Prenatal exposure to endocrine disruptors: a developmental etiology for polycystic ovary syndrome

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PRENATAL EXPOSURE TO ENDOCRINE DISRUPTORS:
A DEVELOPMENTAL ETIOLOGY FOR POLYCYSTIC OVARY SYNDROME

by

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Prenatal Exposure to Endocrine Disruptors: A Developmental Etiology for Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common and complex endocrinopathies among reproductive-aged women. Symptom heterogeneity among clinical cases and in sibling studies suggests that PCOS is a multifactorial disease with environmental components. Investigators hypothesize that endocrine disruptors may contribute to the pathophysiological origins of PCOS in utero. The goal of this literature review is to present and compare the available research investigating the developmental origins of PCOS deriving from prenatal exposure to three major classes of endocrine disruptors: bisphenol A (BPA), phthalates, and androgenic endocrine disruptors.

Considerable evidence has been found to suggest links between fetal exposure to endocrine disruptors and PCOS. Rodent studies reported that maternal BPA exposure dysregulates postnatal development and sexual maturation. In rats, gestational exposure to dibutyl phthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP) resulted in polycystic ovaries among first and third generation offspring. Additionally, serum DBP concentrations are higher among women with PCOS. Regarding DEHP, animal studies have shown that prenatal exposure results in an endocrinological profile similar to PCOS. Nicotine and 3,4,4’-trichlorocarbanilide (TCC) are known androgenic endocrine disruptors. Inducing prenatal exposure to excess androgens in animal models has
successfully recreated a PCOS-like phenotype that includes abnormal ovarian function, anovulatory cycling, impaired fertility, and increased visceral fat distribution.

Based on the literature, endocrine disruptors appear to have an etiological role in PCOS development through prenatal exposure and thus may pose one of the greatest hazards to fetal health and development.
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17-OHP.................................................................. 17-Hydroxyprogesterone
A......................................................................... Androstenedione
AMH................................................................... Anti-Müllerian Hormone
AR...................................................................... Androgen Receptor
ATP.................................................................. Adenosine Triphosphate
ATSDR................................................................. Agency for Toxic Substances Disease Registry
BPA.................................................................. Bisphenol A
CBG................................................................... Corticosteroid Binding Globulin
CDC.................................................................. Centers for Disease Control
DBP.................................................................. Dibutyl Phthalate
DDT.................................................................. Dichlorodiphenyltrichloroethane
DEHP................................................................. Di(2-ethylhexyl) Phthalate
DHEA.................................................................. Dehydroepiandrosterone
E1...................................................................... Estrone
E2...................................................................... Estradiol
EPA.................................................................. Environmental Protection Agency
F1...................................................................... First Generation
F3...................................................................... Third Generation
FSH.................................................................. Follicle-Stimulating Hormone
GABA.................................................................. Gamma Aminobutyric Acid
GABA AR............................................................ Gamma Aminobutyric Acid A Receptor
GnRH .............................................................. Gonadotropin-Releasing Hormone
IGF-I .............................................................. Insulin-Like Growth Factor-I
IGF-II .............................................................. Insulin-Like Growth Factor-II
IGFBP-I .......................................................... Insulin-Like Growth Factor Binding Protein-I
LH ................................................................. Luteinizing Hormone
LOAEL .......................................................... Lowest Observed Adverse Effect Level
MCPP ............................................................ Mono(3-Carboxypropyl) Phthalate
MEHP ............................................................. Mono(2-Ethylhexyl) Phthalate
MEP ............................................................... Mono-Ethyl Phthalate
EPA ............................................................... Environmental Protection Agency
NHANES ........................................................ National Health and Nutrition Examination Survey
NOAEL .......................................................... No Adverse Effect Level
OC ................................................................. Organochlorine
OP ................................................................. Organophosphate
P ................................................................. Progesterone
PBDE .............................................................. Polybrominated Diethyl Ether
PCB ............................................................... Polychlorinated Biphenyl
PCOS ............................................................ Polycystic Ovary Syndrome
P ................................................................. Progesterone Receptor
SHBG .......................................................... Sex Hormone-Binding Globulin
T ................................................................. Testosterone
TCC .............................................................. 3,4,4’-Trichlorocarbanilide/Triclocarbon
INTRODUCTION

Polycystic ovary syndrome (PCOS) is a hyperandrogenic and cardiometabolic disorder in women. As one of the most common endocrine disorders among women of reproductive age, the estimated prevalence of PCOS ranges between 4-12% worldwide (Asuncion et al., 2000; Azziz et al., 2004; Chang, 2014; Diamanti-Kandarakis et al., 1999; Ehrmann, 2005). After excluding for related disorders, the most recently established diagnostic criteria suggest that a diagnosis of PCOS includes two of the following three criteria: 1.) oligo- or anovulation, 2.) a clinical or biochemical observation of hyperandrogenism, and 3.) polycystic ovaries on ultrasonography (Rotterdam, 2004). Despite the syndrome’s namesake, a finding of polycystic ovaries is no longer required for diagnosis. A large contribution to this change in diagnostic criteria came from research which showed that polycystic ovaries were present in about 20% of asymptomatic women (Polson, Reed, Scanlon, Quiney, & Franks, 1988). A more recent study reported a prevalence of 58% for asymptomatic women presenting with polycystic ovaries (Kristensen et al., 2010). Oligo- or anovulation, on the other hand, leads to irregular menstruation, amenorrhea, and ovulation-related infertility. Additionally, the hormonal imbalance caused by hyperandrogenism generally results in acne and hirsutism. Polycystic ovary syndrome is also associated with insulin resistance as well as metabolic dysfunction (Apridonidze, Essah, Iuorno, & Nestler, 2005; Dokras et al., 2005). The adverse impact of such hormonal and metabolic dysregulation exaggerates the syndrome’s clinical presentation and promotes the incidence of chronic sequelae for
women in the long term. For example, insulin resistance is associated with a higher risk of developing non insulin-dependent diabetes mellitus (NIDDM), hypercholesterolemia, and obesity (Strauss & Barbieri, 2014). Polycystic ovary syndrome is also associated with metabolic syndrome (Caserta et al., 2014).

**Background**

*Luteinizing Hormone Hypersecretion*

The origins of hyperandrogenism in PCOS cases are believed to derive from the pituitary hypersecretion of luteinizing hormone (LH). A majority of PCOS cases have been shown to have higher serum levels of LH as well as increased frequencies of LH pulse secretion (Figure 1) (Balen, 1993; Rebar et al., 1976). As part of normal hormonal physiology, ovarian theca cells are stimulated to produce and secrete androgens (mostly androstenedione (A) relative to testosterone (T)), which traverse to ovarian granulosa cells. In response to pituitary secretion of follicle-stimulating hormone (FSH), granulosa cells are stimulated to produce and secrete aromatase which converts the theca-sourced androgens to estrogens via aromatization (estrone (E1) and estradiol (E2)) (Figure 1). The increase in ovarian androgen production and secretion in PCOS pathophysiology may additionally be due to a number of causes which add to the disorder’s complexity such as theca cell hypertrophy in response to LH hypersecretion, or an increased expression of the LH receptor on theca cells (Jakimiuk, Weitsman, Navab, & Magoffin, 2001). In the classical PCOS case, the increase in androgens is due to increased LH which is
potentiated by either hyperinsulinemia, dysregulated hypothalamic gonadotropin-releasing hormone (GnRH) secretion, or both.

Hyperinsulinemia

Insulin resistance and subsequent hyperinsulinemia are common among PCOS cases (Dunaif, 1997; Legro, Finegood, & Dunaif, 1998). Hyperinsulinemia has been shown to lead to decreased levels of sex hormone-binding globulin (SHBG) and insulin-like growth factor binding protein – I (IGFBP-I), as well as increases in free androgens (Lebovic, Gordon, & Taylor, 2005). While serum concentrations of total insulin-like growth factor-I (IGF-I) levels are normal in PCOS cases, decreases in IGFBP-I result in increased concentrations of circulating, free IGF-I. In conjunction with insulin-like growth factor-II (IGF-II), IGF-I appears to augment the actions of LH-stimulated androgen production and secretion in ovarian theca cells. Insulin itself may stimulate ovarian androgen production and secretion as well (Barbieri, Makris, & Ryan, 1984; Nestler et al., 1998). However, whether it acts directly to stimulate ovarian production and secretion at the level of the theca cell or it acts indirectly to stimulate LH secretion at the level of the anterior pituitary or both remains to be determined.

Hyperandrogenism

As previously mentioned, increased LH secretion from the anterior pituitary results in theca cell dysfunction. As part of this dysfunction, theca cells produce and
secrete high levels of T, A, dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone (17-OHP) (Lebovic et al., 2005). The resulting increases in A lead to increased production and secretion of E1; however, ovarian E2 secretion is relatively normal (Figure 1) (DeVane, Czekala, Judd, & Yen, 1975). Despite normal secretion levels, decreased levels of SHBG lead to increased levels of free estrogens, including E2, as well as increased levels of free androgens. Hyperandrogenism has been hypothesized to contribute to excess LH secretion by lowering the efficacy of feedback inhibition by progesterone (P) (Figure 2) (Eagleson et al., 2000). As part of normal physiology, P, synergistically with E2, inhibits the pulsatility of GnRH secretion, which subsequently inhibits LH secretion. Without this inhibition, GnRH will continue to stimulate LH secretion, thereby maintaining this self-perpetuating pathophysiological cycle.
Figure 1. Hormonal Levels in Polycystic Ovary Syndrome. Average serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrone (E1) and estradiol (E2) were collected among 19 women with polycystic ovary syndrome (PCOS) and compared against average serum hormonal concentrations from 10 normal women between day 2 and day 4 of their menstrual cycle. Overall, PCOS women had significantly higher LH and E1 levels. Adapted from DeVane et al., 1975.
Figure 2. Pathophysiological Concept of Polycystic Ovary Syndrome (PCOS). Luteinizing hormone (LH) hypersecretion stimulates increased production and secretion of androgens in ovarian theca cells. While estradiol (E2) secretion is normal, increased levels of circulating androgens appear to inhibit negative feedback at the pituitary. Increased androgens are also associated with visceral fat deposition, obesity, and dyslipidemia. Hyperinsulinemia leads to increased levels of free insulin-like growth factor I (IGF-1) which also stimulates ovarian androgen production. Adapted from Chang et al., 2014.
**Ovarian Dysfunction**

Whether the self-perpetuating cycle of excess androgen production at the level of the ovary induces PCOS or is an effect of another pathophysiological origin remains to be determined. In addition to increased ovarian androgen production, LH hypersecretion promotes theca cell hyperplasia and increases the thickness of the ovarian stroma. Consequently, these effects prevent the maturation of ovarian follicles (Lebovic et al., 2005). However, circulating levels of FSH in PCOS sustainably stimulate follicular growth. More specifically, preovulatory follicles are constantly recruited to replace atretic follicles. However, without proper hormonal stimulation, these follicles fail to complete the process of maturation, which normally culminates in ovulation. The clinical result is oligo- or anovulation. Increased levels of ovarian androgens may also be a direct cause of follicle maturation inhibition (Lebovic et al., 2005).

**Clinical Presentation**

Despite the relatively high prevalence of PCOS, the specific pathophysiology is complex and remains largely elusive. As a syndrome, PCOS is characterized by variable clinical presentations. The redaction of polycystic ovaries as a requisite criterion for diagnosis is one reflection of the nonspecific nature of the syndrome’s clinical and pathophysiological manifestation (Diamanti-Kandarakis et al., 1999). The initial signs and symptoms of PCOS typically present during or just prior to the onset of puberty. Indeed, research has shown that the presentation of precocious pubarche as well as
adolescent hyperandrogenemia with or without insulin resistance may in fact be a manifestation of the syndrome within its early stages (Fruzzetti, Perini, Lazzarini, Parrini, & Genazzani, 2009; Ibanez, Lopez-Bermejo, Diaz, Marcos, & de Zegher, 2011; McCartney et al., 2006).

Additionally, the phenotype has been shown to change as a woman progresses through life (Hsu, 2013). These dynamic changes over time add to the complexity of this syndrome, thereby making a clinical diagnosis difficult to accurately establish and complicating prognostic determinations. For example, many disorders have clinical presentations that are similar to PCOS such as congenital adrenal hyperplasia, hyperprolactinemia, or Cushing’s syndrome (Diamanti-Kandarakis et al., 1999). Such difficulties have prompted researchers to look toward finding a cause for PCOS in an effort to better understand its pathophysiology and elucidate any potential determinants of its development. This effort is comprised of two major objectives: to determine the specific point within a woman’s life cycle when PCOS actually begins to develop, and to ascertain whether there is a genetic or environmental influence or a combination of both that contributes to the biological onset of PCOS.

**Biological Onset of Polycystic Ovary Syndrome and Fetal Programming**

Regarding the time point of biological onset, polycystic ovaries and functional ovarian hyperandrogenism have been observed in prepubertal girls, which supports the hypothesis that the particular origin of PCOS may depend on the establishment of ovarian morphology and function at an early stage of development (Battaglia et al., 2002; Siklar,
Ocal, Adiyaman, Ergur, & Berberoglu, 2007). More specifically, the developmental origin of adult disease during fetal life is believed to stem from fetal programming, a process where biological or exogenous signals or insults at critical, and therefore susceptible, stages of development induce permanent changes in tissue structure or function. These alterations are the manifestation of adaptive responses to such adverse signals or insults, and therefore influence the long-term risk of disease. In relation to the development of PCOS, a study conducted by Webber et al. (2003) proposed that these adaptations take place in utero during ovarian development and oogenesis. By nature, these changes in tissue structure or function, which are carried out through fetal programming, would not necessarily be observable at birth but may result in altered physiological responses later in life as a consequence of these fetal adaptive responses.

It is well known that the critical developmental stages that take place in utero are times of significant cellular proliferation, differentiation, and functional maturation. Therefore, intrauterine exposures may have profound effects based not only upon what the agent actually is but also upon the timing of exposure, even at low doses. For example, it is well known that exposure to insults during the early stages of gestation, most notably when organogenesis and differentiation occur, has the potential to cause structural defects. On the other hand, adverse prenatal exposures during the later stages of gestation (i.e. at the time of fetal maturation) have been shown to lead to functional alterations.

Fetal programming, however, is not solely a pathophysiological event. For example, hormonal programming is normally involved in the establishment of sexually
dimorphic traits by sex steroids during perinatal development. Studies have demonstrated an association between the surge of sex steroid secretion that takes place during this process and the neuroanatomical as well as functional changes in the forebrain (Simerly, 2002). This process was also associated with the changes in the expression of steroid-metabolizing enzymes in the liver and the development of sexual behavior (Collaer & Hines, 1995; Hutchison, 1997).

However, just as low nutrient or oxygen supply can significantly alter anabolic and catabolic homeostasis, suboptimal intrauterine conditions brought upon by exposure to adverse exogenous agents have been proposed to induce programming adaptations, and thus influence the development of disease in adult life. For example, fetal exposure to an excess of glucocorticoids, in conjunction with an adverse intrauterine environment caused by inadequate maternal nutrition, may result in physiological programming and ultimately lead to impaired fetal growth and cardiometabolic disease later in life (Harris & Seckl, 2011; Seckl, 2008).

Additional findings in support of this hypothesis derive from animal studies, which have shown that experimentally induced androgen excess during fetal development, may lead to both reproductive and metabolic abnormalities in later life similar to those exhibited in women with PCOS. For example, studies using the rhesus monkey as an animal model have shown that fetal exposure to very high levels of exogenous androgens in maternal circulation eventually results in many of the characteristic features of PCOS when the female offspring reach adolescence (Dumesic, Abbott, Eisner, & Goy, 1997; Eisner, Barnett, Dumesic, & Abbott, 2002; Eisner,
Dumesic, Kemnitz, Colman, & Abbott, 2003). Such features include disordered ovarian function, delayed menarche, irregular anovulatory cycles, and thus impaired fertility. As adults, the female rhesus monkeys developed enlarged ovaries with a number of moderately-sized antral follicles (Eisner et al., 2002). Endocrine dysregulation was another prominent feature with hypersecretion of LH resulting from increased ovarian and adrenal androgen secretion as well as insulin resistance (Abbott, Tarantal, & Dumesic, 2009).

Findings from these studies additionally hypothesize that prenatal androgenization of female rhesus monkeys may promote alterations in the distribution of adipose tissue. Investigators observed a preferential accumulation of abdominal and visceral fat as the offspring reached adulthood, which were independent of obesity and reflected a relatively masculine distribution (Eisner et al., 2003). Excess androgen also appeared to differentially alter the secretion of insulin and its action on tissues depending on the timing of exposure during gestation (Eisner, Dumesic, Kemnitz, & Abbott, 2000). More specifically, fetal androgenization during early gestation led to impaired pancreatic beta cell function, while the same exposure during late gestation altered insulin sensitivity (Eisner et al., 2000). Other studies conducted in male monkeys have demonstrated similar effects on glucose-insulin homeostasis (Varlamov et al., 2012).

The alterations in visceral fat accumulation as well as the changes in insulin secretion have been hypothesized by investigators as potential contributors to the pathophysiologically development of insulin resistance (Xita & Tsatsoulis, 2010). More specifically, visceral adiposity, also known as central obesity, has been associated with
insulin resistance (Tosi et al., 2015). Therefore, alterations in fetal programming from androgen excess may result in impaired insulin secretion. A study conducted by Bruns et al. (2004) proposed that this programming occurs at the level of potassium-dependent adenosine triphosphate (ATP) channels on the surface of pancreatic beta cells. Such programming would impair pancreatic beta cell response to glucose, thereby hampering insulin secretion. Taken together, impairment in insulin secretion from impaired beta cell function and insulin resistance increase the likelihood of developing NIDDM in adulthood (Bruns et al., 2004). This synergy of metabolic dysfunction has been shown to lead to the premature onset of NIDDM in women with PCOS (Book & Dunaif, 1999; Dunaif, 1993; Dunaif & Finegood, 1996). Findings from animal studies in male rhesus monkeys also suggest that programming takes place during fetal development, as prenatally androgenized male offspring did not continue to have elevated levels of androgens into adulthood (Bruns et al., 2004).

Many studies provide support for the developmental origin hypothesis of PCOS. A majority of these studies propose that a particular subset of PCOS development in adult life is mediated through intrauterine exposure, and ultimately induces its metabolic and reproductive sequelae (Abbott, Barnett, Bruns, & Dumesic, 2005). At the level of the ovary, studies conducted in prenatally androgenized ewes have reported that such exposure influences early ovarian follicular development (Forsdike et al., 2007). A notable characteristic of a significant proportion of PCOS cases is polycystic ovaries, which present with a disordered pattern of folliculogenesis. Correspondingly, exogenous
androgens have also been shown to affect ovarian follicular development in the rhesus monkey (Vendola et al., 1999).

Additional ovarian features that can present in women with polycystic ovaries are an increased number of antral follicles. However, women with anovulatory cycles may actually exhibit halted antral follicle development (Franks, Mason, & Willis, 2000). Studies have also shown that early preantral follicle development can be disturbed in polycystic ovaries. In a study conducted by Webber et al. (2003), small preantral follicle density was significantly higher in ovarian tissue samples from anovulatory women with polycystic ovaries than in tissues collected from participants with normal ovaries. The proportion of follicles for both ovulatory and anovulatory polycystic ovary tissue collections at different stages of early growth differed from normal collections. More specifically, women with polycystic ovaries had a relatively smaller proportion of primordial follicles and a higher proportion of primary follicles (Webber et al., 2003).

Studies have investigated the interplay of anti-müllerian hormone (AMH) in this observation. Anti-müllerian hormone is known to exert different effects in different tissues and at different points in the human life cycle. For example, AMH plays an important role in the development and sexual differentiation of the male fetus. More specifically, AMH is secreted from sertoli cells and inhibits the development of mullerian ducts, which takes place during female fetal development. This inhibition gives rise to the wolffian duct system and the process of male development proceeds (Behringer, Finegold, & Cate, 1994). However, AMH has been shown to have an influence in female reproductive development and physiology, particularly during folliculogenesis. At around
the fifth month of gestation, the female fetal ovary possesses several million primordial follicles each containing a primary oocyte (Wallace & Kelsey, 2010). At the time of menarche, the first few follicles will begin the process of folliculogenesis in which multiple stages of follicular growth occur. Many primordial follicles will not complete this process and will ultimately undergo apoptosis. However, the follicles that evade cell death will successfully develop into primary follicles, followed by secondary follicles, and finally tertiary follicles. Tertiary follicle development is the final stage before ovulation and includes the development of a fluid-filled cavity known as an antrum. The granulosa cells of primordial follicles steadily secrete AMH; however, secretion increases as the follicle begins folliculogenesis. Secretion reaches a maximum when the follicle begins to develop an antrum and then subsequently diminishes (Andersen & Byskov, 2006; Durlinger, Visser, & Themmen, 2002).

Folliculogenesis is a dynamic growth process, thus the ovary will possess multiple follicles that are at different stages of development. This pattern of secretion and the overall serum level of AMH have been identified as useful biomarkers because they are associated with the number of antral follicles (i.e. tertiary follicles) present within the ovaries (Fanchin et al., 2003; Muttukrishna et al., 2005; van Rooij et al., 2004). Anatomic investigations of polycystic ovaries have shown that the number of preantral and small antral follicles can be up to six times greater compared to the number of the same follicles in normal ovaries (Webber et al., 2003). Correspondingly, women with PCOS have been found to have higher serum concentrations of AMH compared to women possessing normal ovaries (Homburg & Crawford, 2014; Homburg et al., 2013).
Additionally, while the rate of folliculogenesis is constant among follicles within normal ovaries, follicles of polycystic ovaries appear to develop at an accelerated rate as there are a higher proportion of follicles in the later stages of development (i.e. preantral and small antral follicles) (X. Huang, Liu, & Wang, 2007). Studies conducted in rats have also shown that offspring that had fetal exposure to excess androgens had a higher density of preantral and antral ovarian follicles compared to offspring that were not exposed (Tehrani, Noroozzadeh, Zahediasl, Piryaeei, & Azizi, 2014). Furthermore, a study conducted in sheep showed that prenatal T exposure increased the secretion of AMH from antral follicles (Veiga-Lopez, Ye, & Padmanabhan, 2012). Such findings indicate that the increased level of serum AMH and the associated increase in the number of preantral and antral follicles could be the result of altered fetal programming.

**Association of Environmental Factors with PCOS**

PCOS has been observed to be familial in a large proportion of clinical cases; however, investigations of potential genetic polymorphisms or aberrations have not yet produced consistent findings (Homburg & Crawford, 2014). Additionally, an increased prevalence of PCOS has been identified in populations where genetic variability has remained relatively constant (Diamanti-Kandarakis et al., 1999). While many clinical and scientific findings also contribute to the hypothesis that environmental exposure plays a role in the etiology of PCOS, investigators have not ruled out the involvement of genetic inheritance. As described previously, numerous studies have been able to successfully replicate a permanent, PCOS-like phenotype in animal models through exogenous
exposure to excess androgens. However, other studies have demonstrated an increased prevalence of PCOS in women with congenital adrenal hyperplasia due to a 21-hydroxylase deficiency (Dumesic, Goodarzi, Chazenbalk, & Abbott, 2014). Starting the third month of fetal life, this genetic defect results in increased androgen production and ultimately increased levels of free androgens in a manner not unlike the exogenous administrations employed in the animal studies (White & Speiser, 2000; Xita & Tsatsoulis, 2006).

From a developmental perspective, environmental exposure could contribute to the altered fetal programming hypothesis as a potential modifier of the phenotypic expression of PCOS later on in life. Thus, environmental exposures could possibly act as determinants for symptom severity. More specifically, environmental exposures could potentially explain the significant variability of symptom severity and clinical presentation observed among women with PCOS. When genetic traits are also taken into consideration, environmental exposures may actually increase the risk of a manifestation of the PCOS phenotype among women who are potentially genetically predisposed (Diamanti-Kandarakis, Christakou, & Marinakis, 2012).

**Endocrine Disruptors**

It is well known that exposure to developmental hazards is prevalent within the modern living environment. One of the most concerning agents are endocrine disrupting chemicals, otherwise known as endocrine disruptors. As the name implies, endocrine disruptors are defined by the Environmental Protection Agency (EPA) as “exogenous
agents that disturb the synthesis, secretion, metabolism, binding action, or metabolism of natural blood-borne hormones that are responsible for homeostasis, reproduction, and developmental processes” (Diamanti-Kandarakis et al., 2009). Endocrine disruptors can be natural or man-made substances. Such examples include pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls (PCBs), pesticides such as dichlorodiphenyltrichloroethane (DDT), and plasticizers such as bisphenol A (BPA). Due to their widespread prevalence in a number of materials, endocrine disruptors are present in many consumer products including plastic bottles, metal food cans, detergents, food, toys, and cosmetics.

Concern over endocrine disruptors as a health hazard has increased as research findings have shown that endocrine disruptors may pose the greatest risk during prenatal development (Schug, Janesick, Blumberg, & Heindel, 2011). As this is a time of significant organ and neural system development, it is one of the most susceptible periods of human growth. To add to this susceptibility, protective measures such as DNA repair, an adequate immune system, detoxifying enzymes, and the blood/brain barrier are not fully developed in the fetus (Newbold, Padilla-Banks, Snyder, Phillips, & Jefferson, 2007). The higher metabolic rate relative to adults may also render the fetus susceptible to developmental toxicity (Newbold et al., 2007). Furthermore, such exposure during a critical period of development has been proposed to result in irreversible changes to differentiating tissue, not excluding the brain (Bigsby et al., 1999). For example, a longitudinal cohort study of prenatal exposure to polybrominated diethyl ethers (PBDEs), a common flame retardant compound, found that children with higher levels of prenatal
exposure scored lower on mental and psychological developmental assessment tests compared to children with low levels of prenatal PBDE exposure (Bigsby et al., 1999). Numerous studies report similar adverse effects including behavioral deficits in association with prenatal exposure to pesticides, especially organophosphates (OPs) (Gonzalez-Alzaga et al., 2014).

In addition to lower intelligence quotients, children with relatively higher levels of prenatal exposure to PCBs had a higher incidence of neuromuscular and neurodevelopmental delay within the first 24 hours of life compared to children with average levels of prenatal exposure (Jacobson & Jacobson, 1996). Research findings also propose that endocrine disruptors interfere with fetal adipocyte development, thereby increasing the risk of obesity and metabolic disorders later in life (Ashley-Martin et al., 2014).

Prospective cohort analyses have shown that the effects of prenatal BPA and phthalate exposure on metabolic function appear to be sex-specific (Ashley-Martin et al., 2014; Chou et al., 2011). A longitudinal birth cohort study conducted in Canadian mothers reported an inverse relationship between maternal exposure to BPA during the first trimester. The study also reported an inverse relationship between this exposure and umbilical cord blood levels of adiponectin among male infants as well as a markedly elevated risk of high levels of leptin in umbilical cord blood among male infants with moderate to high levels of maternal mono (3-carboxypropyl) phthalate (MCP) exposure (Ashley-Martin et al., 2014). Another prospective study conducted in a Taiwanese birth cohort found that maternal exposure to BPA was associated with increased odds of low
adiponectin levels in male infants, but decreased odds in female infants (Chou et al., 2011). However, both low and high levels of maternal BPA exposure were associated with high leptin levels in female infants (Chou et al., 2011). Additionally, increased levels of maternal BPA exposure were associated with higher odds of significantly elevated umbilical cord blood leptin levels in both male and female infants (Chou et al., 2011).

As studies have shown that low as well as high levels of both of adiponectin and leptin are associated with potentially adverse outcomes, these results illustrate the potential impact of intrauterine exposure on the regulation and function of fetal metabolism (Karakosta et al., 2011; Romano, Savitz, & Braun, 2014; Walsh, Byrne, Mahony, Foley, & McAuliffe, 2014). Both leptin and adiponectin are integral to the metabolic process. Leptin is a hormone secreted by adipocytes to signal satiety to the brain and inhibit feeding behavior. As a part of normal metabolic homeostasis and fat storage regulation, leptin levels typically remain stable, but are also known to increase with age (Balasko, Soos, Szekely, & Petervari, 2014; Li, Matheny, Nicolson, Tumer, & Scarpace, 1997). As elevated leptin levels correlate with obesity, high leptin levels at birth could be associated with obesity and its comorbidities later in life such as NIDDM. The protein adiponectin, on the other hand, regulates glucose levels as well as fatty acid metabolism. As adiponectin levels are highest at birth, lower levels during this time appear to be associated with an increased risk of metabolic dysfunction in adulthood (Mazaki-Tovi, Kanety, & Sivan, 2005).
Endocrine disruptors can also interfere with cyclic processes regulated by endogenous hormones, including female reproductive function. The female reproductive system is highly susceptible to perturbation as normal function greatly relies upon the finely controlled modulation of ovarian steroidogenesis in order to properly execute folliculogenesis and ovulation. As endocrine disruptors are ubiquitous in nature as well as in man-made products, the opportunities of perturbing this sensitive system are extensive. Research shows that exposing the female reproductive system to commonly encountered endocrine disruptors such as PCBs can alter the menstrual cycle (Cooper, Klebanoff, Promislow, Brock, & Longnecker, 2005). Pesticides are a subgroup of reproductive endocrine disruptors reported to exert a variety of effects. A cohort study conducted in female agricultural workers showed that women with occupational exposure to pesticides with suspected hormonal activity had 60 to 100% increased odds of experiencing prolonged menstrual cycles, missed periods, as well as inter-menstrual bleeding compared to women without occupational exposure (Farr, Cooper, Cai, Savitz, & Sandler, 2004). A study conducted in the same cohort found an association between these symptoms and infertility (Rowland et al., 2002).

Given the altering effects of endocrine disruptors on developmental, metabolic, and reproductive processes, such exogenous agents could potentially be involved in the development of PCOS. Accordingly, an increasing body of research shows that endocrine disruptors may impact fetal development by altering fetal programming thereby inducing the development of PCOS later on in life.
Specific Aims

This literature review will present and compare the available research on individual endocrine disruptors and their pathophysiological roles in the development of PCOS. As such, this investigation will include studies and experimental approaches that specifically focus on the developmental effects of exposure to endocrine disruptors during fetal life.

This discussion will proceed with a comprehensive analysis of fetal exposure to three major classes of endocrine disruptors: BPA, phthalates, and endocrine disruptors with androgenic activity. Compared to other endocrine disruptors, the agents selected for this review have been the most extensively studied in regard to the relationship between fetal exposure and the development of PCOS later in life. Data on exposure assessment for each endocrine disruptor will be detailed to include their major sources of contact, the media and microenvironments in which they reside, their primary routes of exposure, as well as preliminary research that prompted investigators to assess their involvement in the fetal origins of PCOS. Examinations of each individual endocrine disruptor will conclude with an overall evaluation of the current standing of the research and will provide recommendations for further studies ascertaining its role in the etiology of PCOS. In an effort to elucidate the current and emerging findings related to this area of study as a whole, this review will discuss the challenges encountered and reported in the published research and propose future directions modeled after novel research methodologies that show promise for further investigation.
In summary, this literature review will aim to determine the potential impact of endocrine disruptors on fetal programming through a discussion of the available literature investigating the developmental origins of PCOS deriving from fetal exposure to three major classes of endocrine disruptors. This report will therefore serve to inform investigators about current findings and provide recommendations for future studies.
**PUBLISHED STUDIES**

**Bisphenol A**

Bisphenol A is a man-made chemical substance largely used in the making of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used to make a multitude of consumer goods including plastic water bottles and baby bottles, impact-resistant safety equipment, and medical devices. Epoxy resins are often applied to metal products such as food cans, bottle caps, and water supply pipes (National Toxicology Program, 2010). Additionally, BPA has also been detected in a number of thermal paper products such as cash register and ATM receipts, as well as in certain dental composites and sealants. Findings from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control (CDC) reported that 93% of Americans aged six years or older had detectable urinary levels of BPA (Lakind & Naiman, 2008). Accordingly, human exposure to BPA is considered to be ubiquitous.

The high prevalence of BPA body burden in human populations is explained by the facility with which BPA enters the body. BPA largely comes from dietary sources, thus the major route of exposure is through ingestion of contaminated food and liquids (National Toxicology Program, 2010). Several *in vivo* studies have found that the effects of BPA exposure are significant. For example, BPA has been shown to act in these studies as an estrogen agonist as well as an estrogen antagonist (Alonso-Magdalena, Morimoto, Ripoll, Fuentes, & Nadal, 2006; Melzer et al., 2011; Steinmetz, Brown, Allen, Bigsby, & Ben-Jonathan, 1997; Susiarjo, Hassold, Freeman, & Hunt, 2007). *In vivo* studies have additionally demonstrated that BPA acts as an androgen and thyroid
hormone antagonist (Fini et al., 2007; Lee, Chattopadhyay, Gong, Ahn, & Lee, 2003; Takahashi & Oishi, 2001).

Currently, BPA has been reported to be present at safe levels in consumption products; however, due to its estrogenic activity, it has been identified as a possible hazard to fetal development (United States Food and Drug Administration, 2010). While the placenta confers a protective barrier against the transmission of infectious agents, the fetus may still be susceptible to BPA bioaccumulation during the first half of fetal development. Indeed, BPA has been identified in fetal serum and full-term amniotic fluid, confirming passage through the placenta (Ikezuki, Tsutsumi, Takai, Kamei, & Taketani, 2002). Additionally, the BPA concentration in amniotic fluid assayed at 15 to 18 weeks gestation was found to be five times higher than maternal serum samples collected during early pregnancy, as well as maternal serum samples collected during late pregnancy (Ikezuki et al., 2002). While the precise mechanism of the metabolic clearance of BPA is unknown, BPA likely accumulates in amniotic fluid into mid-term gestation due to the immature liver function of the fetus, thus subjecting it to significant exposure. However, amniotic fluid concentrations of BPA were observed to decrease as fetuses reached full term, possibly indicating that the fetus gradually metabolized BPA as liver functionality matured (Ikezuki et al., 2002).

While exposure may diminish over the second half of fetal life, rodent studies report that prenatal BPA exposure nonetheless leads to morphological and physiological alterations of the reproductive system (Table 1). For example, BPA administered during pregnancy at doses typically encountered in the environment passes on to fetuses and has
been shown to alter postnatal development as well as sexual maturation (Howdeshell, Hotchkiss, Thayer, Vandenbergh, & vom Saal, 1999). Another study investigating in utero exposure of BPA in rodents found that exposure during early ovarian development resulted in decreased fertility among the first generation (F1) offspring (Wang, Hafner, & Flaws, 2014). Such exposure has also been proposed to span across generations as impaired oocyte meiotic maturation has also been reported to occur (Lawson et al., 2011; Susiarjo et al., 2007).

As BPA has been associated with both metabolic and reproductive dysregulation, researchers have begun to investigate its potential contribution to the development of PCOS, a condition known for its presentation of metabolic and reproductive aberrations. Experimental studies in women found that serum BPA concentrations in those diagnosed with PCOS were significantly higher compared to women without PCOS (Kandaraki et al., 2011; T. Takeuchi & Tsutsumi, 2002). Additionally, serum BPA levels positively correlated with BMI, total T, and free T for both women with and without PCOS; however, no such correlation between serum BPA and any other sex hormone-related concentrations was found (Kandaraki et al., 2011; T. Takeuchi & Tsutsumi, 2002; Toru Takeuchi, Tsutsumi, Ikezuki, Takai, & Taketani, 2004). Studies have also reported a positive correlation between BPA levels and the severity of insulin resistance in women with and without PCOS (Kandaraki et al., 2011; Tarantino et al., 2013). More specifically, women with PCOS were found to have higher serum BPA concentration levels along with more severe cases of insulin resistance compared to women without PCOS (Tarantino et al., 2013). Increased insulin resistance has been reported to be
prevalent among PCOS cases as it is present in a majority of obese women and up to 30% of non-obese women with the syndrome (Awwad & Abiad, 2013; Rajkhowa, Bicknell, Jones, & Clayton, 1994).

Findings from studies conducted at the level of neonatal exposure have demonstrated effects similar to those conducted solely at the level of prenatal exposure. For example, results from mouse models have proposed that neonatal exposure to BPA may also be involved in the pathogenesis of insulin resistance as it is associated with alterations in glucose metabolism and insulin secretion (Alonso-Magdalena et al., 2006). Neonatal exposure to BPA in rats has been shown to exert endocrine and reproductive alterations in a manner similar to PCOS. A study conducted by Fernández et al. (2010) found that, in comparison to controls, newborn rats injected with BPA from postnatal day 1 to postnatal day 10 at doses lower than the Lowest Observed Adverse Effect Level (LOAEL) established by the EPA had higher serum T and E2 levels, but decreased serum P levels. When examined at the level of hypothalamic/pituitary secretion, these levels of BPA were also associated with alterations in GnRH secretion. Investigators from previous studies have suggested that abnormal levels of serum sex hormone concentrations could dysregulate the pulsatility pattern of GnRH secretion (Marshall, Eagleson, & McCartney, 2001). Correspondingly, the time intervals between GnRH pulse secretions in neonatally exposed rats at levels below the LOAEL were decreased compared to controls (Fernandez, Bianchi, Lux-Lantos, & Libertun, 2009; Fernandez et al., 2010).
Reduced time between pulses indicates that the frequency of GnRH secretions, as well as downstream hormone secretions regulated by GnRH such as LH and FSH, has increased. Rats exposed at these low levels were also found to be subfertile as they subsequently had fewer offspring compared to controls (Fernandez et al., 2010). Rats that received BPA doses at two times the No Adverse Effect Level (NOAEL) defined by the EPA were also found to have these effects as well as a significant number of cystic ovarian follicles (Fernandez et al., 2010). The alterations in fertility at this dosage level of neonatal exposure in rats, however, were much more significant as they did not ovulate or go on to produce any offspring (Fernandez et al., 2010). These alterations produced at the hypothalamic-pituitary unit as well as the ovarian level resemble the dysregulation observed in PCOS cases. The pulsatile frequency of GnRH/LH secretion has been observed to be increased in PCOS (Marshall et al., 2001). As LH acts to support ovarian theca cells, which secrete androgens, this increased pulsatility has been proposed as a potential cause for elevated serum T levels seen in both animal studies and in PCOS cases (Fernandez et al., 2010). Regarding a more direct mechanism induced by exposure, it has been hypothesized that BPA acts to dysregulate 17 beta-hydroxylase, an enzyme involved in ovarian steroid biosynthesis, thereby resulting in androgen overproduction at the level of the ovary and thus contributing to the development of PCOS (Barrett & Sobolewski, 2014; Diamanti-Kandarakis et al., 2012). However, for many chemical hazards, the time of exposure that poses the greatest risk to health and future development is during the prenatal period when organ systems and physiological homeostasis are being established (Table 1). For example, prenatal, not neonatal,
exposure of female rats to NOAEL concentrations of BPA were observed to have a greater percentage of relatively longer estrous cycles compared to controls (Schonfelder, Friedrich, Paul, & Chahoud, 2004). Additionally mouse studies of prenatal exposure at levels far less than the NOAEL for BPA found that female offspring had a significantly earlier onset of puberty (Honma et al., 2002; Howdeshell et al., 1999).

While the findings from animal studies appear to correlate with the research on adult BPA exposure and the incidence of PCOS, the effects of human prenatal exposure to BPA remain largely unknown. As of this review, no human studies of prenatal BPA exposure have been published to longitudinally investigate its potential association with PCOS development during adolescence or adulthood. However, studies of other endocrine disrupting chemicals have shown that those with estrogenic activity may affect the development of estrogen-sensitive organs (Nguyen et al., 2011). Taking into account the findings from animal studies that indicate that BPA impacts prenatal programming and thus leads to alterations in endocrine and reproductive function, future research should longitudinally examine the relationship between in utero exposure in humans and the development of PCOS. As one approach, investigators could utilize developmental biomarkers, particularly those involved in reproductive development, to explore this association in female infants and children in a longitudinal cohort (Barrett & Sobolewski, 2014).
Table 1. Reported Effects of Prenatal Exposure to Bisphenol A (BPA) in Rodent Models. The effects of BPA exposure during fetal development vary by dosage in micrograms or milligrams per kilogram per day (µg/kg/day or mg/kg/day) and prenatal developmental stage of exposure. Reported reasoning for dosage and timing of exposure are listed, as available. *Exposure measured over multiple lengths of time. Results apply to all durations of BPA exposure.

<table>
<thead>
<tr>
<th>Species</th>
<th>Developmental Stage of Exposure</th>
<th>Dosage</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Day 11-17 (Organogenesis)</td>
<td>2.4 µg per kg/day (environmentally relevant)</td>
<td>Advanced onset of first estrous</td>
<td>Howdeshell et al., 1999</td>
</tr>
<tr>
<td>Mouse</td>
<td>Day 11-Birth (Ovarian Development)</td>
<td>0.5 µg per kg/day (Mimics exposure from bottle feeding)</td>
<td>Decreased number of primordial follicles, Shortened estrous period, Decreased fertility (Impaired Ovulation)</td>
<td>Wang et al., 2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 µg per kg/day (Previously shown to disrupt oocyte meiosis)</td>
<td>Decreased number of primordial follicles, Shortened estrous period, Decreased fertility (Impaired Ovulation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 µg per kg/day (EPA referenced safe dose)</td>
<td>Decreased number of primordial follicles, Advanced puberty onset, Decreased fertility (Impaired Gestation)</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Day 11.5-18.5 (Oocyte Maturation)</td>
<td>20 µg per kg/day</td>
<td>Increased incidence of meiotic aberrations in oocytes</td>
<td>Susiarjo et al., 2007</td>
</tr>
<tr>
<td>Mouse</td>
<td>Day 11-12, 12.5, 13.5, and 14.5</td>
<td>20 µg per kg/day</td>
<td>Changes in oocyte gene expression within 24 hours of exposure onset, Increased incidence of meiotic aberrations in oocytes</td>
<td>Lawson et al., 2011*</td>
</tr>
<tr>
<td>Rat</td>
<td>Day 6-21 (NOAEL)</td>
<td>50 mg per kg/day</td>
<td>Longer estrous cycles</td>
<td>Schönfelder et al., 2004</td>
</tr>
<tr>
<td>Mouse</td>
<td>Day 11-17</td>
<td>20 µg per kg/day</td>
<td>Advanced onset of first estrous</td>
<td>Honma et al. 2002</td>
</tr>
</tbody>
</table>
Phthalates

Phthalates are used to provide flexibility and soften numerous materials such as plastic and vinyl (Heudorf, Mersch-Sundermann, & Angerer, 2007). They can be widely found in numerous consumer products such as cosmetics, toiletries, shower curtains, wallpaper, as well as food packaging (Heudorf et al., 2007). Additionally, phthalates are used to manufacture a number of industrial products such as solvents, insecticides, building materials, adhesives and detergents (Heudorf et al., 2007). Phthalates are easily released into the air and water as part of the manufacturing process (Bertelsen et al., 2013). They are also released into the same environmental media as well as food sources when consumers use products that contain them (Heudorf et al., 2007). Regarding dietary exposure, foods with relatively higher fat content such as dairy products and meats have been found to be a major food source (Fromme et al., 2007). Inhalation of household dust contaminated with phthalates is another route of exposure (Heudorf et al., 2007). The prevalence of phthalate exposure is considered to be widespread in the United States as most people in the general population have been found to have detectable urine levels of phthalate metabolites (Crinnion, 2010). In rodent studies, phthalate exposure has been found to be associated with adverse metabolic and reproductive effects such as increased visceral adiposity and reduced fertility (X. F. Huang et al., 2012; Schmidt, Schaedlich, Fiandanese, Pocar, & Fischer, 2012). Studies also indicate that the developing fetus is vulnerable to phthalate exposure as these chemicals are able to cross the placental barrier (Hart et al., 2014). Additionally, an increased incidence of pubertal abnormalities have
been detected in up to three generations following fetal phthalate exposure (Manikkam, Tracey, Guerrero-Bosagna, & Skinner, 2013).

Given that the effects of phthalate exposure are similar to the symptoms seen in PCOS, investigators have begun to examine the potential association between fetal phthalate exposure and the development of PCOS. The current literature has reported limited findings on the following exposures: dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP).

*Dibutyl Phthalate*

Dibutyl phthalate is primarily used as a plasticizing agent to increase plastic flexibility. DBP is also commonly found in cosmetics and dye solvents such as printing inks. On average about 95% of people are exposed to less than 10 micrograms of DBP per kilogram of bodyweight per day (µg/kg/day) (Kohn et al., 2000). Interestingly, DBP levels were found to differ among age groups and between sexes as reproductive-aged women were found to have the highest levels of DBP urine metabolites (Blount et al., 2000). Investigators have proposed that the higher level of exposure among this group may be due to more frequent usage of personal care products containing DBP such as nail polish, perfume, and hair spray (Blount et al., 2000). Studies of high-level DBP exposure in rodents have shown that exposure during pregnancy was associated with reproductive system and organ abnormalities in female offspring (National Toxicology, 2003). Additionally, findings from animal studies indicate that rodents are more susceptible to the adverse reproductive effects of DBP when they are exposed during prenatal
development compared to adult exposure (National Toxicology, 2003). A study in rats gestationally exposed to plastic-derived phthalates (DBP and DEHP) as well as BPA conducted by Manikkam and colleagues (2013) found that all of the females among the F1 and third generation (F3) offspring had a significantly higher incidence of polycystic ovaries (Figure 2).
Figure 3. Incidence of Polycystic Ovaries among Rats with Control, Plastics, or Lower Dose (LD) Plastics Lineages. Percentages of first (F1) and third (F3) generation offspring arising from ancestral lineages prenatally exposed to plastics derived endocrine disruptors (BPA, DEHP, and DBP) compared to ancestral rats without prenatal exposure (Control). The actual number of rats with polycystic ovaries/total number of rats in each group is shown above each respective bar graph. Ancestral gravid rats designated as “Plastics” received intraperitoneal injections of BPA, DEHP, and DBP at 50 mg/kg/day, 750 mg/kg/day, and 66 mg/kg/day, respectively over embryonic day 8-14 of gestation. Ancestral gravid rats designated as “LD Plastics” were exposed over the same period of gestation as “Plastics” rats, but at half the original dose for each endocrine disruptor. ***p < 0.001. Taken from Manikkam et al., 2013.

Conversely, F1 and F3 rats without ancestral gestational exposure did not develop polycystic ovaries (Manikkam et al., 2013). While these results indicate that such exposure contributes to a transgenerational establishment of PCOS in offspring, the study did not verify whether these effects were associated with the combined exposure to both phthalates and BPA, or to DBP exposure alone. Additionally, the dosage of exposure was
over 10,000 times the average daily human exposure encountered in the environment (Manikkam et al., 2013). However, in a study of reproductive-aged women that assessed serum phthalate levels, women with PCOS had significantly higher levels of DBP compared to controls (Xu, Lin, Zhao, & Zhang, 2011). Additionally, DBP levels were higher than any other phthalate concentration present in serum (Xu et al., 2011).

Findings from prior research report that serum DBP levels positively correlate with serum E2 levels (X. Huang et al., 2007). As one of the principle estrogens, one of the primary actions of E2 is to stimulate the release of GnRH from the hypothalamus, which subsequently leads to increased secretion of LH. LH levels in women with PCOS are much higher compared to women without PCOS (Rotterdam, 2004). Additionally, administration of pharmacological analogs of E2 has been shown to induce PCOS-like characteristics in animal models (Brawer, Munoz, & Farookhi, 1986). In light of these results, the published research on DBP promotes further investigation. More specifically, additional animal studies should be conducted that exclusively assess the effects of gestational exposure to DBP at environmentally relevant exposure levels.

 מווהה

Di(2-ethylhexyl)phthalate

Di(2-ethylhexyl)phthalate is a common plasticizer used in the manufacturing of many consumer products such as wall coverings, floor tiles, food packaging, and furniture upholstery (Agency for Toxic Substances Disease Registry (ATSDR), 2002). Studies show that DEHP is a reproductive and developmental toxicant in humans and animals (Grande et al., 2007; Koch, Drexler, & Angerer, 2003; Lyche et al., 2009). The
current literature has produced conflicting findings in regard to the actions of DEHP on female reproduction. Prior animal studies have shown that DEHP exposure is associated with altered ovarian steroidogenesis and low levels of P, an endocrinological profile similar to PCOS cases (Davis, Maronpot, & Heindel, 1994). Furthermore, in vivo studies show that DEHP exposure decreases serum E2 levels, and stops ovulation in adult rats (Lovekamp-Swan & Davis, 2003). These actions are hypothesized to be carried out by the major metabolite of DEHP, mono(2-ethylhexyl) phthalate (MEHP), which has been proposed to directly inhibit ovarian production of E2, resulting in anovulation (Lovekamp-Swan & Davis, 2003). Polycystic ovaries also have been described in adult female rats after exposure to DEHP (Davis et al., 1994).

Very few studies have examined the association between prenatal DEHP exposure and the development of PCOS. Overall, the results are conflicting with studies reporting that DEHP is negatively associated with PCOS incidence in offspring, and other studies reporting a positive association in offspring with PCOS-like symptoms and similar ovarian morphology. Indeed, the study conducted by Manikkam et al. (2013) mentioned in the previous section which included DEHP as one of the gestational phthalate exposures found an increased incidence of polycystic ovaries in F1 and F3 offspring (Figure 2). A study of gestational exposure to DEHP at environmentally relevant levels in mice found that the ovarian weights of female offspring were approximately 35% higher compared to offspring without prenatal DEHP exposure (Pocar et al., 2012). This finding correlates with the classical presentation of enlarged ovaries in PCOS cases (Chang, 2014).
Conversely, phthalates have been shown to exert anti-androgenic effects in humans. A study conducted by Main et al. (2006) found that serum T concentration in infants was negatively associated with serum phthalate concentrations. In another human study, patients with relatively higher urinary concentrations of MEHP had lower odds of having PCOS in comparison to controls with normal levels of the metabolite (Vagi et al., 2014). Additionally, the only cohort study known thus far to examine the impact of in utero phthalate exposure on PCOS development in offspring found that relatively higher maternal serum levels of phthalate metabolites, including MEHP, were associated with a lower prevalence of PCOS among their daughters (Hart et al., 2014). However, this study also found that maternal levels of another phthalate metabolite, mono-ethyl phthalate (MEP), were negatively associated with levels of AMH, a hormone secreted by maturing follicles to inhibit additional primordial follicle activation, in female offspring (Hart et al., 2014). These findings suggest that a decrease in AMH contributes to the increased activation of primordial follicles, potentially stimulating an alternative pathway of polycystic ovary development. As described previously, increased AMH levels have also been shown to be associated with PCOS. In regard to DEHP and MEHP, rodent studies have shown that they accelerate the rate of primordial follicle recruitment (Hannon & Flaws, 2015). Furthermore, DEHP was associated with an increased number of mature ovarian follicles (Hannon & Flaws, 2015). Classic PCOS cases exhibit an ovarian morphology consisting of many peripheral small antral follicles with relatively larger stroma compared to normal ovaries. Another characteristic distinctive of PCOS cases are an increased number of primary as well as preantral follicles (also termed secondary and
tertiary follicles) (Hughesdon, 1982). Findings regarding the impact of DEHP exposure on follicle numbers, however, are also conflicting. While multiple animal studies have shown that DEHP exposure is associated with an increased number of preantral ovarian follicles, a number of other studies have shown that DEHP exposure is associated with decreases in both preantral and primary follicle numbers (Hannon & Flaws, 2015).

The common morphologies exhibited in the polycystic ovary are a multitude of antral follicles encircled by a hyperplastic layer of theca cells, the primary source of androgen hyperproduction in the ovary. This hyperplastic layer arises from the dysregulated, increased secretion of LH (Chang, 2014). However, such morphology does not appear to be reflected in DEHP-exposed animals. In a recent study of in utero DEHP exposure in rats, the ovaries of exposed female offspring exhibited relatively thin theca cell layers relative to unexposed offspring (Meltzer, Martinez-Arguelles, Campioli, Lee, & Papadopoulos, 2014). In addition, exposure resulted in increased FSH levels, and no apparent changes in offspring fertility (Meltzer et al., 2014). Such findings imply that DEHP does not appear to be implicated in the pathogenesis of PCOS at the level of in utero exposure. However, the study was not able to accurately assess the levels of serum LH or the patterns of LH secretion in offspring. As increased levels of LH, along with a decreased periodicity of its secretion, are a hallmark of PCOS, additional studies are warranted.

Given the discrepancies from the available literature, further animal studies are needed in order to better establish the effects of prenatal phthalate exposure on ovarian folliculogenesis in offspring.
Androgenic Endocrine Disruptors

Hyperandrogenism is a hallmark feature of PCOS. Correspondingly, a multitude of research has shown that increased exposure to androgens in utero is associated with an increased incidence of PCOS in both animal and human studies. Aside from investigations on the impact of BPA, the role of endocrine disruptors in creating a hyperandrogenic environment for fetal exposure has not been well characterized in the literature. Overall, more endocrine disruptors have been found to exert anti-androgenic activity at the level of androgen receptors (ARs). However, some agents have been identified as potential androgenic endocrine disruptors. For example, a study conducted in women with PCOS by Cupisti et al. (2010) found that smokers had increased free T levels compared to nonsmokers. A study in rats has also shown that prenatal exposure to nicotine results in chronically increased levels of serum T in female offspring (Smith, Cloak, Poland, Torday, & Ross, 2003). The pathophysiology underlying the resultant increase in serum T has yet to be determined. However, studies suggest that the metabolism of nicotine by cytochrome P450 in the liver may have some effect on T biosynthesis in the liver, as this also involves cytochrome P450 family of proteins (Enea, Boisseau, Diaz, & Dugue, 2008; Ray, Tyndale, & Lerman, 2009). Another agent with androgenic activity is 3,4,4’-trichlorocarbanilide, also known as triclocarban (TCC). Triclocarban is an antimicrobial compound used in a variety of manufactured household and personal sanitizing products such as detergents, soaps, body washes, and cleansing lotions (Sapkota, Heidler, & Halden, 2007). Alone, TCC has little to no androgenic
activity; however, in the presence of T, TCC amplified the effects of T at the level of the AR by increasing AR-mediated transcriptional activity (Chen et al., 2008). Such a synergistic effect increases the bioactivity of endogenous T, implying a resulting physiological environment not unlike that created by increased serum androgen concentrations.

Numerous animal studies have successfully recreated a PCOS-like phenotype via gestational administration of excess androgens. In summary of what has been previously described, studies in female rhesus monkeys prenatally exposed to excess androgen had abnormal ovarian function, a later menarcheal onset, anovulatory cycling, impaired fertility, enlarged ovaries, as well as increased visceral fat distribution (Dumesic et al., 1997; Eisner et al., 2002; Eisner et al., 2003). In addition to insulin resistance, these offspring also possessed an endocrinological profile similar to that of PCOS cases including hypersecretion of LH, and adrenal and ovarian androgens (Abbott et al., 2009). Furthermore, in rats, female offspring exposed to excess androgens in utero developed cystic ovarian follicles (Tehrani et al., 2014). The PCOS phenotype has also been recreated in sheep, with prenatally androgenized ewes meeting the diagnostic criteria for PCOS, in addition to having other abnormalities characteristic of PCOS including insulin resistance, LH hypersecretion and reduced negative feedback of P secretion (Padmanabhan & Veiga-Lopez, 2014).

Additional research in animals has been conducted to better ascertain the effects of prenatal androgen exposure on female reproductive morphology and physiology. Studies have shown that the timing of in utero exposure to excess androgens may
critically impact whether PCOS manifests in adulthood. More specifically, the reproductive systems of female offspring with early fetal exposure were developmentally and morphologically abnormal relative to unexposed offspring, but hormonal physiology remained normal (Tehrani et al., 2014). Conversely, female offspring exposed during later stages of fetal development demonstrated abnormal hormonal profiles, yet maintained relatively normal reproductive system morphology (Tehrani et al., 2014). The authors indicated that the latter finding was more representative of classical PCOS cases. This assertion was supported by further results showing that the offspring with later fetal exposure had increased serum T concentrations, thereby indicating increased ovarian sensitivity to LH secretion (Tehrani et al., 2014). Additionally, this particular subset of offspring had decreased serum FSH and serum estrogen concentrations (Tehrani et al., 2014). These results are consistent with findings from clinical studies as collected serum FSH concentrations are significantly lower in PCOS cases compared to normal women, indicating that PCOS is associated with decreased secretion of FSH during the early follicular phase of the menstrual cycle (Rebar et al., 1976).

The pathophysiology responsible for decreased FSH secretion in PCOS remains to be characterized; however, studies have proposed that the negative feedback effect of chronically unopposed estrogen secretion could be the underlying mechanism involved (Chang, Mandel, Lu, & Judd, 1982; Yen, 1980). The reduction in serum FSH may also stem from the hypothalamic secretion of GnRH. More specifically, the increased frequency of pulsatile GnRH secretion could lead to a down-regulation of FSH gene expression and an up-regulation of LH gene expression, thereby potentially creating the
hormonal profile seen in prenatally androgenized rodents and PCOS cases alike (Chang, 2014). Furthermore, it has been reported that a high pulse frequency of GnRH stimulates LH secretion whereas a low pulse frequency of GnRH secretion stimulates FSH secretion (Sullivan & Moenter, 2004). At the level of the hypothalamic regulation of GnRH secretion, a study in women with PCOS conducted by Eagleson et al. (2000) found that elevated plasma androgens decreased the sensitivity of the hypothalamic GnRH pulse generator to feedback inhibition by E2 and P. Interestingly, this sensitivity was restored when women were treated with an AR antagonist, flutamide (Eagleson et al., 2000). Findings of increased P and LH secretion as well as an increased frequency and amplitude of LH secretion in prenatally androgenized rats also imply a defect in feedback inhibition (Figure 3) (Wu et al., 2010).
Figure 4. Amplitude and Frequency of Luteinizing Hormone (LH) Secretion in Prenatally Androgenized Rats Compared to Controls. Rats were prenatally exposed from day 16 to 19 of gestation to 3 mg of either testosterone (pT) or dihydrotestosterone (pDHT). The amplitude and frequency of LH secretion measured between 60-70 days after birth over the span of two hours were higher in pT and pDHT rats compared to controls, which received no prenatal exposure. Data points are ± the standard error of the mean. Taken from Wu et al., 2010.

Taking into account the findings from Sullivan and Moenter (Sullivan & Moenter, 2004), this decreased sensitivity and subsequent increase in GnRH pulse frequency may be responsible for the high levels of LH secretion, which subsequently stimulates
increased androgen production at the level of the theca cells in the ovary. The increases in androgen levels would result in decreased sensitivity of GnRH feedback inhibition, thereby implying the presence of a self-perpetuating pathophysiological cycle.

The precise mechanism by which excess androgens alter hormonal physiology and induce fetal reprogramming remains to be understood; however the magnitude of effect is likely related to serum levels of plasma carrier proteins, particularly SHBG. Normally, approximately 98% of sex steroids in plasma are bound to carrier proteins derived from the liver. Plasma E2 and T (primarily) bind SHBG and albumin. Corticosteroid-binding globulin (CBG) binds cortisol, P, and a smaller percentage of plasma T compared to SHBG and albumin. Testosterone has a greater affinity for SHBG compared to E2; therefore, in women, SHBG binds to approximately 78% of T and only 58% of E2 circulating in plasma (Chang, 2014). Albumin accounts for the remaining percentages of bound T and E2. As SHBG largely serves to prevent circulating sex steroids from entering target tissues, it strongly influences the amount of bioavailable sex steroids. Accordingly, increases or decreases in SHBG production can profoundly affect the magnitude of action by sex steroids as well as alter metabolism. In turn, prenatal androgen exposure has been shown to exert lifelong effects on genetic expression in the liver (Hammond, 2011).

The role of SHBG in fetal development remains unclear; however, it is believed to protect female fetuses from androgen exposure and to control androgen action in male fetal sex differentiation (Hammond, 2011). In PCOS models represented by prenatally androgenized female rats, serum total T levels and free T index (i.e. the ratio of
circulating free T to circulating bound T) were higher compared to controls (Wu et al., 2010). Reductions in SHBG may lead to significant hyperandrogenism in women. Clinical findings have shown that women presenting with hyperinsulinemia, excessive weight gain, or hyperandrogenism have also had decreased levels of circulating SHBG, and have subsequently gone on to develop hirsutism (Chang, 2014). Therefore, all of these conditions may be present at the same time in PCOS, thereby augmenting the effects of excess androgen production seen clinically. During development, prenatal exposure to excess T in ewes leads to hyperinsulinemia, which not only suggests that reprogramming takes place at the target tissues of insulin (i.e. liver, muscle, and adipose tissue), but also proposes the presence of a cyclical pathophysiology (Recabarren et al., 2005). More specifically, excess androgen leads to hyperinsulinemia, which, in turn, may reduce levels of SHBG (Chang, 2014). Therefore, reduced levels of SHBG leads to excess androgen and thus hyperandrogenism. SHBG also appears to affect the hypothalamic-pituitary-ovarian axis. Indeed, reduced levels of SHBG resulting from hyperinsulinemia and hyperandrogenemia leads to increased levels of free E2, thereby enhancing its effect of feedback inhibition at the level of the hypothalamus. More specifically, increased E2 levels ultimately reduce the level of FSH secretion relative to LH secretion (Lebovic et al., 2005).

Ovulatory dysfunction and menstrual disorders fall under the diagnostic criterion of oligo- or anovulation for PCOS (Rotterdam, 2004). This common PCOS feature has been replicated in animal models. Female rats with prenatal androgen exposure have been shown to develop irregular estrous cycles, have a decreased number of preovulatory
ovarian follicles, as well as have higher levels of LH and P (Wu et al., 2010). From these physiological findings, the authors confirmed that the prenatally exposed female rats had a significant reduction in ovulation in relation to unexposed female rats (Wu et al., 2010). The estrous cycles of the prenatally androgenized female mice were also longer compared to controls. Similar findings have also been reported in rhesus monkeys. Relative to female rhesus monkeys without prenatal exposure, female rhesus monkeys exposed to excessive androgens during the earlier or later stages of fetal life had ovulation dysfunction as well as decreases in menstrual cycle length by 40%-50% (Abbott et al., 2005). As these findings are analogous to the clinical presentations of PCOS patients, who have been shown to have reductions in ovulation and abnormal menstrual cycles, the hypothesized impact of excess androgen exposure during fetal life on the subsequent development of PCOS is further supported.

The effect of excess androgens on progesterone receptor (PR) expression levels has been explored as a potential contributor to ovulatory dysfunction in PCOS. As previously described, during the follicular phase of normal hormonal cycling, the effect of feedback inhibition by P on GnRH pulse secretion progressively decreases, thereby resulting in a gradual increase in GnRH pulse secretion. Pulsatile secretion of GnRH continues to increase until it peaks late in the follicular phase. During that point in time, the rising levels of E2 secreted by the dominant ovarian follicle exerts a positive-feedback effect on the already increased GnRH pulse generator, thereby stimulating increased LH secretion, otherwise known as the LH preovulatory surge. E2 and P produced and secreted from the corpus luteum following ovulation act to decrease GnRH
pulsatile frequency. This ultimately results in increased FSH secretion, which begins the next cycle of ovarian follicular development. It has been shown that the preovulatory peak in GnRH secretion responsible for the LH surge relies on the coupling of ovarian-secreted E2 with a circadian neural signal (Chappell, Lee, & Levine, 2000; Wu et al., 2010). Investigators have proposed that this coupling is negotiated by hypothalamic PR expression (Chappell et al., 2000). This hypothesis is supported by findings from rodent studies reporting that decreases or complete cessations (in PR knockout mice) of PR expression in the hypothalamus effectively prevent the E2-induced increase in GnRH pulsatility, and thus the LH surge (Chappell & Levine, 2000; Chappell et al., 1999). The same effect has also been demonstrated with the usage of a PR antagonist (Bellido, Gonzalez, Aguilar, & Sanchez-Criado, 1999). In addition to an association of higher levels of LH with oligoovulation, prenatally androgenized female rats have been reported to have decreases in hypothalamic PR expression in adulthood compared to unexposed female rats (Wu et al., 2010). Additionally, in both rats and mice, prenatal androgen excess was found to inhibit the LH surge by interfering with the positive feedback effect of rising E2, which also resulted in oligoovulation as well as anovulation (Moore, Prescott, & Campbell, 2013; Wu et al., 2010).

The establishment of gonadotropin release that occurs during fetal and perinatal life in rats appears to follow a sexually dimorphic developmental pathway that is affected by the presence of androgens. A lack of androgen exposure will form a female brain, which will result in a cyclical pattern of preovulatory gonadotropin release maintained by hypothalamic regulation in adulthood. Conversely the presence of T will lead to the
development of a male brain, and thus will lack this adult cyclical pattern of secretion (Horvath, Cela, & van der Beek, 1998; Wu et al., 2010). Taking the findings reported by Wu et al. (2010) regarding decreased hypothalamic expression of PR in adulthood resulting from prenatal androgen exposure into account, it appears that this cyclical pattern of preovulatory gonadotropin secretion meant to be programmed during female fetal development was inhibited. Thus, exposure to excess androgens may induce fetal reprogramming, which ultimately results in anovulation in adulthood.

However, the mechanism by which androgens suppress PR expression remains to be fully characterized. E2 has been observed to stimulate hypothalamic tissue-expression of PR (Foecking & Levine, 2005). However, in female mice, prenatal androgen exposure, and the resulting activation of AR, appear to inhibit PR generation by E2 (Foecking & Levine, 2005). The mechanism by which AR activation reprograms hypothalamic regulation that leads to the development of increased GnRH pulsatility in adulthood is still relatively unknown. Findings from studies in animal models propose potential mechanistic pathways. For example, synaptic connections to GnRH neurons in adult ewes that had excess prenatal androgen exposure have been shown to be markedly decreased compared to ewes without excess prenatal androgen exposure; however, the number of synaptic contacts were found to be comparable to those observed in adult males (Kim, Foster, & Wood, 1999). These results appear to indicate that increased androgens may alter the synaptic activity of GnRH neurons by decreasing the number of synaptic connections. The reason behind the increased number of synaptic connections in normal females has yet to be identified. Therefore, it is unknown as to whether increased
synaptic connections confer an inhibitory effect thereby regulating GnRH secretion or enhance the preovulatory increase in GnRH. However, other studies have investigated the synaptic input to GnRH neurons from gamma aminobutyric acid (GABA) neurons as another target of AR activation.

Previous studies have demonstrated that GnRH neurons are directly regulated by actions at a GABA A receptor (GABA_A_R) (Sullivan & Moenter, 2004). While GABA is known to exhibit an inhibitory effect on most neurons, this has not been verified in GnRH neurons at the level of GABA_A_R activation. However, in a study of female adult mice, those that had prenatal androgen exposure had an increased GABAergic drive to GnRH neurons as well as an increased level of GnRH response (determined by the relative frequency and size of post-synaptic GnRH current, respectively) to GABA_A_R activation compared to those that had no exposure (Sullivan & Moenter, 2004). Furthermore, evidence of the involvement of AR activation as administration of flutamide, an AR antagonist, to exposed female mice resulted in post-synaptic GnRH current frequencies and sizes that were similar to unexposed mice (Sullivan & Moenter, 2004). The efficacy of flutamide in restoring feedback inhibition of GnRH pulsatility by E2 and P in PCOS cases also implies that increased androgens have a role in the dysregulation of GnRH secretion (Eagleson et al., 2000). Despite these findings, further insight on the direct involvement of AR activation remains elusive as ARs are rarely found on GnRH neurons (Sullivan & Moenter, 2004). Therefore, further investigation on the presence and the potential role of AR in presynaptic GABA neurons is warranted.
While the published research using animal models largely indicate that fetal exposure to excess androgens contributes to PCOS development later in life, the literature of such exposure in humans is very limited and presents inconsistent findings. A major barrier in fully investigating this potential association is the lack of a safe method to quantify the level of human fetal exposure (Abbott & Bacha, 2013). Therefore, studies of prenatal exposure to excess androgen have been executed via indirect assessments or reviewing postnatal outcomes. The concentration level of androgens in umbilical cord blood has been used in multiple studies as an indirect assessment, but has produced mixed results. A study of female infants born to mothers with PCOS found that their blood concentrations of T in their umbilical veins were significantly higher compared to female infants born to mothers without PCOS (Barry et al., 2010). The gestational exposure presented in mothers with PCOS is not unlike exposure to excess androgen as increased maternal circulating levels of T are coming in contact with the fetal environment (Sir-Petermann et al., 2002). However, two studies have reported that PCOS gestation is associated with lower androgen concentrations in umbilical cord blood of female offspring (Anderson et al., 2010; Maliqueo et al., 2013). The limitations presented with cord blood collection may account for the mixed results. For example, investigators have proposed that the variable decreases in umbilical cord blood androgen concentrations that occur at the onset of labor may explain why elevated levels have not been observed in some studies (Abbott & Bacha, 2013). Another limitation with this indirect measurement is that umbilical cord blood of both fetal and maternal origin and collection at birth is outside the critical period of neural and ovarian differentiation in
humans (Padmanabhan, Veiga-Lopez, Abbott, & Dumesic, 2007). If the ovary is the target of excess androgen exposure with its differentiation occurring during midgestation, any potential hormonal abnormalities may not be present, and thus undetectable, at birth (Abbott & Bacha, 2013).

In amniotic fluid analyses collected at midgestation, female fetuses of PCOS gestations had higher levels of T compared to female fetuses of mothers without PCOS (Palomba et al., 2012). Additionally, increased maternal T levels at midgestation have been shown to predict increased AMH levels in female offspring at adolescence, a clinical feature characteristic of both adolescents and adults with PCOS (Hart et al., 2010; Sir-Petermann et al., 2009). Increased AMH levels are also prevalent among infants born to mothers with PCOS (Crisosto et al., 2012; Sir-Petermann et al., 2012). While daughters born to women with PCOS have an increased risk of developing the syndrome as adults, the relevant literature has not yet found a relationship between midgestational androgen levels in female fetuses and the adulthood development of PCOS (Abbott & Bacha, 2013; Goodarzi, Dumesic, Chazenbalk, & Azziz, 2011). As with umbilical cord blood analyses, the timing of collection may account for the lack of association. In a prospective cohort study, investigators found no correlation between midgestational maternal blood concentration of androgens and the development of PCOS traits in female offspring during adolescence (Hickey et al., 2009). Gestational levels of T in male fetuses, however, are increased from about six to sixteen weeks of gestational age and subsequently decrease to a low level until birth (Rouiller-Fabre et al., 2009). Another study conducted by Fowler et al. (2011) reported that male fetal programming of
masculinization by androgen approximately occurs before eleven to thirteen weeks of gestation. As maternal blood collection occurred during the eighteenth week of gestation in the study by Hickey et al. (2009), fetal reprogramming and any potential abnormalities in hormonal levels may have already taken place and were no longer measurable (Padmanabhan & Veiga-Lopez, 2013).

Other investigators have proposed that excess androgen outside of fetal origin are unlikely to contribute to the development of PCOS in adulthood. During gestation, elevated levels of SHBG in maternal circulation and placental androgen metabolism effectively function to protect the fetal environment from excess exposure (Abbott, Dumesic, & Franks, 2002). However, this does not exclude endocrine disruptors, which may bypass or compromise maternal protection to the fetus. Of the previously discussed androgenic endocrine disruptors, TCC has been detected in umbilical cord plasma, and nicotine has been shown to cross the placental barrier and accumulate in amniotic fluid at a concentration 88% greater than in maternal plasma (Pycke et al., 2014; Wickstrom, 2007).
DISCUSSION

This review has provided a comprehensive overview of the current literature on the role of prenatal exposure to endocrine disruptors in the development of PCOS. While PCOS itself has been subject to widespread investigation, there is a paucity of research that seeks to ascertain its etiology as well as its developmental origins. Multiple gene-association studies have been conducted to characterize a potential genetic basis for PCOS; however, reported findings regarding genetic linkage have been mixed and no candidate gene has been characterized as a marker for the PCOS phenotype (Abbott et al., 2005). The heterogeneity in the presentation of the PCOS phenotype in sibling studies has provided evidence in support of the contribution from environmental factors (Diamanti-Kandarakis, Kandarakis, & Legro, 2006). Indeed, endocrine disruptors have had a role in the etiology of many chronic and complex diseases such as obesity, NIDDM, and cardiovascular disease (Diamanti-Kandarakis et al., 2009). The present literature review examined three classes of endocrine disruptors and their potential involvement in the pathogenesis of PCOS beginning in fetal life. BPA, an agent commonly found in many plastics and other consumer products has been shown to have estrogenic activity. Indeed, BPA levels appear to be higher in women with PCOS (Kandaraki et al., 2011; Toru Takeuchi et al., 2004). Additionally, prenatal exposure to BPA in animal models was shown to increase GnRH as well as LH secretion in a hormonal profile similar to that seen in PCOS cases. Serum BPA levels were also positively associated with levels of free T and the severity of insulin resistance.
The review of phthalates included investigations on the influence of DBP and DEHP. Regarding DBP, its serum levels have also been reported to be higher in women with PCOS. Prenatal exposure to DBP, in conjunction with DEHP and BPA, resulted in an increased incidence of polycystic ovaries in F1 and F3 offspring. In animal models, DBP levels were also positively associated with levels of E2, which may account for the increases in GnRH and LH secretion seen in PCOS cases. Prenatal exposure to DEHP, a well-established reproductive and developmental toxicant, has been shown to decrease E2 levels, thereby inhibiting ovulation in animal models due to the lack of positive feedback on GnRH secretion. The findings reported from animal models remain controversial as human studies appear to be contradictory and show a decreased incidence of PCOS among daughters with phthalate exposure (Hart et al., 2014).

While many studies have documented the effect of fetal exposure to excess androgen in creating a PCOS phenotype, the available research on specific endocrine disruptors with androgenic activity is severely limited. The present literature review discussed two potentially applicable endocrine disruptors: nicotine and TCC. Prenatal exposure to excess androgens in animal models has resulted in phenotypes that include abnormal ovarian function, delayed menarche, anovulatory cycling, fertility impairment, enlarged/polycystic ovaries, and visceral fat distribution. In a mechanism that remains to be characterized, it appears that excess androgens have a role in decreasing the sensitivity of GnRH feedback inhibition, leading to its unregulated, increased secretion and the associated increase in LH secretion. The proposed pathophysiological mechanisms in the setting of increased androgen exposure during fetal life imply that they are driven in a
self-perpetuating cycle. However, research involving human fetal exposure to excess androgens has yielded inconsistent results. A significant portion of this outcome may be due to limitations in data collection, as exposing a fetus to excess androgen is unethical and collecting biological samples of the fetal environment throughout the gestational period is unsafe. Therefore, investigators have had to rely on more indirect sources of exposure assessment.

In regard to future research, other endocrine disruptors may also have a role in PCOS development through fetal exposure. One example is organochlorine (OC), an agent commonly used in pesticides. Pesticides that were OC-based were used worldwide until they were banned in the 1970s. However, due to the relatively prolonged duration of enzymatic degradation and their hydrophobic properties, OCs have been detected in living organisms and have been shown to bioaccumulate over a span of years. As endocrine disruptors, OCs have been shown to exert estrogenic activity. Interestingly, adipose tissue concentrations of OCs have been found to be greater among women with breast cancer compared to women without breast cancer. Their role in the development of a PCOS phenotype is currently unknown. It is possible that the role of OCs in PCOS development could be similar to the actions of other estrogenic endocrine disruptors such as BPA.

Additionally, as this area of research is still in its infancy, further investigations are necessary including studies to resolve currently discrepant findings, explore other pathways of biological influence, clarify mechanisms of action, and determine more effective methods of exposure and outcome assessment. Regarding the selection of
animal models, investigators may benefit from assessing the relative level of precociousness among potential animal subjects. Mammals that have completed sexual differentiation and have well-developed neural systems at birth (such as marmosets, rhesus monkeys, sheep, and guinea pigs) usually do not exhibit ovulatory dysfunction when they are exposed to androgen excess unless it occurs before birth (Wallen & Baum, 2002). With altricial animal models such as rats, a larger proportion of overall brain development takes place during fetal development compared to precocial animals (Wallen & Baum, 2002). Use of altricial animals to study the fetal origins of PCOS may therefore be a less accurate model of pathophysiological development in response to fetal exposure, as the rate of maturation and rapid brain growth are relatively higher compared to precocious models (Wallen & Baum, 2002).

In order to execute indirect measurements of exposure that are more accurate, future human studies should better characterize the critical periods in fetal development when fetal reprogramming is hypothesized to occur. Longitudinal cohort studies could show promise in efforts to analyze associations between fetal exposure to particular endocrine disruptors and the incidence of PCOS.

**Conclusion**

In consideration of the available literature, endocrine disruptors appear to have an etiological role in the development of PCOS at the level of fetal exposure. However, more research is required in order to gain a better understanding of how PCOS originates and the mechanisms of action that endocrine disruptors exert that potentially result in
hormonal dysregulation. Because they are ubiquitous in the modern living environment, endocrine disruptors could potentially pose one of the largest hazards to maternal and fetal health. Therefore, as PCOS is of the most common endocrine disorders in women, it is of great importance to better characterize this condition’s origins in an effort to introduce effective methods of prevention for the generations of tomorrow.
## LIST OF JOURNAL ABBREVIATIONS

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University of Michigan, Ann Arbor, MI
Bachelor of Science, Neuroscience, May 2009
• Senior Thesis: “Sex Differences in Compensatory Response to a 6-Hour Sleep Deprivation in a Diurnal Rodent, Octodon Degu.”

WORK EXPERIENCE

Division of Sleep Medicine, Brigham and Women’s Hospital
Boston, MA • Dec 2010–Aug 2013
Technical Research Assistant

Michael S. Aldrich Sleep Disorders Center, University of Michigan
Ann Arbor, MI • Aug 2009– Dec 2010
Clinical Research Associate/Sleep Technician

Chronobiology & Neuroendocrinology Laboratory, University of Michigan
Ann Arbor, MI • Sept–Apr 2010
Research Assistant

Learning Resource Center, University of Michigan Medical School
Ann Arbor, MI • August 2006 – May 2009
Computer Operator
RESEARCH EXPERIENCE

Department of Obstetrics and Gynecology, Boston Medical Center
Boston, MA • July 2014–Present
Research Assistant, Advisor: Shruthi Mahalingaiah, MD

Sleep and Chronobiology Research Laboratory, Brown University
Providence, RI • May–Aug 2009
Behavioral Science Research Apprentice/Sleep Technician

Chronobiology & Neuroendocrinology Laboratory, University of Michigan
Ann Arbor, MI • Aug 2008–May 2009
Senior Thesis Research, Advisor: Theresa Lee, PhD

Chronobiology & Neuroendocrinology Laboratory, University of Michigan
Ann Arbor, MI • Jun 2007–Aug 2008
Undergraduate Research Assistant, Advisor: Theresa Lee PhD

Cardioprotection Research Laboratory, University of Michigan
Ann Arbor, MI • Sept 2006–Apr 2007
Undergraduate Research Opportunity Program (UROP) Student

MEDICALLY RELEVANT EXPERIENCES

Korle-Bu Teaching Hospital
Accra, Ghana, West Africa • Mar–Apr 2013
Hospital Volunteer

Beth Israel Deaconess Medical Center
Boston, MA • Jan–Aug 2013
Physician Shadowing

VOLUNTEER EXPERIENCES

Read to A Child – Boston
Boston, MA • Mar 2011–Jun 2013
Reading and Mentoring Program Volunteer

Washtenaw County American Red Cross Club
Ann Arbor, MI • Sep 2006–Apr 2009
American Red Cross Club Member and Committee Chair

HONORS, AWARDS, SCHOLARSHIPS & FELLOWSHIPS
William C. Dement Behavioral Sciences Research Fellowship Program  
May 2009–Aug 2009

Neuroscience Honors Senior Thesis Program, University of Michigan  
Jan 2009–May 2009

Student Recognition Award, University of Michigan  
May 2005–May 2009  
*Full-tuition scholarship recipient*

**SKILLS**

**Administrative**
- Experience with reviewing and developing case report forms (CRFs), serious adverse event (SAE) forms, participant contact, and taking inventory of study supplies.
- Proficient in assembling research grants and proposals.

**Laboratory**
- Small animal handling experience with rodents including injections, blood collection, anesthesia, and cranial surgery techniques.
- Trained polysomnography technician
- Knowledgeable with using SAS and SPSS.
- Proficient with multiple types of sleep diagnostic software including ActiView, ICELUS, Pursuit Sleep, EncorePro 2. Complete training in Compumedics software.

**HOBBIES**
- Rock climbing, running, tennis, sailing, and figure skating.