Effect of maternal anastrozole treatment on ovarian follicle development in neonatal mouse: A morphologic study

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Abstract

Introduction: The origin of neonatal oocyte development is unknown. However, estrogen plays an essential role during development of the female reproductive system. Anastrozole is used as both ovulation stimulating and an anticancer drug. The aim of this study was to evaluate the impact of Anastrozole on follicular development and differentiation in mice.
Materials and methods: In the present study, 30 adult female and 15 adult male mice were used. Then, two female mice (at their sterous cycle) were kept with one male mouse in a cage for mating. After observing the vaginal plug (considered as first day of pregnancy) female mice were divided into two groups of control and experimental. In the experimental group, on the 14th day of pregnancy the mice received anastrozole (50 mg/Kg, i.p. injection). After delivery 16 pups were selected in each group. Then 2, 4 and 7 day pups were studies for primordial, primary and growing follicles number.
Results: According to the morphometric studies, in the 2 day pup, the breakdown was not complete in treatment group. However, the number of primordial, primary and growing follicles of 4 and 7 day pups were not significantly difference in the control and experimental groups.
Conclusion: According to the studies, estrogen is necessary for follicular breakdown and maternal anastrozole can reduce the primordial follicles. However, maternal anastrozole and estrogen depression couldn't effect on the histology of ovarian follicle in neonatal mouse.

Keywords: Anastrozole, Ovarian follicles, Neonatal mouse, Histology

Introduction

In ovarian differentiation of female mouse, the clusters are the place of germ cells and were called oocyte nest or germline cysts. Mouse germ cells in clusters develop and dissociate after birth and an individual oocytes are surrounded by somatic pregranulosa cells and forming primordial follicles (1). Two thirds of the oocytes die by the mechanism of apoptosis, at the same time (2). In this way, in the neonatal mouse ovary, estrogen regulates oocyte nest dissociation and primordial follicle formation by multiple pathways (3). It has been suggested that sex differentiation in vertebrates is steroid hormones dependent. Estrogen plays a critical role in ovarian

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differentiation. Male sex differentiation can occur to the absence of estrogen (4). This hormone is associated with reproductive organs, mammary gland and sexual behavior in female (5).

Estrogens play important roles and several effects on the ovarian follicle level (6). Also, studies have shown that estrogen had protective effects on the follicular atresia in the adult mammalian ovaries (7). This hormone can regulate the granulosa cells proliferation and differentiation, increasing the gonadotropin action, initiation of gap junctions and acceleration of steroidogenesis. In addition, it has several extragonadal effects and important role in endometrial proliferation and biosynthesis of cervical mucus (6). In addition, it has been shown that estrogen plays basic roles in the regulation of follicle/oocyte maturation (8).

Aromatase, the product of the CYP 19 gene, is an enzyme complex that catalyzes the rate-limiting step in the production of estrogens. The conversion of adrenal androstenedione to estrogen was performed through three hydroxylation steps. his enzyme was expressed in some tissues such as liver, muscle, fat and ovary (9, 10).

Because aromatase is the terminal step in biosynthetic sequence, it can be a good target for selective inhibition of estrogen biosynthesis. Aromatase inhibitors (AIs) have been used in clinical applications more than 20 years (11). The studies have shown that the use of AIs for adjuvant therapy in breast cancer are superior to the tamoxifen (9, 12).

The lack of specificity and the unfavorable toxicity profile of first-generation AIs led to research for more selective AIs. Third generation aromatase inhibitors, including anastrozole and letrozole, have been approved for the treatment of breast cancer (13). Letrozole and anastrozole are two main members of non-steroidal AIs (14). These aromatase inhibitors have a short half-life (approximately 45 hours), few side effects, and more potency (15).

Anastrozole has been approved in North America and Europe and is a first selective and high potency drug with 96.7% aromatase inhibition in subjects receiving 1mg/day orally (16).

This study was designed to determine whether estrogen is required for follicular development in mouse model estrogen absence using anastrozole.

Materials and methods

All tests were performed according to the experimentation at Department of Medical Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

In this experimental study, 9 mature male and 18 mature female BALB/c mic (15-25 gr weight, six week age) were housed for mating in the 12h light/12h dark cycle and controlled temperature (21-22°C). Food and water were available for animals during the study. The animals were kept for three weeks in the laboratory before the beginning the experiments.

After presenting the vaginal plaque, the female mice were divided into two groups of control and experimental and this day was considered as the first day of pregnancy. In experimental group, anastrozole (50 mg/kg, i.p.) was injected on the 14^{th} day of pregnancy. Also, the control group was received normal saline i.p. any treatment on the 14^{th} day of pregnancy.

After the delivery, 16 female pups were separated randomly in each group. In the 2^{nd}, 4^{th} and 7^{th} day after delivery, eight pups from each group for age time were sacrificed, respectively and the ovaries were carefully removed and were fixed in the 10% formalin. After tissue processing, the 5um sections were prepared and H&E staining were performed. The primordial, primary and growing follicles were counted and compared in each groups.

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Data analysis

Data were analyzed by SPSS-16. ANOVA test were used for comparing the groups and $P < 0.05$ was considered as significant level.

Results

The number of primordial, primary and growing follicles was compared in the control, sham and treated groups.

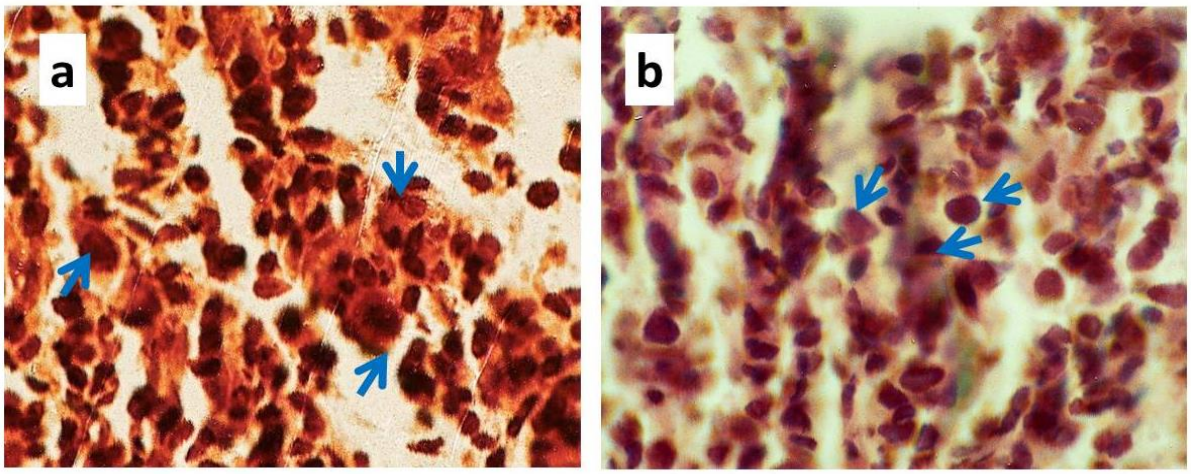


Figure 1. The follicular development in the pups from mice treated by anastrozole. (a) 2 day Control group, (b) 2 day treatment group, blue arrows show the oocytes (H&E staining, 100×).

According to the Figure 1, the 2 day ovaries were studied. As showed in this photography, oocytes in control groups were separated (Figure1a), however in treatment group oocytes are in clusters (Figure1b). Table 1 shows the number of primordial, primary and growing follicles in the ovary of five day pups. There wasn't significant differences in the mean number of primordial, primary, growing follicles of 4 day pups (P=0.092, P=0.121 and P=0.129, respectively, Table 1, Figures 2a and 2b).

Table 1. The mean number of primordial, primary and growing follicles in five day pup. The comparison between control, sham and treated groups.

		Control	Treatment	P value
Primordial follicles	4th day	15.65±2.35	13.56±2.89	0.092
	7th day	19.24±3.21	17.32±3.12	0.076
Primary follicles	4th day	10.56±3.76	11.76±3.71	0.121
	7th day	13.24±3.4	14.32±3.13	0.099
Growing follicles	4th day	3.56±2.61	3.56±2.6	0.129
	7th day	6.24±2.49	5.61±2.13	0.136

In addition, There wasn't significant differences in the mean number of primordial, primary, growing follicles pf 7 day pups (P=0.076, P=0.099 and P=0.136, respectively, Table 1, Figures 2c and 2d).

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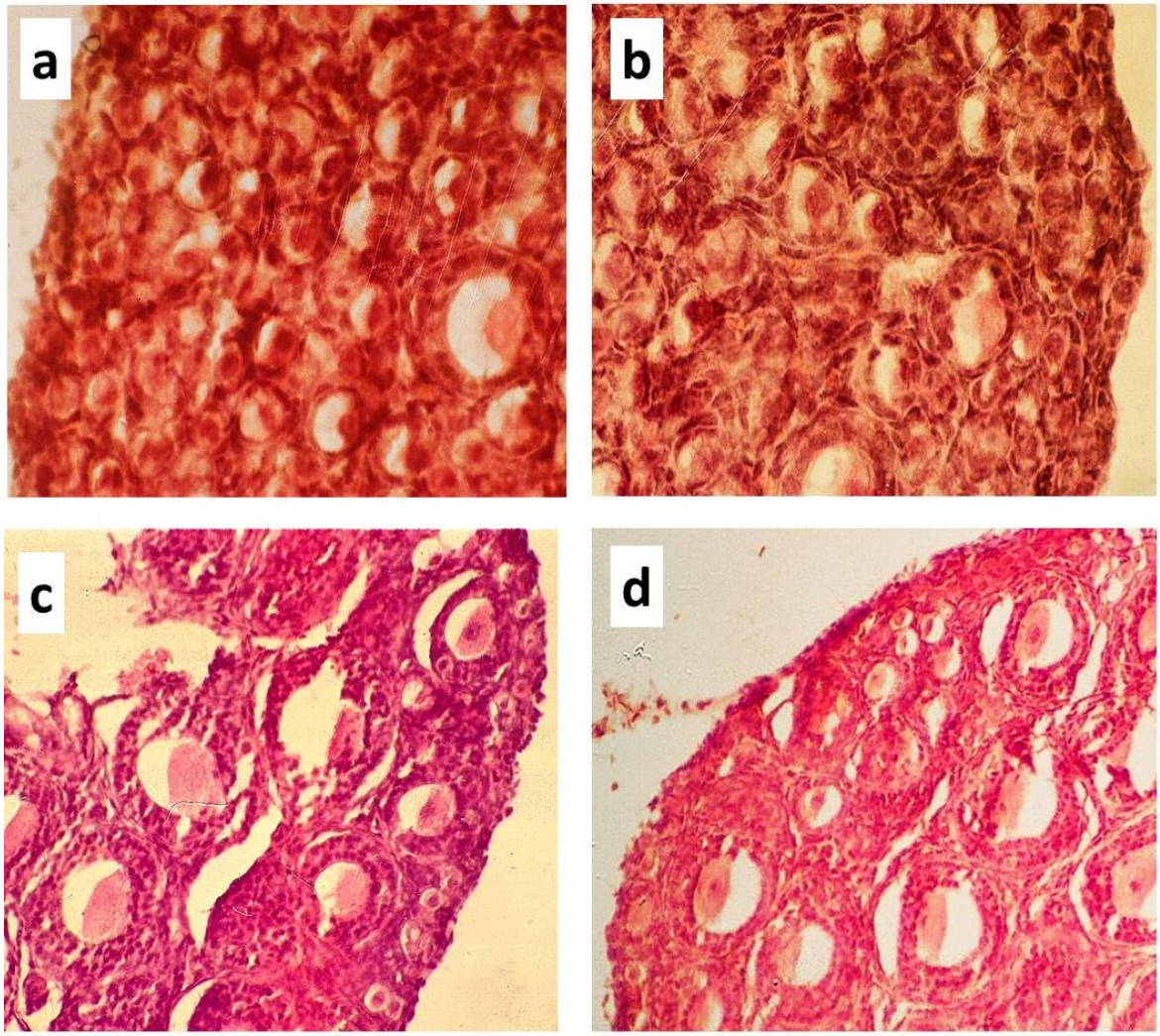


Figure 2. The follicular development in the pups from mice treated by anastrozole. (a) 4 day Control group, (b) 4 day treatment group, (c) 7 day Control group, (d) 7 day treatment group. (H&E staining, 100×).

Discussion

Estrogen plays an important role in sexual characteristics and essential in sex organ development in female (5). It has important effect on ovarian follicles developments and protect them from atresia in adult mammalian ovaries (6, 7). In addition, it has been shown that estrogen plays basic roles in the regulation of follicle/oocyte maturation (8). Aromatase is the last step in biosynthetic sequence and can be a good target for selective inhibition of estrogen biosynthesis (17). AIs such as anastrozole are used for treatment of hormone-sensitive breast cancer (13). AIs were used for blocking the estrogen biosynthesis in animal models (6). In these studies, the number of healthy follicles was decreased and estrogen presentation in the folliculogenesis environment was necessary to lead the normal oocyte maturation.

Accordingly, this drug can be used for evaluation of estrogen effects on the development of ovary.

In this study, anastrozole was injected into the pregnant mice and the number of several follicles (Primordial, primary and growing follicles) of ovaries was compared within the 2, 4 and 7 day pups between control and treatment groups.

According to the results, oocytes were observed in clusters in the 2 day pups of treatment group. These results indicate that

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maternal anastrozole can affect the breakdown of the clusters and formation of primordial follicles.

In addition, it can be suggested that maternal estrogen absence can affect these processes and estrogen has important role in the breakdown of nest. Chronic administration of anastrozole decreased the capacity of the ovaries and anastrozole exposure can affect folliculogenesis at the initiation stage.

Primordial follicles formation is performed in a process known as ovarian follicular breakdown process. In this process, individual primordial follicles separate from 'nests' of oocytes which have complete mitotic proliferation and entered into meiosis (18). However, during nest breakdown process, some oocytes disappear by apoptosis (19).

Estrogen presence in follicular fluid had important roles in follicular breakdown process.

In addition, Guo et al. (2004) evaluated the estrogen deprivation effects on follicle/oocyte development in mice. Their results showed that estrogen plays basic roles in the regulation of follicle/oocyte maturation (8).

In this study mice were used as a model of ovarian follicular development. In rodents, nest breakdown process occurs after their birth (18, 20). Accordingly, rodents are suitable models for studying the external materials on ovarian follicular development.

Because of Anastrozole quick effects on estrogen production, is a selective anti-hormonal treatment for the hormone dependent breast cancers in postmenopausal women (21). Anastrozole can reduce the level of estrogen by inhibiting the conversion of androgen to estrogen (22).

Steroidal products present in high levels in the follicular fluid, during the follicular phase and its role in oocyte maturation and embryo development is unknown (6).

Roshangar et al. (2010) evaluated the maternal tamoxifen effects on the neonatal oocyte differentiation. In their study, tamoxifen was used to study the estrogen deprivation effects on the ovarian follicular development. Their result showed that in the 2 day pups oocytes were in the cluster formation (23). Their results confirm the results of present study.

In addition, Fatum et al. (2006) evaluated the anastrozole effect on the 3-4 weeks mice. Their results showed that estrogen couldn't effect on folliculogenesis and it might be an independent process (6).

The results of present study showed the treatment of the mothers with anastrozole and the absence of estrogen can't effect on the development of follicles in 4 and 7 day pups. This study was designed on histological findings in different days of birth and the molecular change weren't considered. According to the results, it is suggested that after birth the development of ovaries in mice can be affected by the neonatal estrogen. Therefore, the differences were not observed. However, the deprivation of estrogen can effect on ovaries and change its regular follicular development.

Dutta et al. (2014) showed that in hormonal deprivation, maternal estradiol remain high and showed that neonatal ovaries were the source of hormones. However, in late fetal phases, the production of hormones stopped (24).

In several studies, the effect of different aromatase inhibitors was evaluated. Zelinski-Wooten et al. showed that the 1, 4, 6-androstatrien-3, 17-dione (ATD; Steraloids, Wilton, NH) could reduce the estradiol levels during the late follicular phase. However, the total number of follicles was not changed (25).

In other study conducted by Moudgal et al. (1995), fadrozole as an aromatase inhibitor was evaluated in hamster, rabbits and monkeys. Their results showed that fadrozole couldn't affect the normal and hCG/LH-induced ovulations in these types of animals (26). However, Roshangar et al. (2010) showed that estrogen deprivation could effect on the

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follicular number and diameter in the 3, 6 and 7 day old litters and chronic administration of tamoxifen the could decrease the number and diameter of oocytes and dramatically increased the ratio of primordial follicles to primary follicles they suggested that such tamoxifen exposure could suppress the ovarian functional capacity (23). The result of this study was in contrast with the results of present study in older pups.

Conclusion

According to the studies, estrogen is necessary for oocyte maturation and follicular breakdown and blocking it with an aromatase inhibitor, like anastrozole, can reduce the primordial follicles. However, maternal anastrozole and estrogen depression couldn't effect on the histology of ovarian follicle in neonatal mouse.

References

1. Jefferson W, Newbold R, Padilla-Banks E, Pepling M. Neonatal genistein treatment alters ovarian differentiation in the mouse: inhibition of oocyte nest breakdown and increased oocyte survival. Biol Reprod. 2006;74(1):161-8.
2. Chen Y, Jefferson WN, Newbold RR, Padilla-Banks E, Pepling ME. Estradiol, progesterone, and genistein inhibit oocyte nest breakdown and primordial follicle assembly in the neonatal mouse ovary in vitro and in vivo.Endocrinology.2007;148(8):3580-90.
3. 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. J Endocrinol. 2009;202(3):407-17.
4. Lance VA, Bogart MH. Disruption of ovarian development in alligator embryos treated with an aromatase inhibitor. Gen Comp Endocrinol. 1992;86(1):59-71.
5. Hegele-Hartung C, Siebel P, Peters O, Kosemund D, Müller G, Hillisch A, et al. Impact of isotype-selective estrogen receptor agonists on ovarian function. Proc Natl Acad Sci USA. 2004;101(14):5129-34.
6. Fatum M, Gyo Y, Diana P, Laufer N, Simon A. Is estradiol mandatory for an adequate follicular and embryo development? A mouse model using aromatase inhibitor (anastrozole). J Assist Reprod Genet. 2006;23(11-12):407-12.
7. Billig H, Furuta I, Hsueh A. Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. Endocrinology. 1993;133(5):2204-12.
8. Guo Y, Guo K-J, Huang L, Tong X-G, Li X. Effect of estrogen deprivation on follicle/oocyte maturation and embryo development in mice. Chinese Med J. 2004;117(4):498-502.
9. Hirakawa H, Yokoyama Y, Yoshida H, Mizunuma H. Inhibitory effects of aromatase inhibitor on estrogen receptor-alpha positive ovarian cancer in mice. J Ovarian Res. 2014;7(1):4.
10. Steinkampf MP, Mendelson CR, Simpson ER. Regulation by follicle-stimulating hormone of the synthesis of aromatase cytochrome P-450 in human granulosa cells. Mol Endocrinol. 1987;1(7):465-71.
11. Karaer Ö, Oruç S, Koyuncu FM. Aromatase inhibitors: possible future applications. Acta Obstet Gynecol Scand. 2004;83(8):699-706.
12. Wong Z, Ellis M. First-line endocrine treatment of breast cancer: aromatase inhibitor or antioestrogen&quest. Br J Cancer. 2004;90(1):20-5.
13. Buzdar A, Howell A. Advances in aromatase inhibition clinical efficacy and tolerability in the treatment of

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breast cancer. Clin Cancer Res. 2001;7(9):2620-35.
14. Angelopoulos N, Barbounis V, Livadas S, Kaltsas D, Tolis G. Effects of estrogen deprivation due to breast cancer treatment. Endocr Relat Cancer. 2004;11(3):523-35.
15. Bhatnagar A, Häusler A, Schieweck K, Lang M, Bowman R. Highly selective inhibition of estrogen biosynthesis by CGS 20267, a new non-steroidal aromatase inhibitor. J Steroid Biochem Mol Biol. 1990;37(6):1021-7.
16. Gangadhara S, Bertelli G. Long-term efficacy and safety of anastrozole for adjuvant treatment of early breast cancer in postmenopausal women. Ther Clin Risk Manag. 2009;5:291.
17. Casper RF, Mitwally MF. Aromatase inhibitors for ovulation induction. J Clin ndocrinol Metab. 2006;91(3):760-71.
18. Pepling ME. From primordial germ cell to primordial follicle: mammalian female germ cell development. Genesis. 2006;44(12):622-32.
19. Pepling ME, Spradling AC. Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. Develop Biol. 2001;234(2):339-51.
20. Nilsson EE, Skinner MK. Progesterone regulation of primordial follicle assembly in bovine fetal ovaries. Mol Cell Endocrinol. 2009;313(1):9-16.
21. Fabian C. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract Suppl. 2007;61(12):2051-63.
22. Di Leo A, Malorni L. Polyendocrine Treatment in Estrogen Receptor–Positive Breast Cancer: A "FACT" Yet to Be Proven. J Clin Oncol. 2012;30(16):1897-900.
23. Roshangar L, Rad JS, Afsordeh K. Maternal tamoxifen treatment alters oocyte differentiation in the neonatal mice: Inhibition of oocyte development and decreased folliculogenesis. J Obstetr Gynaecol Res. 2010;36(2):224-31.
24. Dutta S, Mark-Kappeler CJ, Hoyer PB, Pepling ME. The steroid hormone environment during primordial follicle formation in perinatal mouse ovaries. Biol Reprod. 2014: 114.119214.
25. Selvaraj N, Shetty G, Vijayalakshmi K, Bhatnagar A, Moudgal N. Effect of blocking oestrogen synthesis with a new generation aromatase inhibitor CGS 16949A on follicular maturation induced by pregnant mare serum gonadotrophin in the immature rat. J Endocrinol.994;142(3):563-70.

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