Prenatal Exposure to Acrylamide Differently Affected the Sex Ratio, Aromatase and Apoptosis in Female Adult Offspring of Two Subsequent Generations

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Summary

In the present study, we investigated the effect of acrylamide (ACR) exposure during pregnancy on the ovary of female adult offspring of two subsequent generations. Sixty-day-old Wistar albino female rats were given different doses of ACR (2.5 and 10 mg/kg/day) from day 6 of pregnancy until giving birth. Females from the first generation (AF1) were fed *ad libitum*, and thereafter, a subgroup was euthanized at 8 weeks of age and ovary samples were obtained. The remaining females were maintained until they reached sexual maturity (50 days old) and then treated in the same way as the previous generation to obtain the second generation of females (AF2). The histopathological examination indicated a high frequency of corpora lutea along with an increased number of antral follicles that reached the selectable stage mainly at a dose of 2.5 mg/kg/day. Interestingly, ACR exposure significantly increased the mRNA levels of *CYP19* gene and its corresponding CYP19 protein expression in AF1 females. The TUNEL assay showed a significantly high rate of apoptosis in stromal cells except for dose of 2.5 mg/kg/day. However, in AF2 females, ACR exposure significantly increased the number of degenerating follicles and cysts while the number of growing follicles was reduced. Moreover, in both ACR-treated groups, estradiol-producing enzyme CYP19A gene and its corresponding protein were significantly reduced, and an excessive apoptosis was produced. We concluded that the ovarian condition of AF1 females had considerable similarity to the typical early perimenopausal stage, whereas that of AF2 females was similar to the late perimenopausal stage in women.

Key words

Reproductive toxicity • Pregnancy • Acrylamide exposure • Fetal development • Menopause • Aging • Fertility

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Introduction

Information reported by epidemiological studies indicates an increase in female infertility, which may be caused by environmental exposure to endocrine disrupting chemicals (EDCs) [2-4]. Indeed, the latter has an effect on ovarian stocks, oocyte quality, sexual hormone levels, and thus fertility [5]. Studies have indicated that synthesized, secreted, transported, bound, or degraded hormones could be affected by endocrine disruption as a result of chemical exposure in the environment or during occupational exposure [6-8].

Polyacrylamide, which results from polymerization of ACR under certain temperatures or upon UV irradiation, is used in the synthesis of cosmetics and skin creams, in food packaging, in pulp and paper making, in the processing of metals and in textile manufacturing [15]. It has been found in fertilizers [19], tobacco industry products and plastics [20]. The total amount of acrylamide in smoke from one cigarette is

approximately 1 µg or higher [21]. ACR can enter the body through absorption by the skin (e.g. *via* cosmetics), digestive system (ingestion) and respiratory system (inhalation), as from smoking [22,23]. Because of the small size of the ACR molecule and its hydrophilic nature, it is able in theory to diffuse passively and target all tissues and induce carcinogenesis [24]. ACR can also be formed in food that contains carbohydrates and amino acids when exposed to high temperature [25,26]. The European Food Safety Authority (EFSA) reported that the level of ACR in food ranges from under 30 µg/kg to 4700 µg/kg, depending on the product [27,28]. As many products have a high content of asparagine, including asparagus, cocoa beans and cereals, most people are exposed to the effects of ACR. Our recent study found that ACR can damage ovarian function and thereby female fertility [29]. The aim of the present study was therefore to investigate the early life exposure to acrylamide on 1) the histopathological ovarian changes, 2) the expression levels of some key genes and proteins involved in the regulation of folliculogenesis and steroidogenesis, and 3) the ovarian apoptotic cell death in female adult offspring of two subsequent generations.

Materials and Methods

Animal treatment and sampling

This study was approval by the Scientific Research Ethics Committee at King Saud University, Riyadh, Saudi Arabia (Reference No: KSU-SE-20-21). Thirty pubertal virgin females (50 days old) were weighed and housed individually in mating cages with males and provided a diet of standard laboratory chow (23 % protein, 4.5 % fat, 3030 kcal/kg; lab diet 5001, Brentwood, MO). The pregnant females at day 0 of gestation (GD 0) were divided into three groups, and treatment was administered beginning at GD 6 and until delivery. The first group of females was considered a control and was gavaged with distilled water (n=5). The second group of females (n=5) received an ACR by oral treatment at a dose of 2.5 mg/kg. The third group of females (n=5) received an ACR by oral treatment at a dose of 10 mg/kg. [30]. After parturition, we obtained the first generation of offspring obtained from ACR-treated mothers; these were called ACR animals of the first generation (AF1), and those obtained from the control were called the control group of the first generation (CF1). A subgroup of AF1- and CF1-offspring females were euthanized when they reached 8 weeks old (adult age). Ovaries were collected, cleaned with distilled water, quickly measured, labeled according to their group of origin and fixed in the corresponding fixative (10 % Neutral Buffer Formalin (NBF) for histopathological and TUNEL studies and RNA later for gene and protein expression analyses). The remainder of the AF1 and CF1-females were allowed to reach sexual maturity and were mated with males and given the same treatments as the previous generation. After parturition of AF1 and CF1-females, the second generation offspring were obtained from ACR-treated AF1 mothers and called ACR offspring of the second generation (AF2), while those obtained from the control were called the control of the second generation (CF2). The AF2 and CF2 females were euthanized when they reached 8 weeks old (adult age) and ovaries were collected, labeled according to their groups of origin and fixed as described above.

Histological preparation

Obtained ovary samples were fixed for 24 h in NBF and cut using a microtome in sections of 5-7 µm thickness, then transferred to glass slides containing warm water (3 °C) and albumin glycerol fixative for adhesion on a hotplate. Wrinkles were removed, and hematoxylin and eosin were used to stain the sections.

Immunofluorescence staining

Sections were placed on hotplates (60 °C) and deparaffinized with xylene. Then, they were rehydrated by passed in descending concentrations of ethanol and washed with distilled water and 1× phosphate-buffered saline (PBS). After that, the tissue sections were treated with 0.1 % Triton X-100 with 0.1 % sodium citrate and then with blocking buffer (1 % Bovine Serum Albumin (BSA) in PBS). The polyclonal rabbit primary antibody CYP19 (1:100, DGpeptides Co., Ltd. China) was applied on a flat, balanced surface overnight at 4 °C. After washing with PBS, the fluorescein (FITC) goat anti-rabbit antibody (dilution 1:2000, ab6717, MA, USA) was added at room temperature in the dark for 45 min. Sections were washed with PBS and then TE buffer prior to the addition of the Hoechst solution (diluted 1:15000, Hoechst 33342, Life Technologies, Waltham, MA, USA). Finally, the slides were washed with TE buffer, dried and covered with coverslips in one drop of solution 50 % glycerol/ Tris-EDTA (TE) buffer, the edges of coverslip were sealed with nail polish. For imaging, spinning disk confocal microscope from Zeiss was used. The signal intensity was analyzed and used for the quantification of protein expression with the Zen 3.1 service (ZEN lite).

Analysis of gene expression

Total RNA was extracted by using a Qiagen RNeasy Mini Kit (Qiagen, Westburg, The Netherlands) from RNA-stabilized tissue (30 mg), including on-column DNase treatment *via* Qiagen RNase-Free DNase. Real-time PCR was implemented with a SYBR Green and Applied Biosystems 7500 Fast Real-time PCR system (Carlsbad, CA) using the *CYP19A* gene-specific primers (Forward: TGAGTCTCCCAAGGTCATCC, Reverse: GGGTTCAGCATTTCCAAAAA). An iScript™ cDNA synthesis kit (Applied Biosystems, Carlsbad, CA) was used to produce cDNA of these samples with multiple sets of primer cDNA designed to amplify the cDNA for relative quantification RT-PCR. For each sample, this procedure was repeated three times.

TUNEL assay

The ovaries samples were fixed for 24 h in NBF then preserved in alcohol 70 %. Paraffin sections with a thickness of 3 µm were mounted on coated slides at room temperature. The sections were dewaxed, rehydrated and washed in PBS. Then, the tissue sections were incubated at 37 °C with a Proteinase K working solution and permeabilized in 0.1 % Triton X-100 with 0.1 % sodium citrate. TUNEL staining was applied following the manufacturer's instructions with an In Situ Cell Death Detection Kit, TMR red (12156792910, Roche Diagnostics, Mannheim, Germany). The nuclei were stained with Hoechst solution (diluted 1:15000, Hoechst 33342, Life Technologies, Waltham, MA, USA). For imaging, we used a spinning disk confocal microscope from Zeiss. The signal intensity was analyzed and used for the quantification of protein expression with the Zen 3.1 service (ZEN lite).

Statistics

For statistical comparisons was used one-way analysis of difference followed by Tukey's multiple comparison (GraphPad Prism version 9.3.0). All values are presented as the mean ± standard deviation (SD). Significance was positioned at a P value less than 0.05.

Results

Effect of acrylamide on offspring sex ratio

There were no significant differences in the sex ratio of the treated groups of the first-generation AF offspring compared to the control group (Fig. 1A). However, the sex ratio of the second-generation AF2 offspring showed a significant increase at the ACR dose of 10 mg/kg/day, while those that received the dose of 2.5 mg/kg/day did not show any significant effect compared to the control CF2 (Fig. 1B).

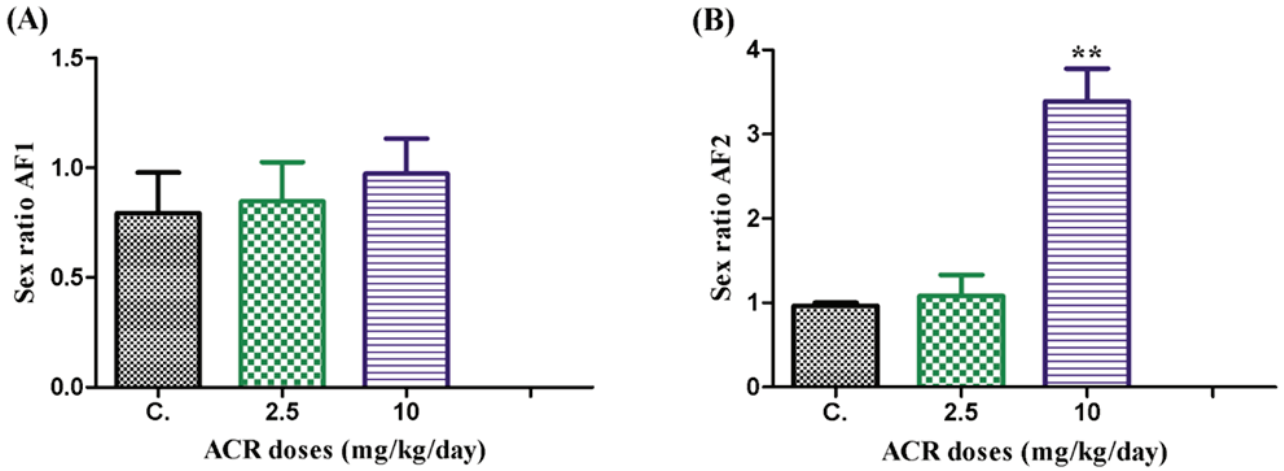

Fig. 1. (**A**) The sex ratio of offspring in the first generation AF1 compared to that in control CF1. No significant difference between the control CF1 and other treatment groups. (**B**) The sex ratio of offspring in second generation AF2 compared to that in control CF2. ACR (10 mg/kg/day) caused significant elevation, while the dose of ACR (2.5 mg/kg/day) did not show any different from the control CF2. Values are means ± S.E.M. ** P<0.001.

Effect of acrylamide on ovarian histopathological changes

While the ovaries from control 8-week-old females showed a normal number of growing follicles and corpora lutea, ovaries from treated 8-week-old females in AF1 showed a decrease in the number of growing follicles that was inversely proportional to the increase in the dose of ACR. The number of corpora lutea increased with increasing doses of ACR, and even the

few growing follicles that were found were characterized by pyknosis in granulosa cell nuclei and oocyte vacuolization (Fig. 2D-I Plate I).

Microscopic examination of the ovarian sections in 8-week-old ACR-treated female AF2 mice showed an increase in the number of degenerating follicles and cysts at doses of 2.5 and 10 mg/kg/day, while the number of growing follicles was reduced or almost absent compared to that in the control, indicating early ovarian aging (Fig. 2D-I, Plate II).

Effect of acrylamide on estradiol-producing enzyme CYP19A

Compared with that in the control group (Fig. 3A-C), the protein expression level of CYP19 was significantly increased with increasing doses of ACR in the 8-week-old AF1 groups (Fig. 3D-I and 3P), while the green fluorescence intensity of CYP19 in the 8-week-old AF2 groups was significantly decreased (Fig. 3J-O and 3R).

In accordance with aromatase protein levels, RT-PCR results showed that the relative mRNA levels of CYP19 were significantly increased in the 8-week-old AF1 females in the 2.5 and 10 mg/kg/day groups compared with the control CF1 group (Fig. 3Q). However, CYP19 mRNA levels in the ovaries from 8-week-old AF2 females were reduced significantly in all treated groups compared to the control group CF2 (Fig. 3S).

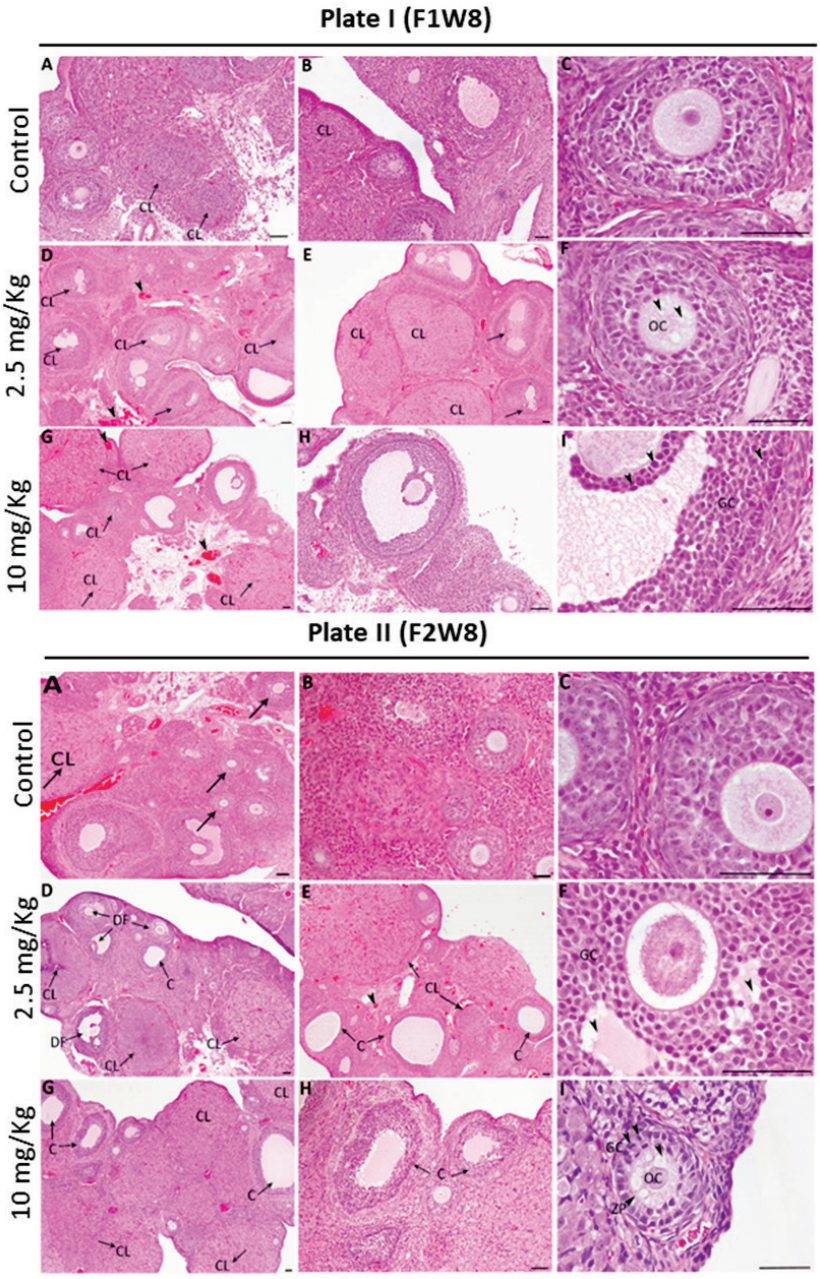

Fig. 2. Plate I: Histological sections from 8-week-old females of the first generation (F1W8) from treated and control groups. Ovaries from control (CF1) displayed normal growing follicles and few corpora lutea (CL) (arrows) (**A-C**). In ovaries from females AF1, proportional increase of the number of corpora lutea (CL) with vacuolations and degenerative luteal cells (arrows), blood vessel congestion and vacuolated oocyte (OC) (arrowheads) can be observed with the dose (2.5 mg/kg) (**D-F**). Rising number of corpora lutea (CL) (arrows), blood congestion and pyknosis in granulosa cells (GC) (arrowheads) can be seen in the dose 10 mg/kg (**G-I**). Scale bar=60 µm. Plate II: Histological evaluation of ovary structure in 8-week-old females of the second generation (F2W8). Ovaries from control (**A-C**) CF2 show normal growing follicles and few corpora lutea (CL) (arrows). AF2 females treated with the dose (2.5 mg/kg) showed an increased number of corpora lutea (CL), degenerated follicles (DF) and cysts (C) (arrows), reduction in growing follicles. Congestion of blood vessels and vacuolation of granulosa cell's layers (arrowheads) can also been seen (**D-F**). In (10 mg/kg) ovary showing rising in cysts (C) and corpora lutea (CL) number (arrows). Oocytes (OC) vacuolation, irregular thinning zona pellucida (ZP) and pyknosis in the granulosa cells nuclei (arrowheads) (**G-I**). Scale bar=60 µm.

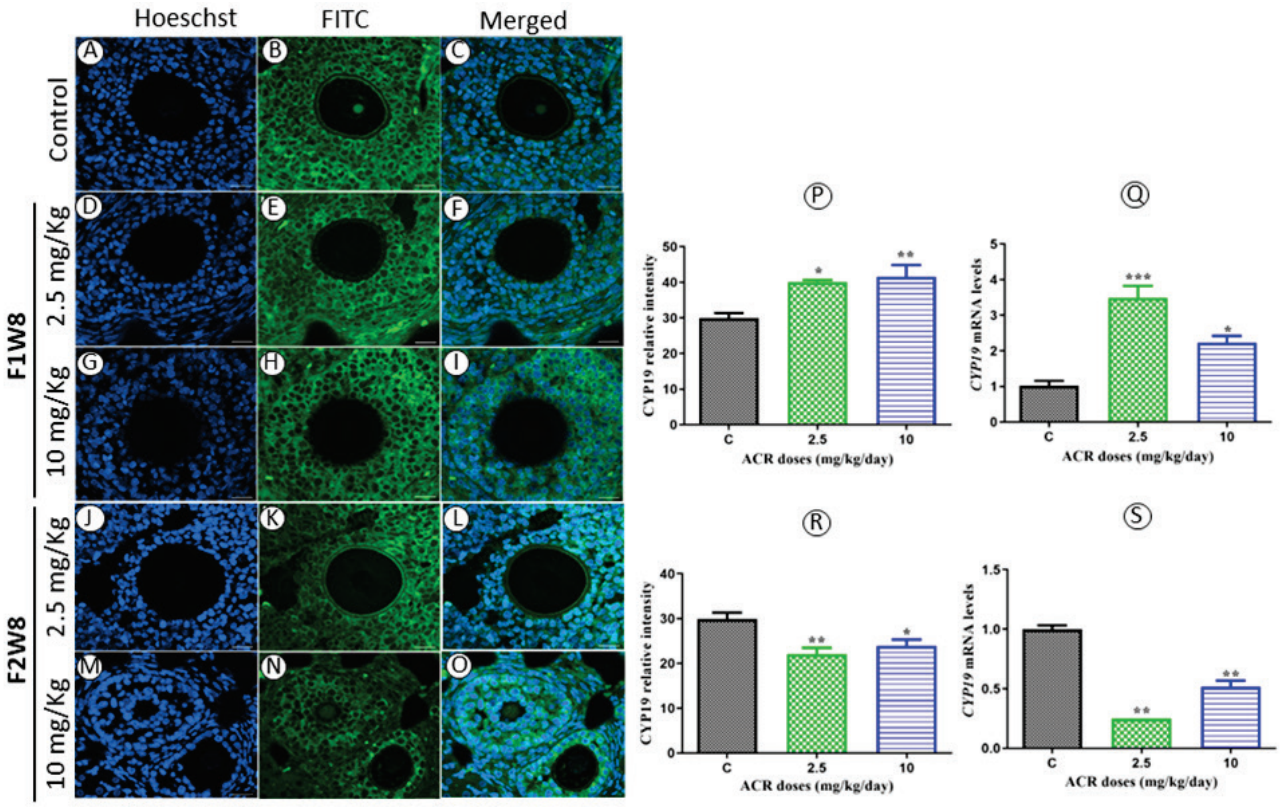

Fig. 3. The ovarian CYP19 localization and expression in 8-week-old female offspring from the first (F1W8) and the second (F2W8) generation compared to control. F1W8: Stained sections from control CF1 (**A-C**), AF1 females treated with the dose 2.5 mg/kg (**D-F**) and 10 mg/kg (**G-I**). F2W8: Stained sections from AF2 females treated with the dose 2.5 mg/kg (**J-L**) and 10 mg/kg (**M-O**). Sections of ovary was performed by immunofluorescence using specific CYP19 antibody stained with FITC (green), cell nuclei are stained with Hoechst (blue). Scale bar=20 µm. The relative CYP19 intensity in 8-week-old AF1 females and AF2 females compared with control females are presented in the graphs (**P**) and (**R**), respectively. Effect of ACR on the mRNA expression level of the *CYP19* gene in 8-week-old AF1 females (**Q**) and in 8-week-old AF2 females (**S**) compared with control females. Values are means ± S.E.M. * P<0.05; ** P<0.001; *** P<0.0001.

Effect of acrylamide on ovarian cell apoptosis

While no significant number of TUNEL-positive cells was found in the ovaries from 8-week-old AF1 females treated with 2.5 mg/kg ACR (Fig. 4D-F), a significantly greater number of TUNEL-positive cells were observed in the groups treated with 10 mg/kg (Fig. 4G-I) in comparison to the control group (Fig. 4A-C and Fig. 4J). However, ACR treatment led to a significant increase in the number of TUNEL-positive cells in both treated groups of 8-week-old AF2 females (Fig. 4N-S) compared to the control group (Fig. 4K-M and Fig. 4T).

Discussion

In the present study, female rats were orally exposed to 2.5 and 10 mg/kg ACR during pregnancy and the ovary function and structure in the F1 and F2 female offspring at week 8 of age were analyzed. We found that ACR does not have any effect on sex ration of the AF1 female offspring, while it significantly increased only for the dose of 10 mg/kg/day in AF2 female offspring. Although the sex of a species is genetically normally inherited and fixed for life, this critical demographic parameter can be induced by environmental factors during early steps of development [35], and affects the growth rate and the evolutionary trajectories of populations. In fact, the primary genetic sex can be challenged during early development in response to environmental factors, commonly reported in insects, fish, amphibians and reptiles [36]. This phenomenon may be attributed to epigenetic mechanisms, notably DNA methylation, as reported in many previous studies [37].

The histological study indicated the high frequency of corpora lutea along with an increased number of antral follicles that reached the selectable stage mainly at a dose of 2.5 mg/kg/day and the occurrence of pyknosis in granulosa cell nuclei of the antral follicles. The TUNEL assay showed a significantly high rate of apoptosis in stromal cells, except in the 2.5 mg/kg/day group. These characteristics of reproductive aging remind

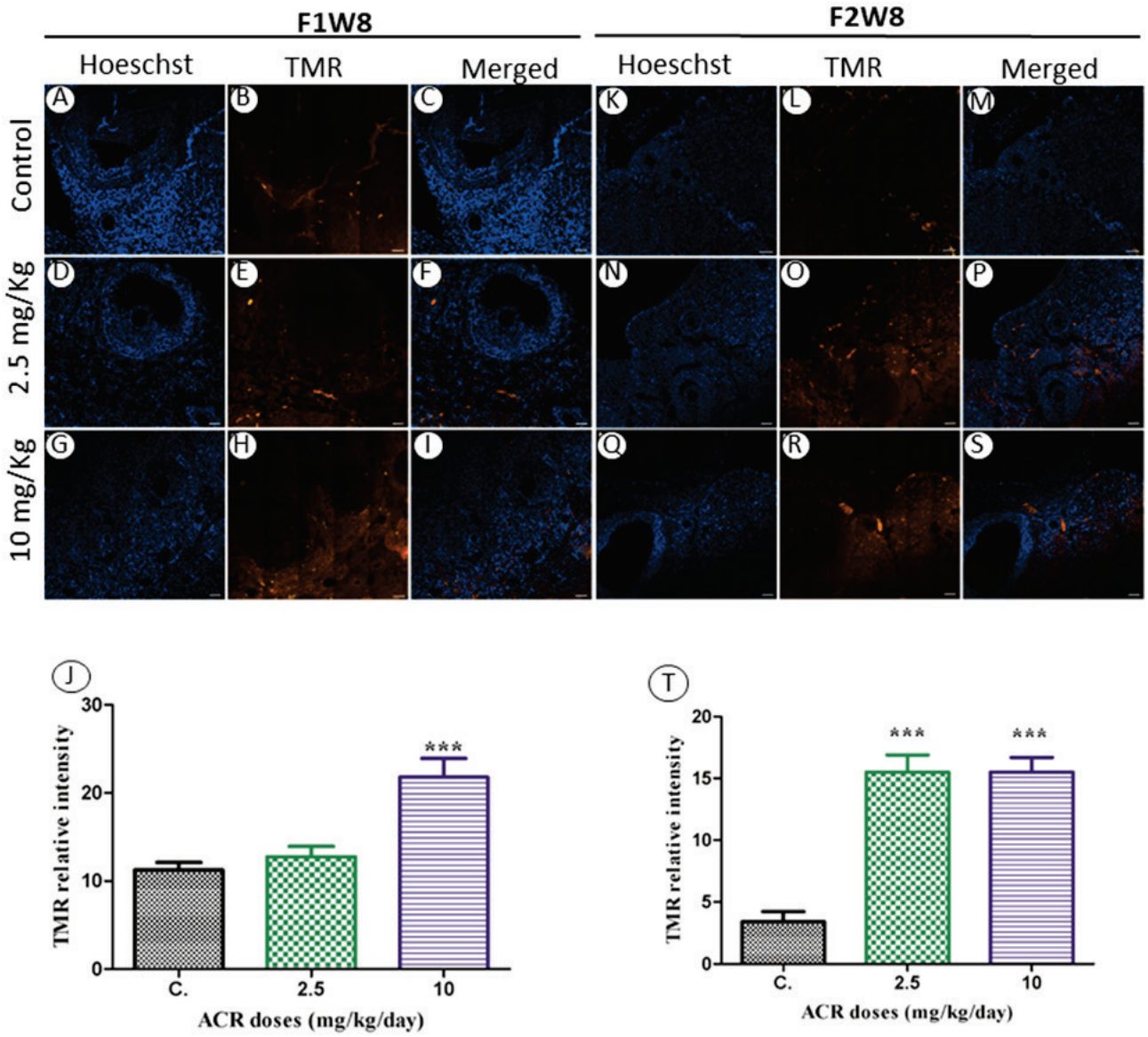

Fig. 4. The TUNEL reaction in 8-week-old female offspring from the first (F1W8) and second (F2W8) generations compared to control. Stained sections from control CF1 (**A-C**), AF1 females treated with the dose 2.5 mg/kg (**D-F**) and the dose 10 mg/kg (**G-I**). (**J**) The TMR relative intensity in 8-week-old AF1 females compared with control CF1. The TUNEL reaction in 8-week-old females of control CF2 (**K-M**), AF2 females treated with the dose 2.5 mg/kg (**N-P**) and the dose 10 mg/kg (**Q-S**). Sections of ovary apoptosis was performed by confocal images of follicles with TUNEL fluorescence in both granulosa and oocyte nuclei stained in (red) TMR, cell nuclei are stained with Hoechst (blue). Scale bar=50 µm. The TMR relative intensity in 8-week-old AF2 females compared with control CF2 (**T**). Values are means ± S.E.M. *** P<0.0001.

us of a specific stage of the female reproductive lifespan that precedes menopause and those previous studies named the menopause transition stage. This stage, also called perimenopause, is defined as the transition from reproductive age to menopause and can be divided into early perimenopause and late perimenopause, ending by the last menstrual period and leading to the beginning of the postmenopause stage [38]. In contrast to the late perimenopausal stage, early menopause is mainly defined by high estrogen levels [39] which is in agreement with our results as we found an upregulation of estradiol-producing enzyme *CYP19A* gene and increased expression of its corresponding protein. This stage is also called the "end of female fertility" (EFF) [40] because it is characterized by a great decrease in fertility but passes unnoticed by women since normal and regular menstrual cycles are maintained [40]. As an example, when menopause occurs at age 45, the EFF had already started at 35, and the beginning of subfertility had already started at 25 [40]. When the EFF period approaches, a gradual disruption occurs, characterized by an increased proportion of growing follicles reaching the selectable

stage and a rise in FSH, accompanied by declines in the levels of the inhibin B and Anti-Mullerian Hormone (AMH) proteins [40-42]. As these biomarkers of ovarian reserve are declining, ovarian aromatase expression increases with age in regularly cycling women compared with younger women, whereas follicular phase estrogen is preserved or increased successively [41]. These findings are consistent with our results, since we found an increase in *CYP19* gene and CYP19 protein expression. It is known that the ovary is the site of dynamic morphogenetic changes that begin during intrauterine life and continue during childhood and adulthood [43]. Thus, our data suggest that ACR exposure during pregnancy may reduce the reproductive lifespan by accelerating gradually in proportion to the dose of ACR, leading to decreased female fertility and premature reproductive aging. In fact, it has been described that high levels of estrogen and progesterone in females are associated with ovarian aging [44,45]. Moreover, in the current study, we showed using the TUNEL assay that the stromal cells are the main source of apoptotic cells. This finding is in agreement with prior published studies that reported that some of the characteristics of aged ovaries are increased level of apoptosis and fibrosis in the ovarian stroma associated with an aberrant accumulation of endocrine cells that highly expressed CYP17a1, CYP19a1, and luteinizing hormone/choriogonadotropin receptor (Lhcgr) [46]. Additionally, the high pyknosis we observed in granulosa cell nuclei is consistent with previous studies that described the decreased number of granulosa cells in antral follicles of aging ovaries [47]. A previous study reported statistically important positive correlations between ACR use and high estradiol levels in premenopausal women of normal weight [34], both ACR exposure and high estradiol have long been linked to an increased risk of premenopausal breast cancer [33,50,51].

We also examined the developmental effects of ACR on AF2 females. We found that if multigenerational exposure to ACR induced ovarian aging in females of the first generation (AF1), it accelerated potential early menopause in females of the second generation (AF2). Indeed, ACR exposure significantly reduced the levels of key ovarian markers, such as the estradiol-producing enzyme CYP19A1. In addition, ACR exposure increased AF2 female ovarian histological abnormalities, such as the appearance of cysts and the near-absence of primordial and growing follicles. The number of apoptotic cells in the stroma was highly elevated. These ovarian conditions have considerable similarity to the typical late menopausal transition patterns, also called late perimenopause, that may occur at any time of the reproductive lifespan of any female [39]. Studies on ovarian aging patterns have documented that women may undergo menopause prematurely due to chemotherapy, genetic disorders or environmental factors [53-55]. Our histological examination indicated an elevation in corpora lutea and the appearance of cysts, while almost no growing follicles were seen. This confirms our hypothesis, since the key aspect of the late menopausal transition stage is the decrease in oocyte number below the threshold required for ovarian function [56]. The increased number of corpora lutea may be due to the gradual increase in proportion of growing follicles reaching the selectable stage and ovulated as this period of late perimenopause approaches, which was reported previously [40,41]. Many chemicals have also been shown to induce follicular atresia, as well as a decline in the reserve of follicle “pools”, leading to premature menopause [52].

Conclusions

The current study demonstrated that ACR exposure during pregnancy may cause decreased female fertility and premature reproductive aging among the first generation of female offspring by inducing endocrine function alterations, histopathological disruptions, an up- and downregulation of estradiol-producing enzyme *CYP19A* gene and protein, and excessive apoptosis. This damage could be most severe among the female offspring of the second generation because successive generations of exposure may have accelerated the occurrence of the early menopausal transition stage, evidencing the transgenerational inheritance effect of ACR on reproductive longevity.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

1. Silvestris E, Lovero D, Palmirotta R. Nutrition and female fertility: an interdependent correlation. Fron Endocrinol (Lausanne) 2019;10:346. https://doi.org/10.3389/fendo.2019.00346
2. Hanson B, Johnstone E, Dorais J, Silver B, Peterson CM, Hotaling J. Female infertility, infertility-associated diagnoses, and comorbidities: a review. J Assist Reprod Genet 2017;34:167-177. https://doi.org/10.1007/s10815-016-0836-8
3. Jalouli M, Mofti A, Elnakady YA, Nahdi S, Feriani A, Alrezaki A, Sebei K, ET AL. Allethrin promotes apoptosis and autophagy associated with the oxidative stress-related PI3K/AKT/mTOR signaling pathway in developing rat ovaries. Int J Mol Sci 2022;23:6397. https://doi.org/10.3390/ijms23126397
4. Tarko A, Štochmaľová A, Hrabovszká S, Vachanová A, Harrath AH, Aldahmash W, Grossman R, Sirotkin AV. Potential protective effect of puncture vine (Tribulus terrestris, L.) against xylene toxicity on bovine ovarian cell functions. Physiol Res 2022;71:249. https://doi.org/10.33549/physiolres.934871
5. Harrath AH, Alrezaki A, Mansour L, Alwasel SH, Palomba S. Food restriction during pregnancy and female offspring fertility: adverse effects of reprogrammed reproductive lifespan. J Ovarian Res 2017;10:1-9. https://doi.org/10.1186/s13048-017-0372-x
6. Harrath AH, Alrezaki A, Jalouli M, Al-Dawood N, Dahmash W, Mansour L, Sirotkin A, Alwasel S. Benzene exposure causes structural and functional damage in rat ovaries: occurrence of apoptosis and autophagy. Environ Sci Pollut Res Int 2022:29:76275-76285. https://doi.org/10.1007/s11356-022-21289-5
7. Harrath AH, Alrezaki A, Jalouli M, Aldawood N, Aldahmash W, Mansour L, Alwasel S. Ethylbenzene exposure disrupts ovarian function in Wistar rats via altering folliculogenesis and steroidogenesis-related markers and activating autophagy and apoptosis. Ecotoxicol Environ Saf 2022;229:113081. https://doi.org/10.1016/j.ecoenv.2021.113081
8. Sirotkin AV, Dekanová P, Harrath AH. FSH, oxytocin and IGF-I regulate the expression of sirtuin 1 in porcine ovarian granulosa cells. Physiol Res 2020;69:461. https://doi.org/10.33549/physiolres.934424
9. Practice Committee of the American Society for Reproductive M. Smoking and infertility. Fertil Steril 2004;81:1181-1186. https://doi.org/10.1016/j.fertnstert.2003.11.024
10. Higgins S. Smoking in pregnancy. Curr Opin Obstet Gynecol 2002;14:145-151. https://doi.org/10.1097/00001703-200204000-00007
11. Wetendorf M, Randall LT, Lemma MT, Hurr SH, Pawlak JB, Tarran R, Doerschuk CM, Caron KM. E-cigarette exposure delays implantation and causes reduced weight gain in female offspring exposed in utero. J Endocr Soc 2019;3:1907-1916. https://doi.org/10.1210/js.2019-00216
12. Rodriguez HA, Santambrosio N, Santamaria CG, Munoz-de-Toro M, Luque EH. Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary. Reprod Toxicol 2010;30:550-557. https://doi.org/10.1016/j.reprotox.2010.07.008
13. Ozel S, Tokmak A, Aykut O, Aktulay A, Hancerliogullari N, Engin Ustun Y. Serum levels of phthalates and bisphenol-A in patients with primary ovarian insufficiency. Gynecol Endocrinol 2019;35:364-367. https://doi.org/10.1080/09513590.2018.1534951
14. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. J Agric Food Chem 2003;51:4504-4526. https://doi.org/10.1021/jf030204
15. Aldawood N, Alrezaki A, Alanazi S, Amor N, Alwasel S, Sirotkin A, Harrath AH. Acrylamide impairs ovarian function by promoting apoptosis and affecting reproductive hormone release, steroidogenesis and autophagy-related genes: An in vivo study. Ecotoxicol Environ Saf 2020;197:110595. https://doi.org/10.1016/j.ecoenv.2020.110595
16. Tilson HA. The neurotoxicity of acrylamide: an overview. Neurobehav Toxicol Teratol 1981;3:445-461.
17. Smith EA, Oehme FW. Acrylamide and polyacrylamide: a review of production, use, environmental fate and neurotoxicity. Rev Environ Health 1991;9:215-228. https://doi.org/10.1515/REVEH.1991.9.4.215
18. Adler ID, Gonda H, Hrabe de Angelis M, Jentsch I, Otten IS, Speicher MR. Heritable translocations induced by dermal exposure of male mice to acrylamide. Cytogenet Genome Res 2004;104:271-276. https://doi.org/10.1159/000077501

19. Żyzelewicz D, Nebesny E, Oracz J. Acrylamide - formation, physicochemical and biological properties. (Article in Polish) Bromat Chem Toksykol 2010;3:415-427.
20. Szczerbina T. Acrylamide as probable carcinogen in food. (Article in Polish) Kosmos 2005;54:367-372.
21. Moldoveanu SC, Gerardi AR. Acrylamide analysis in tobacco, alternative tobacco products, and cigarette smoke. J Chromatogr Sci 2011;49:234-242. https://doi.org/10.1093/chrsci/49.3.234
22. Carere A. Genotoxicity and carcinogenicity of acrylamide: a critical review. Ann Ist Super Sanita 2006;42:144-155.
23. Vesper HW, Bernert JT, Ospina M, Meyers T, Ingham L, Smith A, Myers GL. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. Cancer Epidemiol Biomarkers Prev 2007;16:2471-2478. https://doi.org/10.1158/1055-9965.EPI-06-1058
24. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. Am J Clin Nutr 2008;87:1428-1438. https://doi.org/10.1093/ajcn/87.5.1428
25. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 2002;50:4998-5006. https://doi.org/10.1021/jf020302f
26. Becalski A, Lau BP, Lewis D, Seaman SW. Acrylamide in foods: occurrence, sources, and modeling. J Agric Food Chem 2003;51:802-808. https://doi.org/10.1021/jf020889y
27. EFSA. Results on the monitoring of acrylamide levels in food. EFSA Scientific Report. EFSA J 2009;7:2-26. https://doi.org/10.2903/j.efsa.2009.285r
28. Mojska H, Gielecińska I. Assessment of exposure of children and youth to acrylamide in fast foods and snacks. Probl Hig Epidemiol 2012;93:613-617.
29. Aldawood N, Alrezaki A, Alanazi S, Amor N, Alwasel S, Sirotkin A, Harrath AH. Acrylamide impairs ovarian function by promoting apoptosis and affecting reproductive hormone release, steroidogenesis and autophagy-related genes: An in vivo study. Ecotoxicol Environ Saf 2020;197:110595. https://doi.org/10.1016/j.ecoenv.2020.110595
30. Lai SM, Gu ZT, Zhao MM, Li XX, Ma YX, Luo L, Liu J. Toxic effect of acrylamide on the development of hippocampal neurons of weaning rats. Neural Regen Res 2017;12:1648-1654. https://doi.org/10.4103/1673-5374.217345
31. Zhang Y, Huang M, Zhuang P, Jiao J, Chen X, Wang J, Wu Y. Exposure to acrylamide and the risk of cardiovascular diseases in the National Health and Nutrition Examination Survey 2003-2006. Environ Int 2018;117:154-163. https://doi.org/10.1016/j.envint.2018.04.047
32. Allam AA, El-Ghareeb AW, Abdul-Hamid M, Bakery AE, Gad M, Sabri M. Effect of prenatal and perinatal acrylamide on the biochemical and morphological changes in liver of developing albino rat. Arch Toxicol 2010;84:129-141. https://doi.org/10.1007/s00204-009-0475-2
33. Nagata C, Konishi K, Tamura T, Wada K, Tsuji M, Hayashi M, Takeda N, Yasuda K. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. Cancer Epidemiol Biomarkers Prev 2015;24:249-254. https://doi.org/10.1158/1055-9965.EPI-14-0935
34. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM. Associations between dietary acrylamide intake and plasma sex hormone levels. Cancer Epidemiol Biomarkers Prev 2013;22:2024-2036. https://doi.org/10.1158/1055-9965.EPI-13-0509
35. Nagahama Y, Chakraborty T, Paul-Prasanth B, Ohta K, Nakamura M. Sex determination, gonadal sex differentiation, and plasticity in vertebrate species. Physiol Rev 2021;101:1237-1308. https://doi.org/10.1152/physrev.00044.2019
36. Capel B. Vertebrate sex determination: evolutionary plasticity of a fundamental switch. Nat Rev Genet 2017;18:675-689. https://doi.org/10.1038/nrg.2017.60
37. Shao C, Li Q, Chen S, Zhang P, Lian J, Hu Q, Sun B, Jin L, Liu S, Wang Z. Epigenetic modification and inheritance in sexual reversal of fish. Genome Res 2014;24:604-615. https://doi.org/10.1101/gr.162172.113
38. Prior JC. Perimenopause: the complex endocrinology of the menopausal transition. Endocr Rev 1998;19:397-428. https://doi.org/10.1210/edrv.19.4.0341
39. Prior JC. Ovarian aging and the perimenopausal transition: the paradox of endogenous ovarian hyperstimulation. Endocrine 2005;26:297-300. https://doi.org/10.1385/ENDO:26:3:297

40. te Velde ER, Scheffer GJ, Dorland M, Broekmans FJ, Fauser BC. Developmental and endocrine aspects of normal ovarian aging. Mol Cell Endocrinol 1998;145:67-73. https://doi.org/10.1016/S0303-7207(98)00171-3
41. Shaw ND, Srouji SS, Welt CK, Cox KH, Fox JH, Adams JA, Sluss PM, Hall JE. Compensatory increase in ovarian aromatase in older regularly cycling women. J Clin Endocrinol Metab 2015;100:3539-3547. https://doi.org/10.1210/JC.2015-2191
42. Klein NA, Battaglia DE, Fujimoto VY, Davis GS, Bremner WJ, Soules MR. Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. J Clin Endocrinol Metab 1996;81:1038-1045. https://doi.org/10.1210/jcem.81.3.8772573
43. Nicosia S. The aging ovary. Med Clin North Am 1987;71:1-9. https://doi.org/10.1016/S0025-7125(16)30878-1
44. Beckers NG, Macklon NS, Eijkemans MJ, Fauser BC. Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for in vitro fertilization exhibit follicular phase characteristics suggestive of ovarian aging. Fertil Steril 2002;78:291-297. https://doi.org/10.1016/S0015-0282(02)03227-2
45. Hamdine O, Macklon NS, Eijkemans MJ, Laven JS, Cohlen BJ, Verhoeff A, van Dop PA, ET AL. Elevated early follicular progesterone levels and in vitro fertilization outcomes: a prospective intervention study and meta-analysis. Fertil Steril 2014;102:448-454.e1. https://doi.org/10.1016/j.fertnstert.2014.05.002
46. Umehara T, Richards JS, Shimada M. The stromal fibrosis in aging ovary. Aging (Albany NY) 2018;10:9-10. https://doi.org/10.18632/aging.101370
47. Abdollahifar MA, Azad N, Sajadi E, Shams Mofarahe Z, Zare F, Moradi A, Rezaee F, Gholamin M, Abdi S. Vitamin C restores ovarian follicular reservation in a mouse model of aging. Anat Cell Biol 2019;52:196-203. https://doi.org/10.5115/acb.2019.52.2.196
48. Welt CK. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin Endocrinol (Oxf) 2008;68:499-509. https://doi.org/10.1111/j.1365-2265.2007.03073.x
49. Cordts EB, Christofolini DM, Dos Santos AA, Bianco B, Barbosa CP. Genetic aspects of premature ovarian failure: a literature review. Arch Gynecol Obstet 2011;283:635-643. https://doi.org/10.1007/s00404-010-1815-4
50. Endogenous Hormones and Breast Cancer Collaborative Group, Key TJ, Appleby PN, Reeves GK, Travis RC, Alberg AJ, Barricarte A, ET AL. Sex hormones and risk of breast cancer in premenopausal women: a collaborative reanalysis of individual participant data from seven prospective studies. Lancet Oncol 2013;14:1009-1019. https://doi.org/10.1016/S1470-2045(13)70301-2
51. Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjonneland A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. Int J Cancer 2008;122:2094-2100. https://doi.org/10.1002/ijc.23359
52. Klinger FG, Rossi V, De Felici M. Multifaceted programmed cell death in the mammalian fetal ovary. Int J Dev Biol 2015;59:51-54. https://doi.org/10.1387/ijdb.150063fk
53. Marder W, Fisseha S, Ganser MA, Somers EC. Ovarian damage during chemotherapy in autoimmune diseases: broad health implications beyond fertility. Clin Med Insights Reprod Health 2012;2012:9-18. https://doi.org/10.4137/CMRH.S10415
54. Folsom LJ, Fuqua JS. reproductive issues in women with Turner syndrome. Endocrinol Metab Clin North Am 2015;44:723-737. https://doi.org/10.1016/j.ecl.2015.07.004
55. Yu K, Zhang X, Tan X, Ji M, Chen Y, Tao Y, Liu M, Yu Z. Transgenerational impairment of ovarian induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) associated with Igf2 and H19 in adult female rat. Toxicology 2019;428:152311. https://doi.org/10.1016/j.tox.2019.152311
56. Thomford PJ, Jelovsek FR, Mattison DR. Effect of oocyte number and rate of atresia on the age of menopause. Reprod Toxicol 1987;1:41-51. https://doi.org/10.1016/0890-6238(87)90070-0
57. Abbott DH, Dumesic DA, Eisner JR, Colman RJ, Kemnitz JW. Insights into the development of polycystic ovary syndrome (PCOS) from studies of prenatally androgenized female rhesus monkeys. Trends Endocrinol Metab 1998;9:62-67. https://doi.org/10.1016/S1043-2760(98)00019-8
58. 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. https://doi.org/10.1210/en.2002-0131

59. 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;148:3580-3590. https://doi.org/10.1210/en.2007-0088
60. Haney AF, Newbold RR, McLachlan JA. Prenatal diethylstilbestrol exposure in the mouse: effects on ovarian histology and steroidogenesis in vitro. Biol Reprod 1984;30:471-478. https://doi.org/10.1095/biolreprod30.2.471
61. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev 2009;30:465-493. https://doi.org/10.1210/er.2009-0006
62. Nelson SM, Telfer EE, Anderson RA. The ageing ovary and uterus: new biological insights. Hum Reprod Update 2013;19:67-83. https://doi.org/10.1093/humupd/dms043