Published in final edited form as:
Fertil Steril. 2008 February ; 89(2 Suppl): e47–e51. doi:10.1016/j.fertnstert.2007.12.029.

Fetal and Early Postnatal Environmental Exposures and Reproductive Health Effects in the Female

Teresa K. Woodruff, Ph.D.[1] and **Cheryl Lyn Walker, Ph.D.**[2]

1*Northwestern University, Department of Neurobiology and Physiology, 2205 Tech Drive, Hogan 4-150, 4-160, 5-140, Evanston, IL 60208-3520*

2*The University of Texas M.D. Anderson Cancer Center, Department of Carcinogenesis, PO BOX 389, Smithville, TX 78957*

Abstract

This short review presents current research into the role of the environment in normal female reproductive function and pathogenesis, specifically focusing on the ovary and uterus.

Introduction

The environment influences not only the development of the reproductive system, but also impacts adult reproductive function. Environmental cues such as the light:dark cycle, caloric signals and pheromones are integrated at the central nervous system and, in combination with endogenous endocrine and paracrine signals, create a permissive or non-permissive environment for the initiation and maintenance of the normal reproductive cycle. On the other hand, exposure to environmental agents can have profound negative effects on the development and function of the reproductive tract. The developmental programming hypothesis proposes that at critical times during development, exposure of developing tissues to an adverse stimulus or insult can permanently reprogram normal physiological responses, and so give rise to metabolic and hormonal disorders later in life (1–5). The female reproductive tract has been shown to be a target for developmental programming as a result of environmental hormone exposure. This short review presents current research into the role of the environment in normal female reproductive function and pathogenesis, specifically focusing on the ovary and uterus.

Ovary

Ovarian Follicle Development

The ovarian follicle is the functional unit of the ovary. Mammalian folliculogenesis is one of the most dynamic and intricately regulated developmental processes in biology. Ovarian follicles are comprised of an oocyte surrounded and supported by the somatic granulosa and theca cells. In the human, primordial follicle formation is initiated around 21 weeks gestation (6–9), while in the mouse this process occurs shortly after birth (10). Between 10–12 dpc in male and female mouse embryos, germ cells tend to cluster in the indifferent gonad and extensively multiply. The dividing germ cells are held together by intracellular bridges, as a result of incomplete cytokinesis, to form clonal clumps of 32 cells that tend to go through

mitosis synchronously (11,12). The cytoplasmic bridges contain mitochondria, ribosomes and other organelles that are freely exchanged between these dividing cells (13). Shortly after the sex-specific differentiation of the gonad, the transformation from primordial germ cells (mitotic and migratory), to oogonia (mitotic, but immobile) to oocytes (meiotically active) begins (14). In the mouse, the first wave of oogonia enter meiosis around 13.5 dpc and eventually arrest as diplotene oocytes near birth (19.5 dpc) (10,15,16). Meiotically active oocytes progress through the stages of prophase I and arrest in diplotene until ovulation, at which time meiosis is resumed. Within 24–48 of birth, somatic cells invade the cytoplasmic space and surround individual oocytes; thus creating primordial follicles. The precise mechanisms involved in early ovarian follicle formation are not known, but are essential in organizing the fetal ovary and establishing the postnatal follicle number that will provide the female with enough oocytes for a lifetime of fertility.

Two factors that are known to impact oocyte and follicle development include estrogenic compounds and activin. Estrogen plays an important role in regulating ovarian follicle development and function (17,18). Aromatase knockout mice, which are estrogen deficient, demonstrate decreased primordial and primary follicle numbers, blockage of follicle development at the antral stage and absence of corpora lutea (19,20). When the synthetic estrogen diethylstilbestrol (DES) or the natural form of estrogen, estradiol (E_2), is administered to adult or pre-pubertal hypophysectomized rats or mice, stimulation of follicle growth and proliferation and reduction in follicle atresia have been observed (18). In fact, the trophic effect of estrogen has been employed to prime granulosa cells for primary cell cultures *in vitro* (21, 22).

Activin is a member of the transforming growth factor-β (TGF-β) superfamily. Activin and its functional antagonist inhibin were originally isolated from gonadal sources based on their ability to stimulate (activin) or suppress (inhibin) the synthesis and secretion of FSH (23–29). Recently, autocrine and paracrine roles for activin and inhibin have been described in the regulation of ovarian follicle development (30–34). Activin has been shown to regulate ovarian granulosa cell proliferation and differentiation (30,33–35), promote ovarian follicle atresia (36), increase FSH receptor expression in undifferentiated granulosa cells (37,38) and stimulate oocyte maturation *in vitro* (39). Mice that lack activin receptor type II are infertile and exhibit a block in follicle development at the antral stage, and have very few corpora lutea (40). Overexpression of follistatin, an activin antagonist, also blocks follicle development at the secondary follicle stage (41). Previous studies in our laboratory using 2 different transgenic animal models demonstrated that abrogating the activin signaling pathway leads to the formation of multi-oocytic follicles (MOFs) and epithelial cell lined cysts (42,43).

Neonatal Exposure to Estrogen and Activin

Based on the fact that estrogen and activin signals are both important for normal follicle formation, it is not surprising that aberrant development and ovarian pathologies are observed in mice exposed to neonatal estrogen or activin. Ikeda et al demonstrated delayed follicle and interstitial development at days 14 and 21 of age in rats exposed to neonatal estradiol benzoate (44). Forsberg reported a lack of corpora lutea in adult mice exposed neonatally to diethylstilbestrol (DES) or E_2 (45), suggesting that these effects persist beyond reproductive tract development and impact fertility in the adult. Neonatal DES, E_2 or the phytoestrogen genistein exposure in mice also induces formation of MOFs (46–48), which have also been reported in alligators exposed to environmental estrogenic contaminants (49). Additionally, we have demonstrated that activin administered during the critical postnatal period of primordial follicle formation changes the number of postnatal follicles. Of note, a subset of follicles is affected by this neonatal insult, rather than the entire follicle population, suggesting

that prepubertal follicles may be divided into sensitive or 'susceptible' populations and insensitive or 'privileged' populations (50).

Current studies are investigating the possibility that neonatal estrogen exposure may alter activin signaling in the ovary, thereby leading to ovarian pathologies (51). We have examined the effect of neonatal DES and E_2 exposure on the mRNA and protein levels of the key factors involved in activin signaling in the mouse ovary. Preliminary results demonstrate that neonatal estrogen exposure decreases activin subunit gene expression and impacts activin signaling, indicating that activin genes are targets of estrogen action in the mouse ovary. Future studies will further characterize the mechanisms underlying the effects of premature estrogen and activin exposure on adult ovarian and follicular function.

Uterus

Environmental Factors in Uterine Pathologies

From the 1940s to 1970s, the xenoestogen DES was extensively prescribed to pregnant women at risk for miscarriage. Women exposed to DES in utero during critical periods of reproductive tract development developed several types of reproductive tract abnormalities, as well as an increased incidence of cervical-vaginal cancer later in life (50). Animal studies that simulate the human DES experience have since shown that exposure of the developing reproductive tract of CD-1 mice to DES imparts a permanent estrogen imprint that alters reproductive tract morphology, induces persistent expression of the lactoferrin and c-fos genes and induces a high incidence of uterine adenocarcinoma (51–53). Since DES is readily metabolized and cleared within days after exposure, the persistent alterations resulting from developmental DES exposure cannot simply be explained by residual body burden of the compound (54,55). DES exposure also induces changes in the expression of several uterine genes involved in tissue patterning, such as Wnt7a, Hoxa9, Hoxa10 and Hoxa11, contributing to changes in tissue architecture and morphology (56–58). DES-induced developmental programming appears to require the estrogen receptor α (ERα) (59), suggesting that signaling through this receptor is crucial for establishing developmental programming. These initial observations with DES firmly established the developmental period as a window of susceptibility during which an inappropriate xenoestrogen exposure can induce developmental programming and increase risk for diseases, including cancer, later in life.

We have recently demonstrated that developmental programming can increase the risk for developing uterine leiomyoma in adulthood the adult (60). Utilizing rats carrying a germline defect in the tuberous sclerosis complex 2 (*Tsc-2*) tumor suppressor gene that are predisposed to uterine leiomyomas, we found that an early life exposure to DES during development of the uterus increased risk for uterine leiomyoma from 65% to greater than 90% and increased tumor multiplicity and size in genetically predisposed animals, but failed to induce tumors in wild-type rats. Importantly, we found that DES exposure imparted a hormonal imprint on the developing uterine myometrium in both wild-type and carrier rats, causing an increase in expression of estrogen-responsive genes prior to the onset of tumors. Thus, when developmental programming of estrogen-responsive genes was combined with the presence of the Tsc-2 tumor suppressor gene defect, the result was an increased risk of developing hormone-dependent leiomyoma in adult animals. These data suggest that exposure to environmental factors during development can permanently reprogram normal physiological tissue responses and thus lead to increased tumor suppressor gene penetrance in genetically susceptible individuals. Developmental programming occurred as a result of the hormonal imprint imparted on the developing uterus by the brief early life exposure to DES.

Window of Susceptibility in the Uterus

In contrast to humans, in which reproductive tract development occurs primarily *in utero*, the majority of rodent uterine differentiation and maturation occurs postnatally (61). At birth, the rat uterus is comprised of the luminal epithelial layer of the endometrium and a randomly ordered, undifferentiated uterine mesenchyme. From birth through the onset of puberty (~day 35 in the rat), the uterine mesenchyme follows an ordered pattern of differentiation, which results in the formation of the uterine myometrium and endometrial stroma and glands. During early postnatal life, uterine development is estrogen-independent, even though estrogens are present in neonatal blood. This is due to high levels of estrogen binding proteins, such as alpha-feto protein (AFP) (62), which bind to and inactivate endogenous estrogen, thus protecting developing tissues from estrogen exposure (63–65). The neonate begins to produce endogenous estrogen near the end of the first week of postnatal life, but AFP levels do not begin to decline until between neonatal days 12–16, when it is cleared by the liver. Upon AFP clearance, the uterus becomes exposed to circulating estrogen and begins to acquire estrogen responsiveness as it prepares for the onset of puberty (63).

To understand what defines the critical risk period for developmental programming in the uterus, we determined the sensitivity of the female reproductive tract to this epigenetic phenomenon during various stages of neonatal development (66). Eker rats were exposed to 10 μg/kg DES, on postnatal days 3–5, 10–12 or 17–19, three important periods of reproductive tract development and differentiation. Developmental programming resulted in increased tumor suppressor gene penetrance in rats exposed to DES at day 3–5 and day 10–12, with tumor incidence increasing significantly to 95% and 100%, respectively, relative to vehicle controls. In contrast, although animals exposed to DES at day 17–19 had a tumor incidence of 85%, the risk of uterine leiomyoma in these females was not significantly higher than vehicle controls, suggesting that the window of susceptibility for increased cancer risk was closing by this time. Tumors from animals exposed earlier in development were also more numerous, larger and more proliferative than tumors exposed on day 17–19.

To confirm that developmental programming differed between early (day 3–5 and day 10–12) and later (day 17–19) periods of uterine development, gene expression analysis was performed, which revealed that the expression of estrogen-responsive genes (calbindin and progesterone receptor [PR]) had been reprogrammed in adult females exposed to DES at day 3–5 and day 10–12 but not in those exposed at day 17–19. Reprogramming in response to DES exposure during early development resulted in hyper-responsiveness to ovarian hormones and could be prevented by ovariectomy prior to sexual maturity. Real-time PCR demonstrated that in animals exposed to DES during this early, susceptible period (day 3–12), expression of calbindin and PR was elevated, and more importantly, was induced even when estrogen levels were low, indicating a hyper-responsiveness to estrogen, which was not observed by similar exposure during the later, resistant period (day 17–19). Interestingly, the timing of the resistant period coincided with the time at which the uterus becomes exposed to functional endogenous estrogen, suggesting that the myometrium was most susceptible to developmental programming by a xenoestrogen during the period in which it was normally in an estrogen-naïve state. However, the ability of the uterus to respond to estrogens is not impaired while the uterus is maintained in this estrogen-naïve state. DES was able to induce estrogen-responsive gene expression at all times tested (ie, during both sensitive and resistant neonatal periods). Recall, however, that developmental programming only occurs when the uterus is exposed at day 3–5 or day 10–12. This indicates that the resistance to developmental programming observed at the day 17–19 period was not due to a change in genomic function, (ie, transactivation of gene expression in the nucleus), suggesting that DES was triggering mechanism(s) other than transactivation of gene expression to induce developmental programming.

Developmental Programming by Genestein and Bisphenol A

These studies have now been extended beyond DES to demonstrate that other xenoestrogens can also program gene expression in the uterus. Like DES, genistein and bisphenol A (BPA) exposure at days 10–12 (the period for maximum sensitivity to developmental programming) induce the expression of the estrogen-responsive genes calbindin and PR. Interestingly, in contrast to DES, exposure to these xenoestrogens does not disrupt ovarian function in adult females, which continue to cycle normally. When estrogen-responsive gene expression was quantitated and controlled for stage of estrus in the adult animals that been neonatally exposed to BPA, estrogen-responsive gene expression was attenuated. In contrast, genistein exposed females exhibited even higher levels of expression of estrogen-responsive genes than even DES-exposed females, suggesting that this xenoestrogen was even more effective at inducing developmental programming than DES. These data indicate that developmental programming is a general phenomenon that is induced by a variety of environmental estrogens.

Conclusions

The female reproductive tract is susceptible to environmental factors that can have a profound impact on not only development of reproductive tissues, but also adult reproductive function. Through developmental programming, prenatal exposure to environmental factors can modify normal cellular and tissue function such that women may have a higher risk of reproductive pathologies later in life. New research is describing the effects of environmental estrogen exposure on normal reproductive development of the ovaries and the uterus, and the link to specific disease states in the adult. In addition to providing important insights into female reproductive function and the factors that may make certain women more susceptible to disease, these studies may lead to strategies that can reduce women's risk and ensure reproductive health throughout their life.

Acknowledgement of Financial Support

This work was supported in part by grants as follows: NIEHS (ES07784), NCI (CA63613), NIEHS (ES08263), NICHD (HD046282) to CLW and NICHD (HD021921) and NICHD (HD044464) to TKW.

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