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Minireview

Effects of Endocrine-Disrupting Chemicals on the Ovary[1]

Shreya Patel, Changqing Zhou, Saniya Rattan, and Jodi A. Flaws[2]

Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois

ABSTRACT

Endocrine-disrupting chemicals (EDCs) are found abundantly in the environment, resulting in daily human exposure. This is of concern because many EDCs are known to target the female reproductive system and, more specifically, the ovary. In the female, the ovary is the key organ responsible for reproductive and endocrine functions. Exposure to EDCs is known to cause many reproductive health problems such as infertility, premature ovarian failure, and abnormal sex steroid hormone levels. Some EDCs and their effects on adult ovarian function have been studied extensively over the years, whereas the effects of others remain unclear. This review covers what is currently known about the effects of selected EDCs (bisphenol A, methoxychlor, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, phthalates, and genistein) on the adult ovary and the mechanisms by which they act upon the ovary, focusing primarily on their effects on folliculogenesis and steroidogenesis. Furthermore, this review discusses future directions needed to better understand the effects of EDCs, including the need to examine the effects of multiple and more consistent doses and to study different mechanisms of action.

*2,3,7,8-tetrachloro-*p*-dibenzodioxin, bisphenol A, folliculogenesis, genistein, methoxychlor, ovary, phthalates, steroidogenesis*

INTRODUCTION

Environmental toxicants are chemical compounds found in the environment due to a variety of processes, including manufacturing, combustion, leaching from products, and human contamination (reviewed in [1]). Some environmental toxicants also occur naturally in plants. Many environmental toxicants are known endocrine-disrupting chemicals (EDCs) [2]. This is of concern because women are exposed to EDCs on a daily basis, and some EDCs are known to target the ovary and cause reproductive health problems such as infertility, premature ovarian failure, and abnormal sex steroid hormone levels. Infertility is a public health concern because it affects millions of women worldwide, decreases quality of life, and results in large medical costs. Premature ovarian failure is a public health concern because it leads to early infertility and is associated with an increased risk of osteoporosis, depression, cardiovascular disease, and early death [3, 4]. Abnormal sex steroid hormone levels are of public health concern because they can cause infertility and are associated with other serious conditions including osteoporosis, depression, and cardiovascular disease [1, 5].

The purpose of this minireview is to review the recent research on the impact of selected EDCs on the adult ovary. We focused on the adult ovary because adult humans are exposed to EDCs, and several recent reviews have already discussed developmental exposure to EDCs [6–9]. Furthermore, we chose to review the effects of EDCs on ovarian folliculogenesis, follicle/oocyte health, and steroidogenesis because they are key factors required for normal ovarian function (Figs. 1 and 2). We also present information on effects of the selected EDCs bisphenol A (BPA), methoxychlor (MXC), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), phthalates, and genistein. We focused on BPA, MXC, and phthalates because they have been identified as contaminants present in human tissue and the environment [10–17]. We selected TCDD because it is widely used as a model EDC, is present in human tissues, and has been shown to be an extremely toxic chemical [18]. We review genistein because women are widely exposed to it, and it is used as a model compound for phytoestrogen studies [19, 20].

BISPHENOL A

BPA is a plasticizer used commonly in a wide range of consumer products such as food and drink containers, epoxy resins, plastics, baby bottles, thermal receipts, and dental sealants (reviewed in [21]). As a result, humans are constantly exposed to BPA via oral and dermal routes of exposure. BPA has been measured repeatedly in serum [22–24], plasma [25, 26], urine [27], sweat [27], breast milk [28, 29], amniotic fluid [30–32], placental tissue [33], fetal serum [30, 33], and umbilical cords [34]. The currently accepted lowest observed adverse effect level for BPA is 50 mg/kg/day, and the current U.S. Environmental Protection Agency (EPA) reference dose is 50 μg/kg/day, but actual daily human exposure to BPA may be much higher [35]. This raises concern because BPA exposure has been associated with female fertility problems [10], polycystic ovary syndrome [36], and endometriosis [37]. Moreover, in women undergoing fertility treatments, urinary BPA levels have been associated with decreased antral follicle counts and a reduction in the number of oocytes retrieved [38, 39].

BPA has also been shown to reduce fertility [10, 40], reduce the primordial follicle pool [40], lead to premature ovarian failure [40], disturb the estrous cycle [10, 40], and disrupt steroidogenesis in a variety of animal models [10, 40]. Furthermore, studies have shown that adult exposure to BPA

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[2]Correspondence: Jodi A. Flaws, University of Illinois at Urbana-Champaign, Department of Comparative Biosciences, 2001 S. Lincoln Ave., Urbana, IL, 61802. E-mail: jflaws@illinois.edu

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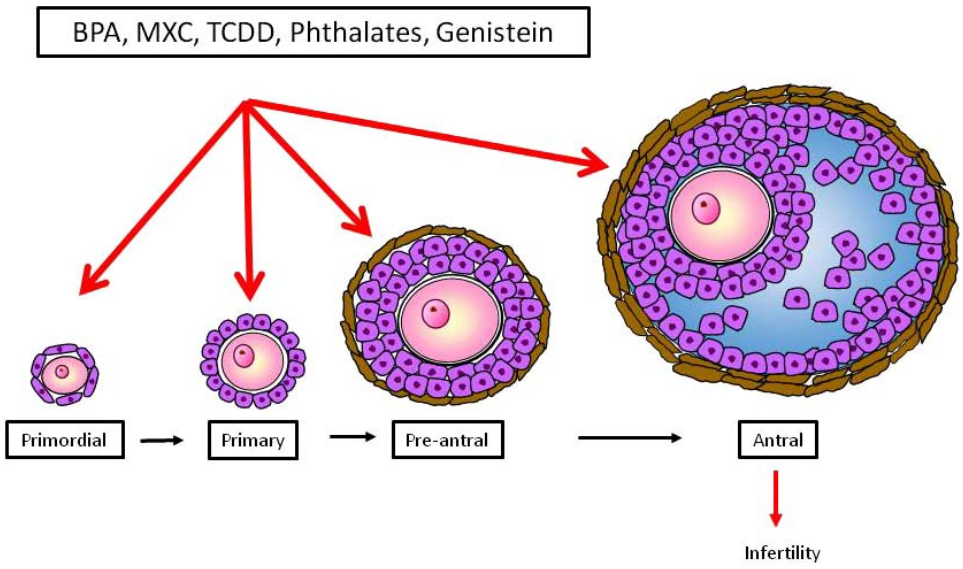

FIG. 1. Endocrine-disrupting chemicals can affect folliculogenesis. Females are born with a finite pool of immature primordial follicles, and over time, some will grow and mature to primary, preantral, and then antral follicles. The most mature follicle is the antral follicle. It is the only follicle type capable of ovulating and producing sex steroid hormones. Exposure to EDCs affecting primordial follicles can lead to permanent and early infertility. Effects of EDCs on the antral follicle can result in infertility and altered hormone levels.

(110, 219, and 440 μM) inhibits antral follicle growth in isolated mouse antral follicles in vitro (Fig. 1) [41–43]. It is likely that BPA-induced inhibition of follicle growth is mediated by BPA-induced disruption in steroidogenesis [41], interference with the aryl hydrocarbon receptor (AHR) pathway [42], and dysregulation of cell cycle regulators [43]. It is unlikely, however, that the ability of BPA to inhibit follicle growth is mediated by estrogen receptors because estradiol and ICI 182,780 (a high-affinity estrogen receptor antagonist) co-treatments failed to protect follicles from BPA-induced inhibition of growth [43].

Follicle atresia can also be induced by BPA exposure. In mouse antral follicles, BPA (100 μg/ml) increased the expression of the proapoptotic factor BCL2-associated X protein (*Bax*) to the antiapoptotic factor B cell lymphoma 2 (*Bcl2*), and transformation-related protein 53 (*Trp53*), leading

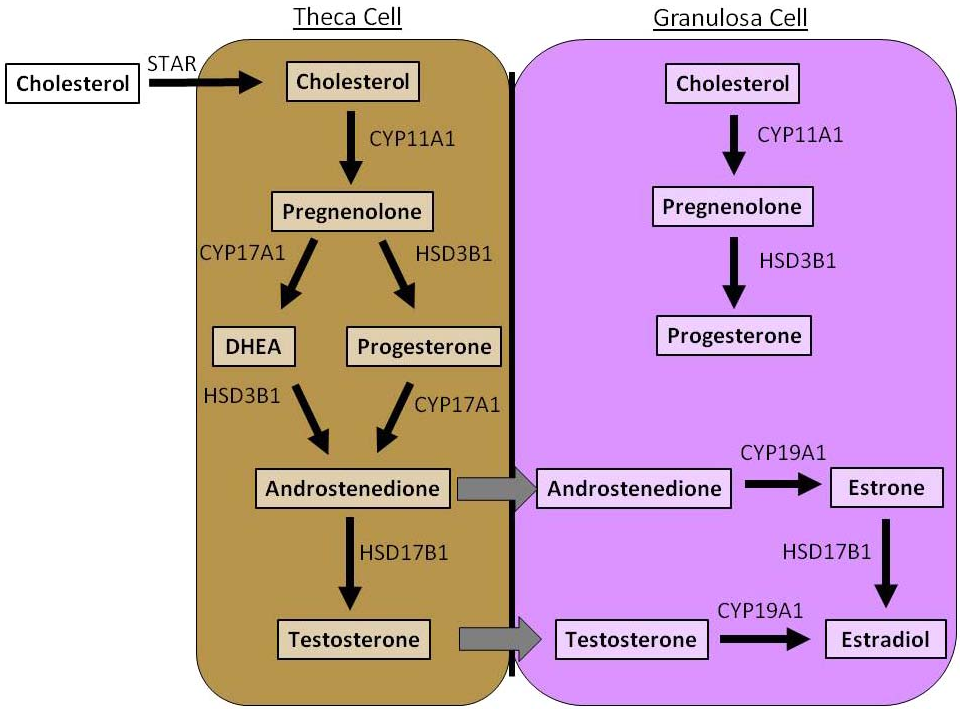

FIG. 2. Steroidogenic pathway. The antral follicle is responsible for producing female sex steroid hormones, and it does so by the process known as steroidogenesis. Steroidogenesis requires both theca and granulosa cells. Steroidogenic enzymes present in these cells are responsible for metabolizing cholesterol to 17-β estradiol and other necessary sex steroid hormones. EDCs can alter the expression, protein level, or enzyme activity of steroidogenic enzymes, thus resulting in altered sex steroid hormones.

to follicle atresia [43]. In murine granulosa cells, BPA treatment (100 μM) elevated the *Bax*:*Bcl2* ratio at both the protein and the mRNA levels and caused G_2-to-M arrest, leading to DNA damage [44]. In an in vivo study in which adult female rats were dosed with BPA for 90 days, BPA treatment (0.001 and 0.1 mg/kg/day) increased follicle atresia and luteal regression by inducing caspase-3-associated apoptosis [45]. Although BPA has been shown to bind to estrogen receptor alpha (ESR1) [46], overexpression of ESR1 did not affect the susceptibility of follicles to BPA-induced atresia, suggesting that BPA-induced follicle atresia is independent of ESR1 [43]. Although some studies have shown that BPA may act independently of ESR1 [43], others have shown that BPA may act through estrogen receptor beta 2 (ESR2) [47] or the nongenomic G protein-coupled receptor (GPCR) [48].

BPA exposure has also been associated with decreased oocyte quality in multiple studies [49–51]. Hunt et al. [49] first reported that BPA exposure (20, 40, and 100 ng/g/day) causes meiotic defects in the oocytes harvested from mice. Subsequently, Trapphoff et al. [50] showed that BPA exposure (3 nM) induces epigenetic changes, which can lead to meiotic errors in cultured mouse follicles. BPA (100 μM) has also been shown to decrease hyaluronic acid in the extracellular matrix of the oocyte cumulus matrix and to adversely affect oocyte meiotic maturation [51].

BPA exposure also adversely affects sex steroid hormone levels by interfering with steroidogenesis in several animal models. However, the specific effects of BPA on steroidogenesis differ by species and doses. In rat ovarian theca interstitial cells and granulosa cell cultures, BPA exposure (0.1–10 μM) significantly increased testosterone and progesterone levels by increasing the expression of several key cytochrome p450 steroidogenic enzymes such as 17-α hydroxylase (*Cyp17a1*), cholesterol side chain cleavage enzyme (*Cyp11a1*), and steroidogenic acute regulatory protein (*Star*) [52] (Fig. 2). Conversely, BPA exposure (1–100 μM) decreased estradiol levels by decreasing the expression of aromatase (*Cyp19a1*) [52]. In porcine granulosa cells, BPA exposure (0.01–10 μM) increased the basal levels of progesterone but inhibited follicle-stimulating hormone (FSH)-induced estradiol production [53]. In contrast, another study conducted with porcine ovarian granulosa cell cultures showed that exposure to BPA for 48 h decreased progesterone production (0.1–10 μM) and stimulated estradiol production at low BPA concentrations (0.1 μM) but inhibited estradiol production at higher BPA concentrations (1 and 10 μM) [54]. In the two studies using porcine granulosa cell cultures, the dose ranges were similar, but the culture time lengths were different. Thus, differences in results obtained from the two studies may be due to the different lengths of the cell culture time. In mouse antral follicle cultures, BPA (10 μg/ml and 100 μg/ml) also inhibited steroid hormone production by disrupting expression of steroidogenic enzymes [55]. Specifically, BPA (10 μg/ml and 100 μg/ml) acutely decreased *Cyp11a1* expression as early as 18 h, leading to a decrease in progesterone levels at 24 h and further leading to decreases in androstenedione, testosterone, and estradiol, as well as the expression of *Star* at 72 h [55]. Interestingly, some of the effects of BPA on steroidogenesis in mice are reversible with removal of BPA [55] or supplementation with pregnenolone, a precursor hormone in the ovarian steroidogenesis pathway [41]. In vivo studies also indicate that BPA can disrupt steroidogenesis in the adult ovary. In rats, BPA (0.001 mg/kg/day and 0.1 mg/kg/day) significantly decreased serum estradiol levels by decreasing the protein levels of CYP19A1 and StAR in granulosa cells and theca interstitial cells [45]. Together, these studies show that BPA exposure disrupts normal steroidogenesis in the ovary by affecting several steroidogenic enzymes and upstream hormone levels in both in vitro and in vivo settings.

The mechanisms by which BPA exerts ovotoxic effects are still not fully understood. Oxidative stress, glucose metabolism, and insulin signaling have been shown to impair testicular functions, induce toxicity, and lead to infertility in adult male animals [56–59], but limited information is available on whether such processes are involved in BPA-induced ovarian toxicity. In one study, BPA at 25 mg/kg/day caused oxidative damage in ovarian tissues [60], but the study only used one dose of BPA and thus, there is a need for additional studies using a wide range of BPA doses. Such studies will be helpful in understanding how BPA disrupts female reproduction.

METHOXYCHLOR

MXC is an organochlorine pesticide used in many countries against insects that attack fruits, vegetables, and home gardens. It is present in food and water samples [11–13], and as a result, humans are exposed to MXC. Studies have shown that 35% of agricultural commodities contain pesticide residues including MXC [13]. Badach et al. [12] showed that MXC levels ranged from 0.0165 to 1.1507 μg/L in well water and from 0.0177 to 0.9660 μg/L in samples from water mains. Although MXC production was stopped in the United States due to the failure of the manufacturer to properly register its production with the EPA, it is still an important chemical to study because it is currently used on agricultural products in many other countries that are also imported into the United States and other countries, resulting in global human exposure. Furthermore, Golovleva et al. [14] showed that MXC is persistent in soil and that its residues are present even 18 mo after soil treatment using microorganisms that biodegrade MXC, and Shegunova et al. [15] showed that MXC residues are present even in areas where the chemical has not been used, and they propose this is due to atmospheric redistribution.

MXC is metabolized predominantly to 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl) ethane (MOH) and the bisphenolic compound 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl) ethane (HPTE) by cytochrome p450 enzymes in the body [61]. Several studies have consistently shown that MXC, as well as its metabolites, interferes with folliculogenesis. For example, studies have shown that treatment with MXC (100 mg/kg) in vivo inhibits folliculogenesis (Fig. 1), likely by reducing the expression of *Esr2* and by increasing the expression of anti-Müllerian hormone (*Amh*) in preantral and early antral follicles [16, 62]. Studies have also shown that treatment with MXC (1–100 μg/ml) or its major metabolites in vitro inhibits growth of isolated antral follicles, likely by reducing the expression of G_1-S phase cell cycle regulators such as cyclin D2 (*Ccnd2*) and cyclin-dependent kinase 4 (*Cdk4*), by decreasing the antiapoptotic factor *Bcl2* and increasing the proapoptotic factor *Bax* [63, 64]. In addition, MXC exposure in vivo (32 and 64 mg/kg/day) induces oxidative stress in mouse ovaries [65, 66].

Multiple studies have also shown consistently that MXC and its metabolites inhibit ovarian steroidogenesis. For example, MXC (1–100 μg/ml) inhibits the production of estradiol, testosterone, and androstenedione in isolated mouse antral follicles [67]. The effects of MXC (1–100 μg/ml) on steroid hormone levels likely stem from its ability to inhibit the expression of key factors in the estradiol biosynthesis pathway (i.e., *Cyp19a1*, *Cyp17a1*, *Cyp11a1*, *Star*, 17β-hydroxysteroid dehydrogenase-1 [*Hsd17b1*], and 3β-hydroxysteroid dehydrogenase-1 [*Hsd3b1*]) (Fig. 2), along with its ability to induce

expression of cytochrome P450 1b1 (*Cyp1b1*), an enzyme that metabolizes estradiol [67]. The MXC metabolite HTPE (500 nM) inhibits *Cyp11a1* activity, leading to decreased progesterone production by cultured rat ovarian follicular cells [68]. Similarly, HPTE (1–10 μM) reduces FSH-stimulated synthesis of progesterone and estrogen in cultured rat granulosa cells by decreasing the expression levels of *Cyp11a1* and *Cyp19a1* [69]. The MXC metabolite MOH inhibits steroidogenesis both by reducing the availability of pregnenolone [70] and by inhibiting the expression levels of *Cyp11a1*, *Cyp17a1*, and *Cyp19a1* mRNA in mouse antral follicles in vitro [71].

The mechanisms by which MXC and its metabolites interact with ovarian cells to inhibit folliculogenesis and steroidogenesis are unclear. Some studies show that MXC and its metabolites may bind to estrogen receptors and exert estrogenic, antiestrogenic, and antiandrogenic properties, depending on the receptor subtype with which it interacts [72, 73]. This possibility is supported by studies that have shown that fetal and neonatal exposure to MXC (20 μg/kg and 100 mg/kg) alters methylation on the promoter region of *Esr1*, suppressing the expression of *Esr1* and causing ovarian dysfunction in the rat [16, 74]. It is also supported by studies by Paulose et al. [75] that show that ESR1 overexpression in antral follicles was more sensitive to toxicity induced by MXC and its metabolites than control antral follicles, suggesting that disruption in the equilibrium between ESR1 and ESR2 in the ovary may alter the response of the ovary to estrogenic chemicals. In addition, some studies suggest that MXC may interact with pathways that cross-talk with estrogen receptors. Specifically, evidence provided by Basavarajappa et al. [76] suggests that MXC may act through the AHR pathway to inhibit follicle growth and induce atresia in mouse antral follicles.

Although studies consistently show that MXC inhibits follicle growth and steroidogenesis, little is known about the effects of MXC on female fertility. Thus, future studies should examine whether MXC exposure during adulthood causes subfertility or infertility. Furthermore, little is known about whether other organochlorine pesticides affect folliculogenesis and steroidogenesis. Therefore, future studies should examine the effects of organochlorine pesticides as well as mixtures of organochlorine pesticides on the ovary and female reproductive system.

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

TCDD is a persistent environmental contaminant inadvertently produced as a by-product of herbicide and pesticide manufacturing, bleaching process at tree pulp and paper mills, and burning of municipal solid waste [77–79]. TCDD is the most toxic member of the dioxin class of chemicals. It also has a long environmental half-life, accumulates in the food chain [18], and is found in human fat tissue [18], blood serum [18, 80], breast milk [81], and ovarian follicular fluid [82].

Studies consistently show that TCDD exposure targets the ovary and leads to altered folliculogenesis (Fig. 1). However, the effects of TCDD on folliculogenesis differ by species. In mice, TCDD (0.1–100 nM) exposure does not affect the growth of antral follicles in vitro, suggesting that it does not affect proliferation of granulosa cells [83]. In pigs, however, TCDD (0.1 and 10 nM) reduces the percentage of proliferating cells in follicles over time [84]. Similarly, in rats, TCDD exposure decreases the number of antral follicles without increasing in atresia, suggesting that TCDD has an antiproliferative effect on the rat ovary [85]. Reasons for species differences in response to TCDD are unclear but may stem from differences in the abilities of species to metabolize TCDD as well as species differences in expression of the AHR, the primary receptor for TCDD [86].

TCDD exposure has also been shown to reduce or block ovulation in rodents in vivo [87–89]. The mechanism by which TCDD (32 μg/kg body weight) blocks ovulation likely involves the ability of TCDD to reduce the numbers of granulosa cells in S phase and inhibit the levels of cyclin dependent kinase 2 (*Cdk2*) and *Ccnd2* following equine chorionic gonadotropin treatment in immature rats [90].

In addition to interfering with folliculogenesis and ovulation, TCDD can interfere with ovarian steroidogenesis (Fig. 2). Karman et al. [83, 91] showed that TCDD exposure (0.1–100 nM) decreased progesterone (1 nM), androstenedione (0.1 and 1 nM), testosterone (0.1 and 1 nM), and estradiol (0.1–10 nM) levels in a nonmonotonic dose response manner in isolated mouse antral follicles. Furthermore, Karman et al. [83] showed that the addition of pregnenolone substrate (10 μM) restored hormone levels to control levels, suggesting that TCDD may act prior to pregnenolone formation to decrease hormone levels in mice. The effects of TCDD on ovarian steroidogenesis appear to be due to the ability of TCDD to inhibit key steroidogenic enzymes (*Hsd17b1* and *Cyp19a1*), leading to reduced steroidogenic capacity of antral follicles [91].

These data are consistent with those in other studies in other species and culture systems, which also show that TCDD inhibits ovarian steroidogenesis and that it often does so in a nonmonotonic fashion [84, 92–94]. Specifically, TCDD inhibits estradiol levels in female mice in vivo [95], and oral administration of TCDD (20, 50, and 125 ng/kg/day) weekly for 29 wk reduces estradiol levels in Sprague-Dawley rats [96]. In addition, TCDD exposure (90 ng/kg/day) increases the testosterone-to-estradiol ratio in juvenile mice [97], and TCDD decreases estradiol levels in the chicken ovary [98]. Furthermore, TCDD exposures of 0.1 nM or 10 nM reduce estradiol levels, whereas only 10 nM exposure significantly reduces progesterone levels in isolated porcine thecal and granulosa cell co-cultures [99]. These results suggest that, like hormones and other environmental contaminants that act as endocrine disruptors, TCDD may have multiple modes of action in antral follicles, depending on the dose.

PHTHALATES

Phthalates are synthetic chemicals found ubiquitously in the environment and are known to have endocrine-disrupting characteristics (reviewed in [17]). More than 18 billion pounds of phthalates are used each year, predominantly as plasticizers in polyvinyl chloride (PVC) products such as upholstery, table cloths, shower curtains, pesticides, solvents, and infant toys [100]. Phthalates are used to impart flexibility to plastics but leach from plastic products into the environment over time due to their noncovalent bonds [17]. Phthalates can also be found in air, sediments, agricultural and urban soil, wastewater, and natural bodies of water [101–103].

Di-(2-ethylhexyl) phthalate (DEHP) is the plasticizer most commonly used for PVC and is approved for use in medical devices such as tubing, blood bags, and dialysis equipment [104]. It is also used to manufacture disposable medical examination and sterile surgical vinyl gloves [104]. Dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and diethyl phthalate (DEP) are also produced in high volumes and are commonly used in consumer products [17]. The estimated range of daily human exposure to DEHP is 0.71–4.6 μg/kg/day, 0.84–5.22 μg/kg/day to DBP, 0.26–0.88 μg/kg/day to BBP, and 2.32–12 μg/kg/day to DEP [105].

Phthalates have been shown to alter follicle development and growth and impair follicle functionality (Fig. 1). Studies in mice have shown that exposure to phthalates may lead to acceleration of primordial follicle recruitment (Fig. 1). Specifically, exposure to DEHP (20 μg–750 mg/kg/day) in vivo for 10 to 30 days accelerates primordial follicle recruitment through a mechanism that involves overactivation of the phosphatidylinositol 3-kinase (PI3K) pathway [106]. In contrast, exposure to DEHP in vitro did not alter folliculogenesis, but exposure to mono-(2ethylhexyl) phthalate (MEHP), the bioactive metabolite of DEHP, in vitro, accelerates primordial follicle recruitment [107]; thereby indicating that DEHP accelerates folliculogenesis through its metabolite MEHP. Exposure to DBP (1000 μg/ml) in vitro inhibits growth of antral follicles by disrupting the cell cycle [108]. Specifically, DBP inhibits the expression of *Ccnd2*, cyclin E1 (*Ccne1*), cyclin A2 (*Ccna2*), and cyclin B1 (*Ccnb1*) and increases expression of cyclin-dependent kinase inhibitor 1A (*Cdkn1a*) [108]. These changes indicate DBP exposure may result in cell cycle arrest, thereby inhibiting antral follicle growth in vitro [108]. Collectively, these studies provide evidence that exposure to certain phthalates affects ovarian folliculogenesis, but additional studies are required to determine if other phthalates inhibit follicle growth and to determine the mechanisms by which they do so.

Phthalates also adversely affect the health of follicles. Exposure to DEHP (600 mg/kg) by oral gavage for 60 consecutive days decreases primary and secondary follicle numbers and increases atretic follicles [109]. This may have been caused by a DEHP-induced increase in apoptotic granulosa cells [109]. MEHP has been shown to affect follicle health by inducing oxidative stress by increasing reactive oxygen species levels and disrupting the expression and activity of the antioxidants superoxide dismutase 1 (SOD1) and glutathione peroxidase (GPX), thereby, resulting in MEHP-induced inhibition of antral follicle growth [110]. BBP (1 μM) has been shown to reduce human granulosa cell viability most likely by increasing mRNA and protein levels of the AHR, aryl hydrocarbon receptor nuclear translocator (ARNT), and CYP1B1 [111].

Although these studies collectively indicate that phthalates directly affect follicle health, future studies are needed to determine whether the effects of phthalates on the ovary significantly impact fertility and the timing of reproductive senescence. In addition, future studies are needed to examine the impact of other phthalates on follicle and oocyte health and to determine the mechanisms by which phthalates induce ovarian toxicity.

Like BPA, MXC, and TCDD, phthalates have been shown to alter steroidogenesis (Fig. 2). In vivo studies have shown that DEHP exposure (300 and 600 mg/kg) in rats decreases serum estradiol levels [109]. This is likely the result of decreased *Cyp19a1* expression [109]. Similar results are seen in vitro as well. Studies showed that exposure to DEHP (10 and 100 μg/ml) decreased estradiol levels in mouse antral follicles [112, 113]. The same studies showed that DEHP exposure (100 μg/ml) reduced *Cyp19a1* expression [112, 113]. Exposure to MEHP (0.1–10 μg/ml) decreased testosterone, estrone, and estradiol levels in cultured mouse antral follicles [107]. These effects are likely due to the ability of MEHP to inhibit the expression of *Cyp17a1*, *Hsd17b1*, and *Cyp19a1* [107]. Similar to the in vitro mouse study, a study by Reinsberg et al. [114] showed that increasing MEHP exposure decreases estradiol levels and aromatase activity in human granulosa cells. In contrast, Inada et al. [115] found that exposure to MEHP (10, 30, and 100 μg/ml) increased the combined levels of progesterone, androstenedione, testosterone, and estradiol in cultured rat antral follicles. The increases in steroid hormones are likely attributable to overactivation of progesterone and testosterone synthesis. Reasons for differences between the effects of MEHP on rat and those on mouse follicles are unknown but could stem from species differences in metabolism of MEHP. Studies also show that exposure to DBP (1000 μg/ml) significantly reduced the amount of accumulated estradiol in cultured mouse antral follicles, although the exact mechanism by which this occurs is not fully understood [108].

Collectively, these studies provide evidence that phthalate exposure during adulthood alters ovarian steroidogenesis, but future studies need to examine whether similar effects of phthalates occur in humans and whether the ability of phthalates to alter steroid levels leads to infertility and other adverse outcomes such as cardiovascular disease and osteoporosis. Furthermore, future studies should focus on biologically relevant dose ranges, and attempt to mimic normal human exposure by examining the effects of exposure to phthalate mixtures as well as mixtures of other ubiquitous toxicants on the ovary.

GENISTEIN

Genistein is an isoflavone phytoestrogen found naturally in plant structures such as soy beans, chickpeas, sunflower seeds, and lentils [116]. Genistein, like other phytoestrogens, is also consumed as a supplement to treat numerous ailments including cancer, kidney disease, neuronal injury, sexual dysfunction, depression, and menopausal symptoms [117, 118]. Humans are exposed to genistein primarily through consumption of soy-based dietary products such as soy milk, tofu, and soy flour, and thus, genistein is considered an important environmental estrogen in the human diet [117, 119]. Genistein, like other phytoestrogens, has been shown to bind to and signal through estrogen receptors [120–122] with a greater affinity to ESR2 than to ESR1 [121], thereby making the estrogen receptor-rich ovary a major target tissue for genistein.

Genistein exposure has been shown to alter folliculogenesis in rats in vivo [123, 124] (Fig. 1), but the results have varied depending on age, strain, and dose. Genistein exposure (50 mg/kg) decreases healthy primordial, primary, and secondary follicles but increases the amount of antral follicles in 18-day-old Wistar rats, suggesting that genistein exposure accelerates follicle recruitment [123]. Additionally, the same study showed that genistein exposure (50 mg/kg) increases the number of atretic secondary and antral follicles [123], suggesting that genistein-induced accelerated follicles may not survive to produce viable oocytes. Conversely, other studies indicate that genistein exposure (160 mg/kg) increases primordial follicles and decreases antral follicles in 3-mo-old Sprague-Dawley rats [124], suggesting that genistein inhibits follicle growth. Studies using a mixture of soy isoflavones (SIF) with a 60% concentration of genistein have shown that SIF at 200 mg/kg increases the number of apoptotic cells in primary and antral follicles and corpora lutea in rats [125]. Furthermore, 100 mg/kg SIF also increases the number of apoptotic cells in antral follicles and corpora lutea of rats [125]. This phenomenon is likely due to increased protein levels of the apoptotic factors caspase 3, FAS, and BAX combined with decreased protein levels of antiapoptotic factor BCL2 [125]. The reasons for differences in the ability of genistein to accelerate or inhibit follicle growth are unclear. Future studies should be conducted with a wider range of doses, various dosing periods, and ages and should examine the possible mechanisms by which genistein increases or decreases folliculogenesis in vivo.

The effects of genistein exposure on steroidogenesis have been studied extensively in vitro. Studies showed that exposure to genistein alters progesterone levels, but the actual effect depends on the dose and species. One study showed that genistein (45 μM) decreases prolactin-induced progesterone in porcine theca cells [126]. In another study, exposure to genistein (1–50 μM) decreased progesterone levels in human granulosa cells [127]. Furthermore, genistein exposure (50 μM and 100 μM) decreased production of progesterone from pregnenolone in human granulosa cells [127, 128]. Studies done with rat granulosa cells have also provided results that varied by dose. In one study, genistein exposure (0.5–50 μM) reduced basal levels of progesterone and FSH-induced progesterone levels (10 μM) [129]. Another study showed that low concentrations of genistein (0.1–3 μM) increased FSH-induced progesterone levels, but high concentrations of genistein (30–100 μM) decreased FSH-induced progesterone levels [130]. Genistein exposure (30 and 100 μM) also reduced FSH-induced estradiol levels in rat granulosa cells [130]. Similarly, genistein (50 and 100 μM) decreased estradiol levels in human granulosa cells, possibly due to altered levels in precursor hormones or changes in steroidogenic enzymes [127, 128].

It is possible that the effects of genistein on sex steroid hormone levels may be due to dysregulation of steroidogenic enzymes. For example, genistein exposure (10 and 50 μM) reduces mRNA expression and activity of CYP19A1 in human granulosa cells [131]. Because *Cyp19a1* is the key enzyme responsible for transforming precursor hormones to estradiol (Fig. 2), down-regulation of this enzyme may explain the decreased estradiol levels seen in many studies. Genistein exposure (1–50 μM) also decreases CYP11A1 protein levels and *Hsd3b1* expression in porcine granulosa cells [132]. These two enzymes are responsible for transforming cholesterol and pregnenolone to progesterone (Fig. 2). Thus, inhibition of these enzymes may cause the genistein-induced decrease in progesterone levels.

The reasons for the different effects of genistein on sex steroid hormone levels are unclear. Future studies should examine the potential mechanisms by which genistein affects estradiol levels. In addition, studies should be conducted using whole antral follicles because steroid hormone production relies heavily on both the theca and granulosa cells and most previous studies have been conducted solely on isolated theca or granulosa cells.

CONCLUSIONS

Adult women are exposed to several EDCs, including BPA, phthalates, MXC, TCDD, and genistein. These EDCs have been shown to target the ovary and adversely affect folliculogenesis, follicle/oocyte health, and steroidogenesis (Figs. 1 and 2). This is of concern for public health because the selected EDCs are used extensively in a wide variety of commonly used items or they are present as contaminants in the environment, resulting in ubiquitous human exposure. Furthermore, the effects of EDCs on folliculogenesis, follicle/oocyte health, and steroidogenesis can have lasting effects on reproductive and nonreproductive health because folliculogenesis, follicle/oocyte health, and steroidogenesis are essential for fertility, maintenance of appropriately timed reproductive senescence, and regulation of skeletal, cardiovascular, and brain health [1–5].

Although recent studies indicate that EDCs are ovarian toxicants, we still have limited information about the impact of many EDCs in women and in animal models. Furthermore, we have limited information on the mechanisms by which EDCs exert ovarian toxicity. Thus, future studies are needed to fully understand the impact of EDCs on the ovary and to determine the mechanisms by which they exert ovarian toxicity. Additionally, future studies should examine the effects of EDCs on the hypothalamic-pituitary-gonadal axis, as a whole, to see if EDCs indirectly affect the ovary by affecting the hypothalamus or pituitary. By better understanding the impact of EDCs on the adult ovary, we may be able to develop better policies for preventing EDC-induced ovarian toxicity. Increased comprehension of the mechanisms by which EDCs inhibit folliculogenesis, decrease follicle/oocyte health, and alter steroidogenesis may help us develop novel treatments for EDC-induced ovarian damage and thus, develop novel treatments for EDC-induced abnormalities in reproductive and nonreproductive health.

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