Do prenatal exposures pose a real threat to ovarian function? 
Bisphenol A as a case study

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Abstract

Fetal development represents a time of potential vulnerability due to rapid cell division, organ development and limited fetal kidney/liver activity for detoxification and metabolism of exposures. Health effects of prenatal toxicant exposure have previously been described, but there is little cohesive evidence surrounding effects on ovarian function. Using bisphenol A (BPA) as a case study, we seek to examine whether a prominent prenatal environmental exposure can pose a real threat to human ovarian function. To do so, we broadly review human oogenesis and menstrual cycle biology. We then present available literature addressing prenatal bisphenol A and diverse outcomes at the level of the ovary. We highlight relevant human cohorts and mammalian models to review the existing data on prenatal exposures and ovarian disruption. Doing so suggests that while current exposures to BPA have not shown marked or consistent results, there is data sufficient to raise concerns regarding ovarian function. Challenges in the examination of this question suggest the need for additional models and pathways by which to expand these examinations in humans.


Introduction

Developmental and fetal programming can be conceptualized as the cumulative result of numerous influences during the perinatal period (Padmanabhan et al. 2016). These influences include exposures to environmental factors such as toxicants, air pollution and endocrine-disrupting chemicals (EDCs). The latter can be encountered by direct contact, ingestion, inhalation, maternal-fetal transfer and intravenous administration in the medical setting (Gore et al. 2014). However, data capture on prenatal exposure to EDCs, especially in humans, remains a limited subject. Furthermore, there are significant challenges in this body of work, including (1) heterogeneity in capturing or measuring exposures, (2) nonlinearity in dose-response, (3) concurrent exposures to multiple agents and (4) limited (though growing) studies that examine the preconception and prenatal windows and subsequent adult life. There is additional challenge in harmonizing across and comparing studies that were designed to understand safety (i.e. regulatory studies) versus academic studies designed to test hypotheses on potentially novel end points, not considered in the regulatory lexicon. An added complexity is source of funding for either type of study; studies funded by industry sponsors may require an additional level of disclosure and transparency.

Insights from academic and regulatory studies are important and inform very different aspects of the discussion on which chemical pose which type of threat to human health. In this review, we present only one model compound as a means of example. Finally, the potentially inadequate screening procedures of chemicals for everyday use by regulatory bodies, introduction of new untested substitute chemicals or the relaxation of current policy guidelines are other emergent areas of great concern. However, effects noted at the gamete level should be taken seriously with great contemplation. Gamete-level changes may be involved in the process of natural selection and loss of reproductive capacity.

These challenges are magnified when trying to examine ovarian function. Ovarian development and function represents a complex coordination of processes, starting early during the prenatal window. Early aberrations have potential to carry through the female reproductive life span. Accurate ascertainment of dysfunction is challenged by the multitude of outcomes that may be examined. These examinations may be anatomical, histological, quantification of gene expressions or hormone measurements. Outcomes may

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include timing of sexual maturation, estrus cyclicity, oogenesis, follicular dynamics or fertility indices.

Here we seek to use bisphenol A (BPA) as a case study to examine the question as to whether prenatal exposures have the potential to disrupt ovarian function. BPA is a commonly encountered agent in modern life, including in pregnant women (Woodruff et al. 2011). Most recently, statements by the Food and Drug Administration have sought to highlight its minimal risks to humans, based on early studies/results from the CLARITY-BPA cohort (2018). Yet, it has previously been identified to have potential reproductive health effects (Gore et al. 2014, 2015, Peretz et al. 2014, Ziv-Gal & Flaws 2016). We hypothesize that there may be mounting evidence for early and potentially subclinical effects on ovarian function and ovulation from BPA at doses lower than current safety standards.

**Human toxicant exposures and EDCs**

Human toxicant exposures may be discussed in the context of biologic attacks or critical events in which single, intentional, large-volume exposures lead to significant population health effects. Examples of this include the Yusho and Yucheng rice oil contaminations (Masuda & Schecter 2012), the Love Canal waste site (Austin et al. 2011) and phthalate contamination of food products in Taiwan (Tsai et al. 2017). However, EDCs are rarely encountered in large-scale/large-volume contaminations. More often, exposure to endocrine disruptors occurs in smaller doses over longer time periods, via a number of routes, including enteral consumption, contact with skin, inhalation, maternal-fetal transfer and intravenous administration due to contact materials (Fig. 1) (Gore et al. 2015). These exposures can even occur in the most cautious of settings – even infants in the neonatal intensive care units demonstrated significantly increased urinary BPA measurements, especially when exposed to higher numbers of medical devices (Duty et al. 2013).

In 2012, an expert panel for the United Nations Environment Programme (UNEP) and World Health Organization (WHO) defined disruptors as ‘chemicals or chemical mixtures, that interfere with normal hormone action’ (Bergman et al. 2012). Similarly, the most recent Endocrine Society statement characterizes EDCs as ‘exogenous chemicals or mixture of chemicals that interfere with any aspect of hormone action’ (Gore et al. 2015). However, in 2002 the International Programme on Chemical Safety (IPCS), a collaboration between WHO, UNEP and the International Labour Organization, noted that an endocrine disruptor is ‘...an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations. A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations’ (Damstra et al. 2002). The difference between EDC definitions that require interference only vs demonstrated adverse health effects is a critical issue when considering what bases to regulate EDCs. It is unclear whether the inclusion of adverse effects in the definition helps to exclude chemicals for which statistically significant but biological important changes cannot be demonstrated enabling regulators and scientists to focus attention on chemicals that are more likely to pose a health risk.

The Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA) program is a partnership between academic scientists and US Food and Drug Administration (FDA), the National Toxicology Program (NTP) and the National Institutes of Environmental Health Sciences. The goal is to have shared data for regulatory decision making, including tissue samples. In some cases initial findings were not replicated, and in other cases differences of data interpretation resulted in the FDA declaring that BPA is safe in March 2018. While the FDA determination included sources of data from industry-sponsored studies as well, the main concern is that without the appropriately designed study for certain outcomes, we will not be able to have definitive data to make certain conclusion given different points of view within the social, political, economic and environmental context. For example, there is a paucity of data on prenatal exposures to EDCs and adult alterations in ovarian reserve, menstrual cyclicity/ovulatory function and reproductive potential. Given restrictions of resources, decisions to reduce human/ecosystem burden of EDCs based on demonstrated altered endocrine system activity

**Figure 1** Windows and modes of exposures. Over the course of the reproductive life span, exposures shift from those of the mother, to direct environmental exposures. Not illustrated are potential transgenerational and delayed effects that may be present with certain agents.
in model systems may be required, in the perspective of the authors of this article.

**EDCs and ovarian function**

EDCs previously identified as of concern to ovarian function include, but are not limited to, BPA, phthalates, polychlorinated biphenyls, dichlorodiphenyltrichloroethane (DDT), diethylstilbestrol (DES) and perfluorooctanoic acid (PFOA). These compounds can be further classified by both structure (phenolic, non-phenolic) or function (estrogenic, anti-estrogenic, anti-androgenic) and increasingly are recognized as having notable and differential effects at both low and high levels of exposure. Prominent historic examples have highlighted their potential impacts on reproductive health, particularly with prenatal exposure. As seen in the example of thalidomide and DES, the fetus may have marked effects from exposures that have no overt negative effects in mothers. These effects may span generations, as with DES where daughters of exposed women showed increased risk of infertility, breast malignancies and range of pregnancy complications (Trowbridge et al. 2014). More recently, fetal exposures to commonly used analgesics (ibuprofen, acetaminophen) have also been suggested to affect germ cell number and gene expression (Hurtado-Gonzalez et al. 2018). A conceptual figure of windows of exposure of EDCs is included as Fig. 1.

**Selection of BPA as a model compound**

BPA is used in many industrial processes (epoxy resins, polycarbonate plastic production) and in lining food cans (https://www.factsaboutbpa.org/bpa-overview/products-bpa). BPA is currently detectable in surface water and soil sediments (Yan et al. 2017) and is noted in thermal paper receipts (Liao & Kannan 2011) among other sources of dermal exposure. While the greatest exposure is dietary from canned foods and plastic containers (Yang et al. 2011), dermal exposure is a second smaller route of exposure (Von Goetz et al. 2017). We selected BPA as a model EDC for this paper because (1) BPA, in its monomeric form, is considered to be a weak estrogen receptor agonist compared to the endogenous 17-β estradiol in *in vitro* studies of receptor binding and displacement, reporter gene and cell proliferation/transcriptomics (Hewitt & Korach 2011) and (2) BPA has been studied within the context of prenatal exposure and adult disease, albeit mostly in nonhuman model systems. The estrogen receptor exists in cell membrane and cytosolic compartments, though it is primarily considered a nuclear receptor (Razandi et al. 1999). The nuclear receptors are present in isoforms alpha (ERα), beta (ERβ) and in almost every tissue (Heldring et al. 2007) with different relative concentrations across tissues (Kuiper et al. 1997), and may contribute to tissue-specific thresholds and cell-specific action (Friedman et al. 1990). In receptor-binding studies, BPA has higher affinity for ERβ but binds both isoforms (Kuiper et al. 1997, Matthews et al. 2001). There is great debate regarding downstream cellular responses, such as post-binding coactivator recruitment and gene-level responses. Finally, other studies note that BPA exposure elicits a rapid (i.e. within 15 min of exposure) and potent estrogenic effects via nonclassical estrogen-triggered pathways (Alonso-Magdalena et al. 2012, Shearer et al. 2012).

Derived from early rodent studies, current regulatory bodies report the no observed adverse effect level as 5 mg/kg/day (75 parts per million in diet) for systemic effects and 50 mg/kg/day (750 parts per million in diet) for reproductive effects (Tyl et al. 2002, 2008). There is debate whether typical human exposures to BPA in the non-medicalized general population would cause endocrine disruption via estrogen receptor-related effects (Teeguarden et al. 2013). Additionally, a study found that 83% of samples measuring urine and serum BPA concentration in non-pregnant women exposed to a high canned food diet were below the limit of detection (Teeguarden et al. 2011). However, that study did not include exposures from routine use of any other BPA-associated materials. In humans, BPA is rapidly absorbed from the gastrointestinal tract, conjugated in the liver (to its major metabolite BPA glucuronide) and excreted in the urine often within less than 24 h (Völk et al. 2002). Another study from the Teeguarden group noted that in pregnancy, the dominant endogenous estrogens (estradiol, estriol and estrone) dominated receptor occupancy compared to BPA in an *in vitro* study (Pande et al. 2018). Nonclassical tests for estrogenicity were not employed in that study.

However, in the fetus where hepatic maturation and renal function are not yet at adult human levels, BPA which enters the amniotic fluid volume may not be eliminated in such an expedient manner and may have post-receptor effects at the level of gene expression that may be of concern during ovarian organogenesis. There has been evidence suggesting prenatal and childhood exposure to BPA may be associated with neurobehavioral and cardiometabolic effects (Harley et al. 2013, Hoepner et al. 2016, Vafeiadi et al. 2016, Braun et al. 2017a, b).

Finally, there are multiple exposures which may individually, or in combination, have health effects of concern. Phthalates are also commonly encountered, thus making population-based studies more challenging to have discreetly isolated associations with BPA without controlling for other exposures, such as phthalates (Benjamin et al. 2017).
Ovarian development and function

Ovarian development/oogenesis/follicular life cycle

Germ cells migrate to the genital ridge by 6 weeks gestational age. This is followed by initiation of meiosis at 8 weeks, and subsequent follicular maturation and thecal cell development, which ultimately gives rise to the identifiable fetal ovaries by 10 weeks gestational age in humans (Smith et al. 2014, Snyder 2014). A maximum number of oocytes is reached by approximately 20 weeks gestational age (Wallace & Kelsey 2010), with subsequent atresia progressively decreasing these numbers over the life span. By birth, oocytes number around 1–2 million, then 400,000 by puberty and eventually approximately 400–500 complete the process of ovulation, with depletion of this pool at menopause (Table 1) (Baker 1963, Sarraj & Drummond 2012, Strauss & Williams 2014, Rosen & Cedars 2018).

Ovulation/ovarian function

After the initiation of puberty with thelarche, peak growth velocity and the culmination of menarche, oocytes undergo meiosis and are recruited into a follicular pool to become primary oocytes. Pulsatile release of gonadotrophin-releasing hormone (GnRH) directs the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which influences follicular development and ovulation.

Ovulation disorders can take the form of oligo- or anovulation and disordered bleeding. These symptoms may reflect hypothalamic, pituitary or gonadal pathology, but can also be due to disease of the thyroid, pituitary and adrenals. Other systemic diseases including renal or liver failure and certain medication effects can also impact ovulatory status.

Ovarian function outcomes

Taken together, it is clear that to examine disruptions of development and function at the level of the ovary, there are numerous potential outcomes. Organ weights are often reported in toxicology studies. This typically takes the form of absolute or relative (organ weight/total body weight) organ weights. Many researchers cite value addition by the latter measurement, such as in minimizing inconsistencies due to body weight fluctuations (Michael et al. 2007). We suspect changes in ovarian weight may suggest changes in ovarian composition (such as with cyst formation or follicular content). Follicular distribution and dynamics may also be examined directly with histology or ultrasonography. Immunohistochemistry can assess ovarian protein enzyme or receptor expression, while immunofluorescence studies can examine meiotic function. Gene expression studies may elucidate receptor expression, steroidogenic function and even apoptotic activity. Serum hormone levels may be measured directly or examined phenotypically with age of sexual maturation and estrus cyclicity. And downstream, fertility and fecundability (the capacity to conceive) may be an ultimate measure of ovarian function. Figure 2 illustrates the many potential outcomes surrounding ovarian function, beyond just the level of the ovary.

Methods

To present a narrative review, we performed a directed literature search in PubMed between September 2017 and June 2018, to examine existing literature surrounding prenatal exposure to BPA. The directed literature review was initially conducted in September 2017 and updated in June 2018. The date range for inclusion was from 1999 - 2018 before exclusions. For this discussion, we selected specific outcomes to include in the search, based on clinical experience and knowledge of existing literature. We did specifically focus at the level of the ovary. We have previously detailed a model for endocrine disruptors and polycystic ovary syndrome (PCOS) development (Hewlett et al. 2016) and did not look for specific ovarian disorders/diseases as part of the scope of this paper. We utilize in vivo animal studies, given the relative dearth of human studies and the use of rodent models in most toxicity studies.

The ultimate search input for BPA-eligible studies was as follows:

‘Search (((((folliculogenesis) OR "Estrogens"[Mesh]) OR ((("Reproduction"[Mesh])OR"Sexual Development"[Mesh])) OR ((("Ovarian Function Tests"[Mesh] OR "Ovarian Reserve"[Mesh] OR "Ovarian Follicle"[Mesh] OR "Menstrual Cycle"[Mesh]) OR ("Ovulation"[Mesh] OR Ovulations)))))) AND ("(bisphenol A" [Supplementary Concept] OR benzhydryl compounds OR phenols OR 4,4'-dihydroxy-2,2-diphenylpropane OR diphenylolpropone OR 2,2-bis(4-hydroxyphenyl)propone OR bisphenol A, sodium salt OR bisphenol A, disodium salt)) AND ("(Prenatal Exposure Delayed Effects"[Mesh]) OR "Maternal Exposure"[Mesh] OR in utero OR neonatal)."

This yielded 769 articles with an additional seven articles added from outside the search query. Articles were excluded if they were not available in English, or not primary investigations. They were also excluded if they did not include the relevant compounds, did not examine the compounds individually (rather than as part of mixtures), were in vitro or did not include prenatal exposure. Studies that had prenatal with neonatal exposures were included, though not if the exposure was only neonatal. Final exclusion criteria were if articles did not have data on female offspring or an outcome that was related to

<table>
<thead>
<tr>
<th>Reproductive period</th>
<th>Estimated oocyte population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal: 20-week gestational age</td>
<td>4–5 million</td>
</tr>
<tr>
<td>Birth</td>
<td>1–2 million</td>
</tr>
<tr>
<td>Puberty</td>
<td>400,000</td>
</tr>
<tr>
<td>Reproductive maturity</td>
<td>400–500 s</td>
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</tbody>
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Table 1 Oocyte populations over the female life span in humans.

Adapted from Greenspan’s Basic and Clinical and Endocrinology and Sarraj & Drummond (2012).
ovarian function, as judged by prior clinical knowledge. This yielded a final article count of 36 (Fig. 3). Search strategies are mapped according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) template (Liberati et al. 2009) and included as Fig. 4.

BPA and ovarian outcomes

Here we specifically highlight the current knowledge surrounding prenatal exposures and specifically ovarian outcomes in rodent and human models (Fig. 3).

Human studies

There is limited human data surrounding prenatal effects of BPA on ovarian measures. As such, we present three cohorts that evaluated (1) prenatal exposure and peripubertal hormone levels, (2) prenatal exposures and anogenital distance at birth and (3) a cross-sectional evaluation of cord blood BPA and hormones at birth. The Early Life Exposures in Mexico to Environmental Toxicants study evaluated third trimester measurement of urinary BPA (and correction for urine specific gravity), followed by examination of sex steroid concentrations and Tanner staging and menarchal status in 8- to 13-year-old female offspring. They did not find associations with maternal BPA concentration and examined outcomes (Watkins et al. 2014). One concern in exposure assessment is that measured exposures in the third trimester may or may not correlate with exposures in the first trimester corresponding to oogenesis and fetal ovary formation. Henceforth, expansion of this work included assessment of associations in relation to maternal BPA concentrations during specific gestational periods. In this follow-up study, there was an association of total BPA (following enzymatic deconjugation) exposure in the second trimester and increased peripubertal testosterone levels (33% change/interquartile range, 95% CI 0.3, 77.0) (Watkins et al. 2017), which may have implications for adult onset disorders of ovulation, such as PCOS. While not significant, there was also a suggested association with total BPA in the second trimester and increased peripubertal estradiol (14.9% change/interquartile range, 95% CI –1.9, 34.6); as well with total BPA in third trimester and dehydroepiandrosterone sulfate (DHEAS) (14.6% change/interquartile range, 95% CI –9.3, 44.6), though both cross 0, with wide confidence intervals (Watkins et al. 2017). DHEAS is an adrenal androgen associated with PCOS, the most common cause of menstrual irregularity and ovulation disorders in women. While they separately examined peripubertal measurements of BPA, the findings still may not fully account for the individual exposures of examined girls throughout childhood and is limited by relatively low sample sizes. Another notable cohort was The Infant Development and the Environment Study (TIDES), a multicenter study. Using this, Barrett et al. examined the relation of first trimester urinary total BPA measurements (similarly adjusted for urine specific gravity) and anogenital distance in infant girls. After utilizing two separate measurements (anus to clitoris and anus to fourchette), they found that once adjusted for covariates, BPA concentration was inversely associated with anus to clitoris measurements (Barrett et al. 2017). However, this again was a relatively small cohort, with potential for selection bias, and examining only one window of prenatal exposure. Furthermore, while changes in anogenital distance may reflect shifts in hormonal/androgen balance, it is hard to parlay this measurement for implications on ovarian function. The Sapporo Cohort of the Hokkaido Study on Environment and Children’s Health allowed another group to examine simultaneous measurement of cord blood BPA, sex steroids and other hormones at the time of delivery. This did not find associations between BPA concentrations and levels of estradiol, progesterone or testosterone (though did note a weakly positive association with prolactin) in infant girls (Minatoya et al. 2017). Yet, given the relatively rapid metabolism of BPA, cord blood measurements may be a limited mechanism of assessing exposure, possibly reflected in the reports that only 83.2% of samples had detectable BPA levels.

Ultimately, these few cohorts demonstrate models by which to study ovarian function outcomes in humans. However, there is significant room for expansion in scope as well as
### Figure 3

Outcomes related to ovarian function in offspring exposed to prenatal bisphenol A in human, rodent, sheep and primate models. This figure summarizes the notable outcome variables of each included study. Though this review focuses on outcomes at the ovary level, notation of many relevant outcomes such as adrenal androgens and gonadotrophins have been included in this figure.

| Study                  | Rodent (R) or Human (H) Model | Ovarian weight | Ovarian Histologic Features | Germ Cells | Oocytes | Oogenesis | Ovarian Follicular Distribution/Dynamics | Ovarian Atresia | Estrous Cycle | Testosterone | Total Estradiol | Progesterone | Inhibin B | DHEAS | LH | FSH | AMH | Progesterone | Estrogen Receptor Expression | Androgen Receptor Expression | Gonadotrophin Expression | Ovarian Steroidogenic Protein Expression | Ovarian Steroidogenic Gene Expression | Ovarian Apoptosis | Anogenital Distance/Index | Fertility Indices/Reproductive Capacity |
|------------------------|-------------------------------|----------------|-----------------------------|------------|---------|----------|------------------------------------------|-----------------|--------------|-------------|-----------------|-------------|-----------|-------|----|-----|------------------|-----------------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|-------------------------------|-------------------------------|---------------------------------------|
| Kwon et al. 2000       | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Lawson et al. 2011     | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Ma et al. 2017         | R                             | x              |                             | x          |         |          |                                          | x               |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Newbold et al. 2009    | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Patel et al. 2017      | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Ryan et al. 2010       | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Santamaria et al. 2016 | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Santamaria et al. 2017 | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Savabieasfahani et al. 2006 | S                     |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Suzuki et al. 2007     | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Tiel et al. 2002       | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Veiga-Lopez et al. 2013| R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Veiga-Lopez et al. 2014| R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Wang et al. 2014       | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Xi et al. 2013         | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Yoshida et al. 2004    | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Zhang et al. 2012 (A)  | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Zhang et al. 2012 (B)  | R                             |                |                             |            |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |
| Zhu-Gai et al. 2015    | R                             | x              |                             | x          |         |          |                                          |                 |              |             |                 |             |           |       |    |     |                  |                             |                                        |                                        |                                        |                                        |                               |                               |                                        |

1. Sex-hormone binding globulin (SHBG)
2. Dehydroepiandrosterone (DHEAS)
3. Follicle stimulating hormone (FSH)
4. LH
5. Anti-Mullerian hormone (AMH)
6. Cord blood measurements
numbers of these cohorts, further highlighting the need to review animal models. There are several markers of ovarian reserve, generally thought to facilitate diagnosis in the setting of infertility. Currently, even novel markers such as anti-Müllerian hormone do not have a predictive function in the absence of infertility. While historically, ovarian reserve testing and ovulation have required daily monitoring with urine and blood tests, newer methods involving real-time tracking of menstrual cycles via mobile health apps and prediction of ovulation, and the availability of immune-histochemical tests that interface with smartphones may allow more wide-scale population-based studies in humans. This is one reason why the authors have undertaken a planning study entitled the Ovulation and Menstruation Health Study. http://sites.bu.edu/pcos/. The challenge remains, however, to have cohorts of sufficient length to measure exposures in each reproductive time window, starting with conception and prenatal exposures.

Mammal studies

Aforementioned safety data largely derives from rodent studies conducted and published by Tyl et al. (2002, 2008), where reproductive toxicity was based on examination of organ anatomy, histology, anogenital distance, estrus cyclicity, vaginal opening and fertility indices. These two- and three-generation studies found decreased absolute/relative ovarian weights and delayed vaginal puberty at 7500 ppm (~500 mg/kg body weight/day), leading to current recommendations. However, continued rodent studies have expanded this body of knowledge. Notably, the design of these studies can be highly variable in almost every facet – but they do allow for a broad view of current knowledge of BPA and ovarian outcomes.

Fertility indices

Finally, a downstream measure of ovarian function is fertility. Ziv-Gal et al. previously reviewed the body of work surrounding potential BPA-mediated effects leading to infertility (Ziv-Gal & Flaws 2016), including studies that included fertility outcomes between 2007 and 2016. In 2001, Ema et al. examined these outcomes following maternal doses of BPA 0.2–200 µg/kg/day, as well as direct exposure via gastric intubation; fertility indices did not significantly change for F1 rats, with treatment group rates of 92–96% compared to 100% in controls (Ema et al. 2001). Also prior to the time period of Ziv-Gal and co-authors, Honma et al. mated gestationally exposed females (2 or 20 µg/kg/day subcutaneous injections) with unexposed males and found no significant changes in number of offspring as compared to control (14.9 ± 0.8) (Honma et al. 2002). Also with subcutaneous exposures (10 or 100 mg/kg/day) of BPA, now in rats, Suzuki et al. did not find differences in number of offspring (control: 11.9 ± 0.8, 10 mg: 10.7 ± 0.9, 100 mg: 10.8 ± 1.0) or percent giving birth out of those mated (control: 91%, 10/11, 10 mg: 100%, 10/10, 100 mg: 82%, 9/10) (Suzuki et al. 2002).

Ovarian weight

As in the seminal Tyl et al. articles, many studies examine both absolute and relative ovarian weights, following prenatal BPA exposures. A large group of these studies with prenatal BPA doses up to 2500 mg/kg body weight/day did not demonstrate changes in absolute ovarian weight (Suzuki et al. 2002, Tinwell et al. 2002, Kobayashi et al. 2010, Christiansen et al. 2014, Ziv-Gal et al. 2015, Ma et al. 2017); however, some studies had different time points for measurement, and without data consistently reported for harmonization and comparison across studies. In contrast, Kobayashi et al. did observe an increase in relative ovarian weight in rat models at dietary exposures of 3.3 (0.5 mg/kg/day) and 33 ppm (5 mg/kg/day) with mean weights of 42.0 ± 7.3 mg and 40.5 ± 4.9 mg respectively, compared to mean weight of 34.6 ± 6.3 mg ovarian weight in their unexposed counterparts (P < 0.05). Notably this difference was seen in a 5-week old F1 offspring but did not persist when assessed at 3 months (Kobayashi et al., 2012), and direct measurements of food consumption were not made. Another study showed significant decreases (P < 0.05) in absolute wet weights of rat ovaries at postnatal day 90, following prenatal doses 0.5 and 50 µg/kg/day (Santamaria et al. 2016), though these used an alternate modality of subcutaneous injections rather than typical oral exposure. Ema et al. looked at this outcome in a multigenerational study with exposure through gastric intubation, from mating through lactation. They did not find changes in ovarian weights at most BPA exposures (2, 20, 200 µg/kg/day), though did note a significant decrease in absolute (not relative) ovarian weight in F1 females following 0.2 µg/kg-day (controls: 123 ± 14 µg, 0.2 µg/kg: 110 ± 15 µg, P < 0.05). Nor did they find any differences in...
ovarian weights in the F2 generation (Ema et al. 2001). Another multigenerational study was also complicated by inconsistent decreases in absolute and relative ovarian weight, depending on ovarian laterality and dose exposures (0–1000 mg/kg/day), but did report effects in F2 females who did not receive direct prenatal BPA exposure (Hiyama et al. 2011).

Oogenesis/oocyte survival

Following 1 week of exposure to BPA doses of 20 µg/kg/day (via continuous implant) Susiarjo et al. examined meiosis in fetal oocytes isolated at 18.5 days postconception. They found a significant increase in synaptic abnormalities in oocytes from BPA-exposed females (placebo: 16.0%, BPA: 52.0%, \( \chi^2 = 134.8; P < 0.0001 \)), as well as increases in recombination (via MLH loci numbers) and consequent aneuploidy (Susiarjo et al. 2007). Zhang et al. found that prenatal oral BPA exposure of 0.08 mg/kg/day increased total oocyte numbers per histologic sections (295.566 compared to 193.77 in control, presumed \( P < 0.01 \)) on postnatal day 3 (Zhang et al. 2012a). They also reported increased percentages of oocytes in the germ cell cysts was higher than controls with this dose and time point (control: 22.14%, 0.08 mg/kg: 69.17%, \( P < 0.01 \)). They did not observe significant changes at other time points or with the 0.02 and 0.04 mg/kg/day doses (Zhang et al. 2012a). With immunostaining, they examined chromosomes and found shifts in meiotic stages, suggestive of significant delays following prenatal BPA exposure of 0.08 mg/kg/day (Zhang et al. 2012a). Following prenatal exposures by subcutaneous injections of 10 and 100 mg/kg body weight/day, Suzuki et al. did not find changes in prevalence of polycystic follicles (Suzuki et al. 2002).

In their experiments with prenatal oral doses of BPA, 0.5 and 50 µg/kg/day, Santamaria et al. did not find differences in oocyte survival between treatment groups and controls (Santamaria et al. 2016). Nor did they find changes in numbers of ovulated oocytes following gonadotrophin therapy (Santamaria et al. 2017).

Using primates, Hunt et al. examined both continuous BPA implants and oral BPA exposure. With these experimental models, they found an increase in recombination events (via increase in number of MLH1 foci on synaptonemal complexes of pachytene cells) in BPA groups compared to controls (with insufficient samples to characterize BPA oral exposure). They additionally noted high levels of synaptic defects and slight (not significant) decreases in normal pachytene following both BPA exposure modalities (Hunt et al. 2012). They also noted an increase in multi-oocyte follicles following oral BPA exposure, and distinct phenotypic oocyte appearances within follicles following BPA exposure (Hunt et al. 2012). They also found that the single oral dose of BPA significantly shifted distribution of follicles with single and multiple oocytes, while continuous maternal exposure via implant only significantly increased the number of follicles with 4–5 or >5 oocytes (Hunt et al. 2012).

Histologic changes and follicular distribution/dynamics

Yoshida et al. examined rat ovarian sections for incidence of atrophy, cystic follicles and absent corpora lutea after prenatal and postnatal doses of 0, 0.006 and 6 mg/kg/day, without significant differences found (Yoshida et al. 2004). Following exposure to 0, 0.1, 1, 10, 100 and 1000 µg/kg/day subcutaneous exposure prenatally in mice, Newbold et al. found that there seemed to be an increased percentage of ovarian cysts with this exposure, this was only statistically significant in offspring exposed to 1 µg/kg dosing (67% or 8/12 samples compared to 25% or 4/16 in controls, \( P < 0.05 \)). Notably, this was limited by the fact that sufficient histologic sections were not available in all mice (Newbold et al. 2009). Ma et al. used prenatal exposure doses of 0, 50, 500 and 2500 mg/kg/day of BPA and found histologic sections of exposed pups demonstrated increased vacuoles, disorganization of granular layers and decreased follicle detection. These differences were not quantified but were associated with decreased levels of B-cell lymphoma-2 (BCL2) and increases levels of Bcl-2-associated X (BAX) proteins by immunohistochemistry, corresponding to increasing doses of BPA (Ma et al. 2017).

Following prenatal exposures by subcutaneous injections of 10 and 100 mg/kg body weight/day, Suzuki et al. did note significant decrease in corpora lutea presence in mice at 30 days of age (4/9, at dose of 10 mg/kg weight/day BPA dosing, \( P < 0.05 \)), compared to controls (7/9), with 100 mg dosing also showing nonsignificant decrease (5/8) (Suzuki et al. 2002). Zhang et al. enumerated primordial follicles at multiple time points in mice following exposures of BPA at 0, 0.02, 0.04 and 0.08 mg/kg/ day (Zhang et al. 2012a). At postnatal day 3, primordial follicle percentages were significantly lower following BPA treatment of 0.08 mg/kg/day (28.5%) compared to control (67.4%) with \( P \text{-value}<0.01 \). At postnatal day 90, Santamaria et al. reported decreased numbers of growing follicles following prenatal BPA exposure of 0.5 µg (5.013 ± 0.550) and 50 µg/kg/day (5.101 ± 0.485) compared to controls (7.634 ± 0.983), with \( P < 0.05 \), largely at the expense of primary follicles, without effects on preantral or antral follicles. They also demonstrated higher numbers of corpora lutea (5/9) in both treatment groups, but again did not include positive controls. Using BPA exposure of 50 µg/kg/day, the same group also examined follicular dynamics following stimulation with exogenous gonadotrophins (pregnant mare serum gonadotrophin (PMSG) + human chorionic gonadotrophin (hCG)) (Santamaria et al. 2017). Following PMSG treatment on day 32 with a day 33 sacrifice, they noted an increase in growing follicles in the BPA-exposed group (11.33 ± 1.31) compared to control (7.91 ± 1.12, \( P < 0.05 \)), due to increased numbers of small antral follicles. Following the addition of hCG, there was no significant change in the number of oocytes or corpora lutea, at 14 or 22 h, with accompanying increase in atresia of small antral follicles, though all potentially limited by small numbers of experimental animals and lack of positive controls.

Positive controls are utilized by the CLARITY-BPA study, from which Patel et al. report ovarian follicle outcomes. They found significant decreases in primordial follicles (at 250 mg/kg/day BPA), primary follicles (at 2.5 and 250 mg/kg/day doses) and preantral follicles (at 2.5 mg/kg/day BPA) at postnatal day 21 (\( P < 0.05 \)), which was not seen in ethinyl estradiol controls. These early differences were not maintained on subsequent assessments at 90 days, 6 months and 1 year of age (Patel et al. 2017). In another
Prenatal BPA and ovarian function

multigenerational study, Berger et al. also looked at similar outcomes at different time points, using lower doses of BPA. At postnatal day 4, they did not find significant changes in germ cell breakdown percentages or primordial follicles in the F2 and F3 generations following BPA exposures of 0.5, 20 and 50 µg/kg body weight/day (Berger et al. 2016). However, at post-day 21 in the F2 generation, they did observe significantly decreased primary and increased preantral follicle percentages in the 0.5 µg/kg/day treatment group, as compared to controls. Also in the F2 generation, doses of 20 and 50 µg/kg/day decreased primordial follicles and 20 µg/kg/day increased preantral follicles compared to control. They did not observe these changes in the F3 generation.

In sheep, Veiga-Lopez did not find differences following gestational BPA exposure (0.05, 0.5, 5 mg/kg/day) in ovulatory/non-ovulatory follicular duration or mean number on ultrasonography (Veiga-Lopez et al. 2014). They did note that by dividing follicle groups by size, shifts in counts of each size group did differ between treatment groups (particular with follicles 2–3 mm and 4–5 mm). This was also accompanied by inconsistent follicular wave dynamics with BPA treatment, though they report a significant decrease in the number of ≥2 mm follicles in specific waves, though the clinical value of these latter measurements is less clear.

Sex steroids/steroid receptors

Along with examinations of anatomy and histology, several studies investigate the effects of prenatal BPA exposure on sex steroids, especially estradiol, and their receptor expression. Following prenatal BPA exposures of 0.03, 0.3, 3, 30 or 300 ppm, as well as with 17α-estradiol-positive controls, Kendig et al. did not find significant changes in serum estradiol levels in offspring during estrus (Kendig et al. 2012). Following oral BPA exposures from gestational day 11 to birth, one study followed three generations of offspring for estradiol levels along with germ cell and follicular changes. In F1 mice on postnatal day 4, BPA 20 µg/kg/day significantly increased estradiol levels (control: 5.65 ± 0.50; BPA 0.5 µg/kg/day: 6.59 ± 0.14; BPA 20 µg/kg/day: 7.30 ± 0.61; BPA 50 µg/kg/day: 7.09 ± 0.57; pg/mL, P ≤ 0.05), without effects observed in F2 and F3 generations (Berger et al. 2016). However, these findings were derived from samples of 2–7 mice, and was not seen in subsequent time points. Another group examined ‘higher’ doses of prenatal BPA exposure, utilizing doses of 0, 50, 2500 and 25000 mg/kg/day BPA. At these levels, they found dose-dependent increases in estradiol at 8 weeks of age (control: 47.77 ± 0.13, 50 mg/kg: 56.84 ± 0.12, 500 mg/kg: 59.45 ± 0.20, 2500 mg/kg: 90.92 ± 0.20; presumed pg/mL, P < 0.01). Concurrently, they noted BPA exposures significantly increased FSH levels and decreased testosterone levels (though reporting was unclear as to whether these were measured in both/only one sex of offspring) (Ma et al. 2017). Patel et al. further extended out the time periods for hormone measurements, checking levels at postnatal day 1, postnatal day 21, 6 months and 1 year of age, with slightly higher sample sizes (typically 5–10), and in comparison to ethinyl estradiol exposure as a positive control (Patel et al. 2017).

In the prenatal arm of their study, exposures of 2.5, 25, 250, and 25,000 mg/kg body weight/day did not demonstrate effects on estradiol levels at any of the time points, nor did they show effects on progesterone levels (Patel et al. 2017). In their experiments, Santamaria et al. examined sex steroids and expression of sex steroid receptors (via immunohistochemistry) at postnatal day 90 (morning of estrus). Using doses of 0, 0.5 and 50 µg/kg/day from gestational day nine to postnatal day 21, they found no changes in estradiol levels between controls and exposures, but did show significant increases in progesterone levels at both BPA doses (P < 0.01) (Santamaria et al. 2016). Concurrently, examinations of protein receptor expression on staining demonstrated no changes in cyclin-dependent kinase inhibitor 1B (P27) in oocytes and granulosa cells, ERα in granulosa or thecal cells, or ERβ in granulosa cells, at any stage of follicular development. However, when examining androgen receptors of granulosa cells, they noted an increase in expression in primordial follicles at 0.5 mg/kg, decrease in expression in primary follicles at 0.5 µg/kg and decrease at both 50 µg/kg doses in primary, preantral and antral follicles (Santamaria et al. 2016). In their expanded examination following gonadotrophin stimulation with BPA exposure of 50 µg/kg/day, they found no differences in serum estradiol (control: 642.80 ± 129, BPA 50 µg/kg: 665.9 ± 109, pg/mL), progesterone (control: 58.60 ± 0.71, BPA 50 µg/kg: 60.32 ± 1.86, ng/mL) or testosterone (control: 0.86 ± 0.15, BPA 50 µg/kg: 1.08 ± 0.07, ng/dL) (Santamaria et al. 2017). With regard to receptor expression, they noted shifting expression with follicular development, and increased AR and/or ERβ following PMSG treatment. With the addition of hCG therapy, they did see higher estradiol levels in BPA-exposed group (control: 422.40 ± 228.10, BPA 50 µg/kg: 599.80 ± 8.24, pg/mL, P < 0.05), without differences observed in testosterone (control: 62.50 ± 2.14, BPA 50 µg/kg: 62.21 ± 1.46, ng/mL) or progesterone (control: 62.50 ± 2.14, BPA 50 µg/kg: 62.21 ± 1.46, ng/mL). Following hCG, there were no observed changes in hormone receptor expression.

In sheep, gestational treatment with BPA 0.5 mg/kg/day subcutaneous did not show differences in peak progesterone levels. Nor were there differences in luteal progesterone peak levels or duration of increase (Savabieasfahani et al. 2006), though all examined with only one exposure dose of BPA (and separate methoxychlor treatment arms). Using multiple doses of BPA (0.05, 0.5 or 5 mg/kg/day), Veiga-Lopez found a nonsignificant delay in estradiol surge following prostaglandin injections (control: 40.3 ± 1.9 h, BPA 0.05 mg/kg: 44.4 ± 1.8 h, BPA 0.5 mg/kg: 45.3 ± 1.5 h, BPA 5 mg/kg: 44.7 ± 1.6 h). Nor did they find differences in preovulatory peak estradiol levels (control: 6.3 ± 1.1 pg/mL, BPA 0.05 mg/kg: 5.9 ± 0.8 pg/mL, BPA 0.5 mg/kg: 5.2 ± 0.7 pg/mL, BPA 5 mg/kg: 5.3 ± 0.9 pg/mL) or luteal progesterone’s correlation with corpora lutea (Veiga-Lopez et al. 2014).

Age of puberty/vaginal opening/sexual maturation/estrous and estrus cyclicity

Another common method of assessing ovarian function is the examination of sexual maturity and puberty as well as estrus cyclicity. In rodent models, sexual maturity was often identified by age of vaginal opening, first estrus by cornified cells on vaginal smear and subsequent cyclicity by vaginal

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cytology. Following maternal rat exposure to BPA (0, 3.2, 32 and 320 mg/kg/day) from gestation to postnatal day 21, there were no significant differences in age at vaginal opening (control: 33.2 ± 0.6, 3.2 mg/kg: 32.9 ± 0.4, 32 mg/kg: 33.6 ± 0.3, 320 mg/kg: 33.7 ± 0.5, days) or age of first estrus (control: 35.4 ± 0.8, 3.2 mg/kg: 36.3 ± 0.6, BPA 32 mg/kg: 36.7 ± 0.5, BPA 320 mg/kg: 36.1 ± 0.8, days) (Kwon et al. 2000). In the same study, no changes were seen in estrus cyclicity, whereas doses of DES 15 µg/kg/day did lead to disruptions in cyclicity compared to controls. Using mice, Honma et al. examined prenatal subcutaneous doses of 2 and 20 µg/kg/day BPA (as well as exposures to diethylstilbestrol-DES), and found that age of vaginal opening was significantly earlier in BPA 20 µg/kg exposure (approximately 26 days vs >27 days in control, P < 0.05), as well as in all DES groups. This was accompanied by significant decreases in body weight at vaginal opening across treatment groups, as well as earlier age of first estrus with BPA 20 µg/kg/day group. Estrus cycle length was increased at both BPA dose exposures when compared to control (control: 4.5 ± 0.4, BPA 2 µg/kg: 5.8 ± 0.4, BPA 20 µg/kg: 5.5 ± 0.4, all with relatively large sample sizes >45) (Honma et al. 2002). Based on earlier studies examining male offspring, Tinwell et al. studied effects of BPA (20 µg, 100 µg, 500 mg/kg/day) exposure on two strains of rats. In Sprague-Dawley rats, they found no effects of BPA on age of vaginal opening, while in Wistar-derived rats, dose of 50 mg/kg/day led to significant delay in vaginal opening (35.4 ± 0.6 days, litter average) compared to control litter average (33.8 ± 0.8 days). However, this finding was not significant when comparing individual values instead of litter averages (Tinwell et al. 2002). In a similarly designed study, in rat offspring exposed to maternal BPA dosing (2, 20 and 200 µg), there was no significant difference in age of vaginal opening, in contrast to those treated with 5 µg/kg/day of ethinyl estradiol which did demonstrate accelerated vaginal opening (Ryan et al. 2010). Also using a rat model, with doses of 0.006 and 6 mg/kg/day by oral administration from gestational day 2 to weaning, Yoshida et al. did not find significant effects for day of vaginal opening nor for ovulation (by ova in oviduct) (Yoshida et al. 2004). In an examination of the hypothalamic-pituitary-gonadal axis in mice, where pups received in utero, lactational and then direct exposure, Xi et al. also examined estrus cycling without finding significant effect with doses of 25 or 50 mg/kg/day BPA (Xi et al. 2011). Following prenatal dietary exposures of BPA (0.03, 0.3, 3, 30, 300 ppm), also using mice offspring, there was no significant change in age of vaginal opening (though notably several litters exposed to BPA 0.3 ppm did have markedly early time to vaginal opening), nor with ethinyl estradiol control exposures – though with smaller numbers of pups examined (≤10) (Kendig et al. 2012). Wang et al. utilized maternal oral dosing of BPA (0.5, 20, 50 mg/kg/day), and reported no observed effect on age of vaginal opening, though the time between this and first estrus was significantly decreased in BPA 50 µg/kg/day (~less than 2 days compared to ~3 days in controls, P < 0.05), which was also seen in DES 0.05 µg/kg/day controls (Wang et al. 2014). Doses of 20 µg/kg/day also indicated some near significant decreases in time to first estrus compared to the control (P = 0.1). Simultaneously, they noted variable effects on estrus cycle. While the 20 and 50 µg/kg/day doses did not show significant effects, BPA 0.5 µg/kg/day decreased percent time in proestrus and estrus periods, and increased time in metestrus and diestrus (P < 0.05), with the latter finding not seen in DES control (Wang et al. 2014). In the multigenerational study conducted by Ziv-Gal et al. doses of 0.5, 20 and 50 µg/kg/day were used. While there were no difference in F1 and F2 generations, in the F3 generations, there was significant delay in age of vaginal opening in treatment groups of 0.5 and 50 µg/kg/day BPA compared to controls (P < 0.05) (Ziv-Gal et al. 2015). Also in the F3 generation, there was a statistically significant delay in age of first estrus in the 50 µg/kg/day BPA dosing group (P < 0.05) (Ziv-Gal et al. 2015). In an earlier multigenerational study with extended and repeated exposures from mating to lactation, there was no significant changes in age of vaginal opening in F1 or F2 females following BPA exposure of 0.2, 2, 20 or 200 µg/kg/day by gastric intubation (Ema et al. 2001), though there was a significant decrease in the percentage of F1 females with normal estrus cyclicity following 20 µg/kg/day exposure (76% vs 96% in controls, P < 0.05).

Savabieasfahani et al. did not find changes in age of puberty and duration of progestogenic cycles following subcutaneous BPA 0.5 mg/kg/day in pregnant ewes (Savabieasfahani et al. 2006).

Ovarian gene expression

With the advent of techniques for evaluating gene expression, newer studies may examine many of the aforementioned ovarian outcomes through mRNA analyses. These analyses include quantification of expression of steroidogenic enzymes, sex steroid receptors, apoptotic proteins and numerous other processes.

Lawson et al. examined 12-, 12.5-, 13.5- and 14.5-day-old oocytes. Following validation of time-specific expression of meiosis-related genes, they demonstrated maternal doses of 20 ng/g bodyweight of BPA from gestational day 11, and found that the number of genes affected increased over time, with the most meiotic-related genes affected in 14.5-day-old ovaries, though nearly all with less than twofold changes in expression (Lawson et al. 2011). When examined for affected biologic processes, using the most stringent criteria for significance, downregulated genes were most commonly involved in cell cycle (P = 0.0007) and mitosis (P = 0.03). In contrast upregulated genes did not show consistent/significant areas of activity when using these criteria (Lawson et al. 2011). Zhang et al. investigated meiosis-specific gene expression in oocytes at 17.5 days, following maternal BPA exposures of 0.08 mg/kg/day compared to controls (Zhang et al. 2012a). They found decreases in mRNA expression of Stemplated by Retinoic Acid 8 (Strα8), Meiotic recombination protein REC8 (Rec8), Disrupted meiotic cDNA (Dmc1) and synaptosomal complex protein 3 (Spc3), but only a significant decrease in Strα8 (0.689 compared to 1 in control, P < 0.05) (Zhang et al. 2012a). They also noted shifts in DNA methylation of Strα8, at different time points of 13.5, 15.5 and 17.5 days after the same treatment dose of BPA, noting increases in percent methylation at all time points (Zhang et al. 2012a). The same group examined early maternal BPA exposure and changes
in DNA methylation of imprint genes (insulin-like growth factor 2 receptor (Igf2r), paternaly expressed gene 3 (Peg3) and imprinted maternally expressed transcript (H19)) in germ cells at 12.5 days postconception (Zhang et al. 2012b). They found significant decreases in methylation of Cpg sites of Igf2r gene at doses of 80 and 160 µg/kg/day BPA (control: 19.6%, BPA 80 µg/kg: 11.2%, BPA 160 µg/kg: 8.2%, P < 0.01), but not with 40 µg/kg/day (19.44%). With Peg3, all BPA exposure doses led to significant decreases in methylation (control: 36.8%, BPA 40 µg/kg: 10.76%, BPA 80 µg/kg: 6.75% and 160 µg/kg: 6.0 %, P < 0.01). With H19, the only significant decrease in methylation occurred with dose of 80 µg/kg/day of BPA (control: 9.6%, 80 µg/kg: 4.38%, P < 0.01). They further reported changes in mRNA expression of germ cell-specific genes, noting Stra8 (0.297) and Deleted in Azoosperma Protein 1 (Dazl) (0.300) in 160 µg/kg group to be significantly lower and Newborn Ovary Homeobox-Encoding (Nobox) significantly higher than in controls (P < 0.01) (Zhang et al. 2012b). They additionally found that the same dose of BPA increased mRNA expression levels of Era 1.63-fold over control levels (P < 0.01) (Zhang et al. 2012b).

In the context of germ cell nest breakdown, Wang et al. focused attention on genes known to be involved in apoptosis following maternal exposures of 0.5, 20 and 50 µg/kg/day. Following RNA extraction from postnatal day 4, they found BPA 0.5 µg/kg/day significantly decreased the expression of pro-apoptotic factor, Bax (−1.60, P < 0.05), while 20 µg/kg/day BPA increased the expression of anti-apoptotic factor, Bcl2 (2.42, P < 0.05) and decreased the expression of pro-apoptotic factors, Bax (−1.92, P < 0.05) and BCL2-Antagonist/Killer 1, Bak1 (−2.02, P < 0.05). Maternal exposure to BPA 50 µg/kg/day increased the expression of anti-apoptotic factors, Bcl2 (1.26, P < 0.05) and Bcl21 (1.63, P < 0.05), and decreased the expression of pro-apoptotic factor, Bak1 (−3.08, P < 0.05) (Wang et al. 2014). All doses had variable effects on mRNA expression of genes involved in the tumor necrosis-signaling pathway (Wang et al. 2014).

Ma et al. examined mRNA expression in offspring mice testes and ovaries following high dose maternal exposures (50, 200, 2500 mg/kg/day). They reported significantly increased relative mRNA expression levels of the anti-Mullerian hormone, AMH gene (200 mg: 9.09 ± 0.270, 2500 mg: 6.70 ± 0.355 compared to control: 1.00 ± 0.020, P < 0.01) and decreased expression levels of Kitlg (200 mg: 0.58 ± 0.029, 2500 mg: 0.70 ± 0.017 compared to control: 1.00 ± 0.041, P < 0.01) following the higher dose exposures (Ma et al. 2017). At the level of the ovary, Xi et al. investigated mRNA expression levels of steroidogenic and gonadotrophic receptor enzymes including cholesterol side chain cleavage enzyme (Cypsscc), Cyp17, Cyp19a, StAR, FSH receptor (Fshr) and LH receptor (Lhr) following 12, 25, 50 µg/kg/day maternal exposures to BPA (Xi et al. 2011). They found significantly increased expression levels of Cypsscc at 50 µg/kg/day (P < 0.05) and CYP19a at 12.5, 25 and 50 µg/kg/day (P < 0.05). These were also positively correlated with increases in serum estradiol, though only significantly so with Cyp19a (Xi et al. 2011).

Santamaria et al. also examined ovarian mRNA expression of Cyp11, Cyp17, Cyp19, 3βHSD, Fshr and Lhr following maternal exposure to 0.5 and 50 µg/kg/day BPA (Santamaria et al. 2016). They found significant expression increases in 3βHSD mRNA levels at both BPA doses (P < 0.05), without significant changes in expression of other steroidogenic enzyme or gonadotrophin receptor mRNA (Santamaria et al. 2016). The same group observed significant increases in expression of Cyp17, Cyp19 and Fshr mRNA levels (P < 0.05) following exogeneous gonadotrophins. They also noted BPA-exposed groups also had significant increases in Fshr mRNA expression and significant decreases in Lhr mRNA following therapy with hCG at 7 and 22h respectively (P < 0.05) (Santamaria et al. 2017). In their transgenerational studies, Berger et al. performed gene expression analysis of RNA from snap frozen ovaries of offspring mice following maternal exposures. Looking at antioxidant, autophagy-related, apoptotic, steroidogenic and sex steroid receptor genes, they noted variable, though significant, effects across generations and doses of 0.5, 20 and 50 µg/kg/day (Berger et al. 2016).

In sheep, using doses of 0.5 mg/kg/day subcutaneous BPA, Veiga-Lopez et al. achieved free BPA levels of 2.62 ± 0.52 ng/mL (compared to 0.43 ± 0.09 ng/mL in controls) and examined gene expression at gestational days 65 and 90 in fetuses. They found significant increase in mRNA expression of CYP19 and 5-alpha reductase enzymes at gestational day 65, but not at day 90. They also found various miRNAs downregulated at either day 65 or day 90, but only one, miR-203, downregulated at both days 65 and 90 (Veiga-Lopez et al. 2013).

Discussion

Summary

The prenatal period of increased gene activation/inactivation, organ development and increased sensitivity to chemical exposures represents a ‘window of susceptibility’ in endocrine and reproductive health (Schug et al. 2011, Trowbridge et al. 2014). It is clear that rodent models for these exposures are starting to demonstrate the early and, in cases, transgenerational effects of EDCs on elements of ovulation health. The epidemiologic findings are uncertain and there is sufficient data from animal and mechanistic studies to continue to build the models and pathways by which we study the question in humans. There is mounting evidence for the effects of these exposures in the prenatal period, a particularly vulnerable time of development, with note that exposure ranges in animal studies may not be comparable to human exposures. Whether there are causative associations with human ovulation disorders remains to be elucidated.

Limitations

While not intended to be a traditional systematic review, we do acknowledge limitations of our search. These included the challenges of appropriate ascertainment of relevant studies given the wide breadth of terminology used for both ovarian function and endocrine disruptors. We also acknowledge that in attempts to focus on the review’s scope, we did eliminate notable papers focusing on central (hypothalamus/pituitary) and systemic contributors to ovarian function. Nor did we seek out specific disorders such as polycystic ovarian syndrome...
or premature ovarian failure. Ultimately, while there may be numerous additional search terms that could be utilized to further examine any aspect of our questions, we felt the current strategy yielded a broad overview of current literature on potential disruption of function at the level of the ovary.

Challenges to evaluating environmental toxicants and endocrine disruptors

The traditional model for risk assessment occurs in a series of steps: hazard characterization, exposure assessment, dose-response assessment, risk characterization and subsequent risk management/communication (Futran Fuhrman et al. 2015). In the case of EDCs, this process is challenged by inconsistent definitions and dosing, mixed and subtle exposures, variable administration routes and transgenerational effects (Schug et al. 2011, Futran Fuhrman et al. 2015). This is exacerbated with the complex convergence of anatomy, hormonal axes and metabolic influences involved in ovulatory health.

The ubiquity of EDCs leads to challenges in controlling levels and types of exposures, particularly when studying human populations or examining specific time periods of effect. As previously mentioned, metabolism of EDCs is variable based on compound(s) and individual body composition, and does not consistently follow monotonic dose-response curves (Schug et al. 2011, Lore et al. 2015). Additionally, a combination of multiple agents may show effects where solitary exposures do not (Hass et al. 2012). While animal studies in preceding sections took care to control and standardize doses of various agents, there is no such possibility in human studies, while there is sufficient concern for harm. Those limited studies available have difficulty accounting for the subsequent and inevitable incidental exposures of female offspring, even when urinary measurements for offspring are available. Additionally, the possibility of a true control group and comparison to other estrogenic compounds is near impossible.

Many studies with animal models show highly variable dose effects, occasionally with effects at lower rather than higher exposures. There may also be significant sex-specific variation. Sex-specific differences were not expanded on in our discussions, and studies in humans have only just started to measure these effects.

Finally, functional ovulation reflects not only ovarian status, but the entire hypothalamic-gonadal axis and potentially other endocrine systems, such as the thyroid. With regard to prenatal exposures, there is the added consideration of maternal physiologic adaptations to pregnancy, including increased plasma volume, glomerular filtration rate and increased adipose stores, which may further impact the metabolism and question of EDCs.

Avenues for future research

There is a significant opportunity for expansion of human studies across multiple geographic cohorts and diverse ethnic populations. Furthermore, while existing studies have started this process, there is room for expansion on the measurement of metabolic parameters. In both animal and human studies, examination into the convergence of adipose tissue and EDCs also help elucidate true ovulation effects. Ultimately, it will take large and longitudinal studies to assess the true impact of early anatomical and hormonal changes on ovarian function and moreover, population reproductive health. One potential avenue of exposure ascertainment in large cohorts of youth and adult women includes using personal monitors. In combination with mobile health technology, we may be able to ascertain ovarian function as noted by menstrual cycle length, variability and duration.

Declaration of interest

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

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