

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

## Endocrine Active Compounds Actions during Neonatal Period: Effect on the Ovary

---

Maria Slomczynska, Malgorzata Grzesiak and  
Katarzyna Knapczyk-Stwora

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69220>

---

### Abstract

Many female reproductive disorders observed during adulthood originate from the neonatal period, which is a critical stage toward the reproductive potency. Human and animal fertility are determined by the pool of primordial follicles that are established during fetal or neonatal life. The earliest stages of follicle development are under control of a variety of factors, including sex steroids. Neonatal period is a “critical developmental window” in which organisms are susceptible to the environmental chemicals that may affect the reproductive health. Endocrine active compounds (EACs) are found abundantly in the environment and interfere with sex steroids (predominantly androgens and estrogens) by either mimicking or blocking their functions. This review covers the current knowledge about the effects of selected EACs with androgenic (testosterone propionate), anti-androgenic (flutamide), estrogenic (diethylstilbestrol, bisphenol A, 4-tert-octylphenol, phthalates and genistein), anti-estrogenic (ICI 182,780 and parabens), or mixed activity (methoxychlor) on the ovary of neonates, focusing on their effects on the early stages of folliculogenesis. These chemicals have been shown to affect oocyte survival, follicle formation, and growth, as well as steroidogenic functions. The better cognition of mechanisms underlying the long-term consequences of the neonatal EACs exposure may in future lead to an understanding of human health risks and developing prevention strategies.

**Keywords:** endocrine active compounds, estrogens, anti-estrogens, androgens, anti-androgens, ovary, neonatal window

---

## 1. Introduction

Considerable evidence demonstrates that inappropriate steroid exposure during pre- and postnatal developmental periods may have long-term effects on the adult reproductive functions [1]. Female fertility is determined by the pool of primordial follicles within the ovary that is established during fetal or neonatal life. The earliest stages of follicle development are tightly coordinated by numerous factors, including steroids. Importantly, these fetal/neonatal periods are “critical developmental windows” in which organisms are susceptible to the environmental chemicals that may affect ovarian formation and disturb reproductive functions during adulthood, including conception rates, maintenance of pregnancy, and reproductive disorders [2]. Endocrine active compounds (EACs) comprise a wide variety of synthetic or natural chemicals found in the environment arising from anthropogenic, industrial, agricultural, and domestic sources. EACs may interfere with the natural regulation of endocrine system by either mimicking or blocking the function of endogenous hormones and also they may act directly on gene expression by means of epigenetic modifications [3]. This is of concern because women are exposed to EACs on a daily basis, and some EACs are known to target the ovary and cause reproductive health problems. However, the harmful effects of EACs during neonatal period may occur with exposure to much lower doses than those considered harmful to adults [4]. Although many disrupting chemicals are excluded from the everyday use since their dangerous properties have been described, they may persist in the environment for long periods of time. Furthermore, new products with similar properties are still introduced to the market. Therefore, these compounds have currently been one of the greatest concerns all over the world. To assess the effect of EACs on human ovarian and consequently reproductive functions, the animal models are extensively used in experimental studies.

In this chapter, we aim to point out a possible impact of androgens/estrogens excess or deficiency on neonatal ovary following EACs action. The effect of EACs with androgenic (testosterone propionate), anti-androgenic (flutamide), estrogenic (diethylstilbestrol, bisphenol A, 4-tert-octylphenol, phthalates and genistein), anti-estrogenic (ICI 182,780 and parabens), or mixed activity (methoxychlor) will be discussed.

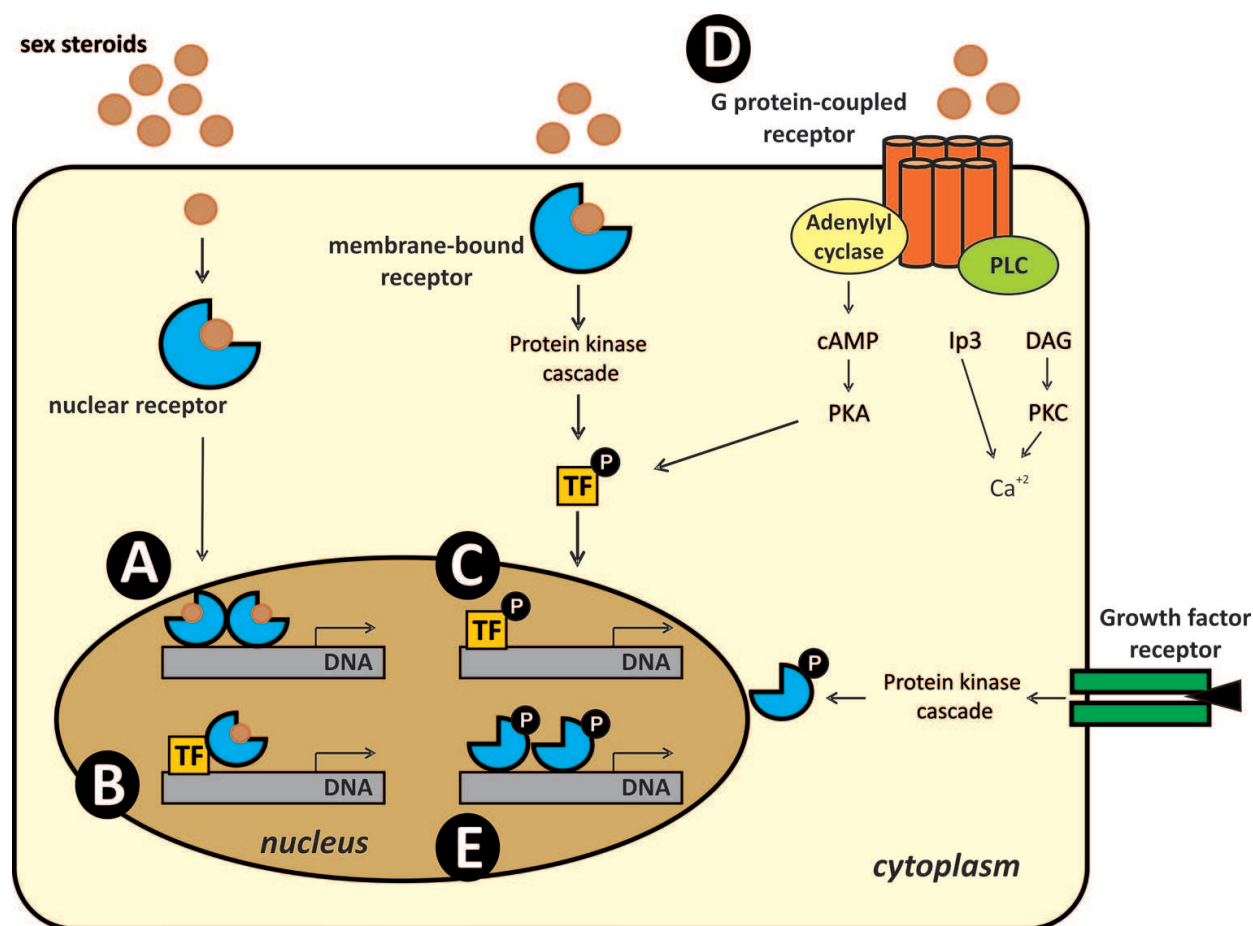
## 2. Mechanism of steroids action

The action of androgens/estrogens, and their agonists or antagonists, depends on the presence of specific receptors in target cells. The types of receptors that are involved in the signal transduction decide on the signaling pathway. The effect of sex steroid hormones action within the cell may be genomic or nongenomic. A genomic response is usually induced by nuclear/cytoplasmic receptors, while nongenomic pathway is activated by membrane receptors, mostly G-protein-coupled receptor. It is now evident that there is crosstalk between nongenomic and genomic signaling pathways [5].

Steroid hormone receptors, which belong to the superfamily of nuclear receptors, are hormone-activated transcriptional factors. They are modular proteins consisting of C-terminal ligand-binding domain (LBD), highly conserved DNA-binding domain (DBD) with centrally located zinc fingers, and N-terminal domain [6]. Activation of these receptors is mediated by two transcriptional activating domains, AF-1 and AF-2. AF-1, characterized by a ligand-independent transcriptional activation, is localized in the N-terminal region of the receptor, while AF-2, activated by ligands, is localized within the LBD. The LBD and DBD of nuclear receptors are conserved regions, whereas the N-terminal domain is highly variable but important for full transcriptional activity [7]. The latter contains many sites for Ser/Thr phosphorylation, which may be involved in mediating cross-talk with other signaling pathways leading to modulation of AF-1 activity and interaction with coregulators [8].

Sex steroid hormones can freely diffuse across the plasma membrane and bind to their cognate receptors inducing dissociation from the heat-shock proteins. Ligand binding to LBD region causes conformational changes followed by the dimerization of receptors and its translocation into the nucleus. The dimer binds to the hormone response elements (HREs) typically located in the promoter of the target gene and leads to the recruitment of coregulators, either coactivators or corepressors [9]. However, some nuclear receptors (i.e., estrogen receptors, and ERs) are capable of binding to DNA sequence, lacking the classic HRE sequence. Such receptors associate with the components of the AP-1 transcription factor complex (i.e., c-Jun and c-Fos). Additionally, in the low steroids concentration, the ligand-independent signaling pathway occurs allowing the activation of receptors. This process depends on the growth factor receptors and involves MAPK/ERKs pathway, which enhances transcriptional activity through direct phosphorylation of steroid receptors [10]. These pathways are known as “genomic pathway” and are characterized by the regulation of the expression of specific sex steroid receptor-regulated genes [11].

Although steroids exert most of their biologic activity through direct or indirect genomic effects, there are several examples that do not fit to this regulatory scheme. This pathway known as “nongenomic” is much more rapid and involves receptors localized in the plasma membrane or in “lipid rafts” [12]. All sex steroid hormones may stimulate rapid effects in signal transduction pathway through the production of second messengers, ion channels transport, and protein kinase cascades. Rapid effects of androgens and estrogens have been reported in many cell types [13, 14]. Conventional steroid receptors located in the membrane trigger the signal transduction cascade by activation of the Src/Ras/Raf/MAPK/ERK1/2 pathway [15]. Furthermore, steroids may mediate rapid signaling by binding to transmembrane receptors unrelated to nuclear hormone receptors (usually, G protein-coupled receptor, GPCR) and to the modified forms of steroid receptor or to receptors for neurotransmitters. The involvement of the type of receptor may depend on the steroid and cell type [16]. GPCR activates a protein kinase cascade or acts at the level of secondary messengers such as PI3K/AKT/mTOR, PI3K/AKT/cAMP/Ca<sup>2+</sup> or ERK-mediated pathways like PI3K/AKT/MAPK-ERK/Elk1 [17]. Among GPCR, there are GPR30 for estradiol, mPR for progesterone, and GPRC6A and ZIP9 for androgens [18–21]. All the abovementioned mechanisms of steroid action are presented in **Figure 1**.



**Figure 1.** Molecular mechanism of the action of sex steroid hormone receptors. (A) Direct genomic effects. (B) Indirect genomic effects. (C) TF activation triggered by membrane receptor. (D) Nongenomic effects triggered by G-protein-coupled receptor. (E) Ligand-independent effects. TF, transcription factor; cAMP, cyclic AMP; PKA, protein kinase A; PLC, phospholipase C; IP3, inositol 1,4,5-triphosphate; DAG, diacylglycerol; PKC, protein kinase C. Based on Laurentino et al. [22].

### 3. The role of androgens and estrogens in the ovarian development

Primordial follicle assembly and the primordial-to-primary follicle transition are the major developmental events within the ovary [23]. The primordial follicle pool represents the total population of germ cells available during reproductive life and, therefore, determines the fertility potential of the female. In mammals, mitosis of oogonia and initiation of meiotic prophase I occur before birth, but the timing of oocyte meiotic arrest, follicle formation, and the initiation of follicle growth varies among species. During fetal ovarian development, primordial germ cells (PGSs) migrate from the endoderm of the yolk sac to the bipotential genital ridges. In mice (reviewed in Ref. [24]), which is a valuable animal model for studying embryogenesis, PGSs migrate into the undifferentiated gonad from around 8.5 day *post coitum* (dpc) to 11.5 dpc. The genital ridge is identical in both males and females and remains bipotential until sex determination (12.5 dpc). PGSs continue to proliferate mitotically during the migration and after arrival in the genital ridge until 12.5–13.5 dpc in mouse and 6th week of pregnancy in human. Then, at 13.5 dpc, in the mouse and between 8 and 13 weeks of gestation in human, meiosis is initiated, allowing oogonia to develop into oocytes that are arrested at the early diplotene phase of meiotic prophase I (reviewed in Ref. [25]). Oocytes



initially develop as large, interconnected clusters of cells called oocyte nests. They are organized into long ovigerous cords surrounded by somatic or pregranulosa cells [24]. In rodents, oocyte nests break down in neonatal ovary that leads to the formation of primordial follicle. This is mediated by oocyte apoptosis starting at 16.5 dpc in mice. In addition, somatic cells can mediate nest breakdown by moving into the nest and intersperse between the remaining oocytes to form primordial follicles [26]. In the mouse ovary, the primordial follicles consist of an oocyte surrounded by a single layer of squamous pregranulosa cells that are formed fairly synchronously between 17.5 dpc and 4 postnatal day. Once assembled, some of the primordial follicles are immediately stimulated to growth, but most of them remain quiescent until selected follicles are gradually recruited into a growing follicle pool throughout reproductive life [27]. The recruitment of primordial follicles into growth (primordial-to-primary follicle transition) involves a change in the shape of the granulosa cells from squamous to cuboidal and the initiation of oocyte growth. The primordial-to-primary follicle transition is an irreversible process. Most of the growing follicles (99.9%) are destined to become atretic, and the rest will ovulate [28]. Around the end of the first postnatal week, some secondary follicles are present in the mice ovary [29].

In contrast, in humans, ruminants, and pigs, follicle formation and the initiation of follicular growth are much less synchronous than in rodents and take place during fetal life. Although cattle and human have similar gestation lengths, follicle assembly in cattle was observed earlier (around 80 dpc) than in human (112–133 dpc). Besides, in bovine ovaries, the first primary and secondary follicles were observed around 140 and 210 dpc, respectively. No secondary follicles were observed in the fetal human ovary [30]. In pig, primordial follicle formation was observed between 70 and 90 dpc, while primordial to primary follicle transition was between 90 dpc and postnatal day 1 [31]. Interestingly, even antral follicles were present before birth in the sheep ovaries [32].

The early stages of folliculogenesis are believed to be gonadotropins-independent. All events related to early follicular development during fetal/neonatal period are mostly regulated by paracrine growth factors originating from the growing oocyte itself and from the somatic cells that surround it [33], and also by ovarian steroid hormones (i.e., progesterone, androgens, and estrogens). Although the pivotal role of androgens in the development of male reproductive organs is well understood, growing evidence supports the direct involvement of these hormones in female reproduction, including early follicular development [34]. The androgen receptor (AR) role in the female is implicated from the studies of various global and tissue-specific AR knockout (ARKO) mouse models. Granulosa cell-specific ARKO (GCARKO) mouse models have demonstrated that granulosa cells are important sites for androgen action and strongly suggested that the AR in these cells is an important regulator of androgen-mediated follicular growth and development. On the other hand, AR inactivation in the oocyte, as shown in the OoARKO female mouse model, appears to have no major overall effect on female fertility [35]. Fowler et al. [36] reported that the oocyte of the primordial follicles was able to synthesize androgens and that pregranulosa cells expressed AR in human fetal ovaries. Initiation of primordial follicle growth was stimulated in mouse, bovine, and primates ovaries by testosterone and dihydrotestosterone [37–39], while in sheep by dehydroepiandrosterone [40]. In general, androgens stimulate the primordial-to-primary follicle transition but impede the later stages of follicle development.

Studies using ER $\alpha$  knockout ( $\alpha$ ERKO) mice have demonstrated that the lack of ER $\alpha$  does not appear to affect folliculogenesis until the preantral stage of follicle development [41]. Similarly, in ER $\beta$  knockout ( $\beta$ ERKO) mice, 17 $\beta$ -estradiol (E2) does not appear to be essential for the establishment of germ cell number or ovarian development [42]. However, Kezele and Skinner [43] have shown that both progesterone (P4) and E2 inhibit oocyte nest breakdown. It has been shown that fetal ovarian P4 and E2 concentrations decreased during the time as follicle assembly was initiated in the bovine ovary, while the maternal blood P4 and E2 levels were stable [44]. Recent work by Dutta et al. [45] also revealed that fetal mice produce their own steroid hormones to coordinate primordial follicle development. Notably, E2 promotes primordial follicle formation in hamster [46]. These data indicate that both P4 and E2 are important regulators of ovarian follicle assembly. Furthermore, E2 is likely to inhibit primordial-to-primary follicle transition, which was revealed in neonatal rat ovaries [43, 47]. In contrast, the lack of E2 in aromatase knockout (ArKO) mice was associated with decreased numbers of primordial and primary follicles [48]. Similarly, the primordial follicles number was reduced in estrogen-depleted fetal baboon ovaries [49]. These results indicate that although E2 is needed for early events in folliculogenesis, its excessive level inhibits these events. Abnormal control of primordial follicle assembly may affect the reproductive potential of the female and can lead to pathological conditions such as premature ovarian failure.

#### 4. Effects of endocrine active compounds within the neonatal ovary

Many female reproductive disorders observed during adulthood originate from the neonatal period, which is a critical stage toward the reproductive potency. Consequently, it is important to study the effects of neonatal exposure to chemicals that mimic or antagonize the effects of estrogens and androgens to establish their role in ovarian function. Recent attention has been especially focused on environmental factors interfering with endogenous steroids action. There are synthetic and natural environmental compounds such as pesticides (i.e., dichlorodiphenyltrichloroethane (DDT); methoxychlor (MXC), vinclozolin, and atrazine); detergents and surfactants (4-tert-octylphenol, nonylphenol, and bisphenol A); plastics (phtalates); industrial compounds (polychlorinated biphenyl, PCB; 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD); and fitoestrogens (genistein and coumestol) [4]. In the light of growing body of evidence that demonstrates the presence of EACs in the environment, understanding the mechanism of selected EACs action within neonatal ovaries provides a basic data for further research of the female reproductive potency. The susceptibility of target tissues is related to the stage of development, the exposure dose, and the individual immune status. Mammals are more sensitive to EACs during fetal and postnatal life than in adulthood. However, environmental influence during fetal and neonatal development may lead to adult onset pathology.

##### 4.1. EACs with androgenic and anti-androgenic properties

Previous data on primates clearly showed the essential role of androgens in promoting early follicular growth [50]. These results were confirmed by *in vitro* study on bovine ovarian follicles, indicating that testosterone stimulated the primary-to-secondary follicle transition

[38]. Importantly, testosterone increased the number of secondary follicles with no impact of estradiol that suggested the direct effect of androgens without conversion to estrogens. Testosterone promotes the growth of bovine follicles *in vitro*, and its stimulatory effect was mediated through ARs in the follicular cells [38]. Thus, reprogramming of early folliculogenesis by androgens excess or deficiency seems to be crucial for understanding their adverse effects on female fertility.

Studies on sheep [51, 52] and primates [53] have shown that excess of androgens in fetal life resulted in reduced ovarian functions in adult life and may provide models for polycystic ovary syndrome (PCOS) in women. Research by Tyndall et al. [54] was undertaken to investigate the effects of different windows of testosterone propionate treatment during fetal or neonatal life in female rats to determine whether and when excess androgen exposure would cause disruption of adult reproductive functions. The findings from fetal exposure to testosterone propionate suggested the possibility of reducing the sensitivity threshold to neonatal androgens exposure in female animals. The results support the concept that androgen programming of adult female reproductive function occurs only during specific time windows in fetal and neonatal life with implications for the development of PCOS in women.

Neonatal androgenization by testosterone propionate induced early misprogramming of ovarian functions in the female rats [55]. The obtained results strongly suggested that transient neonatal hyperandrogenemia led to ovarian dysfunctions marked by altered steroidogenesis and folliculogenesis. Exposure to testosterone propionate resulted in the increase of primary and the decrease of antral follicle frequencies. Moreover, the higher proportion of atretic follicles and the lack of corpora lutea within the ovaries from testosterone propionate-treated rats were observed. Another research on rats revealed that exposure to excess of testosterone in neonatal period increased the LH and testosterone serum levels, the LH/FSH ratio, ovarian theca-interstitial area and expression of steroidogenesis-involved genes, and *Lhr* and *Cyp17a1* in ovaries of adult animals [56]. These results provide some insight into the role of androgens on reproductive development and on the manifestations of clinical disorders such as PCOS.

Among EACs, there is a large group of chemicals exerting anti-androgenic effects and blocking endogenous androgens action. In our previous experiments, the androgen involvement in early stages of folliculogenesis was confirmed using *in vivo* animal model generated for studying androgen deficiency. We have utilized flutamide (2-methyl-N-[4-nitro-30-(trifluoromethyl)-phenyl] propamide), which is a nonsteroidal anti-androgen acting at the AR level and blocking androgen action. Flutamide promotes AR translocation to the nucleus and DNA binding, but nevertheless fails to initiate transcription, inhibiting the AR signaling pathway [57]. Although this is a pharmaceutical compound, it is regarded as a model anti-androgen in experimental studies [58].

In our recent research, flutamide (50 mg/kg body weight) was injected into pregnant gilts during gestational days 20–28 and 80–88 and into female piglets on postnatal days 2–10. We have found ovarian morphological changes indicating delayed folliculogenesis in neonatal pigs exposed *in utero* to flutamide [59]. Abnormal folliculogenesis was also seen in prepubertal pigs, following prenatal period of anti-androgen exposure [60], whereas in adult



animals, only neonatal time of flutamide treatment exerted disturbances marked by altered luteinization and corpus luteum cyst formation [61]. Apart from morphological disturbances, the altered follicular functioning in adulthood was manifested by changes in production of steroids (estradiol and androgens) and gonadotropins (FSH and LH), as well as in expression of genes involved in proper ovarian functioning, including AR, FSHR, aromatase, connexin 43,  $\beta$ -catenin, and aquaporin 5 [61–63]. Furthermore, gestational or neonatal exposure to flutamide affected proliferation and apoptosis rates in large antral follicles of adult porcine ovary, which might influence the normal development of the follicles and pigs fertility as a consequence [64].

It seems that disturbed androgen action during gestational and neonatal periods by using flutamide led to reprogramming of the trajectory of ovarian development in pigs; however, neonatal window of exposure leads to long-term effects observed in adulthood.

#### 4.2. EACs with estrogenic and anti-estrogenic properties

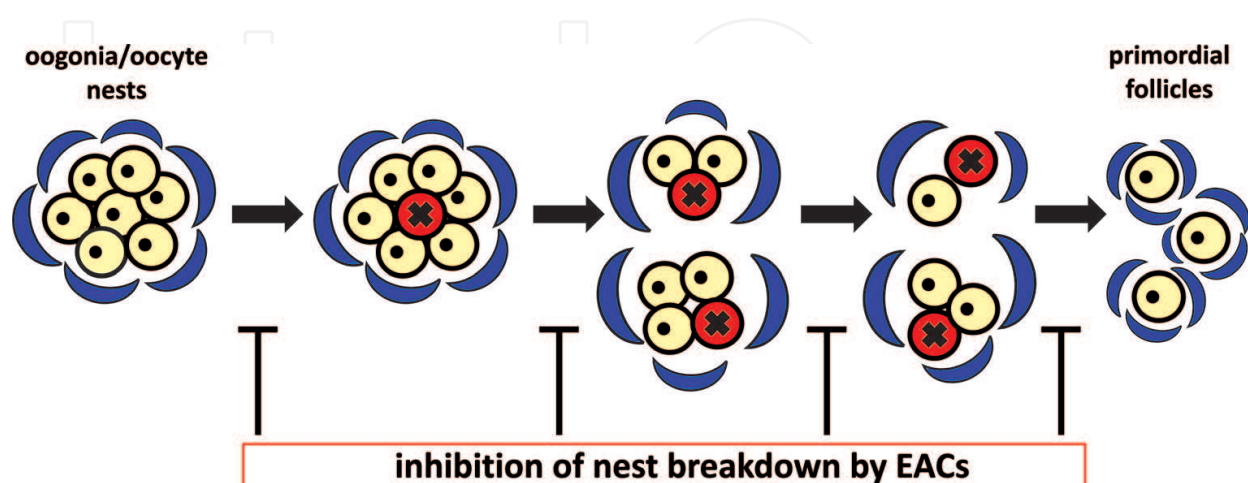
Compounds that mimic estrogens action through an interaction with ERs continue to receive considerable attention. One of them is 4-tert-octylphenol, which belongs to alkylphenol polyethoxylates derivative, which is a nonionic surfactant widely used in a variety of industrial applications [65]. It was demonstrated that pre- or postnatal exposure to 4-tert-octylphenol can advance the onset of puberty in rats [66] and pigs [67]. In addition, in a three-generation study, 4-tert-octylphenol given prenatally to sows induced some effects, including reduction in litter size, observed in the next generations [67]. Furthermore, 4-tert-octylphenol exposure during fetal and postnatal life accelerated the onset of puberty but did not disrupt FSH concentration and the dynamic of ovarian follicular growth in ewes [68]. On the other hand, research on rats revealed that neonatal administration to 4-tert-octylphenol altered hypothalamo-pituitary-ovarian axis resulting in atrophic and polycystic ovaries without corpora lutea [66]. In our most recent experiments using neonatal pigs treated with 4-tert-octylphenol, the advanced folliculogenesis has been shown. We assumed that these specific effects on folliculogenesis in neonatal pigs were characterized by changes in proliferation and apoptosis rates during initial follicular recruitment [69].

Both bisphenol A and diethylstilbestrol are another of the extensively studied EACs with estrogenic activity. Bisphenol A is a plasticizer used commonly in a wide range of consumer products such as food and drink containers, epoxy resins, plastics, baby bottles, thermal receipts, and dental sealants (reviewed in Ref. [70]). Zhou et al. [71] revealed that bisphenol A exposure significantly inhibited germ cell nests breakdown by altering the expression of key ovarian apoptotic genes in cultured neonatal mouse ovaries. Interestingly, exposure of neonates to estrogenic compounds like bisphenol A significantly increases the number of multiple oocyte follicles (MOFs) in adult mice ovaries. It was proposed that neonatal exposure to exogenous estrogens blocked cysts breakdown resulting in oocytes survival [72]. Apart from abovementioned influence of bisphenol A on oocyte nests, additional effects on the follicle development were found. Bisphenol A may interact with multiple factors produced by oocyte and granulosa cells to alter neonatal follicular dynamics. [73, 74]. It was shown that this compound reduced the primordial follicle pool by stimulating the initial recruitment in neonatal

rats associated with an increased proliferation rate, which is likely mediated by an estrogenic pathway [75]. It could also decrease follicle activation in neonatal mice [76]. Additionally, the effect of neonatal exposure to bisphenol A on the reproductive axis was observed in adult rats, which may lead to the development of PCOS [77].

Diethylstilbestrol is a synthetic nonsteroidal estrogen. Studies on neonatal rodents injected with diethylstilbestrol demonstrated results similar to those of bisphenol A. Neonatal exposure to diethylstilbestrol of mice and hamster inhibited germ cell nest breakdown and induced MOFs formation, likely by interfering with the ER $\beta$  pathway and inhibiting programmed oocyte death and germ cell loss [78, 79]. Rodríguez et al. [75] used a relatively low concentration of diethylstilbestrol (20  $\mu$ g/kg body weight) in the rat and found that primordial follicle activation was increased compared to studies of Karavan et al. [80] on mice, where the higher diethylstilbestrol concentration (100  $\mu$ g/kg body weight) decreased the follicle activation. Both diethylstilbestrol and bisphenol A reduced follicle growth and development, resulting in the presence of more primordial follicles and fewer primary and secondary follicles in mouse neonatal ovary [80]. In prepubertal lambs treated with bisphenol A or diethylstilbestrol during neonatal period, the reduction of the primordial follicle pool and increased atresia was observed. That was surprising since nest breakdown in sheep is completed before birth. However, it seems that the reduction of the primordial follicle pool is due to stimulation of their initial recruitment and subsequent development until antral stage. These alterations may affect the ovarian function in the adulthood [81].

Genistein is a natural phytoestrogen with estrogenic activity found in soy products. The belief that estrogens can regulate nest breakdown is supported by a study of Jefferson et al. [82]. They have demonstrated exposure of neonatal mice to genistein, which inhibited the oocyte nests breakdown and increased oocyte survival. Moreover, ovaries from adult mice treated as neonates with genistein have an increased occurrence of MOFs, suggesting incomplete nests breakdown [83]. This indicates that cell survival/death pathways are altered following neonatal genistein treatment. The inhibition of oogonia/oocyte nests breakdown by EACs action was shown in the **Figure 2**.



**Figure 2.** Schematic model of oogonia/oocyte nests breakdown and its inhibition by endocrine active compounds (EACs) with estrogenic activity (bisphenol A, diethylstilbestrol, and genistein).

Phthalates are commonly used as plasticizers in the manufacturing of flexible polyvinyl chloride products. Little information is available on the effects of phthalates on neonatal ovary. These compounds have been actually shown to affect germ cell nest breakdown and primordial follicle assembly in cultured newborn mouse ovaries [84]. In addition, early postnatal treatment with diethylhexyl phthalate accelerated folliculogenesis by decreasing the number of primordial follicles and increasing the number preantral and antral follicles in mice [85]. The decrease in primordial follicle pool indicated estrogenic action of these compounds.

ICI 182,780 is a widely used anti-estrogen that can bind to both ER $\alpha$  and ER $\beta$  with very high affinity and completely antagonize the effect of estrogens [86]. It was found that ICI 182,780 in combination with estradiol allowed oocytes cyst breakdown neonatal rat ovaries [87]. On the other hand, Wang and Roy [46] revealed that ICI 182,780 significantly increased apoptosis and caused a modest reduction in primordial follicle formation in neonatal hamster ovary. Likewise, ICI 182,780 accelerated primordial follicles formation from oocyte nests in the neonatal pig. However, the initial follicle recruitment marked by the number of growing was delayed [69].

The compounds that exert both estrogenic and anti-estrogenic properties are parabens, which are widely used as anti-microbial agents in the cosmetic and pharmaceutical industries. Parabens inhibited the early phase of folliculogenesis and steroidogenesis in the ovaries of neonatal rats [88]. Moreover, it appears that parabens through inhibition of transcriptional repressor Foxl2, regulated the levels of steroidogenic enzymes [88].

#### 4.3. EACs with mixed properties

Methoxychlor (MXC) is an organochlorine pesticide metabolized predominantly to 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl) ethane (MOH) and the bisphenolic compound 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl) ethane (HPTE) in the organism [89]. MXC along with its metabolites acts through ERs and possesses estrogenic, anti-estrogenic, and anti-androgenic activities depending on the receptor subtype [90]. In a variety of experimental studies, MXC administration in early pregnancy or during the prenatal and neonatal periods has been shown to cause adverse abnormalities in adulthood of female rats, such as reduced ovarian functions and ovulatory rates, as well as pregnancy outcome [1]. Uzumcu et al. [91] demonstrated that MXC administration during the primordial-to-primary follicle transition period in mice (postnatal days 3–10) inhibited follicular development and reduced antral follicle numbers. In addition, MXC treatment increased the level of AMH protein production, which in turn inhibited folliculogenesis within the ovary. Another research suggested that exposure to MXC during fetal and neonatal ovarian development led to adult ovarian dysfunction, including an increase in the number of preantral and early antral follicles and a reduced number of corpora lutea and female infertility in rats [92]. Furthermore, there are several reports suggesting the role of MXC in the induction of epigenetic modifications within the ovary. Rats exposed to MXC (20 or 100  $\mu\text{g/kg}$  body weight) between embryonic day 19 and postnatal day 7 revealed altered methylation pattern in the promoter region of ER $\alpha$  that suppressed the ER $\alpha$  expression and caused ovarian dysfunction. Developmental exposure to MXC led to significant hypermethylation in the ER $\beta$  promoter regions, whereas the ER $\alpha$  promoter was unaffected [93].

## 5. Summary

The plausible link between EACs exposure during critical periods of early development and risk of chronic diseases in adulthood, including premature ovarian failure and PCOS, has been reported. The harmful effects of compounds with androgenic, anti-androgenic, estrogenic, and anti-estrogenic activities during neonatal window may occur with exposure to lower doses than those harmful for adults. These chemicals have been shown to target the ovary of neonates and adversely affect oocyte survival, follicle formation, and growth, as well as steroidogenic functions. There is a concern over EACs due to their common use and persistence within the modern living environment. The better cognition of mechanisms underlying the long-term consequences of the neonatal EACs exposure may in future lead to an understanding of human health risks and developing prevention strategies.

## Acknowledgements

This work was supported by National Science Center, Poland, grant no. 2015/17/B/NZ9/01457 (to MS).

## Author details

Maria Slomczynska<sup>1</sup>, Malgorzata Grzesiak<sup>2\*</sup> and Katarzyna Knapczyk-Stwora<sup>1</sup>

\*Address all correspondence to: [m.grzesiak@ur.krakow.pl](mailto:m.grzesiak@ur.krakow.pl)

<sup>1</sup> Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

<sup>2</sup> Department of Animal Physiology and Endocrinology, University of Agriculture, Krakow, Poland

## References

- [1] Vaiserman A. Early-life exposure to endocrine disrupting chemicals and later-life health outcomes: An epigenetic bridge? *Aging and Disease*. 2014;**5**:419-429. DOI: 10.14336/AD.2014.0500419
- [2] Woodruff TK, Walker CL. Fetal and early postnatal environmental exposures and reproductive health effects in the female. *Fertility and Sterility*. 2008;**89**:e47-e51. DOI: 10.1016/j.fertnstert.2007.12.029
- [3] Uzumcu M, Zama AM, Oruc E. Epigenetic mechanisms in the actions of endocrine-disrupting chemicals: Gonadal effects and role in female reproduction. *Reproduction in Domestic Animals*. 2012;**47**:338-347. DOI: 10.1111/j.1439-0531.2012.02096.x



- [4] Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. *Journal of Steroid Biochemistry and Molecular Biology*. 2011;**127**:204-215. DOI: 10.1016/j.jsbmb.2011.08.007
- [5] Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: Convergence of genomic and nongenomic actions on target genes. *Molecular Endocrinology*. 2005;**19**:833-842. DOI: 10.1210/me.2004-0486
- [6] Beato M, Klug J. Steroid hormone receptors: An update. *Human Reproduction Update*. 2000;**6**:225-236. DOI: 10.1093/humupd/6.3.225
- [7] Kumar R, Thompson EB. Transactivation functions of the N-terminal domains of nuclear hormone receptors: Protein folding and coactivator interactions. *Molecular Endocrinology*. 2003;**17**:1-10. DOI: 10.1210/me.2002-0258
- [8] Rochette-Egly C. Nuclear receptors: Integration of multiple signalling pathways through phosphorylation. *Cellular Signalling*. 2003;**15**:355-366. DOI: 10.1016/S0898-6568(02)00115-8
- [9] Khorasanizadeh S, Rastinejad F. Nuclear-receptor interactions on DNA-response elements. *Trends in Biochemical Sciences*. 2001;**26**:384-390. DOI: 10.1016/S0968-0004(01)01800-X
- [10] Bunone G, Briand PA, Miksicek RJ, Picard D. Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO Journal*. 1996;**15**:2174-2183
- [11] Picard D, Bunone G, Liu JW, Donzé O. Steroid-independent activation of steroid receptors in mammalian and yeast cells and in breast cancer. *Biochemical Society Transactions*. 1997;**25**:597-602. DOI: 10.1042/bst0250597
- [12] Simons K, Toomre D. Lipid rafts and signal transduction. *Nature Reviews Molecular Cell Biology*. 2000;**1**:31-39. DOI: 10.1038/35036052
- [13] Falkenstein E, Tillmann HC, Christ M, Feuring M, Wehling MC. Multiple actions of steroid hormones—A focus on rapid, nongenomic effects. *Pharmacological Reviews*. 2000;**52**:513-556
- [14] Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE*. 2002;**2002**:re9. DOI: 10.1126/stke.2002.138.re9
- [15] Liao RS, Ma S, Miao L, Li R, Yin Y, Raj GV. Androgen receptor-mediated non-genomic regulation of prostate cancer cell proliferation. *Translational Andrology and Urology*. 2013;**2**:187-196. DOI: 10.3978/j.issn.2223-4683.2013.09.07
- [16] Leitman DC, Paruthiyil S, Yuan C, Herber CB, Olshansky M, Tagliaferri M, Cohen I, Speed TP. Tissue-specific regulation of genes by estrogen receptors. *Seminars in Reproductive Medicine*. 2012;**30**:14-22. DOI: 10.1055/s-0031-1299593
- [17] Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science*. 2005;**307**:1625-1630. DOI: 10.1126/science.1106943



- [18] Chevalier N, Paul-Bellon R, Camparo P, Michiels JF, Chevallier D, Fénichel P. Genetic variants of GPER/GPR30, a novel estrogen-related G protein receptor, are associated with human seminoma. *International Journal of Molecular Sciences*. 2014;**15**:1574-1789. DOI: 10.3390/ijms15011574
- [19] Thomas P. Characteristics of membrane progesterin receptor alpha (mPRalpha) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progesterin actions. *Frontiers in Neuroendocrinology*. 2008;**29**:292-312. DOI: 10.1016/j.yfrne.2008.01.001
- [20] Pi M, Parrill AL, Quarles LD. GPRC6A mediates the non-genomic effects of steroids. *Journal of Biological Chemistry*. 2010;**285**:39953-39964. DOI: 10.1074/jbc.M110.158063
- [21] Pascal LE, Wang Z. Unzipping androgen action through ZIP9: A novel membrane androgen receptor. *Endocrinology*. 2014;**155**:4120-4123. DOI: 10.1210/en.2014-1749
- [22] Laurentino S, Pinto P, Correia S, Cavaco JE, Canário AVM, Socorro S. Structural variants of sex steroid hormone receptors in the testis: From molecular biology to physiological roles. *OA Biotechnology*. 2012;**1**:4
- [23] McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocrine Reviews*. 2000;**21**:200-214. DOI: 10.1210/edrv.21.2.0394
- [24] Pepling ME. From primordial germ cell to primordial follicle: Mammalian female germ cell development. *Genesis*. 2006;**44**:622-632. DOI: 10.1002/dvg.20258
- [25] Sarraj MA, Drummond AE. Mammalian foetal ovarian development: Consequences for health and disease. *Reproduction*. 2012;**143**:151-163. DOI: 10.1530/REP-11-0247
- [26] Pepling ME, Spradling AC. Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. *Developmental Biology*. 2001;**234**:339-351. DOI: 10.1006/dbio.2001.0269
- [27] McLaughlin EA, McIver SC. Awakening the oocyte: Controlling primordial follicle development. *Reproduction*. 2009;**137**:1-11. DOI: 10.1530/REP-08-0118
- [28] Hsueh AJ, Billig H, Tsafiriri A. Ovarian follicle atresia: A hormonally controlled apoptotic process. *Endocrine Reviews*. 1994;**15**:707-724. DOI: 10.1210/edrv-15-6-707
- [29] Smith P, Wilhelm D, Rodgers RJ. Development of mammalian ovary. *Journal of Endocrinology*. 2014;**221**:R145-R161. DOI: 10.1530/JOE-14-0062
- [30] Fortune JE, Yang MY, Allen JJ, Herrick SL. Triennial reproduction symposium: The ovarian follicular reserve in cattle: What regulates its formation and size? *Journal of Animal Science*. 2013;**91**:3041-3050. DOI: 10.2527/jas.2013-6233
- [31] Ding W, Wang W, Zhou B, Zhang W, Huang P, Shi F, Taya K. Formation of primordial follicles and immunolocalization of PTEN, PKB and FOXO3A proteins in the ovaries of fetal and neonatal pigs. *Journal of Reproduction and Development*. 2010;**56**:162-168. DOI: 10.1262/jrd.09-094H

- [32] McNatty KP, Smith P, Hudson NL, Heath DA, Tisdall DJ, OWS, Braw-Tal R. Development of the sheep ovary during fetal and early neonatal life and the effect of fecundity genes. *Journal of Reproduction and Fertility. Supplement.* 1995;**49**:123-135
- [33] Eppig JJ, Wigglesworth K, Pendola FL. The mammalian oocyte orchestrates the rate of ovarian follicular development. *Proceedings of the National Academy of Sciences of the United States of America.* 2002;**99**:2890-2894. DOI: 10.1073/pnas.052658699
- [34] Gervásio CG, Bernuci MP, Silva-de-Sá MF, Rosa-E-Silva AC. The role of androgen hormones in early follicular development. *ISRN Obstetrics and Gynecology.* 2014;**2014**:818010. DOI: 10.1155/2014/818010
- [35] Chang C, Lee SO, Wang RS, Yeh S, Chang TM. Androgen receptor (AR) physiological roles in male and female reproductive systems: Lessons learned from AR-knockout mice lacking AR in selective cells. *Biology of Reproduction.* 2013;**89**:21. DOI: 10.1095/biolreprod.113.109132
- [36] Fowler PA, Anderson RA, Saunders PT, Kinnell H, Mason JI, Evans DB, Bhattacharya S, Flannigan S, Franks S, Monteiro A, O'Shaughnessy PJ. Development of steroid signaling pathways during primordial follicle formation in the human fetal ovary. *Journal of Clinical Endocrinology and Metabolism.* 2011;**96**:1754-1762. DOI: 10.1210/jc.2010-2618
- [37] 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. *Biology of Reproduction.* 1999;**61**:353-357. DOI: 10.1095/biolreprod61.2.353
- [38] Yang MY, Fortune JE. Testosterone stimulates the primary to secondary follicle transition in bovine follicles in vitro. *Biology of Reproduction.* 2006;**75**:924-932. DOI: 10.1095/biolreprod.106.051813
- [39] Yang JL, Zhang CP, Li L, Huang L, Ji SY, Lu CL, Fan CH, Cai H, Ren Y, Hu ZY, Gao F, Liu YX. Testosterone induces redistribution of forkhead box-3a and down-regulation of growth and differentiation factor 9 messenger ribonucleic acid expression at early stage of mouse folliculogenesis. *Endocrinology.* 2010;**151**:774-782. DOI: 10.1210/en.2009-0751
- [40] Narkwichean A, Jayaprakasan K, Maalouf WE, Hernandez-Medrano JH, Pincott-Allen C, Campbell BK. Effects of dehydroepiandrosterone on in vivo ovine follicular development. *Human Reproduction.* 2014;**29**:146-154. DOI: 10.1093/humrep/det408
- [41] Rosenfeld CS, Murray AA, Simmer G, Hufford MG, Smith MF, Spears N, Lubahn DB. Gonadotropin induction of ovulation and corpus luteum formation in young estrogen receptor-alpha knockout mice. *Biology of Reproduction.* 2000;**62**:599-605. DOI: 10.1095/biolreprod62.3.599
- [42] Kregge JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA, Smithies O. Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *Proceedings of the National Academy of Sciences of the United States of America.* 1998;**95**:15677-15682. DOI: 10.1073/pnas.95.26.15677

- [43] Kezele P, Skinner MK. Regulation of ovarian primordial follicle assembly and development by estrogen and progesterone: Endocrine model of follicle assembly. *Endocrinology*. 2003;**144**:3329-3337. DOI: 10.1210/en.2002-0131
- [44] Nilsson EE, Skinner MK. Progesterone regulation of primordial follicle assembly in bovine fetal ovaries. *Molecular and Cellular Endocrinology*. 2009;**313**:9-16. DOI: 10.1016/j.mce.2009.09.004
- [45] Dutta S, Mark-Kappeler CJ, Hoyer PB and Pepling ME. The steroid hormone environment during primordial follicle formation in perinatal mouse ovaries. *Biology of Reproduction*. 2014;**91**:68:1-12. DOI: 10.1095/biolreprod.114.119214
- [46] Wang C, Roy SK. Development of primordial follicles in the hamster: Role of estradiol-17beta. *Endocrinology*. 2007;**148**:1707-1716. DOI: 10.1210/en.2006-1193
- [47] Ikeda Y, Nagai A, Ikeda MA, Hayashi S. Neonatal estrogen exposure inhibits steroidogenesis in the developing rat ovary. *Developmental Dynamics*. 2001;**221**:443-453. DOI: 10.1002/dvdy.1162
- [48] Britt KL, Saunders PK, McPherson SJ, Misso ML, Simpson ER, Findlay JK. Estrogen actions on follicle formation and early follicle development. *Biology of Reproduction*. 2004;**71**:1712-1723. DOI: 10.1095/biolreprod.104.028175
- [49] Zachos NC, Billiar RB, Albrecht ED, Pepe GJ. Developmental regulation of baboon fetal ovarian maturation by estrogen. *Biology of Reproduction*. 2002;**67**:1148-1156. DOI: 10.1095/biolreprod67.4.1148
- [50] Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *Journal of Clinical Investigation*. 1998;**101**:2622-2629. DOI: 10.1172/JCI2081
- [51] Steckler T, Manikkam M, Inskeep EK, Padmanabhan V. Developmental programming: Follicular persistence in prenatal testosterone-treated sheep is not programmed by androgenic actions of testosterone. *Endocrinology*. 2007;**148**:3532-3540. DOI: 10.1210/en.2007-0339
- [52] Hogg K, McNeilly AS, Duncan WC. Prenatal androgen exposure leads to alterations in gene and protein expression in the ovine fetal ovary. *Endocrinology*. 2011;**152**:2048-2059. DOI: 10.1210/en.2010-1219
- [53] Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome—A hypothesis. *Journal of Endocrinology*. 2002;**174**:1-5. DOI: 10.1677/joe.0.1740001
- [54] Tyndall V, Broyde M, Sharpe R, Welsh M, Drake AJ, McNeilly AS. Effect of androgen treatment during foetal and/or neonatal life on ovarian function in prepubertal and adult rats. *Reproduction*. 2012;**143**:21-33. DOI: 10.1530/REP-11-0239
- [55] Ongaro L, Salvetti NR, Giovambattista A, Spinedi E, Ortega HH. Neonatal androgenization-induced early endocrine-metabolic and ovary misprogramming in the female rat. *Life Sciences*. 2015;**130**:66-72. DOI: 10.1016/j.lfs.2015.03.008

- [56] Marcondes RR, Carvalho KC, Duarte DC, Garcia N, Amaral VC, Simões MJ, Lo Turco EG, Soares JM Jr, Baracat EC, Maciel GA. Differences in neonatal exposure to estradiol or testosterone on ovarian function and hormonal levels. *General and Comparative Endocrinology*. 2015;**212**:28-33. DOI: 10.1016/j.ygcen.2015.01.006
- [57] Kemppainen JA, Lane MV, Sar M, Wilson EM. Androgen receptor phosphorylation, turnover, nuclear transport, and transcriptional activation. Specificity for steroids and antihormones. *Journal of Biological Chemistry*. 1992;**267**:968-974
- [58] O'Connor JC, Cook JC, Slone TW, Makovec GT, Frame SR, Davis LG. An ongoing validation of a Tier I screening battery for detecting endocrine-active compounds (EACs). *Toxicological Sciences*. 1998;**46**:45-60. DOI: 10.1006/toxs.1998.2550
- [59] Durlej M, Kopera I, Knapczyk-Stwora K, Hejmej A, Duda M, Kozirowski M, Slomczynska M, Bilinska B. Connexin 43 gene expression in male and female gonads of porcine offspring following in utero exposure to an anti-androgen, flutamide. *Acta Histochemica*. 2011;**113**:6-12. DOI: 10.1016/j.acthis.2009.07.001
- [60] Kopera I, Durlej M, Hejmej A, Knapczyk-Stwora K, Duda M, Slomczynska M, Kozirowski M, Bilinska B. Effects of pre- and postnatal exposure to flutamide on connexin 43 expression in testes and ovaries of prepubertal pigs. *European Journal of Histochemistry*. 2010;**54**:e15. DOI: 10.4081/ejh.2010.e15
- [61] Durlej M, Knapczyk-Stwora K, Duda M, Kopera-Sobota I, Hejmej A, Bilinska B, Slomczynska M. Prenatal and neonatal exposure to the antiandrogen flutamide alters connexin 43 gene expression in adult porcine ovary. *Domestic Animal Endocrinology*. 2011;**40**:19-29. DOI: 10.1016/j.domaniend.2010.08.003
- [62] Grzesiak M, Knapczyk-Stwora K, Duda M, Slomczynska M. Elevated level of 17 $\beta$ -estradiol is associated with overexpression of FSHR, CYP19A1, and CTNNB1 genes in porcine ovarian follicles after prenatal and neonatal flutamide exposure. *Theriogenology*. 2012;**78**:2050-2060. DOI: 10.1016/j.theriogenology.2012.07.026
- [63] Grzesiak M, Knapczyk-Stwora K, Luck MR, Mobasheri A, Slomczynska M. Effect of prenatal and neonatal anti-androgen flutamide treatment on aquaporin 5 expression in the adult porcine ovary. *Reproduction in Domestic Animals*. 2016;**51**:105-113. DOI: 10.1111/rda.12652
- [64] Durlej M, Knapczyk-Stwora K, Slomczynska M. Prenatal and neonatal flutamide administration increases proliferation and reduces apoptosis in large antral follicles of adult pigs. *Animal Reproduction Science*. 2012;**132**:58-65. DOI: 10.1016/j.anireprosci.2012.04.001
- [65] Giger W, Brunner PH, Schaffner C. 4-Nonylphenol in sewage sludge: Accumulation of toxic metabolites from nonionic surfactants. *Science*. 1984;**225**:623-625. DOI: 10.1126/science.6740328
- [66] Katsuda S, Yoshida M, Watanabe G, Taya K, Maekawa A. Irreversible effects of neonatal exposure to p-tert-octylphenol on the reproductive tract in female rats. *Toxicology and Applied Pharmacology*. 2000;**165**:217-226. DOI: 10.1006/taap.2000.8940



- [67] Bøgh IB, Christensen P, Dantzer V, Groot M, Thøfner IC, Rasmussen RK, Schmidt M, Greve T. Endocrine disrupting compounds: Effect of octylphenol on reproduction over three generations. *Theriogenology*. 2001;**55**:131-150. DOI: 10.1016/S0093-691X(00)00451-9
- [68] Wright C, Evans AC, Evans NP, Duffy P, Fox J, Boland MP, Roche JF, Sweeney T. Effect of maternal exposure to the environmental estrogen, octylphenol, during fetal and/or post-natal life on onset of puberty, endocrine status, and ovarian follicular dynamics in ewe lambs. *Biology of Reproduction*. 2002;**67**:1734-1740. DOI: 10.1095/biolreprod.101.002006
- [69] Slomczynska M, Knapczyk-Stwora K, Grzesiak M, Koziorowski M, Nowak S. Androgen and estrogen imbalance affects folliculogenesis in the neonatal porcine ovary influencing cell proliferation and apoptosis. In: Abstracts from the translational reproductive biology and clinical reproductive endocrinology conference held November 17-20, 2016. *Journal of Assisted Reproduction and Genetics*. 2016;**33**:1693-1708. DOI: 10.1007/s10815-016-0827-9
- [70] Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). *Reproductive Toxicology*. 2007;**24**:139-177. DOI: 10.1016/j.reprotox.2007.07.010
- [71] Zhou C, Wang W, Peretz J, Flaws JA. Bisphenol A exposure inhibits germ cell nest breakdown by reducing apoptosis in cultured neonatal mouse ovaries. *Reproductive Toxicology*. 2015;**57**:87-99. DOI: 10.1016/j.reprotox.2015.05.012
- [72] Suzuki A, Sugihara A, Uchida K, Sato T, Ohta Y, Katsu Y, Watanabe H, Iguchi T. Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. *Reproductive Toxicology*. 2002;**16**:107-116. DOI: 10.1016/S0890-6238(02)00005-9
- [73] Otsuka F, Shimasaki S. A negative feedback system between oocyte bone morphogenetic protein 15 and granulosa cell kit ligand: Its role in regulating granulosa cell mitosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;**99**:8060-8065. DOI: 10.1073/pnas.122066899
- [74] Hutt KJ, McLaughlin EA, Holland MK. Kit ligand and c-Kit have diverse roles during mammalian oogenesis and folliculogenesis. *Molecular Human Reproduction*. 2006;**12**:61-69. DOI: 10.1093/molehr/gal010
- [75] Rodríguez HA, Santambrosio N, Santamaría CG, Muñoz-de-Toro M, Luque EH. Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary. *Reproductive Toxicology*. 2010;**30**:550-557. DOI: 10.1016/j.reprotox.2010.07.008
- [76] Zhang HQ, Zhang XF, Zhang LJ, Chao HH, Pan B, Feng YM, Li L, Sun XF, Shen W. Fetal exposure to bisphenol A affects the primordial follicle formation by inhibiting the meiotic progression of oocytes. *Molecular Biology Reports*. 2012;**39**:5651-5657. DOI: 10.1007/s11033-011-1372-3
- [77] Fernández M, Bourguignon N, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian



- syndrome in adult rats. *Environmental Health Perspectives*. 2010;**118**:1217-1222. DOI: 10.1289/ehp.0901257
- [78] Kim H, Nakajima T, Hayashi S, Chambon P, Watanabe H, Iguchi T, Sato T. Effects of diethylstilbestrol on programmed oocyte death and induction of polyovular follicles in neonatal mouse ovaries. *Biology of Reproduction*. 2009;**81**:1002-1009. DOI: 10.1095/biolreprod.108.070599
- [79] Alwis ID, Maroni DM, Hendry IR, Roy SK, May JV, Leavitt WW, Hendry WJ. Neonatal diethylstilbestrol exposure disrupts female reproductive tract structure/function via both direct and indirect mechanisms in the hamster. *Reproductive Toxicology*. 2011;**32**:472-483. DOI: 10.1016/j.reprotox.2011.09.006
- [80] Karavan JR, Pepling ME. Effects of estrogenic compounds on neonatal oocyte development. *Reproductive Toxicology*. 2012;**34**:51-56. DOI: 10.1016/j.reprotox.2012.02.005
- [81] Rivera OE, Varayoud J, Rodríguez HA, Muñoz-de-Toro M, Luque EH. Neonatal exposure to bisphenol A or diethylstilbestrol alters the ovarian follicular dynamics in the lamb. *Reproductive Toxicology*. 2011;**32**:304-312. DOI: 10.1016/j.reprotox.2011.06.118
- [82] 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. *Biology of Reproduction*. 2006;**74**:161-168. DOI: 10.1095/biolreprod.105.045724
- [83] Jefferson WN, Doerge D, Padilla-Banks E, Woodling KA, Kissling GE, Newbold R. Oral exposure to genistin, the glycosylated form of genistein, during neonatal life adversely affects the female reproductive system. *Environmental Health Perspectives*. 2009;**117**:1883-1889. DOI: 10.1289/ehp.0900923
- [84] Zhang T, Li L, Qin XS, Zhou Y, Zhang XF, Wang LQ, De Felici M, Chen H, Qin GQ, Shen W. Di-(2-ethylhexyl)phthalate and bisphenol A exposure impairs mouse primordial follicle assembly in vitro. *Environmental and Molecular Mutagenesis*. 2014;**55**:343-353. DOI: 10.1002/em.21847
- [85] Zhang XF, Zhang LJ, Li L, Feng YN, Chen B, Ma JM, Huynh E, Shi QH, De Felici M, Shen W. Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. *Environmental and Molecular Mutagenesis*. 2013;**54**:354-361. DOI: 10.1002/em.21776
- [86] Wakeling AE, Dukes M, Bowler J. A potent specific pure antiestrogen with clinical potential. *Cancer Research*. 1991;**51**:3867-3873
- [87] Chen Y, Breen K, Pepling ME. Estrogen can signal through multiple pathways to regulate oocyte cyst breakdown and primordial follicle assembly in the neonatal mouse ovary. *Journal of Endocrinology*. 2009;**202**:407-417. DOI: 10.1677/JOE-09-0109
- [88] Ahn HJ, An BS, Jung EM, Yang H, Choi KC, Jeung EB. Parabens inhibit the early phase of folliculogenesis and steroidogenesis in the ovaries of neonatal rats. *Molecular Reproduction and Development*. 2012;**79**:626-636. DOI: 10.1002/mrd.22070

- [89] Patel S, Zhou C, Rattan S, Flaws JA. Effects of endocrine-disrupting chemicals on the ovary. *Biology of Reproduction*. 2015;**93**:1-9. DOI: 10.1095/biolreprod.115.130336
- [90] 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. *Molecular Pharmacology*. 2000;**58**:852-858. DOI: 10.1124/mol.58.4.852
- [91] Uzumcu M, Kuhn PE, Marano JE, Armenti AE, Passantino L. Early postnatal methoxychlor exposure inhibits folliculogenesis and stimulates anti-Mullerian hormone production in the rat ovary. *Journal of Endocrinology*. 2006;**191**:549-558. DOI: 10.1677/joe.1.06592
- [92] Armenti AE, Zama AM, Passantino L, Uzumcu M. Developmental methoxychlor exposure affects multiple reproductive parameters and ovarian folliculogenesis and gene expression in adult rats. *Toxicology and Applied Pharmacology*. 2008;**233**:286-296. DOI: 10.1016/j.taap.2008.09.010
- [93] Zama AM, Uzumcu M. Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes. *Endocrinology*. 2009;**150**:4681-4691. DOI: 10.1210/en.2009-0499

