Journal of Neuroendocrinology

Journal of Neuroendocrinology, 2014, **26**, 613–624

REVIEW ARTICLE

Long-Term Effects of Early-Life Exposure to Environmental Oestrogens on Ovarian Function: Role of Epigenetics

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Journal of Neuroendocrinology

Oestrogens play an important role in development and function of the brain and reproductive tract. Accordingly, it is considered that developmental exposure to environmental oestrogens can disrupt neural and reproductive tract development, potentially resulting in long-term alterations in neurobehaviour and reproductive function. Many chemicals have been shown to have oestrogenic activity, whereas others affect oestrogen production and turnover, resulting in the disruption of oestrogen signalling pathways. However, these mechanisms and the concentrations required to induce these effects cannot account for the myriad adverse effects of environmental toxicants on oestrogen-sensitive target tissues. Hence, alternative mechanisms are assumed to underlie the adverse effects documented in experimental animal models and thus could be important to human health. In this review, the epigenetic regulation of gene expression is explored as a potential target of environmental toxicants including oestrogenic chemicals. We suggest that toxicant-induced changes in epigenetic signatures are important mechanisms underlying the disruption of ovarian follicular development. In addition, we discuss how exposure to environmental oestrogens during early life can alter gene expression through effects on epigenetic control potentially leading to permanent changes in ovarian physiology.

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Key words: oestrogens, methoxychlor, BPA, DEHP, ovary, epigenetic

doi: 10.1111/jne.12181

Introduction

The gonadal steroid oestradiol (E_2) plays a central role in neurogenesis, synaptogenesis, regulation of neurotrophin expression and sexual differentiation of the brain. Recent evidence suggests that the oestrogenic activity of environmental contaminants is also associated with adverse effects on neurodevelopment and neurobehaviour (1–6). The developing brain undergoes phases of cell neurogenesis, proliferation, differentiation, density, migration and death; the brain also expresses high levels of oestrogen receptors. Although it has been shown that sexual dimorphism exists in brain development, it is clear that, regardless of sex, the actions of oestrogens on the developing brain are typically permanent and range from establishing sex differences to trophic and neuroprotective effects. Gonadal oestrogens affect target organs throughout the body including the brain. Oestrogens can also act as local autocrine or paracrine signals that affect only the microenvironment, including various regions of the brain. The foetal environment is replete with oestrogens originating in the maternal circulation and placenta, as well as from direct synthesis in foetal neurones. All of these parameters occur to varying degrees but, depending on the response to oestrogenic (or androgenic) signals, the cells are either protected from naturally occurring apoptosis or induced to undergo cell death (7).

Oestrogens both promote and prevent synaptogenesis in the developing brain. Oestrogens are involved in the establishment of synaptic patterns that endure into adulthood and subserve the neural networks of steroid-regulated physiology and behaviour. Oestrogens can suppress the formation of dendritic spine synapse in the arcuate nucleus. Oestrogens increase levels of the amino acid transmitter GABA by up-regulating the rate-limiting enzyme glutamic acid decarboxylase. GABA acts on $GABA_A$ receptors of astrocytes and induces process growth and branching, resulting in an

increased stellate morphology (8) compared to those not exposed to oestrogens. On the other hand, oestrogen exposure in the preoptic and ventromedial hypothalamus promotes the development and stabilisation of dendritic spine synapses that involve glutamate and ionotropic glutamate receptors (9). Because oestrogens are an important aspect of neurodevelopment with multifaceted signalling mechanisms that not only influence sex differentiation, but also a myriad of unique and opposing effects in different regions of the brain at different times during development, there is a potential for both direct but also indirect (via ovarian function) effects of environmental oestrogens.

Exposure to endocrine disruptors during foetal and early postnatal life produces different long-term alterations on various brain areas. For example, neonatal exposure to bisphenol A (BPA) decreased the inhibition of gonadotrophic-releasing hormone activity by RFamide-related peptide-3 neurones. This effect may be related with the advanced puberty observed in these animals (10). On the other hand, the perinatal exposure to oestradiol benzoate or methoxychlor (MXC) produced an early reproductive senescence in rats that is related with developmental reprogramming of hypothalamic genes. Specifically, the expression of *Esr1* and *Kiss1* genes was altered in this animal model (11). Other brain areas not related to reproduction could be affected by early exposure to endocrine disruptors. For example, exposure to oestradiol valerate at postnatal day (PND) 1 in rats produced an increase in dopamine content in the striatum, substantia nigra and ventral tegmental area when rats were adult. In addition, these rats lacked of amphetamine induced locomotor activity in the adulthood, possibly because of a decrease of dopamine transporter, the molecular target of amphetamine (12).

As in the brain, exposure to environmental oestrogens produces adverse effects in other oestrogen-sensitive tissues, including the uterus (13) and the ovaries (14–17). Human exposure to environmental contaminants has been widely documented through large-scale biomonitoring (18–24) and epidemiological studies (25–29). Although several different environmental contaminants have been measured in human ovarian follicular fluid (28–31), the impact upon circulating E_2 concentrations and associated health consequences remain largely unknown. However, evidence from studies conducted with wildlife suggests that chemicals in the environment can adversely affect ovarian biology (32,33) across the lifespan.

The ovary is composed of follicles at different stages of development and stroma. Follicles at their advance stages are composed of the theca externa and interna (androgen production), a basement membrane, granulosa cells [sites of E_2 and anti-Müllerian hormone (AMH) synthesis] and an oocyte. Folliculogenesis (follicle recruitment and growth to ovulation) is regulated in a stage-dependent manner by oocyte derived factors (e.g. bone morphogenetic protein-15, growth differentiation factor-9), gonadotrophins [follicle stimulating hormone (FSH) and luteinising hormone in early antral and Graafian follicles] and several growth regulators (e.g. transforming growth factor-β, AMH, inhibin-B, activin, vascular endothelial growth factor, FOXL2 and insulin-like growth factors and binding proteins) (34). Follicles are recruited into the growing pool of follicles where their initial stages of development are gonadotrophin independent. From the time follicles are formed *in utero* (or early postnatal in case of rodents), follicles begin to develop but, in the absence of FSH stimulation, they undergo atresia, with much of the follicle population lost before regular menstrual/oestrous cycles commence with the onset of puberty. Once regular reproductive cycles are established, follicles reach the secondary stage where FSH support from the pituitary provides the trigger to begin steroidogenesis. Steroid production in the ovary involves a two-cell process in which androgens produced in theca cells are transported to the granulosa, where they are converted by aromatase to E_2 or oestrone. Tissue culture studies demonstrate that environmental contaminants can both increase the expression of steroidogenic enzymes such as steroidogenic acute regulatory protein and aromatase (35,36), the rate-limiting enzyme in the conversion of androgens to oestrogens (oestrone and E_2), as well as increase the expression of enzymes involved in the metabolism of gonadal steroids (37,38). Furthermore, animal studies have demonstrated that exposure to chemicals with hormone-like activity changes the functional characteristics of steroid-dependent target tissues (39).

Studies performed in animal models illustrate the potential effects of early exposure to oestrogenic compounds on ovarian development and function. The specific processes that are most vulnerable to oestrogenic compounds are: (i) follicular formation (also known as follicular assembly); (ii) follicular growth; and (iii) follicular maturation and ovulation.

Follicular formation/follicular assembly: In mice, primordial germ cells (PGCs) arrive in the ovary and divide by mitosis between PND 10.5 and PND 13.5. These cells named oogonia enter meiosis I by PND 13.5 and form the oocytes. Then oocytes progress until prophase I and arrest in the diplotene stage. During maturation, oocytes are disposed in clusters and are surrounded by somatic cells forming germ cell cysts (also named oocyte nests). From gestational day 17.5 until PND 5, this germ cell nest breaks down, many of the oocytes undergo programmed cell death and the remaining oocytes are surrounded by pre-granulosa cells. This process, known as follicular formation or follicular assembly, sets the primordial pool of follicles (40,41). A large list of factors, including neurotrophins, growth factors and transcription factors, participate in the follicular assembly (40). Exposure to E_2 or oestrogenic compounds in newborn rodents inhibits and delays the follicular assembly (42,43) and is associated with abnormally assembled follicles, containing more than one oocyte per follicle (multi-oocyte follicles) (44–49). Compounds with oestrogenic activity have been demonstrated to decrease the number of primordial follicles (14,15,46,50), potentially shortening the reproductive lifespan.

Follicular growth: Once formed, most primordial follicles remain quiescent to be recruited throughout the reproductive life, although some immediately start growing prior to puberty. After females reach puberty, groups of follicles are recruited in cohorts to grow up until the early antral stage, independently of central control. Neonatal exposure to oestrogenic chemicals, such as BPA (51), MXC (17,52) and oestradiol valerate (53), has been linked to reduced growth and development of primary, secondary and antral follicles. Because the early stages of follicular development do not depend upon the influence of gonadotrophin; the ovary could be a direct target of disruption by oestrogenic compounds.

Follicular maturation and ovulation: Developmental exposure to oestrogenic compounds could interfere with the final maturation of follicles. Thus, follicles, instead of completing their final maturation to ovulate, form follicular cysts in the ovaries (16,51,54,55). Therefore, environmental oestrogens are considered to be potentially important in the pathogenesis of polycystic ovary syndrome (56,57). This could result from a direct effect in the ovary, as well as indirectly at the level of brain, under the influence of oestrogenic chemicals. The evidence for the disruptive effects of oestrogenic compounds on the differentiation of brain in female has long been known (58–61). Evidence for the direct effects of environmental oestrogens in the ovary exists. For example, prepubertal rats that were exposed to oestrogenic chemicals (e.g. MXC) during foetal and neonatal developmental periods ovulate fewer eggs in response to exogenous gonadotrophins (52), suggesting a reduced gonadotrophin responsiveness in the ovary.

Although exposure to environmental oestrogenic compounds and their effects on human reproductive function remains uncertain, there is a growing support in the literature demonstrating the adverse effects of environmental chemicals in animal models, as well as wildlife species (62). However, because of the inherent limitations of animal models and the assumption that human exposure to environmental chemicals is frequently assumed to be too low to induce adverse health effects in humans, analogous to those documented in animal models, alternative mechanisms are sought to explore the links between exposure and the adverse effects documented in epidemiological studies. There is a growing concern that exposure to environmental oestrogens during early development can produce irreversible long-term programming of physiology, including adverse effects on reproductive physiology and development (Table 1). Recent research has advanced the knowledge of mechanisms that could explain how exposure to oestrogens during foetal and neonatal development produces long-term alterations in the function of different organs. In this context, we review the epigenetic mechanisms of gene expression that regulate ovarian development and function and provide examples of epigenetic reprogramming that could explain long-term alterations in ovarian function produced by exposure to oestrogenic compounds during development (Fig. 1).

Epigenetic mechanisms and their roles in ovarian development and function

The term epigenetics was first coined by Conrad Waddington in 1942 to describe 'the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being' (63). Today, epigenetics is commonly defined as the study of long-lived but reversible DNA modifications that can change gene expression without changing the DNA sequence (64). There are three major epigenetic mechanisms: (i) DNA methylation, which refers to the methylation of cytosine in CpG dinucleotides and is generally associated with a down-regulation of gene expression; (ii) histone modification, which refers to post-translational modifications of various amino acid moieties in histone core proteins, including acetylation and methylation of lysines; some of these modifications (permissive histone marks) render the regulatory regions of genes more accessible to binding of transcription factors and increase gene expression, whereas others (e.g. suppressive histone marks) render it less accessible and decrease gene expression; and (iii) noncoding RNA (ncRNAs), which refers to functional RNAs that do not encode proteins but generally inhibit the expression of other genes. Noncoding RNAs are classified according to their sizes: microRNAs (miRNAs), which are comprised of 21–25 nucleotides; small RNAs, 100–200 nucleotides; and long ncRNAs > 200–10 000 nucleotides. All three mechanisms collectively and cooperatively modify chromatin structures and regulate gene expression (64–69). The present discussion will focus on the specific mechanisms that regulate ovarian development and function, including germ cell differentiation and epigenetic reprogramming (Fig. 2). The role of epigenetic mechanisms in the development and function of the ovary can be viewed from two perspectives: (i) germ cell epigenetic reprogramming and (ii) follicular formation and development.

Germ cell epigenetic reprogramming

During mammalian development, two major epigenetic reprogramming events occur: one in the pre-implantation embryo and the other in PGCs. Epigenetic programming in the pre-implantation embryo refers to the global erasure of DNA methylation patterns in the maternal and paternal genomes in all loci except imprinted genes and tandem repeats such as intracisternal A particles (70), as well as the re-establishment of new DNA methylation patterns according to the cell lineage fate (71). Although epigenetic modifications continue as the cells and tissues further differentiate, these modifications are limited and remain in the trajectory that is initially established.

However, PGCs are an exception and they undergo a global epigenetic reprogramming at later stage of embryonic development. Specifically, around embryonic (E) day 7.5 in mice, PGCs begin to differentiate within the embryo proper (72), and their initial DNA methylation profile resembles that of the embryonal cells from which they differentiate (71). The PGCs gain germ cell-specific expression patterns between E8.5 and E11.5, during which they migrate and colonise the bipotential gonad, an embryonic structure that differentiates into the testis or ovary depending on the genetic sex. Gonadal sex determines the fate of PGCs, which differentiate into either female or male germ cells. Germ cells in both sexes proliferate rapidly until E13–14, when male germ cells enter mitotic arrest and female germ cells enter meiosis and arrest at meiosis I (73,74). Between E8.5 and E12.5, the genome of PGCs, including the imprinted loci, undergoes a major demethylation process. Although the timing of the demethylation process in male and female germs cells is the same, the remethylation timelines are sex-specific. Remethylation in male PGCs takes place *in utero* several days after erasure between E14.5 and E16.5 and is mostly complete by birth (75,76). By contrast, female germ cell remethylation begins postnatally (PND 1–5) and continues throughout the growth of the oocyte. In addition, imprinting patterns are established based on the size of the oocyte rather than the age of the

Table 1. Endocrine Disrupting Chemicals (EDCs): Mechanism, Source and Effects on Ovarian Function.

Compound/potential targets/source	Dose of exposure/species	Main effects	Reference
Bisphenol A (BPA)/ oestrogen receptor (ESR)/plastics industry	500 μg per pup PND 1–10 daily rat s.c. injection	Alters the hypothalamic-pituitary-gonadal axis in female Sprague–Dawley rats and induces development of follicular cysts in the ovary, leading to infertility. Increases testosterone and oestradiol levels and decreases progesterone	51
	20 μg/kg PND 7–14 daily mice hypodermic injection	Inhibits methylation of imprinted genes during oogenesis via the endoplasmic reticulum signalling pathway, affecting the development of oocytes and folliculogenesis	106
	50 mg or 50 μg/kg PC14 to E9.5 or PC14 to E12.5 daily mice diet	Disturbances in tissue-specific DNA methylation and gene expression in imprinted loci, leading to abnormalities in placental development	78
	0.5 mg/kg E30–90 daily ewe sc injection	Decreases microRNAs targeting *SOX* family genes (sex determination and embryonic development), kit ligand (follicular assembly establishment of reserve pool of primordial follicles) and insulin related genes (follicular development and steroid production)	121
	150 μg/pup PND 1–5 daily mice s.c. injection	Induces polyovular follicles or multi-oocyte follicles (having more than one oocyte in a follicle)	47
	0.05 and 20 mg/kg PND 1, 3, 5, and 7 rats s.c. injection	Reduces primordial follicles and increased growing follicles Primordial and recruited showed increased ERβ and proliferation in recruited follicles	15
	50 μg/kg and 50 mg/kg PND 1 rats s.c. injection	Produces early pubertal onset. Large antral-like follicles and follicular cysts, lower numbers of corpora lutea and acyclicity	54
	2, 20, and 200 μg/kg E1–19 daily mice orally	Dose-dependent, brain region-specific and sex-specific effects of BPA on gene expression of ERs, ERα, ERβ and oestrogen-related receptor-γ that varied across brain regions, and changes DNA methyltransferase (DNMT)1 and DNMT3A in the juvenile cortex and hypothalamus Changes in behaviour: hyperactive phenotype in males and hypoactive phenotype in females	104
	4.4, 44 and 440 μM *in vitro* cultured antral follicles PND 32 mice	BPA (440 μM) inhibits follicle growth. BPA (44 and 440 μM) inhibit progesterone, dehydroepiandrosterone, androstenedione, oestrone, testosterone, and oestradiol production	115
	3 and 300 nM *in vitro* cultured Metaphase II oocytes mice	Exposure to 3 nM BPA was associated with slightly accelerated follicle development, increased allele methylation errors in differentially methylated regions of maternally imprinted genes, and decreased histone H3K9 trimethylation and interkinetochore distance	124
	10 and 100 μg per pup PND 1–5 daily mice s.c. injection	Blocks cyst breakdown and rise polyovular follicles and primordial follicles	46
Methoxychlor/ESR1 ESR2 AR/pesticide	50, 100 or 500 mg/kg PND 3–10 daily rats s.c. injection	Inhibits early ovarian development and stimulates AMH production directly in the ovary	17
	100 mg/kg E19 to PND 7 daily rats i.p. or s.c. injection	Induces hypermethylation of the promoter region of *Esr2* in the adult ovary with a decrease in the ESR2 protein and induces *Dnmt3b* expression in the ovary	101
	0.5 or 1 mg per pup PND 1–14 daily mice i.p. injection	Produces ovarian atrophy, relative ovarian weight loss, depletion of corpora lutea and follicular cysts in 0.5-mg doses	55
	20 μg/kg and 100 mg/kg E19 to PND 7 daily rats i.p. or s.c. injection	Hypermethylation and/or down-regulation in: regulatory subunit p85 of phosphoinositide-3-kinase, insulin-like growth factor-1 receptor, Harvey rat sarcoma viral oncogene, insulin receptor and FOXO3 in the ovary	102
	20 μg/kg and 100 mg/kg E19 to PND 7 daily rats i.p. or s.c. injection	Reduces ovulation and induces early puberty, irregular cyclicity, subfertility and premature reproductive ageing	52

(continued)

Table 1 (continued)

Compound/potential targets/source	Dose of exposure/species	Main effects	Reference
Di-ethylhexylphthalate (DEHP)/ESR androgen production/plastic industry medical devices building products containing polyvinyl chloride	20 and 40 μg/kg PND 7–14 daily mice hypodermic injection	Decreases number of the primordial follicles at pubertal and adult age. Decreases methylation of maternal imprinted genes *Igf2* and *Peg3* in oocytes, which leads to abnormalities in oocyte and embryo development	50
Genistein/ESR/ phytoestrogen (soy)	1 nM to 100 μM *in vitro* cultured ovaries for 7 days PND 1 mice	Impairs the formation of the primordial follicular pool in the ovary	42
	10 and 100 μg/pup PND 1–5 daily mice s.c. injection	Increases the number of multi-oocyte follicles	48
Oestradiol valerate and oestradiol benzoate/ ESR/synthetic oestradiol	10 mg/kg PND 1, 7 and 14 rats s.c. injection	Reduces follicular growth and maturation in the ovary, decreases steroidogenesis, disrupts oestrous cyclicity and impairs reproductive performance	53
	5 mg/kg PND 1–14 daily mice i.p. injection	Induces multi-oocyte follicles in adult ovaries	42
	10 μg/kg PN 1–34 day rats s.c. injection	Advances vaginal opening, and induces multi-oocyte follicles in the ovary	49
	0.1 mg per pup PND 1 s.c. injection	Increased ovarian expression of nerve growth factor (*Ngfb*) and p75 low-affinity neurotrophic receptor (*Ngfr*) mRNAs. Early vaginal opening, disrupted cyclicity, appearance of follicular cyst, absence of corpus luteum, infertility and reduced number of primordial follicles	16
	25 μg per pup PND 1 rats s.c. injection	Early pubertal onset. Ovaries undersized and absence of folliculogenesis. Absense of sexual receptivity after ovariectomy and hormone replacement	54
Diethylstilbestrol/ESR/ synthetic oestrogen	0, 0.75, 1.25, 2.5 or 25 μg per pup PND 1–5 daily Rats s.c. injection	Dose-dependent increase in miR-29a, miR-29b, and miR-29c expression; this resulted in a decrease in DNMT1, DNMT3a and DNMT3b expression in the testes	105
	5 μg/kg PND 1–14 daily lambs s.c. injection	Decline in the stock of primordial follicles, increase in the number of multi-oocyte follicles and an increase in the number of antral atretic follicles	14
	0.2 and 20 μg/kg PND 1,3,5 and 7 rats s.c. injection	Produces a decrease in primordial follicles and increased growing follicles. Increases the incidence of multi-oocyte follicles	15
	1 μg per pup PND 1–5 daily mice s.c. injection	Produces a higher incidence in polyovular follicles and the oocytes from polyovular follicles have a smaller capacity for fertilisation	44
	3 μg per pup PND 1 and 2 mice s.c. injection	Induces the appearance of polyovular follicles	45
	10 and 100 μg per pup PND 1–5 daily mice s.c. injection	Blocks the cyst breakdown and rises polyovular follicles and primordial follicles	46

AMH, anti-Müllerian hormone; E, embryonic day; PND, postnatal day; PC, pre-coital day.

animal (77). Thus, the evidence suggests that, as each follicle is recruited into the growing follicular pool, the imprinting patterns of the associated oocyte are established. Any exposure to environmental chemicals such as BPA during imprinting establishment can disrupt the imprinting patterns and the resultant embryo suffers from altered gene expression at these loci and developmental abnormalities, especially in the placenta (78).

DNA methylation and the enzymatic machinery, namely *de novo* DNA methyltransferases (DNMTs) that establish DNA methylation patterns, play major roles in genomic imprinting (65). Therefore, gonadal expression levels of DNMTs closely follow the timing of the establishment of the imprinting marks (79). Some imprinted loci also involve histone marks (80) and noncoding RNAs (81). During germ cell epigenetic reprogramming, besides imprinted loci, some repeat sequences (e.g. LINE1) lose their methylation marks and are *de novo* remethylated along with imprinted sequences (82). However, sequences with high mutagenic potential such as intracisternal A particles are protected from demethylation, which are considered to mediate transgenerational epigenetic inheritance (83).

Follicle formation and development

The main somatic cell types that are associated directly with follicles (granulosa and thecal cells) are regulated by various epigenetic

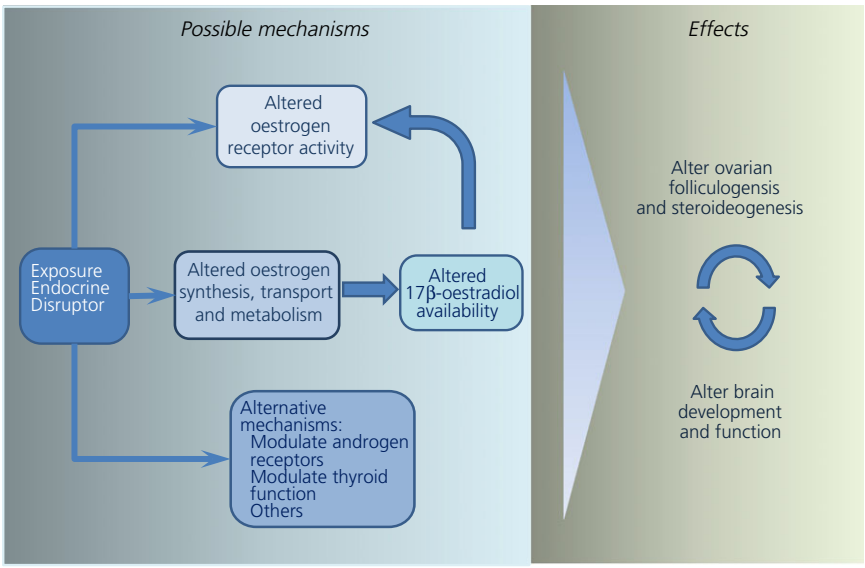

Fig. 1. Developmental endocrine-disrupting chemical exposure and possible mechanisms to alter brain and ovarian functions. Endocrine-disrupting chemicals have different mechanism of actions that mediate the final effects on ovarian function. Among them, the most recognised involve activating oestrogenic receptors either by binding to them or by modifying oestradiol (E_2) availability [e.g. E_2, bisphenol A, methoxychlor (MXC), di(2-ethylhexyl)phthalate (DEHP), diethylstilbestrol (DES)]. Other important mechanisms are the anti-androgenic proprieties of some compounds (e.g. MXC and DEHP) and the thyroid-modulating effects of others (not reviewed). Endocrine-disrupting chemicals can directly target the brain or the ovaries as a result of the presence of oestrogen and androgen receptors in both tissues. However, the mechanisms of regulation between both tissues lead to the possibility that disruption of ovarian physiology affects the brain and vice versa.

mechanisms, including DNA methylation (84), histone modification (85) and noncoding RNA (86) throughout different follicular stages. Steroidogenic factor 1 (*Sf1*), which is expressed in granulosa cells in the embryonic gonad (87), is regulated by promoter DNA methylation at the earliest stage of gonadal differentiation. Although the *Sf1* promoter in the embryonic ovary had no or minimal DNA methylation, it is highly methylated in the embryonic liver or kidney, which reflects the expression patterns of the gene in these organs (88). It is likely that theca cell differentiation also involves epigenetic mechanisms, although relatively little is known regarding the initial cell specification of thecal cell precursors (89).

In the postnatal and adult ovary, epigenetic mechanisms continue to play roles in the differentiation of these two somatic cells. In granulosa cell, down-regulation of inhibin α during luteinisation is closely regulated by histone modification, as well as DNA methylation in the promoter region of this gene (90). By contrast, up-regulation of steroidogenic acute regulatory protein (*StAR*) and down-regulation of aromatase (*Cyp19a1*) following gonadotrophin treatment do not involve DNA methylation but are regulated primarily by histone modification (91). In thecal, cells, when the levels of permissive histone marks are pharmacologically elevated at the promoter region in cytochrome P450 17A1 (*CYP17A1*), gene expression increases, leading to an increase in androgen production (92). By contrast, in bovine thecal cells, the luteinising hormone surge reduces chromatin accessibility of the *CYP17A1* gene and its mRNA levels (93). In addition, patterns of epigenetic marks are determined by the cell types within the follicle, as well as the follicular stage. Although DNA methylation is not involved in preovulatory down-regulation of *CYP19A1* in the follicles, it plays a major role in the permanent silencing of *CYP19A1* in the corpus luteum (94). The roles of miRNAs in various aspects of ovarian development and function, including gonadal differentiation (95), follicle formation (96), selection, maturation (97), ovulation (98) and luteinisation (99), as well as atresia (100), have also been shown.

In summary, the main epigenetic events relating to the ovary that are likely to be vulnerable to adverse effects of environmental factors, including endocrine disruptors, are: (i) the epigenetic reprogramming of female germ cells and the establishment of maternal imprinting patterns and (ii) the initial differentiation and continuous adaptation of somatic components of ovarian follicles to the various stages of folliculogenesis and corpus luteum function. Experimental evidence supporting these potential epigenetic events is provided below. In addition, it is important to note that the epigenetic reprogramming of the early embryo is also vulnerable to the influence of environmental factors, including endocrine disruptors. The consequence of this influence would not only be on the ovary and female germ cells, but also on the entire developing organism (78).

Effects of environmental oestrogenic compounds on epigenetic regulation of gene expression in the ovary and its relationship with ovarian function and development

Emerging evidence suggests changes in epigenetic control of gene expression as a potential mechanism underlying long-term and irreversible alterations in ovarian physiology arising from developmental exposure to endocrine disruptors (62,101,102). Owing to the

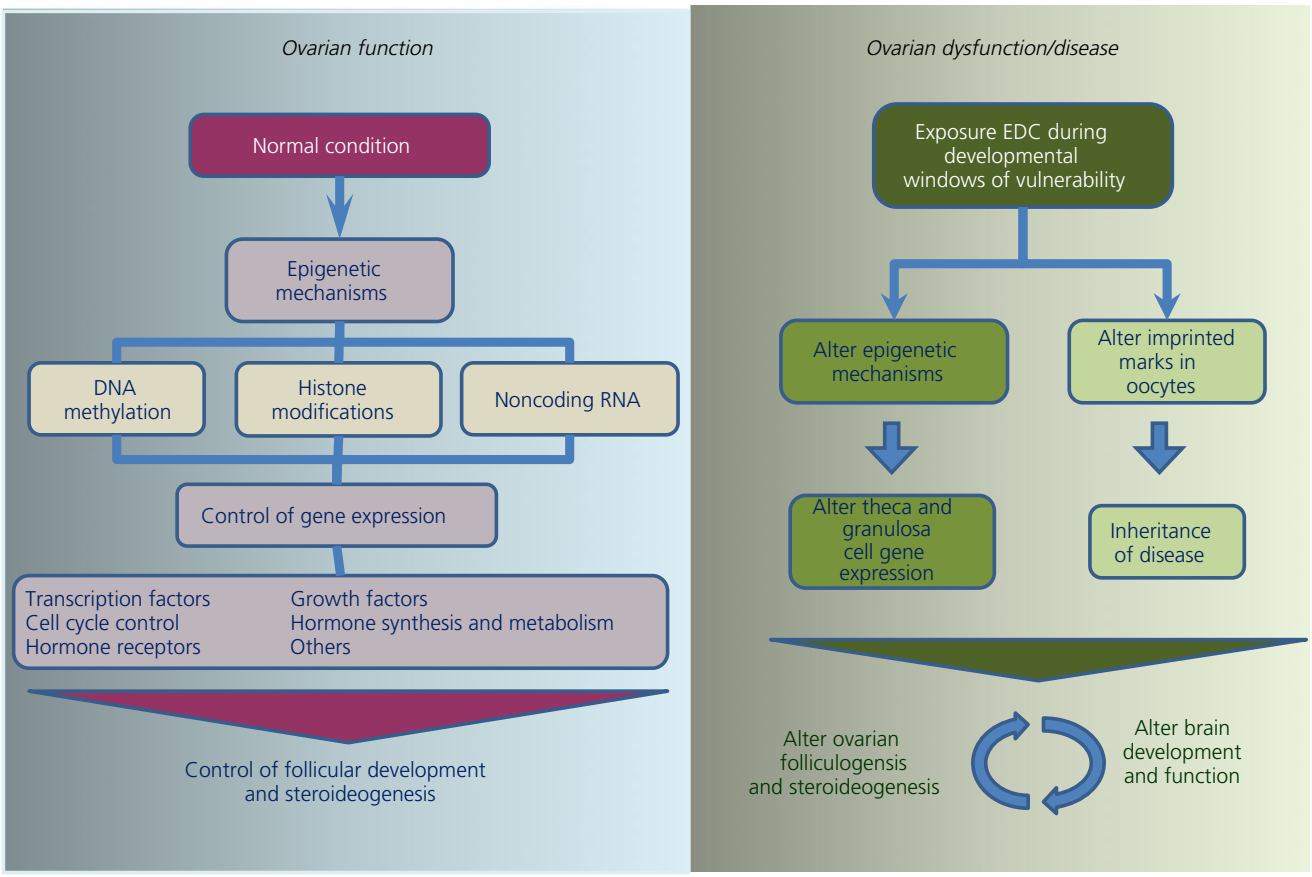

Fig. 2. Developmental endocrine-disrupting chemical (EDC) exposure and potential epigenetic mechanisms in altered brain and ovarian function. The normal ovarian development and function are strictly regulated by precise epigenetic mechanisms (DNA methylation, histone modifications and microRNA) that coordinate the spatiotemporal expression of key genes. These genes are transcription factors, cell cycle proteins, growth factors, hormone synthesis enzymes and receptors, which participate in different stages of development of the follicles. Exposure to environmental oestrogens alters these mechanisms of control, affecting gene expression in the ovary and leading to dysfunction and disease.

central role of oestrogen receptors (ESR) in the ovary during development (103), cells of ovarian follicles are considered as potential targets of disruption by endogenous or environmental oestrogens. Exposure to environmental chemicals with hormone-like activity in early life, such as MXC, BPA, oestradiol benzoate and di(2-ethylhexyl)phthalate (DEHP), produces changes in the expression of DNMT in different tissues (50,101,104–106). DNMT can affect the patterns of methylation of promoter regions of genes, usually resulting in the repression of gene expression. Methylation of CpG island or promoter regions decreases the accessibility of transcription factors to the binding sites in the DNA. If the binding of a stimulating transcription factor to the DNA is blocked, the gene down-regulates. Conversely, if the binding of a transcription repressor is blocked, the gene up-regulates. Ovarian exposure to MXC, BPA and DEHP has been shown to alter the expression of DNMT, resulting in potential alterations to the epigenome (50,101,106). The ovary is regulated by a myriad of transcription factors (107), which in turn regulate the expression of genes related to hormone action, extrinsic innervation and paracrine growth factors. All these regulators act coordinately to promote follicular development, ovulation and ovarian hormone production. As may be predicted, changes in DNMT can deregulate these highly coordinated inputs and alter ovarian physiology. Despite this, the mechanism that explains how an exposure in early life produce a change in the expression of genes that can persist until adulthood, in the absence of the stimuli, remains unknown.

Long-term ovarian alterations produced by different environmental signals during foetal and early postnatal development could result from an adaptive or maladaptive response of the body to the environmental signal. In this context, it was shown that exposure to chemicals with hormone-like activity in early life leads to long-term alterations in expression of critical genes, such as ESR2, in the ovary. One such chemical is chlorinated organic pesticide MXC. Although it is currently de-registered by the Environmental Protection Agency in the USA, MXC is an excellent model endocrine disruptor since its major metabolites (i.e. HPTE and mono-OH-MXC) act as ESR1 agonists, as well as ESR2 and androgen receptor antagonists (108). These activities represent many hormone-like chemicals in the environment. Exposure to MXC during foetal and neonatal development produces hypermethylation of the promoter

region of *Esr2* in the adult ovary with a decrease in the ESR2 protein (101). This decrease in ESR2 could produce a lower response to endogenous E_2 in adulthood and, as a consequence, an alteration in follicular development. Direct effects of E_2 that favour cell proliferation and follicular development have been demonstrated (109); however, much of the paracrine regulators of folliculogenesis and its receptors have oestrogen response elements (EREs). The expression of these paracrine factors can be affected by this permanently altered response to E_2 through the oestrous cycle. For example, AMH, which is produced by granulosa cells of growing pre-antral follicles, has EREs and is increased in adulthood after exposure to MXC or BPA in early life (17,110). E_2 may have a repressive effect on *Amh* expression and the high expression of *Amh* could be the result of lower response to E_2 during the oestrous cycle in adulthood. Alternatively, exogenous oestrogens may stimulate *Amh* expression through the ERE, leading to an increase in AMH (111). AMH has a repressive effect on early follicular development and primordial follicle recruitment, as well as FSH-induced follicular maturation (112–114), which is consistent with the findings of a lower number of developing follicles in different models of oestrogenic disruption (16,17,53,115).

Other genes subject to epigenetic control are important in the regulation of ovarian function and are permanently modified by developmental exposure to oestrogenic compounds (102) including insulin-like growth factor-1 receptor (IGF-1R). IGF-1R agonism leads to activation of phosphoinositide 3-kinase/Akt and mitogen-activated protein pathways, which is related to the protection of cells from Fas-L induced apoptosis and stimulation of granulosa cell proliferation (116,117). IGF-1R is down-regulated in adult rats exposed to MXC in the neonatal period. This is consistent with the phenotype of MXC-treated animals, which show reduced follicular development (17). Interestingly, IGF-1 secretion is influenced by FSH (118) and hence some of the effects of FSH on follicular development are mediated by IGF-1. In this context, the lower expression of IGF1-R in MXC-treated rats could produce a lower response of the ovary to the effects of FSH. Sympathetic innervation of the ovary is also considered to be important in ovarian regulation (119). Norepinephrine activates the β-2 adrenoreceptor and increases the expression of FSHR favouring follicular development (120). The β-2 adrenoreceptor gene is hypermethylated after early exposure to MXC (102). Other genes of intracellular proteins important for cell signalling pathways, such as *Pik3r1*, *Prkar2a*, *Pak3* and *Foxo3a*, are hypermethylated in the adult rat ovary after early exposure to MXC (102). On the other hand, exposure to DEHP decreases methylation of maternal imprinted genes *Igf2* and *Peg3* in oocytes, which leads to abnormalities in oocyte and embryo development (50). In the same way, BPA is also known to alter the methylation pattern of *Igf2* and *Peg3* in oocytes, affecting the development of oocytes and folliculogenesis (106). In addition, daily exposure to 10 mg BPA per kg body weight from 2 weeks prior mating to E9.5 or E12.5, which corresponds to germ cell and early embryonic epigenetic reprogramming, produces a tissue-specific DNA methylation and gene expression disturbances in imprinted loci (*Snrpn*, *Ube3a*, *Igf2*, *Kcnq1ot1*), leading to placental abnormalities at E12.5 in mice. Daily exposure to 10 μg BPA per kg bodyweight, a dose considered to be safe for humans, was also effective in causing alteration at some of these loci. Importantly, the effects of both doses were stage-dependent: a daily exposure between E5.5 and E12.5 had no observable effect (78).

Exposure to environmental oestrogens during pregnancy can also modulate ovarian miRNA expression in the foetus (121). Exposure to BPA in sheep produces a decrease in miRNAs targeting Sry-related high-mobility-group box (*SOX*) family genes, kit ligand and insulin-related genes (121). *SOX* genes are implicated in sex determination and embryonic development (122), whereas Kit ligand signalling is important for follicular assembly and the correct establishment of the reserve pool of primordial follicles (123). Silencing of Kit ligand by miRNA may result in an abnormal follicular assembly and could explain the defects observed in animals exposed to environmental oestrogens. On the other hand, the silencing of insulin-related genes such as those for IGF-1 and IGF-2 could explain the phenotype of reduced follicular growth in animals exposed to environmental oestrogens during foetal and neonatal development. With regard to histone modification, BPA produces a significant decrease in histone H3K9 trimethylation, a repressive histone mark, in cultured follicles (124); however, the *in vivo* effects of developmental exposure to oestrogenic compounds on histone modification in the ovary are not known. Regardless, recent studies have shown that developmental exposure to a phyto-oestrogen, genistein, affects histone marks in the uterus, leading to alteration in gene expression and abnormal growth during adulthood (125).

In addition to the epigenetic reprogramming of ovarian somatic cells after exposure to environmental oestrogens, the PGC can also be a target of epigenetic disruption (50,106). These epigenetic alterations could potentially damage the function of oocytes affecting both oogenesis and embryogenesis. Another consideration of epigenetic modifications of PGCs by environmental oestrogens relates to whether these modifications of the epigenome are hereditable to future generations. There is little information available about the transgenerational inheritance of epigenetic modifications in ovarian follicular development after exposure to oestrogenic compounds (126). The possibility that oestrogens can produce a transgenerational inheritance of ovarian disease through the permanent alteration of germ cell epigenome needs to be considered.

Conclusions

Exposure to environmental oestrogens has been of concern because of its potential impact upon the development and function of the ovaries, reproductive tract and other oestrogen-sensitive tissues, such as the brain. Because foetal and neonatal developmental stages are sensitive periods, exposure to oestrogenic compounds could produce irreversible epigenetic reprogramming of ovarian genes that control ovarian follicular development, which, in turn, might lead to abnormalities in adulthood. Studies in animal models have shown that developmental exposure to environmentally relevant levels of these chemicals can produce changes in epigenetic mechanisms and gene expression changes (50,101,102,106,121,124). In some of these studies, these changes are also associated with ovarian dysfunction (11,50,106). In the future, it will be important

to: (i) establish causal links among epigenetic alterations and gene expression and functional changes in the ovary; (ii) determine whether these chemicals can induce additional epigenetic alterations, gene expression changes and ovarian dysfunction or whether additional environmental chemicals can cause similar changes in the ovary; and (iii) determine whether human females with reproductive diseases/dysfunction display similar patterns of epigenetic alterations that have been demonstrated in animal models. Finally, the possibility that the effects of the oestrogenic chemicals can be transmitted through epigenetic reprogramming of germ cells affecting ovarian follicular development should also be assessed.

Acknowledgements

This work was supported in part by National Institute of Environmental Health Science grant ES017847 and ES017059 and NIEHS Center grant ES005022 to MU, Fondecyt grant 1120147 to AP and Fondecyt grant 11130707 to GC.

Received 13 March 2014,
revised 22 June 2014,
accepted 15 July 2014

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