of 110 girls with precocious puberty and 100 girls without precocious puberty, BPA was associated
with increased uterine and ovarian volume (Qiao et al. 2010). These studies suggest that BPA exposure may not be associated with onset of puberty in girls, but given the limited number of studies, these results should be confirmed in future studies.

In animal models, the effects of BPA on factors such as puberty onset and sexual receptivity are equivocal (Supplemental Material, Table S8). In one study, low dose BPA did not affect the timing of puberty onset as measured by vaginal opening in gestationally and neonatally orally exposed LE rats (Ryan et al. 2010). Conversely, low and high dose BPA exposure accelerated vaginal opening in neonatally exposed ICR mice, SD rats, and LE rats (Adewale et al. 2009; Fernandez et al. 2009; Nah et al. 2011). Low dose BPA also decreased the time spent in estrus (Fernandez et al. 2009; Nah et al. 2011) in neonatally exposed ICR mice and SD rats. However, low dose BPA did not affect estrous cyclicity in gestationally and neonatally exposed CD-1 mice and LE rats (Adewale et al. 2009; Tyl et al. 2008). Further, low dose BPA had no effect on lordosis behavior in gestationally and neonatally exposed LE rats (Ryan et al. 2010) or neonatally exposed female LE rats (Adewale et al. 2009). Collectively, these studies indicate that the effects BPA exposure on the onset of puberty and sexual receptivity in animal models are unclear and likely differ depending on strain and species. Further studies should investigate how strain and animal models influence puberty onset and sexual receptivity.

**Sexual dysfunction**

Only a few studies have examined the association between BPA exposure and sexual dysfunction in epidemiological or experimental studies. One cross-sectional study of 425 men occupationally exposed to BPA had an increased risk of self-reported impaired sexual abilities compared to 284 unexposed men (Supplemental Material, Table S6; Li DK et al. 2010). Some animal studies have also shown that BPA may impair sexual ability (Supplemental Material,
Table S8). Low dose BPA increased the time taken for copulation in neonatally and gestationally exposed Holtzman rats (Salian et al. 2009a, 2009b) and increased latency to insemination in perinatally exposed CF-1 mice (Decatanzaro et al. 2013). Low dose BPA also decreased intromission and ejaculations of unexposed CF-1 mice mated to perinatally exposed females (Decatanzaro et al. 2013). However, low or high dose BPA did not affect the time taken for copulation in gestationally and neonatally exposed adult CD-1 mice or their offspring (Tyl et al. 2008). Given the limited number of studies conducted on BPA and sexual function and the equivocal nature of the results, it is not possible to make firm conclusions on the effects of BPA on sexual function. However, it is important to note that all, but one study conducted to date, suggest that BPA negatively impacts sexual function.

Conclusions

Below we summarize the strength of the evidence for associations between BPA and adverse reproductive outcomes based on literature published from 2007-2013. The data presented in this review build on the overall conclusions of the expert panel report in 2007 (vom Saal et al. 2007) that the widespread effects of BPA in experimental animal studies are a concern for overall human health and may be involved in human reproductive disease. Similar to the 2007 expert panel report, we considered the evidence to be strong when multiple studies in multiples species indicated a similar effect of BPA on a reproductive tissue or endpoint, even if concordance was not 100% across all studies given that species and strain differences can lead to differences in dose response and magnitude of effect. These conclusions, however, are not to be considered definitive without further investigation, especially with the gaps in clear results detailed throughout the review. In the experimental studies, strong, definitive conclusions often were difficult because study designs were so different. Experimental studies rarely utilized the same
doses, timing, positive controls, and exposure routes to compare the effects of BPA on exposure among animal strains and species. However, one unifying strength of these studies related to human health is that the majority of work evaluated in this review used BPA doses below the LOAEL. In the epidemiological studies, strong conclusions were difficult to determine because of study design and exposure parameters. For example, exposure assessments in the majority of human studies rely on a single urine sample, which may introduce exposure misclassification and attenuate associations if they are present. Given the continuous and variable exposure to BPA, a single urine sample may not represent longer term exposure or exposure in the relevant etiological window. Finally, a majority of the human studies were cross-sectional, making it difficult to discern the temporal relationship of exposure with response. These limitations affect the interpretation of human studies. Future human studies need to consider improvements in exposure assessment to represent longer-term BPA exposure assessed at etiologically relevant window(s). Given recent data, it will be critical in future studies to assess effects of different routes of exposure and to analyze effects at different stages in the life of the exposed individual. Additionally, for experimental studies, the addition of positive controls should be considered essential.

However, given the data included in this review, we have drawn some insights and conclusions that add to the conclusions drawn based on reviews of the BPA literature prior to 2007, categorized by the strength of evidence presented.

(1) Strong evidence exists that:

- BPA is an ovarian toxicant in animal models and women. It adversely affects the onset of meiosis in ovaries from both animal models and humans, interferes with germ cell nest breakdown in animal models, accelerates follicle transition in several
animal species, alters steroidogenesis in multiple animal models and women, and reduces oocyte quality in animal models and women undergoing IVF.

- BPA is a uterine toxicant in animal models because it impairs uterine endometrial cellular proliferation, decreases uterine receptivity, alters gene expression, and increases implantation failure in several strains/species. However, human studies have not adequately addressed these endpoints.

- BPA is a prostate toxicant in animal models, impairing the steroidogenic capacity and altering dorsal and ventral lobe morphology, potentially leading to prostate pathogenesis. Human studies, however, are lacking.

- The effects of BPA on the reproductive system are variable and evident at doses below the LOAEL of 50mg/kg and the proposed safe level of 50μg/kg/d.

(2) Limited evidence exists that:

- Relative to impact of BPA on birth rate, birth weight and length of gestation, human data on birth weight are inconsistent; although animal studies suggest such an association, and there are limited human studies on gestational length and pre-term birth.

- BPA exposure is associated with hyperandrogenism, such as in PCOS in women. However, data in rodent models are not supportive of the development of human PCOS symptoms.

- BPA is a testicular toxicant in animal models because it decreases sperm quality, motility, causes oxidative stress, and alters steroidogenesis. Human data, however, are inconsistent.
• BPA is associated with impaired implantation in women undergoing IVF. The associations between BPA and implantation failure in women may be due to the effects of BPA on the embryo, uterus, or both.
• BPA exposure in male rats is associated with implantation failure in non-exposed female rats. Human data, however, are lacking.
• BPA is associated with sexual dysfunction among men exposed to high occupational levels and in experimental studies on males.

(3) There is insufficient evidence to draw conclusions regarding effects of BPA on the oviduct, placenta, and pubertal development.

(4) Future studies need to:
• Consider the critical period of differentiation of the organ system in question and the reproductive life span of the animal model or human.
• Use continuous exposure to BPA in view of the ubiquitous and continuous exposure to BPA in humans.
• Target internal dose levels of BPA that are achieved by human exposures.
• Recognize the potential interaction of BPA with other hormone altering chemicals and life style factors such as diet and stress.
• Distinguish organizational (permanent) vs. activational (transient) effects of BPA.
• Determine whether pre-term and maternal morbidities (e.g., pre-eclampsia, gestational diabetes) as well as paternal factors modify or mediate the effects of BPA on the reproductive system.