

Hypothyroidism and Fertility: An Animal Model follows up in The Second-Generation

Faezeh Panahandeh, M.Sc.¹, Farideh Feizi, Ph.D.^{1,2*}, Mohsen Pourghasem, Ph.D.^{1,2}, Sorya Khafri, Ph.D.³, Zeinab Abedian, Ph.D.^{2,4}, Kaveh Pourghasem, B.Sc.⁵, Zohre Esmaeili, M.Sc.¹

1. Department of Anatomy, Embryology and Histology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
3. Department of Epidemiology and Biostatistics, School of Public Health, Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
4. Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
5. Department of Medical Sciences, Macquarie University, Sydney, NSW, Australia

*Corresponding Address: P.O.Box: 47745-47176, Department of Anatomy, Embryology and Histology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
Email: faridehfeizi@yahoo.com

Received: 25/April/2021, Accepted: 19/September/2021

Abstract

Objective: Hypothyroidism is known as the most common endocrine disorder. The prevalence of hypothyroidism in the female and male population is 2% and 0.2%, respectively. Maternal hypothyroidism is a defect in the thyroid hormones transition from the mother to the fetus. The present study was conducted to find whether maternal hypothyroidism affects the fertility of the second generation.

Materials and Methods: In this experimental study, twelve adult female rats weighting 180-220 g were randomly divided into case and control groups. Hypothyroidism was induced by dissolving 0.1 g/L of 6-n-propyl-2-thiouracil in drinking water toward the end of pregnancy and lactation. At the end of the breastfeeding period, the blood samples of female children were collected. Six healthy, mature, female rats were selected and kept until they reached maturity, and were then mated with male rats. After observing the female rats' delivery, blood samples were collected from their male and female newborns and the healthy rats were selected.

Results: There was a significant difference in the volume and size of ovarian as well as in the number of secondary follicles in comparison with the control group ($P=0.025$). However, there were no significant changes in the other parameters including the number of primary follicles, the number of Graafian follicles and sperm parameters. There was no significant decrease in the testicular volume and size, number of Leydig cells and seminiferous tubules diameter.

Conclusion: Maternal hypothyroidism has no significant effects on testicular tissue function, and sperm parameters in the second generation, but can significantly reduce the rate of secondary follicles in the second generation female rats.

Keywords: Congenital Hypothyroidism, Fertilization, Ovary, Propylthiouracil, Testis

Cell Journal(yakhteh), Vol 24, No 3, March 2022, Pages: 148-154

Citation: Panahandeh F, Feizi F, Pourghasem M, Khafri S, Abedian Z, Pourghasem K, Esmaeili Z. Hypothyroidism and fertility: an animal model follows up in the second-generation. Cell J. 2022; 24(3): 148-154. doi: 10.22074/cellj.2022.8054.

This open-access article has been published under the terms of the Creative Commons Attribution Non-Commercial 3.0 (CC BY-NC 3.0).

Introduction

The presence of thyroid hormones is essential for the development, differentiation, metabolism and control of various types of physiological processes in the body (1). Although their exact mechanism and consequences during pregnancy and lactation are yet to be fully determined, thyroid gland disorders can lead to a wide range of abnormalities. Among these disorders, hypothyroidism can be associated with reproductive disorders, including menstrual disorders, ovulation disorder, hyperprolactinemia, infertility, amenorrhea and spontaneous abortion that are common features in both humans and animals. The clinical manifestations of hypothyroidism are very diverse and dependent on age, duration of disease and severity of thyroid hormone reduction in humans. The prevalence rate of hypothyroidism is 2% of female and 0.2% for male (2). This disorder is also reportedly more prevalent in women with a smaller figure at the birth and during childhood.

Congenital hypothyroidism, one of the most common types of hypothyroidism, is classified into transient and permanent types. Transient type can arise due to maternal abnormalities during pregnancy, such as transplacental passage of maternal thyroid stimulating hormone (TSH) receptor blocking antibodies, while permanent type can occur due to primary or secondary causes such as thyroid dysgenesis (3).

Maternal hypothyroidism during pregnancy is associated with a wide range of disorders, including pregnancy hypertension, placental contractions, preterm delivery, stillbirth (4), impaired brain and nervous system development in the fetus (5-8), fetal genital system developmental disorders (9), congenital hypothyroidism, intrauterine growth restriction (10). Thyroid gland hormones are involved in the body growth regulating during fetal development and after birth alongside stimulating the growth factors production (11). Clinical studies conducted on the hypothyroidism in the pregnant

women have shown that thyroid hormones deficiency during pregnancy can affect the children's emotional functional capacity (12). During pregnancy, the fetus receives thyroid hormones from the mother through the placenta (13). An embryo's thyroid gland is not fully functional until mid-pregnancy, and in the first trimester, it is completely dependent on the thyroxine produced by the mother (14). During pregnancy, the T4 transmitted from the mother to the fetus is present in the amniotic fluid for up to four weeks and protects the fetal brain. Free T4 (FT4) increases rapidly in the fetal fluids, and its concentration is determined by the FT4 from the mother's serum. When the secretion of thyroid hormones begins in the fetus, the mother's T4 transmission continues to play the main role in the T4 concentrations in the fetal serum (12, 15). The metabolism disease will have severe consequences if left untreated.

Here, we conducted an animal model study to investigate the embryonic genital and reproductive systems development in the second generation in infants born from mothers with and without hypothyroidism.

Materials and Methods

Ethical consideration

This research was carried out in accordance with the Animal Ethics Committee guidelines at the Babol Medical Sciences University, Mazandaran, Iran (MUBABOL.REC.1395.38).

Animals

In this experimental study, twelve adult female Wistar rats (weighing 220-280 g, 14 weeks old) were randomly divided into case and control groups. The rats were housed under standard conditions in terms of humidity, temperature, light and access to water and food. Two female rats were allocated to one male rat in the both groups. After confirming the vaginal plaque, the case group received a daily dose of 0.1 g/L of 6-n-propyl-2-thiouracil (PTU) (Iran hormone, Iran) dissolved in their drinking water until the end of their pregnancy and lactation (16). Three days after the administration of PTU, the levels of T4, triiodothyronine (T3), and TSH were measured with an ELISA kit (30K-E30615-17, East Biopharm, China) to confirm hypothyroidism. An approximately threefold increase in the TSH and a nearly twofold reduction in the T4 and T3 compared to the control group were taken as the criteria for hypothyroidism (16, 17). After confirming the mothers' hypothyroidism in the case group, blood samples were taken from their newborn female rats. After puberty, six healthy female rats were randomly selected and kept alongside the male rats for 60 days. Upon detecting pregnancy and delivery, blood samples were taken from infants. Then, 24 healthy rats of both sexes (equal sex ratio; 60 days old) were divided into two equal groups as the second generation. Throughout all the stages of this study, the number of rats in the case group was equal to that of the control.

Hormonal assay

The blood Samples were left for 15 minutes at room temperature for clotting and centrifuged at 2000 (rpm) for 15 minutes. Then, the separated serum was kept frozen at - 20°C till the analysis of the thyroid function test. The levels of T3, T4 and TSH hormones were measured by using an ELISA kit specific for rats) (30K-E30615-17, East Biopharm, China).

The testicular and ovarian weight and volume

A scale with an accuracy of 0.001 was used to measure each testicle and ovary weight. A 100 ml glass measuring cylinder was used to measure volume. Initially, 20 ml of distilled water was poured into a cylinder, then the tissue was placed inside the cylinder. The difference between the initial volume of water and the new volume was considered as the tissue volume.

Sperm collection and count

The caudal epididymis of each rat was minced with an insulin needle in a petri dish containing 10 ml of Ham's F₁₀ (Albuminated) medium (01131, Bioidea, Iran). And allow the sperms to swim up under an incubation at 37°C for 30 minutes. Then, 10 µl of sperm suspension were placed on a glass slide and counted under a light microscope at 40X magnification at room temperature in triplicate for each sample.

Sperm morphology

For the morphological evaluation of sperm, one drop of the sperm suspension was spread on the slide and after fixation with ethanol alcohol 96% (Hamon Teb, Iran) dried at room temperature. The sperm smear was prepared in accordance with the Papanicolaou staining protocol with five repetitions for each sample. In brief, after rehydration by alcohol, the slides were stained with Hematoxylin (0B14695802, Merck, Germany) for 5 minutes and washed with distilled water. After dipping in acid alcohol for 5 seconds, washed with running water for 3 minutes and soaked in the chambers of 96% alcohol twice, 15 seconds for each chamber. Afterwards, the slides were stained with orange G6 dye (OG6, Asiapajohesh, Iran) for 5 minutes and then with 96% alcohols I and II for 15 seconds. After staining with Eosin Azure 50 (EA 50, Asiapajohesh, Iran) for 5 minutes, the slides were dipping in the chambers, two times of 96% alcohol (Hamon Teb, Iran) for 15 seconds and the chamber of 100% alcohol (Merck, Germany) for 1 minute. Finally, the slides were cleared with Xylene and mounted with Entellan. Ultimately, 50-100 sperms were seen under a light microscope at 100X magnification.

Histological assessment

Using 10% neutral buffered formalin (131328.1212 Panreac AppliChem, Germany), the testis and ovary tissues were fixed. The tissues were dehydrated in a series

of graded alcohols (70, 80, 90, 96 and 100), cleared in the Xylene (Asiapajohesh, Iran) and embedded in the paraffin (Asiapajohesh, Iran). The blocks were serially sectioned at 5 micrometers, and every 10 consecutive sections were on one slide, and stained with the Hematoxylin and Eosin (H&E) (0B14695802, Merck, Germany) (18). For the tissues study, using an Olympus optical microscope (BX41TF, Japan) equipped with a Canon camera (PC1587, Japan), pictures were captured at many random locations of slides at 10X and 40X magnification. Using of Motic Image Plus2.0ML (Micro - optic industrial group Co. LTD), we calculated diameter, surface area of the seminiferous tubules cross-sections and number of Leydig cells. For the evaluation of the ovary, after the preparation of the tissue, 10 random sections of each rat were selected randomly and primary, secondary and Graafian follicles were counted.

Statistical analysis

All data were encoded and analyzed using the Statistical Package for the Social Sciences Windows, version 22.0 (SPSS, Chicago, IL, USA). The normality of the variables was estimated by the Shapiro-Wilk test. The t test was used to compare the two groups in terms of the quantitative variables. $P \leq 0.05$ was considered statistically significant.

Results

Hormonal assay

The concentrations of T3, T4, and TSH in the pregnant female rats of both groups showed a significant difference. The mean concentration of T3 was 1098.67 ± 104.2 pg/dl in the control group and 686.67 ± 131.9 pg/dl in the case group ($P=0.00$). The mean concentration of T4 was 16.58 ± 1.8 ng/dl in the control group and 8.00 ± 2.1 in the case group ($P=0.00$). The mean concentration of TSH was 1.4 ± 0.46 mU/l in the control group and 3.1 ± 0.47 mU/l in the case group ($P=0.00$, Fig.1A).

Our results showed no significant variation among the mean concentrations of T3, T4, and TSH in the blood samples

of second generation infants in the control and case groups ($P=0.723$). The mean concentration of T3 in the healthy male infants of the second generation of hypothyroid mothers was 1079.33 ± 71.35 pg/dl, in the healthy female infants was 1064.30 ± 80.7 pg/dl and in the both male and female control group, the mean concentration was 1064.90 ± 78.29 pg/dl ($P=0.64$). The mean concentration of T4 in the healthy infants of the second generation of hypothyroid mothers was 13.3750 ± 1.29 ng/dl for males, and 15.88 ± 1.5 ng/dl for females. Also, in the both control group, the mean concentration was 13.2083 ± 1.35 ng/dl ($P=0.76$). The concentration of TSH in the healthy male and female infants of the second generation of hypothyroid mothers were 1.65 ± 0.27 mU/l, 1.057 ± 7 mU/l respectively, and was 1.75 ± 0.24 mU/l in the both control groups ($P=0.33$, Fig. 1B).

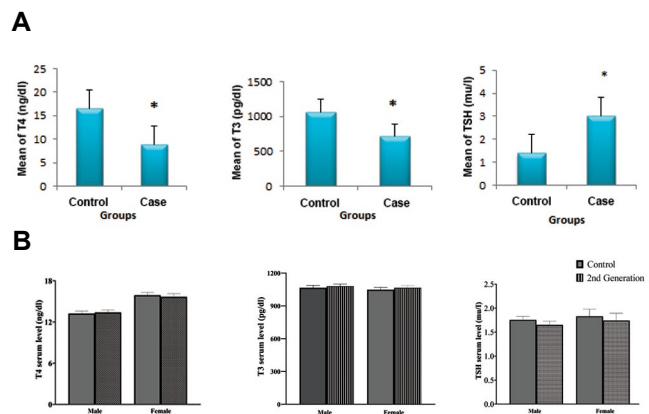


Fig.1: The concentration of T3, T4 and TSH hormones (mean \pm SD). **A.** Case (PTU treated mothers) and controls and **B.** Second generation female and male. TSH; Thyroid stimulating hormone.

Comparing the number of pups in the case and control groups

Examining the number of pups in the second-generation rats born from hypothyroid mothers using the t test showed a reduction in the number of pups in the case group compared to the control group, but this difference was not significant for both male and female ($P=0.633$, Table 1).

Table 1: A glance on the second-generation male rats of hypothyroid mothers

Parameter	Control group	Case group	P value
Number of live births	8.83 \pm 1.169	8.20 \pm 2.588	0.633
Testicular weight (g)	1.5183 \pm 0.04489	1.4867 \pm 0.05087	0.12
Testicular volume (cm ³)	2.7008 \pm 0.04602	2.6767 \pm 0.04793	0.221
Maximum diameter of testicles (mm)	20.9167 \pm 0.514	20.6667 \pm 0.4923	0.237
Minimum diameter of testicles (mm)	11.50 \pm 0.522	11.25 \pm 0.452	0.223
Number of Leydig cells (per microscopic field 40X)	44.752 \pm 2.265	45.959 \pm 1.623	0.148
Seminal tube diameter (μ m)	1110.46 \pm 27.51	1096.04 \pm 28.42	0.220
Area of the seminal tube (μ m ²)	3700.19 \pm 14.34	3692.34 \pm 16.59	0.229
Motility (%)	56.00 \pm 9.74	53.42 \pm 8.73	0.501
Morphology (%)	58.92 \pm 6.73	56.92 \pm 6.77	0.476
Sperm concentration($\times 10^6$ /ml)	44.83 \pm 9.32	40.91 \pm 8.27	0.288

All data are shown in mean \pm SD.

Comparing the testicular and ovarian weight and volume of the second-generation rats in the case and control groups

Examining the testicular weight of the second-generation male rats born from hypothyroid mothers showed weight loss in the case group, but this difference was not significant ($P=0.120$, Table 1). However, a significant decrease in the weight of ovaries for the second-generation female rats born from hypothyroid mothers was seen ($P\leq 0.001$).

Also, a decrease in the testicular volume of the second-generation male rats born from hypothyroid mothers was seen, but this difference was not significant ($P=0.221$, Table 1). Examining the ovarian volume for the second-generation female rats showed a significant decrease in comparison with the control rats ($P\leq 0.001$).

Evaluating male reproductive system

The Leydig cells count, the diameters of the seminal tubes and cross-sectional area of the seminiferous tubules in the case group compared to the control group was the same with no significant differences between the two groups (Table 1).

Sperm parameters (morphology, count, motility)

Examining the number, motility and morphology of the sperms in the second-generation male rats using the t test showed a reduction in the second-generation male rats born from hypothyroid mothers, but this reduction was not notable from the control group ($P>0.05$, Table 1, Fig.2).

Examining the testicular tissue in the second-generation male rats born from hypothyroid mothers found one infertile case with azoospermia (Fig.3).

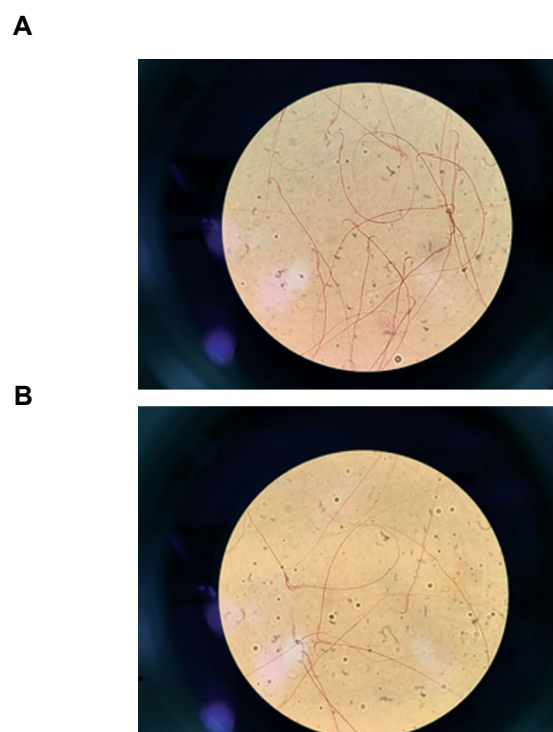


Fig.2: Papanicolaou staining of semen smear. **A.** Control group and **B.** Case group (magnification: 100X).

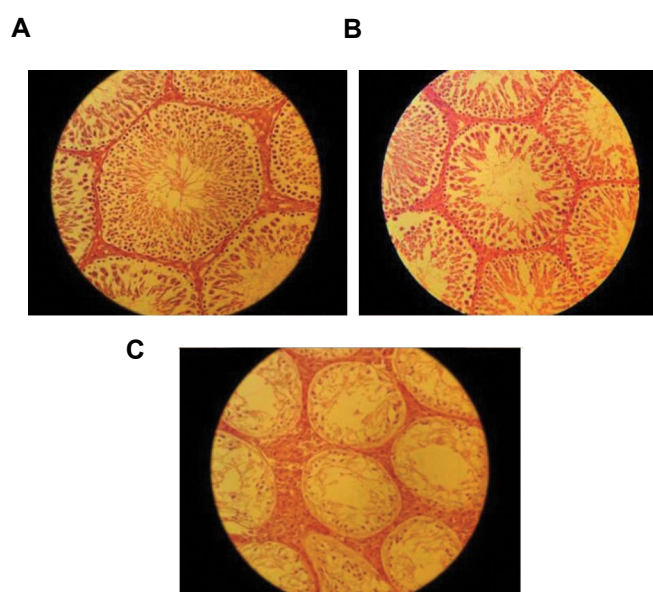


Fig.3: Hematoxylin-Eosin (H&E) Staining of testicular tissue. **A.** Control group, **B.** Case group, and **C.** Azoospermia case (magnification: 40X).

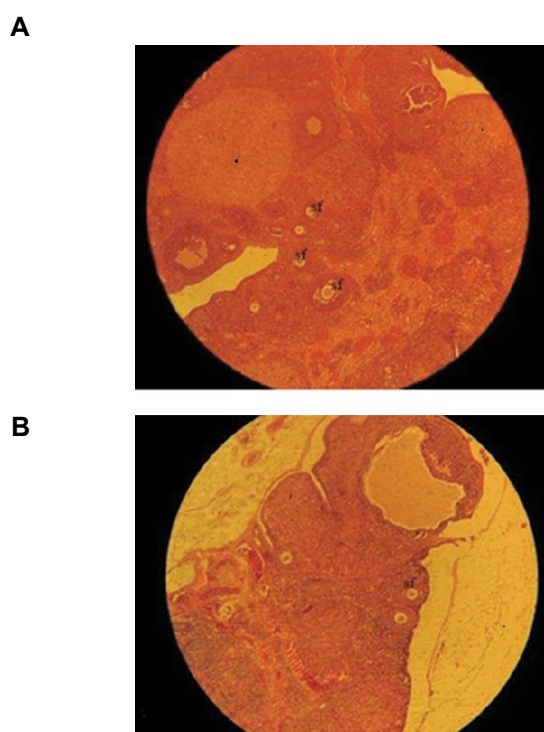


Fig.4: Hematoxylin-Eosin (H&E) staining of ovarian tissue. **A.** Control group and **B.** Case group. (magnification: 10X).

Evaluating the female reproductive system by checking the number of different follicles

Three types of follicles (primary, secondary and Graafian) were counted. The t test analysis showed no significant differences between groups in primary and Graafian follicles count, but the number of secondary follicles was significantly decreased in the case group in comparison with the control group ($P\leq 0.05$, Table 2, Fig.4).

Table 2: A glance on the second-generation female rats of hypothyroid mothers

Parameters	Control group	Case group	P value
Number of live births	9.17 ± 1.329	6.00 ± 3.464	0.13
ovarian weight (g)	0.3350 ± 0.019	0.2400 ± 0.013	0.001
ovarian volume (cm ³)	0.2843 ± 0.013	0.2479 ± 0.011	0.001
Number of primary follicles	16.60 ± 3.738	14.53 ± 4.503	0.197
Number of secondary follicles	6.09 ± 740	5.48 ± 612	0.025
Number of graafian follicles	0.414 ± 0.140	0.342 ± 0.122	0.163

All data are shown in mean ± SD.

Discussion

The lifestyle of pregnant women may have long-term effects on their children's health. Smoking, alcohol abuse and hypothyroidism diseases are their samples that may affect fetal development and health. Although, genetic factors are an important factor of fetal growth environmental factors. In the present study, examining fertility in the male and female rats showed that the case group had reproductive abilities, but the number of children born from the rats in the case group was insignificantly less than the control group.

A study by Dijkstra et al. (19) showed a decrease in the number of antral follicles, minor non-atretic antral follicles and an increase in the number of atretic follicles in the PTU treated rats and concluded that the folliculogenesis disturb is because of thyroid hormone supply deficiency.

Also, Zertashia et al. (20) reported a significant weight reduction in the ovarian tissue of offspring in the postnatal PTU treatment group in comparison with the control group. They didn't see any notable changes in the ovaries diameters, although, observed a significant increase in the diameters of Graafian follicles. The present study showed a decline in the ovarian volume in the case group. Hypothyroidism inhibits the basal luteinizing hormone (LH) release and could cause ovarian atrophy (21). Decreasing of secondary follicles which seen in our study lead to ovarian hypotrophy.

Thyroid hormone deficiency is associated with changes in the menstrual patterns and associates with menstrual cycle frequency decrease and an increase in menses volume. Thyroid hormones and their transporters, and nuclear thyroid hormone receptors have been discovered in ovarian follicles and in oocyte (22). Thyroid hormone receptors are located in the primordial, primary and secondary follicles with minimal expression in the secondary follicles. This could explain why the number of secondary follicles decreased significantly while this change was not significant in the primary follicles. This means that more of the secondary follicles were destroyed in the response to minimal decreasing changes of thyroid hormones in comparison with the primary and graafian

follicles (23).

Hapon et al. reported that hypothyroidism induces various alterations in the hormone profiles of virgin and pregnant rats and induces pseudopregnancy and mammary development in the virgin rats. According Sarkar and Singh (24) study, thyroid hormones play an important role in testicular steroidogenesis and spermatogenesis regulation. In the present study, examining the diameter and the area of the seminiferous tubules indicates no significant differences between the two groups. In a study by Al-Awdan et al. (25) on the testicular tissues of 21-60-day-old rats born from hypothyroid mothers, the diameters of the seminal tubes decreased in the case group, in which the ancestral cells were irregular and the tubes had many degenerated cells. Also, Francavilla et al. (26) study on the structure and function of testicles in the matured rats born from hypothyroid mothers during prenatal and postnatal period showed that they had no effect on their testicular development and morphological changes in adulthood, and the diameter of the tubules was not significantly different between the case and control groups. These researchers also stated that hypothyroidism in the 60-day-old male rats reduces the diameter of the seminal tubes, reduces the number of interstitial cells, and delays spermatogenesis; they also found delayed testicle maturation in the rats who had transient hypothyroidism at birth. In the present study, there was no significant change in the number of Leydig cells between two groups. Opposite to our result, Canale et al. (27) concluded that hypothyroidism in the first generation increases the testicle size without increasing the number of androgens, which is due to the inactivation of the Leydig cells. Valle et al. (28) examined the Leydig cells in the 40-day-old male rats and concluded that hypothyroidism in the young male rats reduces the number of LH receptors on the Leydig cells during puberty. Mendis-Handagama and Ariyaratne (29) also found that hypothyroidism delays the onset of the differentiation of mesenchymal progenitor cells into Leydig cells. In the present study, sperm parameters exhibited a reduction in the case group in comparison with the control rats, but this difference was not significant. Epigenetic information of the male

germ lines will be changed by the alteration in the thyroid hormone level. This information changes can transmit to future generations. This could be applicable for follicles of the ovary (27).

La Vignera et al. (30) study showed thyrotoxicosis and thyroid hormone deficiency are associated with changes that affect the sexual behavior, or reproductive functions. Particularly, the concentration of free and bioavailable testosterone of hyperthyroid patients was lower than the control group. The increasing rate of astheno-zoospermia, oligo-zoospermia, and terato-zoospermia, and high prevalence of sexual disturbances, such as premature ejaculation (PE) was seen in the hypothyroidism group in comparison with the control group. Dick et al. (31) indicated that hyperthyroidism is associated with PE and delaying ejaculation (DE) which can cause fertility issues. A study on the seminal tubes of male rats born from hypothyroid mothers found no sperm in the tubes (25) that has harmony with our study which showed a rat with azospermia in the second generation. Amer's et al. (32) study showed that defective thyroid function was associated with an increased risk of infertility. Koyyada and Orsu (33) mentioned that the occurrence of preterm delivery and abortion is associated with untreated hypothyroidism in pregnant mothers. Nonetheless, there was no study on the effect of maternal hypothyroidism on testicular and ovarian tissue and sperm parameters in second-generation male rats.

Conclusion

It can be concluded that the second-generation offspring of the hypothyroid mothers experienced a reduction in the number of sperm cells, sperm morphology and motility in male rats and reduction in the number of secondary follicles in female rats compared to the control group. This could indicate that these differences in first generation rats due to maternal hypothyroidism could show themselves in the second-generation rats, but with a lesser impact, or in other word, they could be corrected in the following generation.

Acknowledgements

We thank Research and Technology Council of Babol University of Medical Sciences for their kind supporting. This study was funded by the Research and Technology Council of Babol University of Medical Sciences under the No. 9542020. The authors stated no conflict of interest in this study.

Authors' Contributions

F.P., F.F., M.P.; Involved in study plan, data collection, assessment and drafting. S.Kh.; Determined the size of the statistical population, performed statistical analysis and prepared the graphs. F.P., Z.A., Z.E.; Performed all experimental, histological work and

hormonal assay data. F.P.; Performed editing. K.P.; Prepared the scientific resources required for study, performed editing. All authors read and approved the final manuscript.

References

1. El-Sayed MS, El Badawy A, Abdelmoneim RO, Mansour AE, Khalil MEM, Darwish K. Relationship between serum sialic acid concentration and diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus. *Benha Med J*. 2018; 35(2): 257.
2. Poppe K, Velkeniers B, Glinoeer D. Thyroid disease and female reproduction. *Clin Endocrinol (Oxf)*. 2007; 66(3): 309-321.
3. Rastogi MV, LaFranchi SH. Congenital hypothyroidism. *Orphanet J Rare Dis*. 2010; 5: 17.
4. Hou VJ, Yu P, Zhu H, Pan H, Li N, Yang H, et al. The impact of maternal hypothyroidism during pregnancy on neonatal outcomes: a systematic review and meta-analysis. *Gynecol Endocrinol*. 2016; 32(1): 9-13.
5. Pop VJ, Kuijpers JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, et al. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. *Clin Endocrinol (Oxf)*. 1999; 50(2): 149-155.
6. Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? *J Clin Endocrinol Metab*. 2000; 85(11): 3975-3987.
7. Klein RZ, Sargent JD, Larsen PR, Waisbren SE, Haddow JE, Mitchell ML. Relation of severity of maternal hypothyroidism to cognitive development of offspring. *J Med Screen*. 2001; 8(1): 18-20.
8. Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. *Clin Endocrinol (Oxf)*. 2003; 59(3): 282-288.
9. Hamouli-Said Z, Tahari F, Hamoudi F, Hadj-Bekkouche F. Comparative study of the effects of pre and post natal administration of a thyroid drug on testicular activity in adult rat. *Folia Histochem Cytobiol*. 2007; 45 Suppl 1: S51-57.
10. Saki F, Dabbaghmanesh MH, Ghaemi SZ, Forouhari S, Ranjbar Omrani G, Bakhshayeshkaram M. Thyroid function in pregnancy and its influences on maternal and fetal outcomes. *Int J Endocrinol Metab*. 2014; 12(4): e19378.
11. Cabello G, Wrutniak C. Thyroid hormone and growth: relationships with growth hormone effects and regulation. *Reprod Nutr Dev*. 1989; 29(4): 387-402.
12. Patel J, Landers K, Li H, Mortimer RH, Richard K. Delivery of maternal thyroid hormones to the fetus. *Trends Endocrinol Metab*. 2011; 22(5): 164-170.
13. Blazer S, Moreh-Waterman Y, Miller-Lotan R, Tamir A, Hochberg Z. Maternal hypothyroidism may affect fetal growth and neonatal thyroid function. *Obstet Gynecol*. 2003; 102(2): 232-241.
14. Mitchell ML, Klein RZ. The sequelae of untreated maternal hypothyroidism. *Eur J Endocrinol*. 2004; 151 Suppl 3: U45-48.
15. Obregon MJ, Calvo RM, Escobar Del Rey F, Morreale de Escobar G. Ontogenesis of thyroid function and interactions with maternal function. *Endocr Dev*. 2007; 10: 86-98.
16. Hapon MB, Gamarra-Luques C, Jahn GA. Short term hypothyroidism affects ovarian function in the cycling rat. *Reprod Biol Endocrinol*. 2010; 8: 14.
17. Pirahanchi Y, Tariq MA, Jialal I. Physiology, thyroid. *StatPearls*. 2020. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK519566/> (25 Apr 2021).
18. Safarpour S, Zabihi E, Ghasemi-Kasman M, Nosratiyan N, Feizi F. Prenatal and breastfeeding exposure to low dose of diethylhexyl phthalate induces behavioral deficits and exacerbates oxidative stress in rat hippocampus. *Food Chem Toxicol*. 2021; 154: 112322.
19. Dijkstra G, de Rooij DG, de Jong FH, van den Hurk R. Effect of hypothyroidism on ovarian follicular development, granulosa cell proliferation and peripheral hormone levels in the prepubertal rat. *Eur J Endocrinol*. 1996; 134(5): 649-654.
20. Zertashia A, Jalali S, Ahmad L, Mirza A. Effect of hypothyroidism induced by propylthiouracil on ovarian function and structure in offspring from treated mothers (Rats). *J Exp Zool*. 2002; 293(4): 407-413.
21. Ortega E, Rodriguez E, Ruiz E, Osorio C. Activity of the hypothalamo-pituitary ovarian axis in hypothyroid rats with or without triiodothyronine replacement. *Life Sci*. 1990; 46(6): 391-395.

22. Colella M, Cuomo D, Giacco A, Mallardo M, De Felice M, Ambrosino C. Thyroid hormones and functional ovarian reserve: systemic vs. peripheral dysfunctions. *J Clin Med*. 2020; 9(6): 1679.
 23. Aghajanova L, Stavreus-Evers A, Lindeberg M, Landgren BM, Sparre LS, Hovatta O. Thyroid-stimulating hormone receptor and thyroid hormone receptors are involved in human endometrial physiology. *Fertil Steril*. 2011; 95(1): 230-237.
 24. Sarkar D, Singh SK. Effect of neonatal hypothyroidism on prepubertal mouse testis in relation to thyroid hormone receptor alpha 1 (THR α 1). *Gen Comp Endocrinol*. 2017; 251: 109-120.
 25. Al-Awdan AE-WA, Idrees SE, Shalaby SA, Mehlab EM, Mannawy SM. Prenatal and postnatal effects of hypothyroidism and thyroxin replacement on the development of rat testis. *Am J Sci*. 2014; 10(9).
 26. Francavilla S, Cordeschi G, Properzi G, Di Cicco L, Jannini EA, Palmero S, et al. Effect of thyroid hormone on the pre- and post-natal development of the rat testis. *J Endocrinol*. 1991; 129(1): 35-42.
 27. Canale D, Agostini M, Giorgilli G, Caglieresi C, Scartabelli G, Nardini V, et al. Thyroid hormone receptors in neonatal, prepubertal, and adult rat testis. *J Androl*. 2001; 22(2): 284-288.
 28. Valle LB, Oliveira-Filho RM, Romaldini JH, Lara PF. Pituitary-testicular axis abnormalities in immature male hypothyroid rats. *J Steroid Biochem*. 1985; 23(3): 253-257.
 29. Mendis-Handagama SM, Ariyaratne HB. Differentiation of the adult Leydig cell population in the postnatal testis. *Biol Reprod*. 2001; 65(3): 660-671.
 30. La Vignera S, Vita R, Condorelli RA, Mongioi LM, Presti S, Benavenga S, et al. Impact of thyroid disease on testicular function. *Endocrine*. 2017; 58(3): 397-407.
 31. Dick B, Koller C, Herzog B, Greenberg J, Hellstrom WJ. The role of hormones in male sexual function. *Curr Sex Health Rep*. 2020: 1-12.
 32. Amer MH, Thuwain MM, AL-Snafi A. Investigation of thyroids dysfunction among infertile women in Nasiriyah city. *Int J Curr Pharm Res*. 2020; 12(5): 31-34.
 33. Koyyada A, Orsu P. Role of hypothyroidism and associated pathways in pregnancy and infertility: Clinical insights. *Tzu Chi Med J*. 2020; 32(4): 312-317.
-