Published in final edited form as:

Reprod Toxicol. 2007 ; 23(3): 337–352.

Developmental Exposure to Environmental Endocrine Disruptors: Consequences within the Ovary and on Female Reproductive Function

Mehmet Uzunmcu¹ and Rob Zachow^{1,2}

1 Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901

2 Department of Physiology and Biophysics, Robert Wood Johnson Medical School-UMDNJ, Piscataway, NJ 08854

Abstract

Female reproductive function depends upon the exquisite control of ovarian steroidogenesis that enables folliculogenesis, ovulation, and pregnancy. These mechanisms are set during fetal and/or neonatal development and undergo phases of differentiation throughout pre- and post-pubescent life. Ovarian development and function are collectively regulated by a host of endogenous growth factors, cytokines, gonadotropins, and steroid hormones as well as exogenous factors such as nutrients and environmental agents. Endocrine disruptors represent one class of environmental agent that can impact female fertility by altering ovarian development and function, purportedly through estrogenic, anti-estrogenic, and/or anti-androgenic effects. This review discusses ovarian development and function and how these processes are affected by some of the known estrogenic and anti-androgenic endocrine disruptors. Recent information suggests not only that exposure to endocrine disruptors during the developmental period causes reproductive abnormalities in adult life but also that these abnormalities are transgenerational. This latter finding adds another level of importance for identifying and understanding the mechanisms of action of these agents.

1- Introduction

The primary function of an adult ovary is to produce the steroid and protein hormones needed to support (1) the development and maturation of ovarian follicles (i.e., folliculogenesis) including oocytes, (2) ovulation, and (3) the initiation and maintenance of pregnancy in mammals. In order for this to collectively occur, the secretion of ovarian hormones is tightly regulated by the neuroendocrine system and is locally controlled by many intraovarian feedback mechanisms (reviewed in [1]).

Two major developmental events within the ovary are follicular assembly (i.e., the formation of primordial follicles) and the primordial-to-primary follicle transition (the “initial” recruitment) [1]. The initial phase of folliculogenesis is regulated by paracrine growth factors

Correspondence: Dr. Mehmet Uzunmcu, Department of Animal Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, 84 Lipman Drive, New Brunswick, NJ 08901-8525, E-mail: uzumcu@aesop.rutgers.edu, Fax: +1-732-932-6996, Telephone: +1-732-932-6912

Supported by NIH grant R21 ES013854-01A1 (MU) and funds from Robert Wood Johnson Medical School-UMDNJ (RZ).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and influenced by local concentrations of steroid hormones, with a more limited role of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). It is widely accepted that a pool of primordial follicles (i.e., the ovarian follicular reserve) is formed within the developing fetus or neonate, and once established, primordial follicles do not proliferate. Thus, it is the ovarian reserve of primordial follicles that comprises the source of female gametes for life.

The component of folliculogenesis known as follicular transition is one of the most intriguing processes in ovarian biology because little is known about its control mechanisms. This transition enables the growth of some primordial follicles, while others remain quiescent for months, years, or decades depending upon the species [2]. Improper activation of follicular transition is proposed to be the cause of certain reproductive disorders [3].

Recent data using mice suggest that a stem cell population for female germ cell replenishment may exist within the ovary and/or bone marrow [4,5]. Undoubtedly, these experimental findings represent new possibilities in female fertility, while challenging long-standing dogma by suggesting that a finite, exhaustible ovarian follicular reserve may not be the case across mammalian species. However, these studies have been received with skepticism [6,7] and thus need to be further studied by other laboratories and/or in other species, including humans, before being widely accepted. Regardless of whether or not female germ stem cells exist within the postnatal mammalian ovary, the mechanisms which control follicular assembly, and the subsequent primordial-to-primary follicle transition that are discussed in this review, are critical for setting reproductive viability (i.e., puberty) as well as determining the onset of reproductive senescence (i.e., menopause in the human).

Ovarian development, maturation, and ultimately adult reproductive function are subject to regulation by endocrine disruptors (e.g., plant sterols, pesticides, and industrial chemicals) in addition to a plethora of endogenous factors (e.g., hormones, growth factors, and metabolic state). Endocrine disruptors have been shown to affect the aforementioned by mimicking, and therefore disrupting, the normal steroid hormone-dependent control mechanisms within the ovary and neuroendocrine tissues. An example of one such endocrine disruptor is 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane (methoxychlor; MXC) [8]. Methoxychlor is weakly estrogenic [8], but its metabolite 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) exhibits more potent estrogenic, anti-estrogenic, and anti-androgenic activity [9]. This review will discuss the effects of MXC, HPTE, and several other environmental endocrine disruptors within the female reproductive system. The effects of these endocrine disruptors on the developmental stage and/or adult functions that are described in this review are summarized in Table 1.

2- Ovarian development and function

a- Embryonic gonad development

The mammalian ovary consists of two general cell populations: somatic cells and germ cells. The somatic component of the ovary is derived from the embryonic genital ridge. When considering the mouse, which is a common animal model for studying embryogenesis and adult reproductive function, germ cells differentiate from primordial germ cells that originate from extragonadal tissues at 7.0–7.5 day post coitum (dpc) [10]. Primordial germ cells migrate into the indifferent gonad at 9.5 dpc [11], where cells rapidly proliferate until entering meiosis I at 13.5 dpc [12].

One major difference when comparing male and female germ cells is that female germ cells begin meiosis during early development. By comparison, the entrance of male germ cells into meiosis is prevented once cells are enclosed within the testicular cords. Once enclosed, male germ cells continue to proliferate via mitosis until 14 dpc (as occurs in mice), but then remain

quiescent until after birth. The germ cells re-enter mitosis and continue spermatogenesis during the postnatal period [13].

Experimental evidence has suggested that meiotic division is an intrinsic feature of both male and female germ cells; however, meiosis is specifically prevented within the male gonad by unknown mechanisms. This is supported by the observation that in male mice, germ cells which have migrated to ectopic sites, such as the adrenal gland, begin meiosis on 13.5 dpc [14]. Therefore, it seems that local (intratesticular) factors prevent the entry of male germ cells into meiotic growth, while intraovarian mechanisms induce meiosis in female germ cells.

The onset of meiosis appears to be one of the major hallmark events within the female gonad that prevents its differentiation into a testis. One mechanism which guides this developmental course in the female is driven by germ cell meiosis and causes interference of mesonephric cell migration and cord formation, both of which are critical for testis differentiation [15]. It has been proposed that changes in gene expression within the male gonad initiate mechanisms which enable Sertoli cell differentiation and that collectively this and other intratesticular events prevent the start of an intrinsic meiotic clock [16].

Another consideration in the course of the gonadogenesis is the temporal regulation of seminiferous cord formation in the male gonad. As the seminiferous cords are forming in the male embryo, the forming ovary in the age-matched female embryo appears to be morphologically inactive. Although no gross morphologic changes are observed at this embryologic stage, major molecular events do occur within female gonad. For example, gene-profiling experiments suggest that a robust increase in gene expression occurs. It has been estimated that the expression of > 1200 genes is up-regulated in the female gonad between 10.5–13.5 dpc (the period defining gonadal sex differentiation in mice) [17]. Data show that the increased expression of cell cycle inhibitors (e.g., cyclin-dependent kinase inhibitors) and several meiosis marker genes occurs in the female gonad as compared to the male gonad [17, 18]. Through this elaborate pattern of gene expression, it has been proposed that somatic cell proliferation is inhibited while female germ cells enter meiosis in the ovary.

Yet another consideration is the sex-specific timing of germ cell DNA re-methylation. Methylation of DNA functions in parental imprinting [19]. The germ cell genome is normally methylated prior to migrating to the genital ridge [19]. After migration, the genome of primordial germ cells in both sexes is de-methylated during the same time period [20]. Afterwards re-methylation occurs at different times in males and females and in a sex-specific manner. While male germ cell re-methylation starts at 14 dpc and is completed by 16–17 dpc [21,22], female germ cell re-methylation takes place during the postnatal period, (PND 1–5), and continues throughout oocyte growth until the preantral follicle stage [23,24]. Re-methylation requires interaction of somatic and germ cells [20] and appears to be susceptible to alterations by endocrine disruptors [25]. All of these major differences between testicular and ovarian development need to be considered when examining any potential sex-specific mechanisms of action used by exogenous agents such as endocrine disruptors.

Upon entering meiosis, female germ cells are referred to as oocytes. Oocytes are arrested at the early diplotene phase of meiotic prophase I. These cells are initially grouped within large, interconnected clusters known as oocyte nests. Two key developmental processes occur subsequent to the meiotic arrest of the oocytes: (1) the assembly of primordial follicles (follicular assembly), and (2) the recruitment of primordial follicles into a growing cohort identified as primary follicles (primordial-to-primary follicle transition). Both of these processes define the earliest stages of folliculogenesis and are believed to affect the duration of the female reproductive life span [3]. These early events in folliculogenesis are mostly regulated by local growth factors and the predominant ovarian steroid hormones (i.e.,

progesterone, estrogens, and androgens). The pituitary gonadotropins (FSH and LH) are believed to, at best, exert limited control over the processes of follicular assembly and the primordial-to-primary follicle transition [26,27].

b- Follicular assembly

The oocyte nests are broken-down during primordial follicular assembly, apparently due to germ cell apoptosis [28,29]. Apoptosis starts as early as 13.5 dpc and peaks by postnatal day (PND) 2 or 3 in mice [29]. In addition, somatic cells can mediate nest breakdown and the subsequent formation of primordial follicles [28]. Following this thinning of the germ cell reserve, the oocyte clusters are separated by the appearance of pre-granulosa cells so that each primordial follicle is defined by the presence of an oocyte and morphologically discernable pre-granulosa cells (Fig. 1).

As described above, FSH is proposed to have only a limited role in regulating follicular assembly [26,27]. However, evidence does show that FSH exerts some degree of control over this process. For example, in the hamster, the administration of an FSH-neutralizing antibody in utero reduces the number of primordial follicles, and this effect can be reversed by gonadotropin replacement [26]. In FSH receptor (FSHR) knockout mice, fewer naked oocytes are present relative to wild-type control animals; moreover, FSHR knockout mice possess a greater number of primary and secondary follicles [27]. On the other hand, studies using gonadotropin-deficient mice, or gonadotropin receptor-deficient mice (reviewed in [30]), collectively indicate that follicular assembly occurs in the absence of gonadotropin support. In these mice, folliculogenesis proceeds up to the preantral follicle stage. Moreover, ovaries harvested from neonatal mice can undergo follicular assembly in vitro in the absence of gonadotropin stimulation [31]. Collectively then, FSH may exert some species-specific supportive function during the first stages of folliculogenesis; however, data suggest that these events can be gonadotropin-independent.

By way of oocyte-specific transcription factors, the oocyte appears to be the central organizer of ovarian development and function starting with follicular assembly (reviewed in [32]). This is supported by the observation that in the absence of oocytes, follicular assembly does not occur, although structures that resemble ovarian nests or cords do form [33]. One of the oocyte-expressed factors is a basic helix-loop-helix (bHLH) family member factor in germline- α (FIG α). The critical role of FIG α in follicular assembly has been shown since this process does not occur in FIG α -null female mice [34]. In addition, several growth factors and cytokines, including tumor necrosis factor- α (TNF α), neurotrophins (NT), inhibins, and growth differentiation factor-9 (GDF-9), have been shown to control follicular assembly [35–38]. Inhibin also has been implicated, since an increase in inhibin expression is associated with a reduction in the number of primordial follicles in estrogen-depleted fetal baboon ovaries [37]. Several reports have convincingly indicated that GDF-9 stimulates primordial follicle formation in the hamster ovary [38] while others have documented the role of GDF-9 in folliculogenesis only during recruitment and growth of very young preantral follicles in the mouse [39,40].

Steroid hormones can also guide ovarian development, as supported by the data collected in a number of studies. For example, in vivo and in vitro studies have shown that progesterone impairs follicular assembly in the rat [41]. This appears to involve the progesterone-dependent inhibition of apoptosis in oocytes, a process that has been linked with follicular assembly [41]. It has been speculated that progesterone may modulate levels of intraovarian TNF α , which is known to regulate germ cell apoptosis [3].

A slight inhibitory effect of estradiol-17 β (E $_2$) on follicular assembly was observed in the rat [41]. However, the effect of E $_2$ in mice appears to be more obvious. When cultured with E $_2$

(10^{-4} – 10^{-9} M) for 7 days, neonatal mice ovaries had a reduced oocyte nest breakdown, which resulted in a significantly lowered number of primordial follicles [42]. Studies using E_2 -deficient, aromatase knockout (ArKO) mice have demonstrated that the loss of endogenous E_2 results in reduced numbers of primordial and primary follicles [43]. The reduction in follicle number in ArKO mice cannot, however, be corrected by postnatal E_2 treatment, suggesting that lack of E_2 irreversibly affects the earliest phases of folliculogenesis. More examples for the role of estrogens in follicular assembly have been shown in the baboon. Estrogen-depleted fetal baboon ovaries possess an increased number of oocyte nests in conjunction with a reduction in the number of primordial follicles [37,44]. This effect can be blocked by estrogen replacement [37,44]. Similar to E_2 , estrogenic endocrine disruptors affect the process of follicular assembly (Table 1 and Section 3a).

c- Primordial-to-primary follicle transition

Once assembled, most of the primordial follicles remain quiescent (at an arrested state of development) prior to the initiation of reproductive cyclicity, but some transition into the growing follicle pool immediately. Follicles that initially remained quiescent are gradually recruited into a growing follicle pool throughout reproductive life. Follicular growth is critical for the female reproductive life span because once initiated, folliculogenesis ventures through one of two developmental pathways that serve to deplete the follicular reserve: ovulation or death by follicular atresia.

Following recruitment into a growing cohort of primary follicles (Fig. 1), oocyte diameter increases and granulosa cells undergo a morphogenesis, changing from squamous to a cuboidal shape. This early phase of folliculogenesis is accompanied by granulosa cell proliferation. Sometime during later preantral follicular growth, the follicle recruits an identifiable theca cell layer [2]; however, it has been proposed that in some species, a population of pre-theca cells may be present at the onset of follicle growth [45].

By comparison to the limited role of FSH, intraovarian growth factors appear to serve a more important function in the primordial-to-primary transition process. One example is kit ligand (KL) and its receptor, c-kit. The direct role of KL in mediating the primordial-to-primary follicle transition has been shown (reviewed in [3]).

Granulosa cells express KL, while oocytes and theca cells each express c-kit [46,47]. Within growing follicles, this cell-specific pattern of expression of KL and c-kit enables feedback between granulosa cells, theca cells, and oocytes. In response to stimulation by KL, oocytes produce factors such as basic fibroblast growth factor (bFGF), GDF-9, and bone morphogenetic factor-15 (BMP-15), whereas theca cells secrete keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF). In turn, KGF and HGF exert a number of regulatory effects within the growing follicles [3]. When collectively considered, it is plausible that the host of growth factors, which are secreted from the oocyte, granulosa cells, and theca cells, create a complex communication network that modulates folliculogenesis.

Another example of growth factor regulation is seen with GDF-9. Produced by oocytes, GDF-9 regulates granulosa cell proliferation and steroidogenic differentiation. Historically, a putative oocyte-derived growth factor that affected growth and steroidogenesis in granulosa cells and theca cells was evident based upon studies using oocyte coculture, oocyte-conditioned medium, and oocyctomized follicles (reviewed in [48]). This line of research culminated with the discovery of the regulatory effect of GDF-9 in promoting the initial stages of folliculogenesis [39]. Specifically, ovaries in GDF-9-deficient mice possess primordial and primary follicles; however, folliculogenesis does not proceed beyond the primary or early secondary stages of differentiation. [39]. Organ culture experiments have shown that GDF-9 stimulates the growth of primary follicles, but GDF-9 does not support the primordial-to-

primary follicle transition [40]. In contrast, the administration of GDF-9 to neonatal rats increases the number of primary and preantral follicles and decreases the complement of primordial follicles, collectively suggesting that GDF-9 promotes initial recruitment [48].

In addition to KL and GDF-9, other growth factors are potentially involved in the transition process, and these have been reviewed elsewhere [3]. Examples include the neurotrophins, bFGF, leukemia inhibitory factor (LIF), and BMP-4. Neurotrophins and their receptors [36, 49]; bFGF and its receptor FGFR2IIIb [50,51]; LIF and its receptors LIFR-beta and gp130 [52,53]; and BMP-4 and its receptors BMPR-IA, -IB, and -II [54] are all expressed in the ovary. The roles of bFGF [50], LIF [53], and BMP-4 [55] as regulators of various aspects of folliculogenesis beyond follicular assembly (i.e., the primordial-to-primary follicle transition) have been shown in vitro using neonatal rat ovary organ culture.

In contrast to the growth factors mentioned above, anti-Mullerian hormone (AMH; also referred to as Mullerian-inhibiting substance, MIS) appears to have an inhibitory effect on early follicle development. In AMH-null mice, primordial follicle recruitment is increased, and therefore, the primordial follicle reserve is prematurely depleted. This suggests an inhibitory role of AMH in the primordial-to-primary follicle transition [56]. Moreover, when treated with AMH, cultured neonatal mice ovaries contain more primordial follicles but fewer growing follicles, thus indicating that AMH inhibits the initiation of follicular growth [57]. Anti-Mullerian hormone is not expressed in primordial follicles but is expressed in primary follicles, and AMH remains detectable through the early antral stage. However, AMH expression is reduced in large antral follicles [58,59]. It can thus be proposed that larger (antral) follicles produce AMH in order to down-modulate the number of follicles that are recruited during each cycle [58].

In addition to the purported function of numerous growth factors, steroid hormones also modulate folliculogenesis. In a most general sense, ovarian steroidogenesis functions to guide follicle growth and maturation via direct intraovarian actions as well as positive and negative feedback to the hypothalamic-pituitary axis [60]. However, the aberrant production of ovarian steroid hormones (progesterone, E₂, and androgens) can disrupt normal folliculogenesis. It follows that environmental agents and pathogens that mimic the actions of ovarian steroids via the activation of steroid hormone receptors, could disrupt follicle development and/or ovulation. The role of progesterone in regulating ovulation and corpus luteum function is well-established. Experiments have also suggested that progesterone mediates early follicle development. For example, using a rat neonatal organ culture system, it was shown that progesterone reduces the number of growing follicles, with a concomitant increase in the complement of primordial follicles. This suggests that progesterone inhibits both follicular assembly and the primordial-to-primary follicle transition in rats [41].

The predominant bioactive estrogen, E₂, provides numerous levels of regulation within the ovarian-hypothalamic-pituitary axis, which enable the timely secretion of FSH and LH during each reproductive cycle. This endocrine feedback loop works in concert with the intraovarian effects of E₂, which under normal conditions collectively promote follicle growth and differentiation [60].

Estrogen bioactivity is mediated via the activation of estrogen receptors (ER), and two follicular ER isoforms, ER α and ER β , have been localized [61]. Experimental evidence indicates that ER β , but not ER α , mediates the estrogenic control of folliculogenesis. Data also show that ER β can facilitate mechanisms that promote follicle maturation from the early antral to the preovulatory stages [62]. This is of interest because certain endocrine disruptors (discussed in Section 3a) can function as ER agonists and/or antagonists, depending on the specific ER isoform targeted [9].

During embryonic ovarian development, E₂ is likely to influence the primordial-to-primary follicle transition. One example of such regulation has been shown by the inhibition of follicular growth that occurs in newborn female rats in response to an estradiol benzoate (EB; a longer acting form of E₂) challenge [63]. It is interesting that the neonatal exposure to EB, while impairing follicular development, concomitantly results in an elevation of AMH expression in the ovary [64].

The effects of E₂ have been further elaborated by incubating neonatal ovaries without exogenous E₂. In these E₂-depleted cultures, the rate of the primordial-to-primary follicle transition is three times greater when compared to the normal *in vivo* rate. However, when E₂ is included in the ovary cultures, the rate of transition is dramatically reduced, again suggesting that E₂ down-modulates the primordial-to-primary follicle transition [41]. Interestingly, E₂ does not block the primordial-to-primary follicle transition in ovaries collected at a later postnatal stage, thereby indicating that the E₂-dependent effects are restricted to the initial wave of the primordial-to-primary follicle transition [41].

Although E₂ can regulate follicle development, E₂ is not required for oocyte maturation, as was demonstrated using ArKO mice [65]. In one study, ArKO mice were hormonally primed for the purpose of superovulation. Even though these mice did not ovulate, the oocytes that were harvested from these animals underwent normal maturation in the absence of E₂ *in vitro* [65].

The effects of androgens have been demonstrated in the rhesus monkey, in which exogenous testosterone or dihydrotestosterone (DHT) induced an increase in the number of primary follicles in a time-dependent manner [66]. On the other hand, androgens can impede the later stages of follicle development; this effect has been correlated with the induction of follicle atresia [67] and an accumulation of primary and secondary follicles [68].

Interestingly, androgens may not be directly required for female fertility since Tfm mice, in which the androgen receptor (AR) is dysfunctional, remain fertile, albeit with reduced fecundity when compared to wild-type mice [69]. Similarly, AR null mice reach reproductive senescence earlier than control animals [70]. Once again, a correlation can be drawn between certain endocrine disruptors and the steroid hormone-dependent aspects of folliculogenesis. By way of example, both vinclozolin and HPTE (discussed in Section 3b) disrupt ovarian function and have been shown to exert anti-androgenic effects [9,71]. Therefore, it is compelling to propose that these compounds may target the ovarian AR, and in so doing impair the normal androgen-orchestrated events that control folliculogenesis and ovulation.

d- Preantral follicle development

Experiments using juvenile rats have clearly shown the role of gonadotropins during the phases of preantral follicle growth [23]. In addition, there is strong correlation between the relatively rapid growth that occurs during the first wave of follicular development and rising serum FSH levels between PND 10 and PND 20 in rats [72]. The LH and FSH receptors are initially expressed in the rat ovary at PND 7 (when the secondary follicles first appear [73]), once again suggesting that, although gonadotropins stimulate growth and differentiation of preantral follicles, follicles can develop up to the antral stage in the absence of gonadotropins [30]. However, in the advanced secondary stages and beyond, FSH and LH are required for the maturation of preantral follicles into the more mature phases of development.

In response to the waning and waxing levels of FSH and LH, the cohorts of younger follicles undergo further growth and differentiation. Growth probably stops during the mid to late luteal phases but resumes again during the next follicular phase. This stop-and-go pattern of follicular

growth and maturation of a given follicular cohort continues until the dominant follicle is ovulated and the non-selected sister follicles undergo atresia [1].

e- Follicular selection

It is believed that sometime around the tertiary/antral stage of development, one follicle (for monoovulators) or several follicles (for polyovulators) within the recruited cohort is/are selected to complete the full course of folliculogenesis to ovulation and luteogenesis. The biochemical and molecular mechanisms underlying the process of follicular selection remain an enigma. What is known is mostly based on the observations in monoovulatory species including cattle, human, and non-human primates [1]. Within large antral follicles, granulosa cells contain a greater complement of FSHR, and also begin to produce inhibin. Inhibin blocks the secretion of FSH and LH by the pituitary, and gonadotropin levels are further suppressed through inhibin originating within the selected follicle. Theories suggest that the selected follicle remains FSH-responsive [74], while the remaining (non-selected) sister follicles within the growing cohort are *FSH-starved* due to a relatively low number of available FSHR [75]. In effect, this condition of FSH withdrawal favors the onset of apoptosis in granulosa cells, ultimately leading to atresia of non-selected follicles.

Basic mechanisms for follicular selection in polyovulatory species (e.g., rodents) are similar to those occurring in monoovulatory species. However, it is proposed that one reason why numerous follicles are selected and undergo ovulation in polyovulators is due to a lower level of negative regulators (e.g., inhibin) secreted in these species [1].

As the selected follicle grows, its antrum is engorged with follicular fluid, and both theca and granulosa cells proliferate. Within granulosa cells, FSH-orchestrated up-regulation of adenosine 3', 5'-cyclic monophosphate (cAMP)-dependent signal transduction is a major mechanism that supports the expression of steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage (CYP11A), 3 β -hydroxysteroid dehydrogenase type 1 (3 β -HSD), and P450 aromatase (CYP19) (Fig. 2). This signal transduction pathway promotes the step-wise conversion of cholesterol into progesterone, as well as the aromatization of androstenedione and testosterone into estrogens, most notably E₂ [76]. Importantly, the orderly production of aromatizable androgens (within theca cells), E₂, and progesterone is required for the maturation of healthy preovulatory follicles, ovulation, and luteal development. The levels of StAR and the aforementioned steroid biosynthetic enzymes are tightly regulated not only by cAMP-dependent signaling but also by interactions between intra- and extraovarian hormones, growth factors, and cytokines [3,77].

Theca cells represent the other steroidogenic cell population in follicles (Fig. 3). The appearance of morphologically distinct theca cells appears to require LH and occurs concomitant with follicular vascularization. However, granulosa cells enhance the growth and differentiation of theca cells before LH receptor expression; these effects are thus likely to be mediated by granulosa cell-derived growth factors [78].

Theca cells respond to LH by producing androgens (androstenedione and testosterone) [79]. Theca-derived androgens diffuse into granulosa cells where androgens are used as substrates for E₂ production. Cooperation between theca cells and granulosa cells substantially increases E₂ production in the more mature (antral) follicles.

It is during the advanced phase of antral follicle growth when a developmental switch occurs. To describe this process in brief, E₂ production by the selected follicle is markedly elevated, and the elevated serum E₂ exerts a positive feedback effect on gonadotropin secretion. The selected follicle also secretes activin, and activin further stimulates the secretion of FSH. The rise in FSH and LH during the late follicular phase of the cycle cannot rescue the dying (atretic)

non-selected follicles but does support the following: (1) further growth and differentiation of previously recruited (younger) follicles, (2) a robust increase in steroidogenesis by the selected follicle, and (3) initial luteinization of the selected follicle. The feedback dynamics between the selected follicle and the hypothalamic-pituitary axis continue and culminate with the preovulatory gonadotropin surge that stimulates ovulation.

f- Ovulation and luteal phase

Ovulation is dependent upon the gonadotropin surge, most notably a profound increase in LH. The pituitary gonadotropins stimulate the terminal differentiation of granulosa cells within the preovulatory follicle whereby granulosa cells switch from almost the exclusive production of E_2 to the production of both E_2 and progesterone (i.e., luteinization).

Following ovulation, the remnants of what was the selected preovulatory follicle (granulosa cells and theca cells) is stimulated by LH to terminally differentiate into the corpus luteum. The corpus luteum is essential for enabling the initial stages of pregnancy (reviewed in [80]).

In a broader context, decades of research using in vivo and in vitro models have shown that folliculogenesis is regulated by numerous intraovarian growth factors and steroid hormones working in concert with the pituitary gonadotropins. These exquisitely regulated intra- and extraovarian feedback pathways can be disrupted at multiple levels by exogenous agents, such as environmental endocrine disruptors, which mimic endogenous hormone-mediated mechanisms (Table 1 and Section 3).

3- Effects of estrogenic and anti-androgenic endocrine disruptors on ovarian development and function and female fertility

As is clear from the above discussion, ovarian development, folliculogenesis, and steroidogenesis are regulated by local paracrine factors, steroid hormones, and gonadotropins. There are synthetic and natural environmental compounds that mimic and/or antagonize the mechanisms of action of endogenous hormones. Examples of the endocrine disruptors are pesticides (e.g., dichlorodiphenyltrichloroethane, DDT; MXC; vinclozolin; and atrazine), detergents and surfactants (e.g., octyphenol, nonylphenol, and bisphenol-A), plastics (e.g., phthalates), industrial compounds (e.g., polychlorinated biphenyl, PCB; and 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD), and natural plant estrogens (e.g., genistein and coumesterol). By binding to steroid hormone receptors, these compounds have the potential to exert estrogenic, anti-estrogenic, and/or anti-androgenic effects. Based upon the evidence from studies involving long-term exposure to the endocrine disruptors, it is clear that adult ovarian function, and thus female fertility, are affected by these endocrine disruptors [81].

Attention has also been directed to the possibility that environmental influences during neonatal and fetal development may lead to adult-onset pathology. This hypothesis was put forth by Barker and co-workers based on the observation that babies with low birth weight exhibited the highest mortality rate from coronary heart disease during adulthood [82]. To build upon this idea, studies have shown that transient exposure to endocrine disruptors during development leads to abnormal function in adult life [83–85]. These delayed effects have been attributed in part to changes in the pattern of DNA methylation that occurs in certain genes [25,84], perhaps leading to the persistent expression of hormone-responsive genes [84]. Of additional concern is the finding that the effects that were observed in adults [25,86], including altered states of DNA methylation [25], were transmitted to the next generation, apparently via both paternal [25] and maternal germ lines [86,87]. In mice, transgenerational effects of the endocrine disruptor DES through the maternal germ line were shown in male and female offspring [86,87]. Although the molecular mechanisms of these effects were not investigated,

epigenetic mechanisms were suggested as one of the potential modes of transmission [86,87]. A more recent report showed that exposure to MXC or vinclozolin during testis differentiation in the rat (8–15 dpc) increased germ cell apoptosis and reduced both spermatogenesis and fertility in F1 generation males [25]. These effects were associated with changes in DNA methylation of approximately 25 genes. Both male infertility and the altered DNA methylation pattern were transmitted up to four generations via the male germ line [25]. It is suggested that the sex-specific epigenetic programming of male germ cells is permanently altered by the somatic cell-germ cell interactions because of the actions of endocrine disruptors, working through steroid hormone receptors, within somatic cells [25].

a- Estrogenic endocrine disruptors

i. Methoxychlor and HPTE—Methoxychlor is an organochlorine pesticide, which is currently used as a replacement for DDT. Methoxychlor is an estrogenic compound that demonstrates low-affinity binding for the ER [8]. As described above, the major MXC metabolite HPTE can function as an estrogenic, anti-estrogenic, or anti-androgenic compound [9].

In vivo studies have shown that in rats, chronic exposure to MXC (25–200 mg/kg/d) accelerated vaginal opening and the onset of the first estrus and impaired fertility [88]. At highest dose tested (200 mg MXC/kg/d), females showed continuous estrus, and although they mated, these females had no pregnancies. In the same study, it was also discovered that the female offspring (F1) of dams that were exposed to MXC (50 mg/kg/d, through placenta and milk) underwent accelerated vaginal opening, had irregular cycles with reduced fecundity, and experienced early reproductive senescence when compared to unexposed control rats [88]. In another study, pregnant rats were exposed to MXC (5–150 mg/kg/d) for 1 week preceding and 1 week after the delivery. Then the pups were directly exposed to MXC doses until PND 42. All doses used accelerated vaginal opening; the highest doses (50 and 150 mg/kg/d) disrupted the estrous cycle, reduced pregnancy rate, and lowered the number of live pups delivered [89]. Other research has shown that exposure to MXC (0.05–1.0 mg/d) during PND 0–14 leads to irregular estrous cycles and causes the formation of either follicular cysts or ovarian atrophy in adult mice [90]. More recently it has been reported in mice that exposure to a relatively low dose of MXC (32 mg/kg) for 20 days reduces antral follicle number by inducing atresia. Some of these effects were attributed to actions within the hypothalamic-pituitary axis since gonadotropin secretion was impaired [89,90]. However, other studies have disputed any effect of MXC on gonadotropin production [91]. In addition, it has been shown that MXC induces atresia within antral follicles in vitro, and this action appears to be mediated via several mechanisms including the mobilization of Bcl-2- and Bax-mediated signal transduction [91,92] and oxidative stress [93,94] and ER-mediated pathways [95].

Only a paucity of data is available describing the effects of HPTE on ovarian function and follicle growth. In one study, Symonds et al. [96] reported that cultured mouse ovarian surface epithelial (OSE) cells underwent increased proliferation when stimulated with HPTE (3 μ M). Moreover, this stimulatory effect occurred concomitantly with (1) an up-regulation in the expression in several cell cycle genes, and (2) the decreased expression in pro-apoptotic signaling molecules. These responses to HPTE were blocked by the ER antagonist ICI 182,780 in OSE cells. In another experiment, when cultured mouse antral follicles were challenged with HPTE (0.01–10 μ g/ml), follicle growth was impaired while the incidence of follicle atresia was increased [95]. These data indicate that HPTE can, in a cell-specific manner, promote or impair ovarian cell survival and cell proliferation through ER-mediated changes in gene expression.

In addition to studies demonstrating direct actions of MXC and HPTE on intact antral follicles and/or OSE cells [92,97], data from several experiments have suggested that the granulosa cells can be directly affected by both MXC and HPTE. For example, MXC (10 μ M) inhibited

basal, FSH-, and E₂-stimulated progesterone secretion while having no effect on cAMP levels in porcine granulosa cells [98]. Interestingly, MXC (100 and 1000 ng) stimulated basal and cholera toxin-induced CYP11A, a mechanism that in and of itself would be predictive of increased progesterone production [99].

Our laboratory has described inhibitory effects of HPTE on steroidogenesis in immature rat granulosa cells. Data show that HPTE (1 and 10 μ M) inhibits both FSH- and cAMP-stimulated progesterone accumulation [100]. In contrast, HPTE suppressed FSH- but not cAMP-induced E₂ secretion. In HPTE-challenged granulosa cells, FSH-induced CYP11A, 3 β -HSD, and CYP19 expression were collectively blocked by HPTE, with no observed effect of StAR mRNA. Although cAMP-stimulated CYP19 expression was moderately reduced by HPTE, the levels of cAMP-dependent StAR, CYP11A, and 3 β -HSD mRNAs were increased by HPTE. Therefore, it appears that HPTE can impair progesterone and E₂ production in granulosa cells, perhaps by targeting one or more loci within the cAMP-directed cascade that lead to changes in the expression of steroid pathway enzymes [100]. In addition, MXC (50–500 mg/kg/d) stimulated AMH production in the rat ovary, thus manipulating one of the main inhibitory paracrine mechanisms [59]. Furthermore, in vitro experiments using granulosa cells from DES- or PMSG-primed immature rats showed that HPTE (1–5 μ M) stimulates AMH production, suggesting that MXC acts directly on the ovary [59].

ii. Genistein—Genistein is a flavanoid phytoestrogen with proven estrogenic activity in several mammalian species, including humans and pigs [101,102]. When compared to E₂, genistein exhibits substantially less affinity for the ER [103].

Several reports have shown the estrogenic action of genistein in female reproductive tissues in vivo and in vitro. For example, when administered to mice between 15–19 dpc, genistein induced accelerated vaginal opening and irregular estrous cycles [104]. When given to PND 1–5 rats, genistein caused a dose-dependent disruption in the normal estrous cycle pattern, with infertility resulting from the highest dose tested (100 mg/kg/d) [105]. In mice, genistein induced similar effects as were observed in the rat, as well as a dose-dependent reduction in litter size [106].

One of the target organs for genistein appears to be the uterus. This is evident in that embryos implant in the genistein-exposed (50 mg/kg/d) pregnant mouse; however, pregnancies abort prior to term [106]. Of interest is the observation that genistein-treated mice can be induced to super-ovulate, suggesting that ovarian function is normal [106]. Although the number of ovulated oocytes are similar between control and genistein-exposed animals [106], fertilizability and developmental capacity of the fertilized oocytes to the blastocyst stage was adversely affected [107]. In this context, genistein-treated (50 mg/kg/d) mice exhibit a multi-oocyte follicle (MOF) phenotype [105,108]. This has been correlated with a reduction in the number of apoptotic oocytes [108], a process that is required for normal follicular assembly [29].

In pig granulosa cells in vitro, genistein (0.5–50 μ M) inhibited basal and FSH-stimulated progesterone production, but did not alter E₂ accumulation [109]. In isolated rat ovarian follicles, genistein (0.01–1 μ M) decreased cAMP content and blocked testosterone production without lowering E₂ synthesis [110]. The decrease in testosterone, and concomitant unaltered level of E₂, were attributed to the genistein-dependent stimulation of CYP19 activity [110]. In cultured human granulosa cells, genistein inhibited the activities of 3 β -HSD and 17 β -HSD [111]. Finally, genistein (100 μ M) has been shown to block LH-induced ovulation in perfused rat ovaries in vitro due to inhibition of tyrosine kinase-mediated collagenase-3 [112]. In fact, prior to the more current focus on genistein as an environmental endocrine disruptor, this compound was commonly administered as an inhibitor for studying the role of protein tyrosine

kinases in mediating growth factor-dependent steroidogenesis and apoptosis in granulosa cells in vitro [113,114].

iii. Bisphenol A—Bisphenol A (BPA) is monomer that is used to produce polycarbonate plastic and epoxy resins (reviewed in [115]). It is used in numerous manufactured products with a total worldwide production exceeding 3 million tons/yr. Since BPA has been shown to leach from containers into food and beverage products, and from dental resins into saliva, this compound should be considered a potential health risk. Many of studies using various assay systems have clearly shown that BPA has estrogenic properties [115].

Bisphenol A can pass through the placenta and does not bind to α -fetoprotein, so that it can be available to tissues including central nervous system during development [115]. Developmental exposure to BPA is associated with numerous abnormalities in the female including early vaginal opening and first estrus, irregular estrous cycles, MOF, and an increased incidence of ovarian hemorrhagic bursae. These abnormalities correlate with reduced fertility, the onset of reproductive senescence, and the development of pathology later in life [115]. Studies in the mouse have shown that perinatal exposure to environmentally relevant doses of BPA (0.01–100 μ M) leads to defects in brain differentiation [116]. Therefore, it has been proposed that the ovarian dysfunctions associated with developmental exposure to BPA can be attributed, at least in part, to its targeting of the neuroendocrine system.

Evidence of the estrogenic activity of BPA has been demonstrated in ArKO mice. These mice normally exhibit follicular depletion along with uterine and bone maladies resultant from the loss of CYP19 activity. In ArKO mice that were given BPA (0.1 or 1.0% w/w in chow), the ovarian expression of insulin-like growth factor-I (IGF-I), IGF-I receptor, GDF-9, and BMP-15 were collectively increased to normal levels, an effect resembling that achieved in ArKO mice with E₂ replacement [117]. These authors further reported that BPA exerted “little effect” within ovarian and other E₂-dependent tissues of wild-type mice [117].

Although limited information exists, the direct ovarian effect of BPA has also been shown. For example, BPA (10^{-8} – 10^{-5} M) stimulates basal and FSH-induced progesterone production while inhibiting FSH-stimulated E₂ secretion in pig granulosa cells in vitro [118]. The mechanism of action for BPA in granulosa cells has not, however, been reported. In contrast, in mouse granulosa cells, environmentally relevant doses of BPA (100 pM) induced a time- and dose-dependent increase in apoptosis; this effect was coupled with modulation of Bax and Bcl-2 signal transduction [119]. The data obtained in cultured mouse granulosa cells would seem to bring into question what was observed in the study by Toda et al., where no significant morphogenic action of BPA was detected [117]. Perhaps most important to consider is the indication that differences in experimental design and species specificity are important to assess when drawing conclusions regarding the effects of environmental endocrine disruptors.

Another effect of BPA is exerted at the level of oocyte maturation. Bisphenol A, released from damaged animal cages and water bottles that were inadvertently treated with harsh alkaline detergent, induced meiotic disturbances due to congression failure (defect of alignment of chromosomes during the first meiotic division) leading to meiotic aneuploidy [120]. This effect was mimicked when cages were intentionally damaged, or when animals were exposed to a similar dose of BPA (20 ng/g body weight). However, it is not clear whether these effects are mediated by somatic cells or by direct actions within the oocyte.

iv. Diethylstilbestrol (DES)—Diethylstilbestrol is one of the unique endocrine disruptors. DES is a synthetic estrogen with a stronger bioactivity than E₂. Remarkably, DES was widely prescribed to women (from late 1940s and through 1970s) with high-risk pregnancies in an attempt to sustain pregnancy. When prescribed to pregnant mothers, DES adversely affected

development of both female and male offspring, leading to abnormalities within the reproductive, cardiovascular, neuroendocrine, and immune systems. At doses that were prescribed to women, DES has carcinogenic effects; and at lower doses, DES has been used as a model endocrine disruptor for the study of estrogenic environmental endocrine disruptors (reviewed in [121]).

When considering the female reproductive system of DES-exposed individuals, abnormalities were primarily manifested within the reproductive tract (vagina, cervix, uterus, and oviduct). To a limited extent these abnormalities present in the form of malignant tumors, whereas, to larger extent reproductive tract malformations, reproductive dysfunction, and poor pregnancy outcomes have been documented (reviewed in [121,122]).

Mice have been successfully used as an animal model to understand the molecular, cellular, and physiological aspects of the reproductive problems in DES-exposed humans. Mice that were injected (s.c.) with 1µg/kg DES on 15 dpc had accelerated follicular development as observed at PND 7 [123]. Moreover, these animals did not possess corpora lutea but had follicles at various stages of development, as well as atretic follicles, at 7 months of age [124]. Ovarian interstitial abnormalities such as enlargement, vacuolation, excessive lipid droplet accumulation, and clumped pigmented materials within the cells were observed in the DES-treated mice at 7 months of age [124]. Similar interstitial abnormalities, such as an increase in the size of the interstitial compartment, vacuolation, tubular structures, and an increase in the lipid droplets were reported during adulthood when the mice were injected with 100µg/kg/day DES between 9 and 16 dpc. This treatment also resulted in an increase in testosterone production [125].

Injection with 1µg/day DES between 15 and 18 dpc, or PND 1–5, resulted in excessive MOF, also known as polyovular follicles (PF), in mice at the age of 10–34 days [126,127]. Further experiments have shown that PF can be observed when in vitro DES-treated neonatal ovaries are transplanted into untreated females; thus indicating that DES exerts direct effects within the ovary. In addition, the fertilizability and developmental ability of PF oocytes are collectively lower as assessed using in vitro fertilization protocols, suggesting that DES affects oocyte quality [128,129]. Finally, in vitro experiments using follicles isolated from immature rats showed that DES (1µM) inhibited E₂ and testosterone production without affecting cAMP and CYP19 [110]. These data suggests that the effect of DES on oocyte quality may also be mediated through direct effects within granulosa cells.

In contrast to the findings discussed above, the direct effect of DES on the ovary is not a universally accepted mechanism of action. For example, ovaries from mice that were treated with 5µg/day DES functioned normally when transplanted into untreated host mice; furthermore, the host mice produced normal-sized offspring [130]. In addition, the progeny did not exhibit physiologic abnormalities, and themselves produced normal litters. These data suggest that DES does not affect the germ cells nor is its effect on ovarian function transgenerational [131]. Although ovarian effects of DES appear to not be transgenerational, carcinogenic effects of DES may be. For example, a case of rare adolescence ovarian small cell carcinoma was documented in a 15-year-old girl whose maternal grandmother was exposed to DES during pregnancy [132]. As discussed at the beginning of the section 3, the transgenerational carcinogenic actions of DES in mice have also been studied in both sexes. One example of this has been shown in female mice, which were exposed to DES during the fetal or neonatal period. These dams produced male and female offspring expressing reproductive tract tumors; albeit these offspring possessed normal fertility [86,87]. Analysis showed that the reproductive tract maladies were transmitted through the maternal germ line. Findings like these support the urgency to better understand germ line effects of estrogenic endocrine disruptors.

b. Anti-androgenic endocrine disruptors

Some endocrine disruptors show anti-androgenic activity. As previously described, besides being substrate for E₂ biosynthesis, androgens support ovarian development and function. The best example of this observation is that AR-null mice show premature ovarian failure (POF)-like symptoms [70]. Premature ovarian failure is defined as early decline of ovarian function following apparently normal folliculogenesis [70] and is a relatively common reproductive problem in humans. Therefore, it is important to examine the role of anti-androgenic endocrine disruptors such as vinclozolin and p,p'-DDE on the ovary and female fertility.

i. Vinclozolin—The fungicide vinclozolin has known anti-androgenic activity, and its adverse effects on male sexual differentiation and fertility have been documented [71]. Similar to MXC, epigenetic transgenerational effects of vinclozolin on male fertility were described [25]. With regard to its effects on female reproduction, studies have indicated that vinclozolin adversely alters the normal pattern of sexual differentiation and adult sexual function. For example, when 7-week-old female rats were given oral vinclozolin (6.25–100 mg/kg/d), the normal pattern of sex organ development was disrupted. In adult female rats, vinclozolin induced alterations in the serum profiles of LH and the E₂:testosterone ratio, which could collectively account for the prolongation of estrous cycle duration that was measured in these animals [133]. In a two-generation reproductive toxicity study, 40–1000 ppm dietary vinclozolin resulted in hyperplasia of ovarian interstitial cells and vacuolation of lutein cells in females of both generations [134].

In studies using pregnant rats that were given oral vinclozolin (3.0–10 µg/ml), it has been shown that this compound antagonizes the androgenic effects of testosterone propionate in male and female embryos [135]. Further analysis of the in utero effects of vinclozolin revealed that male embryos underwent feminization and female embryos became masculinized when dams were given vinclozolin. As suggested by the data, the morphogenic actions of vinclozolin within embryos could be a result of the effects of this compound on the expression of AR, ER, and/or progesterone receptors [136].

ii. DDE—Another compound possessing anti-androgen activity is the DDT metabolite 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) [137]. Its effects as an anti-androgen have not been definitively demonstrated in the ovary. However, several studies have shown that exposure to DDE does alter ovarian steroidogenesis and may therefore negatively impact fertility.

In pig granulosa cells, DDE (100 µM) inhibited basal and FSH-induced progesterone secretion as well as cAMP production, while stimulating cell proliferation [98]. In a similar study using a porcine granulosa cell line, 3–10 µg/ml DDE inhibited basal- and cholera toxin-stimulated progesterone, cAMP production, and P450_{scc} mRNA level [99].

With regard to the effects of DDE on human reproduction, elevated follicular fluid concentrations of DDE have been correlated with reduced fertilizability of oocytes that were retrieved for in vitro fertilization (IVF) [138]. A mechanistic study using cultures of IVF-primed human granulosa cells indicated that CYP19 activity was increased by DDE (100 ng/ml), with a synergistic effect on CYP19 activity occurring in the presence of both FSH and DDE [139].

A compelling study tracked the fecundability of daughters of women who had measurable levels of DDE (and DDT) within 1–3 days following delivery. Interestingly, elevated maternal DDE concentrations were correlated with increased fecundability in the daughters, although an increase in maternal serum DDT resulted in the opposite effect [140]. Subsequent to the aforementioned research, another group reported that Southeast Asian immigrant women with

increased serum DDE levels experienced a shorter menstrual cycle length, attributable to a decrease in luteal phase duration [141]. This observation was linked with diminished concentrations of progestin metabolites in these women. Whether or not these correlations between serum DDE and effects upon human female reproduction result from the anti-androgenic effects of DDE are at present unknown.

Conclusions

The processes of sexual differentiation and adult reproductive function are under genetic control as well as regulation by numerous hormone-mediated feedback mechanisms. Importantly, both processes, which involve numerous levels of checks and balances, are sensitive to regulation by a plethora of endogenous and exogenous factors, including a list of environmental endocrine disruptors. The sensitivity of pre- and postnatal reproductive development and function becomes exceedingly significant because the system sets in motion during the initial stages of gamete and gonadal differentiation in utero ultimately control reproductive viability in mature offspring and may also be transgenerational. The compounds that have been discussed in this review not only affect the developing embryo but also have been shown to alter adult ovarian function by targeting steroidogenesis. Hence, not only do these agents have the potential to disrupt reproductive cyclicity and/or the normal levels of ovarian steroid hormone production that are required to support implantation and the early stage of pregnancy, but these compounds may also cause transgenerational effects by targeting oocyte maturation and maternal sex chromosomes. As described, there are fundamental differences in developmental processes that control gonadogenesis in males and females. These differences should thus be considered in the design of experiments that will be essential to better understand how environmental endocrine disruptors can affect reproductive viability. To date, only a relatively limited number of mechanistic studies have attempted to model how some of these endocrine disruptors function within the female reproductive system. Therefore, many more questions must be addressed in order to fully understand the reproductive consequences of these agents in the female.

Acknowledgements

The authors wish to thank Dr. Kathy Manger for her assistance in the preparation of the manuscript.

References

1. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 2000;21:200–14. [PubMed: 10782364]
2. Hirshfield AN. Development of follicles in the mammalian ovary. *Int Rev Cytol* 1991;124:43–101. [PubMed: 2001918]
3. Skinner MK. Regulation of primordial follicle assembly and development. *Hum Reprod Update* 2005;11:461–71. [PubMed: 16006439]
4. Johnson J, Bagley J, Skaznik-Wikiel M, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. *Cell* 2005;122:303–15. [PubMed: 16051153]
5. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature* 2004;428:145–50. [PubMed: 15014492]
6. Johnson J, Skaznik-Wikiel M, Lee HJ, Niikura Y, Tilly JC, Tilly JL. Setting the record straight on data supporting postnatal oogenesis in female mammals. *Cell Cycle* 2005;4:1471–7. [PubMed: 16222117]
7. Byskov AG, Faddy MJ, Lemmen JG, Andersen CY. Eggs forever? *Differentiation* 2005;73:438–46. [PubMed: 16351687]
8. Cummings AM. Methoxychlor as a model for environmental estrogens. *Crit Rev Toxicol* 1997;27:367–79. [PubMed: 9263644]

9. Gaido KW, Maness SC, McDonnell DP, Dehal SS, Kupfer D, Safe S. Interaction of methoxychlor and related compounds with estrogen receptor alpha and beta, and androgen receptor: structure-activity studies. *Mol Pharmacol* 2000;58:852–8. [PubMed: 10999957]
10. Saitou M, Barton SC, Surani MA. A molecular programme for the specification of germ cell fate in mice. *Nature* 2002;418:293–300. [PubMed: 12124616]
11. Ginsburg M, Snow MH, McLaren A. Primordial germ cells in the mouse embryo during gastrulation. *Development* 1990;110:521–8. [PubMed: 2133553]
12. McLaren A. Mammalian germ cells: birth, sex, and immortality. *Cell Struct Funct* 2001;26:119–22. [PubMed: 11565803]
13. McCarrey, JR. Development of the germ cell. In: Desjardins, C.; Ewing, LL., editors. *Cell and Molecular Biology of the Testis*. New York: Oxford University Press; 1993. p. 58-89.
14. Zamboni L, Upadhyay S. The contribution of the mesonephros to the development of the sheep fetal testis. *Am J Anat* 1982;165:339–56. [PubMed: 7180819]
15. Yao HH, DiNapoli L, Capel B. Meiotic germ cells antagonize mesonephric cell migration and testis cord formation in mouse gonads. *Development* 2003;130:5895–902. [PubMed: 14561636]
16. Yao HH. The pathway to femaleness: current knowledge on embryonic development of the ovary. *Mol Cell Endocrinol* 2005;230:87–93. [PubMed: 15664455]
17. Nef S, Schaad O, Stallings NR, et al. Gene expression during sex determination reveals a robust female genetic program at the onset of ovarian development. *Dev Biol* 2005;287:361–77. [PubMed: 16214126]
18. Small CL, Shima JE, Uzumcu M, Skinner MK, Griswold MD. Profiling gene expression during the differentiation and development of the murine embryonic gonad. *Biol Reprod* 2005;72:492–501. [PubMed: 15496517]
19. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 2001;293:1089–93. [PubMed: 11498579]
20. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev* 2002;117:15–23. [PubMed: 12204247]
21. Coffigny H, Bourgeois C, Ricoul M, et al. Alterations of DNA methylation patterns in germ cells and Sertoli cells from developing mouse testis. *Cytogenet Cell Genet* 1999;87:175–81. [PubMed: 10702661]
22. Ueda T, Abe K, Miura A, et al. The paternal methylation imprint of the mouse H19 locus is acquired in the gonocyte stage during foetal testis development. *Genes Cells* 2000;5:649–59. [PubMed: 10947850]
23. McGee EA, Perlas E, LaPolt PS, Tsafri A, Hsueh AJ. Follicle-stimulating hormone enhances the development of preantral follicles in juvenile rats. *Biol Reprod* 1997;57:990–8. [PubMed: 9369162]
24. Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. *J Biol Chem* 2002;277:5285–9. [PubMed: 11713250]
25. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005;308:1466–9. [PubMed: 15933200]
26. Roy SK, Albee L. Requirement for follicle-stimulating hormone action in the formation of primordial follicles during perinatal ovarian development in the hamster. *Endocrinology* 2000;141:4449–56. [PubMed: 11108254]
27. Balla A, Danilovich N, Yang Y, Sairam MR. Dynamics of ovarian development in the FORKO immature mouse: structural and functional implications for ovarian reserve. *Biol Reprod* 2003;69:1281–93. [PubMed: 12801993]
28. Coucouvanis EC, Sherwood SW, Carswell-Crumpton C, Spack EG, Jones PP. Evidence that the mechanism of prenatal germ cell death in the mouse is apoptosis. *Exp Cell Res* 1993;209:238–47. [PubMed: 8262141]
29. Pepling ME, Spradling AC. Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. *Dev Biol* 2001;234:339–51. [PubMed: 11397004]
30. Kumar TR. What have we learned about gonadotropin function from gonadotropin subunit and receptor knockout mice? *Reproduction* 2005;130:293–302. [PubMed: 16123236]

31. Eppig JJ, O'Brien MJ. Development in vitro of mouse oocytes from primordial follicles. *Biol Reprod* 1996;54:197–207. [PubMed: 8838017]
32. Pangas SA, Rajkovic A. Transcriptional regulation of early oogenesis: in search of masters. *Hum Reprod Update* 2006;12:65–76. [PubMed: 16143663]
33. Merchant H. Rat gonadal and ovarioan organogenesis with and without germ cells. An ultrastructural study. *Dev Biol* 1975;44:1–21. [PubMed: 1132581]
34. Soyak SM, Amleh A, Dean J. FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. *Development* 2000;127:4645–54. [PubMed: 11023867]
35. Morrison LJ, Marcinkiewicz JL. Tumor necrosis factor alpha enhances oocyte/follicle apoptosis in the neonatal rat ovary. *Biol Reprod* 2002;66:450–7. [PubMed: 11804962]
36. Disson GA, Hirshfield AN, Malamed S, Ojeda SR. Expression of neurotrophins and their receptors in the mammalian ovary is developmentally regulated: changes at the time of folliculogenesis. *Endocrinology* 1995;136:4681–92. [PubMed: 7664689]
37. Billiar RB, Zachos NC, Burch MG, Albrecht ED, Pepe GJ. Up-regulation of alpha-inhibin expression in the fetal ovary of estrogen-suppressed baboons is associated with impaired fetal ovarian folliculogenesis. *Biol Reprod* 2003;68:1989–96. [PubMed: 12606324]
38. Wang C, Roy SK. Expression of growth differentiation factor 9 in the oocytes is essential for the development of primordial follicles in the hamster ovary. *Endocrinology* 2006;147:1725–34. [PubMed: 16384866]
39. Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996;383:531–5. [PubMed: 8849725]
40. Nilsson EE, Skinner MK. Growth and differentiation factor-9 stimulates progression of early primary but not primordial rat ovarian follicle development. *Biol Reprod* 2002;67:1018–24. [PubMed: 12193416]
41. Kezele P, Skinner MK. Regulation of ovarian primordial follicle assembly and development by estrogen and progesterone: endocrine model of follicle assembly. *Endocrinology* 2003;144:3329–37. [PubMed: 12865310]
42. Chen Y, Pepling M. Estradiol inhibits cyst breakdown in neonatal mouse ovary organ culture. *Biol Reprod* 2006;95. [PubMed: 16162875]
43. Britt KL, Saunders PK, McPherson SJ, Misso ML, Simpson ER, Findlay JK. Estrogen actions on follicle formation and early follicle development. *Biol Reprod* 2004;71:1712–23. [PubMed: 15269096]
44. Zachos NC, Billiar RB, Albrecht ED, Pepe GJ. Developmental regulation of baboon fetal ovarian maturation by estrogen. *Biol Reprod* 2002;67:1148–56. [PubMed: 12297530]
45. Hirshfield AN. Theca cells may be present at the outset of follicular growth. *Biol Reprod* 1991;44:1157–62. [PubMed: 1873389]
46. Hutt KJ, McLaughlin EA, Holland MK. Kit ligand and c-Kit have diverse roles during mammalian oogenesis and folliculogenesis. *Mol Hum Reprod* 2006;12:61–9. [PubMed: 16481408]
47. Motro B, Bernstein A. Dynamic changes in ovarian c-kit and Steel expression during the estrous reproductive cycle. *Dev Dyn* 1993;197:69–79. [PubMed: 7691275]
48. Vitt UA, Hsueh AJ. Stage-dependent role of growth differentiation factor-9 in ovarian follicle development. *Mol Cell Endocrinol* 2001;183:171–7. [PubMed: 11604237]
49. Disson GA, Romero C, Paredes A, Ojeda SR. Neurotrophic control of ovarian development. *Microsc Res Tech* 2002;59:509–15. [PubMed: 12467027]
50. Nilsson E, Parrott JA, Skinner MK. Basic fibroblast growth factor induces primordial follicle development and initiates folliculogenesis. *Mol Cell Endocrinol* 2001;175:123–30. [PubMed: 11325522]
51. Berisha B, Sinowatz F, Schams D. Expression and localization of fibroblast growth factor (FGF) family members during the final growth of bovine ovarian follicles. *Mol Reprod Dev* 2004;67:162–71. [PubMed: 14694431]
52. van Eijk MJ, Mandelbaum J, Salat-Baroux J, et al. Expression of leukaemia inhibitory factor receptor subunits LIFR beta and gp130 in human oocytes and preimplantation embryos. *Mol Hum Reprod* 1996;2:355–60. [PubMed: 9238703]

53. Nilsson EE, Kezele P, Skinner MK. Leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition in rat ovaries. *Mol Cell Endocrinol* 2002;188:65–73. [PubMed: 11911947]
54. Erickson GF, Shimasaki S. The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle. *Reprod Biol Endocrinol* 2003;1:9. [PubMed: 12741959]
55. Nilsson EE, Skinner MK. Bone morphogenetic protein-4 acts as an ovarian follicle survival factor and promotes primordial follicle development. *Biol Reprod* 2003;69:1265–72. [PubMed: 12801979]
56. Durlinger AL, Kramer P, Karels B, et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. *Endocrinology* 1999;140:5789–96. [PubMed: 10579345]
57. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. *Endocrinology* 2002;143:1076–84. [PubMed: 11861535]
58. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. *Reproduction* 2002;124:601–9. [PubMed: 12416998]
59. Uzumcu M, Kuhn PE, Marano JE, Armenti AE, Passantino L. Neonatal methoxychlor exposure inhibits folliculogenesis and stimulates anti-Mullerian hormone (AMH) production in the rat ovary. *J Endocrinol*. 2006In Press
60. Strauss, JF., 3rd; Williams, CJ. The Ovarian Life Cycle. In: Strauss, JF., 3rd; Barbieri, RL., editors. *Reproductive Endocrinology*. Philadelphia: Elsevier Saunders; 2004. p. 213–253.
61. Sar M, Welsch F. Differential expression of estrogen receptor-beta and estrogen receptor-alpha in the rat ovary. *Endocrinology* 1999;140:963–71. [PubMed: 9927330]
62. Emmen JM, Couse JF, Elmore SA, Yates MM, Kissling GE, Korach KS. In vitro growth and ovulation of follicles from ovaries of estrogen receptor (ER){alpha} and ER{beta} null mice indicate a role for ER{beta} in follicular maturation. *Endocrinology* 2005;146:2817–26. [PubMed: 15731357]
63. Ikeda Y, Nagai A, Ikeda MA, Hayashi S. Neonatal estrogen exposure inhibits steroidogenesis in the developing rat ovary. *Dev Dyn* 2001;221:443–53. [PubMed: 11500981]
64. Ikeda Y, Nagai A, Ikeda MA, Hayashi S. Increased expression of Mullerian-inhibiting substance correlates with inhibition of follicular growth in the developing ovary of rats treated with E2 benzoate. *Endocrinology* 2002;143:304–12. [PubMed: 11751622]
65. Huynh K, Jones G, Thouas G, Britt KL, Simpson ER, Jones ME. Estrogen is not directly required for oocyte developmental competence. *Biol Reprod* 2004;70:1263–9. [PubMed: 14695903]
66. Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M, Bondy CA. Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. *Biol Reprod* 1999;61:353–7. [PubMed: 10411511]
67. Hutz RJ, Dierschke DJ, Wolf RC. Role of estradiol in regulating ovarian follicular atresia in rhesus monkeys: a review. *J Med Primatol* 1990;19:553–71. [PubMed: 2123257]
68. Maciel GA, Baracat EC, Benda JA, et al. Stockpiling of transitional and classic primary follicles in ovaries of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004;89:5321–7. [PubMed: 15531477]
69. Lyon MF, Glenister PH. Reduced reproductive performance in androgen-resistant Tfm/Tfm female mice. *Proc R Soc Lond B Biol Sci* 1980;208:1–12. [PubMed: 6105650]
70. Shiina H, Matsumoto T, Sato T, et al. Premature ovarian failure in androgen receptor-deficient mice. *Proc Natl Acad Sci U S A* 2006;103:224–9. [PubMed: 16373508]
71. Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray LE Jr. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicol Appl Pharmacol* 1994;126:276–85. [PubMed: 8209380]
72. Ojeda SR, Ramirez VD. Plasma level of LH and FSH in maturing rats: response to hemigonadectomy. *Endocrinology* 1972;90:466–72. [PubMed: 5009330]
73. Sokka T, Huhtaniemi I. Ontogeny of gonadotrophin receptors and gonadotrophin-stimulated cyclic AMP production in the neonatal rat ovary. *J Endocrinol* 1990;127:297–303. [PubMed: 2174455]
74. Xu Z, Garverick HA, Smith GW, Smith MF, Hamilton SA, Youngquist RS. Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. *Biol Reprod* 1995;53:951–7. [PubMed: 8547492]

75. diZerega GS, Hodgen GD. Folliculogenesis in the primate ovarian cycle. *Endocr Rev* 1981;2:27–49. [PubMed: 6795034]
76. Logan KA, Juengel JL, McNatty KP. Onset of steroidogenic enzyme gene expression during ovarian follicular development in sheep. *Biol Reprod* 2002;66:906–16. [PubMed: 11906908]
77. Terranova PF, Rice VM. Review: cytokine involvement in ovarian processes. *Am J Reprod Immunol* 1997;37:50–63. [PubMed: 9138453]
78. Gelety TJ, Magoffin DA. Ontogeny of steroidogenic enzyme gene expression in ovarian theca-interstitial cells in the rat: regulation by a paracrine theca-differentiating factor prior to achieving luteinizing hormone responsiveness. *Biol Reprod* 1997;56:938–45. [PubMed: 9096876]
79. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: a review of structure/function relationships. *Endocr Rev* 1985;6:371–99. [PubMed: 3896767]
80. Henkes LE, Davis JS, Rueda BR. Mutant mouse models and their contribution to our knowledge of corpus luteum development, function and regression. *Reprod Biol Endocrinol* 2003;1:87. [PubMed: 14613537]
81. McLachlan JA, Simpson E, Martin M. Endocrine disrupters and female reproductive health. *Best Pract Res Clin Endocrinol Metab* 2006;20:63–75. [PubMed: 16522520]
82. de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol* 2006;46:4–14. [PubMed: 16441686]
83. Newbold RR, Bullock BC, McLachlan JA. Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res* 1990;50:7677–81. [PubMed: 2174729]
84. Li S, Washburn KA, Moore R, et al. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res* 1997;57:4356–9. [PubMed: 9331098]
85. Uzumcu M, Suzuki H, Skinner MK. Effect of the anti-androgenic endocrine disruptor vinclozolin on embryonic testis cord formation and postnatal testis development and function. *Reprod Toxicol* 2004;18:765–74. [PubMed: 15279874]
86. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA. Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 2000;21:1355–63. [PubMed: 10874014]
87. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA. Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 1998;19:1655–63. [PubMed: 9771938]
88. Gray LE Jr, Ostby J, Ferrell J, et al. A dose-response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. *Fundam Appl Toxicol* 1989;12:92–108. [PubMed: 2925022]
89. Chapin RE, Harris MW, Davis BJ, et al. The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *Fundam Appl Toxicol* 1997;40:138–57. [PubMed: 9398496]
90. Eroschenko VP, Abuel-Atta AA, Grober MS. Neonatal exposures to technical methoxychlor alters ovaries in adult mice. *Reprod Toxicol* 1995;9:379–87. [PubMed: 7496094]
91. Borgeest C, Miller KP, Gupta R, et al. Methoxychlor-induced atresia in the mouse involves Bcl-2 family members, but not gonadotropins or estradiol. *Biol Reprod* 2004;70:1828–35. [PubMed: 14973267]
92. Miller KP, Gupta RK, Greenfield CR, Babus JK, Flaws JA. Methoxychlor Directly Affects Ovarian Antral Follicle Growth and Atresia through Bcl-2- and Bax-Mediated Pathways. *Toxicol Sci* 2005;88:213–21. [PubMed: 16081520]
93. Gupta RK, Miller KP, Babus JK, Flaws JA. Methoxychlor Inhibits Growth and Induces Atresia of Antral Follicles Through an Oxidative Stress Pathway. *Toxicol Sci*. 2006
94. Gupta RK, Schuh RA, Fiskum G, Flaws JA. Methoxychlor causes mitochondrial dysfunction and oxidative damage in the mouse ovary. *Toxicol Appl Pharmacol*. 2006
95. Miller KP, Gupta RK, Flaws JA. Methoxychlor metabolites may cause ovarian toxicity through estrogen-regulated pathways. *Toxicol Sci* 2006;93:180–8. [PubMed: 16760418]

96. Symonds DA, Tomic D, Miller KP, Flaws JA. Methoxychlor induces proliferation of the mouse ovarian surface epithelium. *Toxicol Sci* 2005;83:355–62. [PubMed: 15525693]
97. Gupta R, Schuh RA, Fiskum G, Flaws JA. Methoxychlor causes oxidative damage in the mouse ovary. *Biol Reprod* 2005;(Special Issue):136.
98. Chedrese PJ, Feyles F. The diverse mechanism of action of dichlorodiphenyldichloroethylene (DDE) and methoxychlor in ovarian cells in vitro. *Reprod Toxicol* 2001;15:693–8. [PubMed: 11738522]
99. Crellin NK, Kang HG, Swan CL, Chedrese PJ. Inhibition of basal and stimulated progesterone synthesis by dichlorodiphenyldichloroethylene and methoxychlor in a stable pig granulosa cell line. *Reproduction* 2001;121:485–92. [PubMed: 11226075]
100. Zachow R, Uzumcu M. The Methoxychlor Metabolite, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane, Inhibits Steroidogenesis in Rat Ovarian Granulosa Cells In Vitro. *Reprod Toxicol* 2006;22.
101. Kayisli UA, Aksu CA, Berkkanoglu M, Arici A. Estrogenicity of isoflavones on human endometrial stromal and glandular cells. *J Clin Endocrinol Metab* 2002;87:5539–44. [PubMed: 12466350]
102. Ford JA Jr, Clark SG, Walters EM, Wheeler MB, Hurley WL. Estrogenic effects of genistein on reproductive tissues of ovariectomized gilts. *J Anim Sci* 2006;84:834–42. [PubMed: 16543560]
103. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 1998;139:4252–63. [PubMed: 9751507]
104. Nikaido Y, Yoshizawa K, Danbara N, et al. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol* 2004;18:803–11. [PubMed: 15279878]
105. Nagao T, Yoshimura S, Saito Y, Nakagomi M, Usumi K, Ono H. Reproductive effects in male and female rats of neonatal exposure to genistein. *Reprod Toxicol* 2001;15:399–411. [PubMed: 11489596]
106. Jefferson WN, Padilla-Banks E, Newbold RR. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. *Biol Reprod* 2005;73:798–806. [PubMed: 15930323]
107. Jefferson, WN.; Padilla-Banks, E.; Goulding, EH.; Eddy, EM. Neonatal exposure to the endocrine disruptor genistein adversely affects fertilization rate and oocyte quality; Endocrine Society's 88th Annual Meeting; Boston, MA. 2006.
108. Jefferson W, Newbold R, Padilla-Banks E, Pepling M. Neonatal genistein treatment alters ovarian differentiation in the mouse: inhibition of oocyte nest breakdown and increased oocyte survival. *Biol Reprod* 2006;74:161–8. [PubMed: 16192398]
109. Nynca A, Ciereszko RE. Effect of genistein on steroidogenic response of granulosa cell populations from porcine preovulatory follicles. *Reprod Biol* 2006;6:31–50. [PubMed: 16604150]
110. Myllymaki S, Haaavisto T, Vainio M, Toppari J, Paranko J. In vitro effects of diethylstilbestrol, genistein, 4-tert-butylphenol, and 4-tert-octylphenol on steroidogenic activity of isolated immature rat ovarian follicles. *Toxicol Appl Pharmacol* 2005;204:69–80. [PubMed: 15781295]
111. Whitehead SA, Cross JE, Burden C, Lacey M. Acute and chronic effects of genistein, tyrphostin and lavendustin A on steroid synthesis in luteinized human granulosa cells. *Hum Reprod* 2002;17:589–94. [PubMed: 11870108]
112. Komar CM, Matousek M, Mitsube K, Mikuni M, Brannstrom M, Curry TE Jr. Effects of genistein on the periovulatory expression of messenger ribonucleic acid for matrix metalloproteinases and tissue inhibitors of metalloproteinases in the rat ovary. *Reproduction* 2001;121:259–65. [PubMed: 11226050]
113. Tilly JL, Billig H, Kowalski KI, Hsueh AJ. Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of apoptosis in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism. *Mol Endocrinol* 1992;6:1942–50. [PubMed: 1480180]
114. Gangrade BK, Davis JS, May JV. A novel mechanism for the induction of aromatase in ovarian cells in vitro: role of transforming growth factor alpha-induced protein tyrosine kinase. *Endocrinology* 1991;129:2790–2. [PubMed: 1935810]

115. Markey CM, Rubin BS, Soto AM, Sonnenschein C. Endocrine disruptors: from Wingspread to environmental developmental biology. *J Steroid Biochem Mol Biol* 2002;83:235–44. [PubMed: 12650721]
116. Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. Evidence of Altered Brain Sexual Differentiation in Mice Exposed Perinatally to Low, Environmentally Relevant Levels of Bisphenol A. *Endocrinology*. 2006
117. Toda K, Miyaura C, Okada T, Shizuta Y. Dietary bisphenol A prevents ovarian degeneration and bone loss in female mice lacking the aromatase gene (Cyp19). *Eur J Biochem* 2002;269:2214–22. [PubMed: 11985600]
118. Mlynarcikova A, Kolena J, Fickova M, Scsukova S. Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate. *Mol Cell Endocrinol* 2005;244:57–62. [PubMed: 16225985]
119. Xu J, Osuga Y, Yano T, et al. Bisphenol A induces apoptosis and G2-to-M arrest of ovarian granulosa cells. *Biochem Biophys Res Commun* 2002;292:456–62. [PubMed: 11906184]
120. Hunt PA, Koehler KE, Susiarjo M, et al. Bisphenol a exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 2003;13:546–53. [PubMed: 12676084]
121. Newbold RR. Lessons learned from perinatal exposure to diethylstilbestrol. *Toxicol Appl Pharmacol* 2004;199:142–50. [PubMed: 15313586]
122. Miller KP, Borgeest C, Greenfeld C, Tomic D, Flaws JA. In utero effects of chemicals on reproductive tissues in females. *Toxicol Appl Pharmacol* 2004;198:111–31. [PubMed: 15236949]
123. Wordinger RJ, Derrenbacker J. In utero exposure of mice to diethylstilbestrol alters neonatal ovarian follicle growth and development. *Acta Anat (Basel)* 1989;134:312–8. [PubMed: 2741660]
124. Wordinger RJ, Highman B. Histology and ultrastructure of the adult mouse ovary following a single prenatal exposure to diethylstilbestrol. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1984;45:241–53. [PubMed: 6146217]
125. Haney AF, Newbold RR, McLachlan JA. Prenatal diethylstilbestrol exposure in the mouse: effects on ovarian histology and steroidogenesis in vitro. *Biol Reprod* 1984;30:471–8. [PubMed: 6704476]
126. Iguchi T, Takasugi N, Bern HA, Mills KT. Frequent occurrence of polyovular follicles in ovaries of mice exposed neonatally to diethylstilbestrol. *Teratology* 1986;34:29–35. [PubMed: 3764775]
127. Iguchi T, Takasugi N. Polyovular follicles in the ovary of immature mice exposed prenatally to diethylstilbestrol. *Anat Embryol (Berl)* 1986;175:53–5. [PubMed: 3799991]
128. Iguchi T, Kamiya K, Uesugi Y, Sayama K, Takasugi N. In vitro fertilization of oocytes from polyovular follicles in mouse ovaries exposed neonatally to diethylstilbestrol. *In Vivo* 1991;5:359–63. [PubMed: 1810421]
129. Iguchi T, Fukazawa Y, Uesugi Y, Takasugi N. Polyovular follicles in mouse ovaries exposed neonatally to diethylstilbestrol in vivo and in vitro. *Biol Reprod* 1990;43:478–84. [PubMed: 2271729]
130. Halling A, Forsberg JG. Ovarian reproductive function after exposure to diethylstilbestrol in neonatal life. *Biol Reprod* 1990;43:472–7. [PubMed: 2271728]
131. Forsberg JG, Halling A. Failure to detect a second-generation effect in female mice after neonatal treatment with an estrogen (diethylstilbestrol). *Acta Anat (Basel)* 1992;144:103–6. [PubMed: 1514366]
132. Blatt J, Van Le L, Weiner T, Sailer S. Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. *J Pediatr Hematol Oncol* 2003;25:635–6. [PubMed: 12902917]
133. Shin JH, Moon HJ, Kim TS, et al. Repeated 28-day oral toxicity study of vinclozolin in rats based on the draft protocol for the “Enhanced OECD Test Guideline No. 407” to detect endocrine effects. *Arch Toxicol*. 2006
134. Matsuura I, Saitoh T, Ashina M, et al. Evaluation of a two-generation reproduction toxicity study adding endpoints to detect endocrine disrupting activity using vinclozolin. *J Toxicol Sci* 2005;30:163–188. [PubMed: 16641540]Spec No
135. Wolf CJ, LeBlanc GA, Gray LE Jr. Interactive effects of vinclozolin and testosterone propionate on pregnancy and sexual differentiation of the male and female SD rat. *Toxicol Sci* 2004;78:135–43. [PubMed: 14736997]

136. Buckley J, Willingham E, Agras K, Baskin LS. Embryonic exposure to the fungicide vinclozolin causes virilization of females and alteration of progesterone receptor expression in vivo: an experimental study in mice. *Environ Health* 2006;5:4. [PubMed: 16504050]
137. Andersen HR, Vinggaard AM, Rasmussen TH, Gjermansen IM, Bonefeld-Jorgensen EC. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. *Toxicol Appl Pharmacol* 2002;179:1–12. [PubMed: 11884232]
138. Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. *Arch Environ Contam Toxicol* 2002;43:121–6. [PubMed: 12045882]
139. Younglai EV, Holloway AC, Lim GE, Foster WG. Synergistic effects between FSH and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (P,P'-DDE) on human granulosa cell aromatase activity. *Hum Reprod* 2004;19:1089–93. [PubMed: 15070871]
140. Cohn BA, Cirillo PM, Wolff MS, et al. DDT and DDE exposure in mothers and time to pregnancy in daughters. *Lancet* 2003;361:2205–6. [PubMed: 12842376]
141. Windham GC, Lee D, Mitchell P, Anderson M, Petreas M, Lasley B. Exposure to organochlorine compounds and effects on ovarian function. *Epidemiology* 2005;16:182–90. [PubMed: 15703532]
142. Tomic D, Frech MS, Babus JK, et al. Methoxychlor Induces Atresia of Antral Follicles in ER{alpha}-Overexpressing Mice. *Toxicol Sci* 2006;93:196–204. [PubMed: 16787999]
143. Zachow R, Uzumcu M. The methoxychlor metabolite, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane, inhibits steroidogenesis in rat ovarian granulosa cells in vitro. *Reproductive Toxicology*. 2006
144. Suzuki A, Sugihara A, Uchida K, et al. Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. *Reprod Toxicol* 2002;16:107–16. [PubMed: 11955941]

Figure 1. An overview of ovarian folliculogenesis

Primordial follicles are formed following apoptosis of some oocytes within the oocyte nests. The remaining oocytes are then surrounded by squamous precursor-type granulosa cells (pre-GC). During each reproductive cycle, a cohort of primordial follicles is recruited to enter the growth phase. Subsequent follicular growth and differentiation results in granulosa cell (GC) proliferation, the presence of theca cells (TC), and the onset of steroidogenesis. It is believed that sometime during the later preantral stages of folliculogenesis the dominant follicle(s) is/are selected, but this is uncertain. Depending on whether the female is a monoovulator or a polyovulator, one or a few of the selected follicles undergo ovulation and a corpus luteum forms from the remnants of each ovulated follicle. The stimulatory (+) and inhibitory (-) effects of environmental endocrine disruptors are shown.

Figure 2. Control of granulosa cell steroidogenesis

FSH binds to the FSHR, stimulating the mobilization of stimulatory guanine-nucleotide binding proteins (Gsα). The subsequent activation of adenylyl cyclase and generation of cAMP leads to the phosphorylation of cAMP-dependent protein kinase (pPKA). Substrates for pPKA include StAR protein and a cohort of steroid pathway enzymes and other molecules that collectively mobilize intracellular cholesterol and guide the formation of progesterone from cholesterol and the aromatization of androstenedione and testosterone into E₂. Progesterone, estrone (E₁), and E₂ represent the predominant steroids, which are secreted. The stimulatory (+) and inhibitory (-) effects of the environmental endocrine disruptors reviewed are shown. HDL: high-density lipoprotein; P₅: pregnenolone.

Figure 3. Control of theca cell steroidogenesis

LH binds to the LH receptor, stimulating the mobilization of stimulatory guanine-nucleotide binding proteins ($G_{s\alpha}$). The subsequent activation of adenylyl cyclase and generation of cAMP leads to the phosphorylation of cAMP-dependent protein kinase (pPKA). Substrates for pPKA include StAR protein and a cohort of steroid pathway enzymes and other molecules, which collectively mobilize intracellular cholesterol and guide the formation of progesterone from cholesterol and the synthesis of androstenedione and testosterone. Androstenedione and testosterone are secreted, and within granulosa cells, are aromatized into estrogens as described in the text and Figure 2. The inhibitory (–) effects of genistein and DES are shown. DHEA: dehydroepiandrosterone; HDL: high-density lipoprotein; P₅: pregnenolone.

Table 1

The effect of select endocrine disruptors on ovarian development and function.

Compound (Primary Action)	In vivo/ In vitro	Dose	Species & Route/ Cell type	Duration	Effect Observed or Stage Affected	Potential Mechanism (s)	Reference (s)
MXC (Estrogenic)	In vivo	25– 200 mg/kg/ d	Rat, oral	Weaning-post- partum day 15	No visible CL Luteal phase	Impaired HP axis* No LH surge*	[88]
	In vivo	5–150 mg/ kg/d	Rat, oral	1 wk before to 6 wk after birth	Blocks pre-antral to antral transition, Follicular selection Polycystic ovary, anovulation, no CL Luteal phase	Impaired HP axis, No LH surge*	[89]
	In vivo	0.05– 1.0 mg/day	Mouse, i.p.	PND 0–14	Ovarian atrophy, ↓ folliculogenesis Follicular cysts, no ovulation and no CL Luteal phase	Impaired HPO axis* Low FSH* No LH surge*	[90]
	In vivo	8–64 mg/ kg/d	Mouse, i.p.	PND 39–58	↓ Antral follicles ↑ Antral follicle atresia	Bcl-2/Bax Oxidative stress	[91,93]
	In vivo	1–500 mg/ kg/d	Rat, i.p.	PND 3–10	Blocks pre-antral to antral transition, Follicular selection	↓ FSH and LH** ↑ AMH	[59]
HPTE (Estrogenic, anti- estrogenic, or anti-androgenic)	In vitro	1–100 µg/ ml	Mouse antral follicles	24–96 h	↓ Antral follicle growth ↑ Antral follicle atresia	Bcl-2/Bax, Oxidative stress, ER	[92,94,95, 142]
	In vitro	10µM 0.1– 1.0µg/ml	Pig granulosa cells or cell line	48 h	↓ Basal, FSH-, E ₂ -, and CT- induced P ₄ ↔ cAMP ↑ P450 _{scc} mRNA	Distal to cAMP	[98]
	In vitro	0.1–10 µM	Rat granulosa cells	24/48 h	↓ FSH- & cAMP-induced E ₂ and P ₄ ↑ Basal AMH	↓ FSH-induced steroidogenic enzymes ↑ cAMP-induced steroidogenic enzymes, except CYP19 (↓)	[59,143]
	In vitro	0.01– 10 µg/ml	Mouse antral follicles	24–96 h	↓ Antral follicle growth ↑ Antral follicle atresia	ER	[92,94,95, 142]
	In vivo	50 mg/kg/d	Mouse, s.c.	PND 1–5	↑ Multi-oocyte follicles ↓ Follicular assembly ↓ Oocyte fertilization & embryo development	Apoptosis↓	[107,108]
Genistein (Estrogenic)	In vitro	0.5–50 µM	Pig granulosa cells	48 h	↓ Basal and FSH-induced P ₄ ↔ E ₂		[109]
	In vitro	0.01–1 µM	Rat ovarian follicles	5 d	↓ cAMP ↓ Testosterone ↔ E ₂	CYP19↑	[110]
	In vitro	50 µM	Human granulosa cells	4–24 h	↓ 17β-HSD ↓ 3β-HSD		[111]
	In vivo	20–100 mg/ g	Mouse, oral	7 d	Meiotic abnormalities↓	↑ Chromosomal congression failure	[120]
	In vivo	150 µg/pup	Mouse, s.c.	PND 1–5	↑ Multi-oocyte follicles ↓ Follicular assembly		[144]

Compound (Primary Action)	In vivo/ In vitro	Dose	Species & Route/Cell type	Duration	Effect Observed or Stage Affected	Potential Mechanism (s)	Reference (s)
DES (Estrogenic)	In vitro	0.01– 10 μ M	Pig granulosa cells	72 h	↑ Basal and FSH-induced P ₄ ↓ FSH-induced E ₂	Apoptosis [†] *	[118]
	In vitro	100 pM		24–72 h	Apoptosis [†]	Bcl-2/Bax pathway	[119]
	In vivo	10 or 100 μ g/kg	Mouse, s.c.	15 dpc	↑ Follicular development, Interstitial compartment ↑ Testosterone production No CL		[123–125]
Vinclozolin (Anti-androgenic)	In vivo/ in vitro	1 μ g/d 1 μ g/ ml	Mouse, s.c.	15–18 dpc or PND 1–5	↑ Multi-oocyte follicles ↓ Follicular assembly ↓ E ₂ , testosterone, $\leftrightarrow$ cAMP and CYP19	Apoptosis [†] *	[129]
	In vitro	1 μ M	Rat follicles				[110]
Vinclozolin (Anti-androgenic)	In vivo	40– 1000 ppm	Rat, dietary	Two generations 10 wk	↑ Interstitial cells ↑ Vacuolation of lutein cells		[134]
DDE (Anti-androgenic)	In vitro	100 μ M 3– 10 μ g/ml	Pig granulosa cells or cell line	48 h	↓ Basal and CT-induced P ₄ ↓ Basal, FSH- & CT- induced cAMP ↓ Basal and CT-induced P450 _{sc}	P450 _{sc} ↓	[98,99]
	In vitro	100 ng/ml	Human granulosa cells	24 h	↑ Basal, FSH-induced CYP19		[139]

* Speculated or predicted mechanisms

** Based on unpublished preliminary observations

CT = cholera toxin

CL = corpus luteum

AMH = anti-Mullerian Hormone

PND = postnatal day

dpc = day post coitum

HP = Hypothalamic-pituitary

↓ Inhibition or reduction

↑ Stimulation or increase

$\leftrightarrow$ No effect or change