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Effect of Maternal Hypothyroidism During Gestation and Lactation in Female Rats on Thyroidal and Testicular Functions of Their Male Offspring at Puberty

Sarah J. S. [1, *] and Jassim M. A. Alkalby [2]

[1] College of Pharmacy, Al-Ayen University, Thi-Qar, Iraq.
[2] Department of physiology and pharmacology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Abstract: Maternal hypothyroidism, is a deficiency of the transfer of thyroid hormones from the mother to the fetus. This study was designed to investigate the effect of induced hypothyroidism in female rats during gestation (G), lactation (L) and gestation and lactation (G+L) on thyroid and testicular functions of their male offspring at puberty. The hypothyroidism state was induced by administration of propylthiouracil (PTU) (0.2) mg/kg b.w/day orally to three pregnant female rats' groups during different physiological state as follows: gestational group (treated from day 6 till parturition), lactational group (treated from parturition till weaning) and gestational and lactational (treated from day 6 of parturition till weaning), in addition, to pregnant control group which received distilled water (D.W) only. All offspring were weaned at 30 days age and six male offspring of each group were chosen randomly and left without treatment until puberty (60 days) postnatal. Blood samples were collected to the measurement of serum level of some hormones including thyroid stimulating hormone (TSH), thyroxin (T_4), triidothyronine (T_3), testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Thyroid glands and testes were removed for histopathology study. The results of hormonal analysis revealed a significant ($P \leq 0.05$) increase of TSH concentration in G and L groups and a significant ($P \leq 0.05$) decrease of T_4 in all treatment groups while a significant decrease in T_3 was recorded in the G group compared with the control group. Histopathological results showed histological changes in both thyroid glands and testes in G, L, and G+L groups. The present study concluded that the different maternal hypothyroidism periods have a nearly different impacts on offspring male rat, where the G group was the most affected than others.

Keywords: Hypothyroidism, Gestation and Lactation, Thyroid, Testis, Propythiouracil

Introduction

Thyroid hormone is a decisive regulator of growth, development, and metabolism in almost all tissues, and change thyroid condition affects numerous organs and systems [(1)]. Hypothyroidism refers to the insufficient production of thyroid hormones from the thyroid gland, it may occur due to a disturbance within the thyroid gland itself (primary hypothyroidism) or with the hypothalamic-pituitary-thyroid axis (secondary hypothyroidism) [(2)]. Maternal hypothyroidism also referred to as gestational hypothyroidism, is a deficiency of the transfer of thyroid hormones from the mother to the fetus, offspring thyroid function independently starts at gestation day 17.5-18 in mouse and the second trimester in humans [(3,4)]. While, in rat thyroid starts to function between days 17 and 18 of gestation [(5)]. Thyroid hormones play an important role in fetal normal development [(6)]. Untreated maternal hypothyroidism has serious consequences on the development of offspring, resulting in stunted growth and mental retardation [(7)]. And can lead to preterm birth, low birth weight, and in the neonate [(8)].

Antithyroid drug 6-propyl-2-thiouracil (PTU) suppress the synthesis of thyroid hormones, and conversion of T4 to the biologically active form T_3 in peripheral tissue [(9, 10)]. Hapon et.al., [(7)] found

that the period of gestation in rats was significantly longer with severely impaired of litters growth and increased of pup mortality during lactation in the in propylthiouracil (PTU) treated group. However, thyroid disorders are more common in women and may begin before or during pregnancy, post-delivery, or later in life [11]. Adequate thyroid hormone is essential for maintaining a pregnancy and optimal fetal development [12]. So, changes in the metabolic functioning of hormones occur because of increased metabolic demands during both pregnancy and lactation [13]. The thyroid hormone of maternal origin can cross the placenta and reach the fetus [14]. As the only source of thyroxin (T_4) and triidothyronine (T_3) for the brain of the fetus is the maternal thyroid [15]. The thyroid hormones, T_4 and T_3, are detectable in the fetal circulation from early in gestation and have important developmental, metabolic, and maturational effects in the fetus in all species studied to date including human infants, their bioavailability in fetal plasma and tissues is regulated developmentally and also differs with species, gestational age, availability of nutrients and oxygen, and the endocrine environment in utero [16]. However, thyroid hormones deficiency during intrauterine development impairs growth of the fetus and compromises its adaptation to extrauterine life [17,18].

Despite the previously held belief that thyroid hormones do not affect the male reproductive system, many experimental and clinical studies have shown that thyroid hormones play an essential role in the growth and functions of the testes [1,19, 20]. Changes in the state of the thyroid hormone during development or adult age are related to the abnormal development of the gonads and their function in human and animal models. In a developing testis, abnormal levels of thyroid hormone action lead to disturbances in cell proliferation and differentiation, eventually affecting the size of the testis, spermatogenesis, steroidogenesis, and male fertility [21]. Because of the contradictory results obtained previously, the current study aimed to investigate the effects of hypothyroidism during pregnancy and lactation in the thyroid and testicular functions of offspring at puberty.

Materials and Methods

The present study was carried out in the animal house of College of Veterinary Medicine /University of Basrah. Adult female rats (*Rattus norvegicus*), weighing (188-267) gm were used in this study. Rats were kept under the following standard conditions (12/12 hours of light and dark cycle at 25±2 Cº and they maintained on rat chow and water *ad libitum*). Animals were kept two weeks before mating for adaptation. Mating was done by placing one well experience male with two fertile females in each cage. The presence of spermatozoa in the vaginal smears or vaginal plug at morning is consider as gestational day (GD) 0. Then, the pregnant rats divided into four groups (8 for each group) control group and three treated groups with different treatment periods. Hypothyroidism was induced by administration of 0.2 mg/kg B.W./day PTU orally by gavage. Control group received distal water only orally by gavage, gestation group received PTU on the GD 6 until they gave birth, lactation group received PTU on the 1st day post-natal (PN) to 30th day PN (weaning), and gestation and lactation group received PTU on the GD 6 to the 30th day PN. The hypothyroid state was assured by the significantly lower plasma total T_4 and T_3 and significantly higher plasma TSH in the dams at the end of treatment period. Six male offspring from each group were randomly chosen at weaning (30 days) PN and, left without treatment until puberty (60 days) PN as follows:

1- Control group (**Control**): (n=6) male offspring born from control dams.
2- Gestation group (**G**): (n=6) male offspring born from dams treated with PTU during gestation.
3- Lactation group (**L**): (n=6) male offspring treated with PTU during lactation.
4- Gestation and lactation group (**G+L**): (n=6) male offspring born from dam treated with PTU during gestation and lactation.

At the end of the experiment, the rats were euthanized by chloroform. Blood samples were collected via heart puncture for hormonal analysis and thyroid glands and testes were removed for histopathological study. All efforts were made to reduce the number of animals used and their suffering.

The study was carried out in accordance with the internationally accepted principles for laboratory animal use and care as found in the European community guidelines (EEC Directive of 1986; 86/609/EEC) .

Studied Criteria:

Measurements of hormones concentration:

1- The measurement of serum concentration of TSH is generally regarded as the most sensitive indicator available for the diagnosis of hypothyroidism. ELISA Kit (Monobind Inc. Lake Forest, CA 92630, USA) was used.
2- Measurement of serum concentration of T_3 is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. ELISA kit (Monobind Inc. Lake Forest, CA 92630, USA) was used.
3- Measurement of serum concentration of T_4 is generally regarded as an important diagnostic test for assessing thyroid function. ELISA kit (Monobind Inc. Lake Forest, CA 92630, USA) was used.
4- Measurement of serum concentration of T was intended for the quantitative determination of total T as it is the most potent naturally secreted androgen. ELISA kit (Monobind Inc. Lake Forest, CA 92630, USA) was used.
5- Measurement of serum concentration of LH is generally regarded as a valuable tool in ascertaining the homeostasis of fertility regulation by the hypothalamic–pituitary–gonad axis. ELISA kit (Monobind Inc. Lake Forest CA 92630, USA) was used.
6- Measurement of serum concentration of FSH is also generally regarded as a valuable tool in ascertaining the homeostasis of fertility regulation by the hypothalamic –pituitary –gonad axis. ELISA kit (Monobind Inc. Lake Forest CA 92630, USA) was used.

Histopathological study: Thyroid and testicular tissue were treated for light microscopic studies. The tissue samples were fixated in 10% formaldehyde solution. After that tissue samples were dehydrated in a series of alcohol and clarified in xylol and tissue samples then embedded in paraffin. Embedded tissue in paraffin wax was cut by a rotary microtome (Shandon, Finesse 325, UK) with a thickness of 5 microns and stained using the traditional method of hematoxylin and eosin. The sections were then dehydrated and mounted using DPX medium.

Statistical analysis: The one-way ANOVA (Analyses of Variation) followed by LSD (Least Significant Difference) test was performed for statistical analysis. Data were expressed as mean±SD and statistical significance was set at $P \leq 0.05$. The IBM SPSS Statistics program (V.26) was used.

Results

The results of the present study in **Table 1** showed a significant ($P \leq 0.05$) increase in the TSH serum concentrations of **G** and **L** groups compared with the **control** group. Moreover, an insignificant increase in the serum concentration of TSH was recorded in the **G+L** group but the difference was not significant compared with the control and other treatment groups. On the other hand, a significant ($P \leq 0.05$) decrease in serum concentration of T_4 was observed in all treated groups compared with **control** group, while the lowest significant ($P \leq 0.05$) value was recorded in the male offspring born from dams treated with PTU during pregnancy compared with control and other treated groups. Finally, a significant ($P \leq 0.05$) decrease in serum T_3 concentration was observed in **G** group compared with the **control** group. However, no significant differences in serum T_3 concentrations were found between all treated groups.

Table (1): Effect of Maternal Hypothyroidism on Serum Concentrations of TSH, T_4 and T_3 in Their Male Offspring at Puberty (M±SD) (n=6)

Parameters / Groups	TSH (µlU/ml)	T4 (µg/dl)	T3 (ng/ml)
Control	0.10 ± 0.02 **b**	6.92 ± 0.81 **a**	4.08 ± 0.77 **a**
G	0.20 ± 0.08 **a**	4.01 ± 0.48 **c**	2.80 ± 0.68 **b**
L	0.17 ± 0.05 **a**	5.25 ± 0.95 **b**	3.23 ± 0.72 **ab**
G+L	0.16 ± 0.04 **ab**	5.66 ± 1.10 **b**	3.65 ± 0.99 **ab**
LSD	0.07	1.23	1.28

The different letters refer to significant differences among groups at the level of (p≤0.05).

The results presented in **Table 2** revealed a significant ($P \leq 0.05$) decrease in serum concentration of T in the **G** group compared with **control** group. While, no significant differences in serum concentration of T between all treated groups were observed. The results also showed a significant ($P \leq 0.05$) decrease in serum LH concentration in all treated groups compared with the **control** group.

The results in the same table also indicated that no significant difference in FSH serum concentration among all treated group compared with **control**.

Table (2): Effect of Maternal Hypothyroidism on Serum Concentrations of T, FSH and LH in Their Male Offspring at Puberty (M±SD)
(n=6)

Parameters / Groups	T (ng/ml)	LH (mlU/ml)	FSH (mlU/ml)
Control	11.16 ± 2.58 **a**	0.29 ± 0.05 **a**	0.20 ± 0.06
G	7.29 ± 2.41 **b**	0.14 ± 0.04 **b**	0.31 ± 0.10
L	8.08 ± 2.80 **ab**	0.13 ± 0.08 **b**	0.26 ± 0.11
G+L	9.68 ± 2.56 **ab**	0.18 ± 0.05 **b**	0.22 ± 0.06
LSD	3.87	0.10	N.S

The different letters refer to significant differences among groups at the level of ($p \leq 0.05$).

Result of Histopathological Examination:

Thyroid Gland:

The thyroid gland of male offspring rats (60 days old) in the **control** group showed a clear normal sized thyroid follicle with normal homogenized colloid and surrounded by normally distributed parafollicular cells as shown in **Figure 1** while the thyroid gland of **G** group showed a histpathological
changes including misshaped irregular size thyroid follicles with either empty or sever vacuolated colloid with irregularly arranged parafollicular cells with a clear band of fibrous interfollicular tissue as shown in **Figure 2** whereas the thyroid of rats of **L** group showed less number of different size of thyroid follicles containing severely vacuolated and less homogenized colloid with disturbance of surrounding parafollicular cells also there is interfollicular spaces as shown in **Figure 3**. Moreover, the thyroid gland of rats of the **G+L** group showed either enlarged thyroid follicles or small size thyroid follicles with clear vacuoles formation and increased proliferated parafollicular cells irregularly distributed as shown in **Figure 4**.

Testis:

The testis of male offspring rats (60 days old) in the **control** group showed a normal structure of seminiferous tubule with clear thick interstitial tissue. Very clear Sertoli cells lining the seminiferous tubules with clear nuclei also there are clear spermatogonia cells yielding spermatids normally as shown in **Figure 5** while the testis of **G** group rats showed a histopathological change including clear disarrangement of Sertoli cells and sloughing of them with scattered nuclei and absence of spermatogonia (empty lumen of seminiferous tubule) with decreasing interstitial tissue as shown in **Figure 6**. The testis of **L** group rats, on the other hand, showed distinct thin lining epithelial Sertoli cells with disturbance of Sertoli cells allocation. Little spermatogonia formation with very little interstitial tissue formation as shown in **Figure 7**. The testis of the **G+L** group, however, showed that most of seminiferous tubules which are normal containing large number of spermatogonia but there are very thin irregular lining epitheliums (Sertoli cells). In addition to nearly disappearance of interstitial tissue as shown in **Figure 8**.

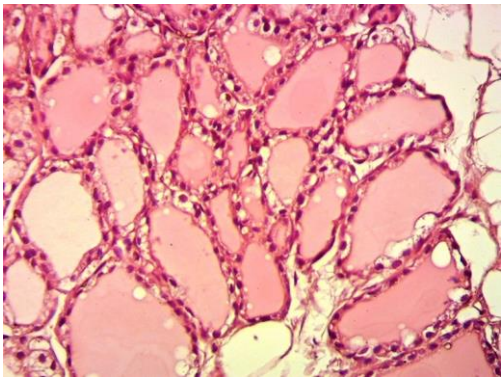

Fig. 1: Thyroid of control rat. Showing structure of thyroid follicle (Tf) filled with homogenous colloid (C) surrounded by normal parafollicular cells (P). Stain (H&E) 400X.

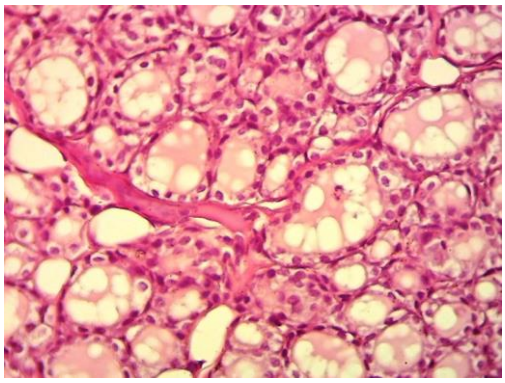

Fig. 2: Thyroid of G group rat. Showing irregular size thyroid follicles (Tf) with either empty or vacuolated (V) colloid (C) with irregularly arranged parafollicular cells (P). Stain (H&E) 400X.

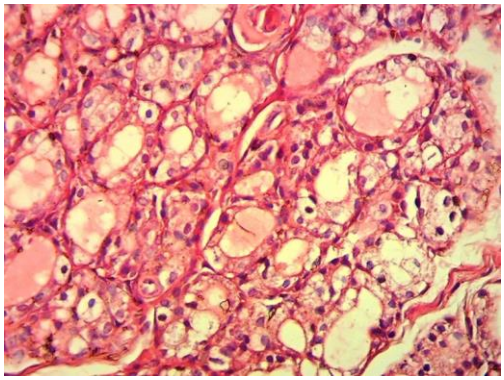

Fig. 3: Thyroid of L group rat. Showing a smaller number of different size of thyroid follicles (Tf), vacuolated (V) and less homogenized colloid (C). Stain (H&E) 400X.

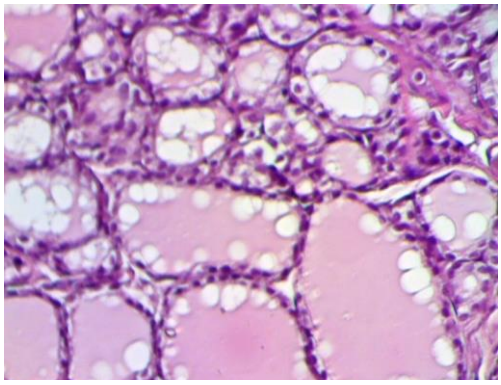

Fig. 4: Thyroid of G+L group rat. Showing different size of thyroid follicles (Tf) with clear vacuoles (V) formation and increased proliferated parafollicular cells (P). Stain (H&E) 400X.

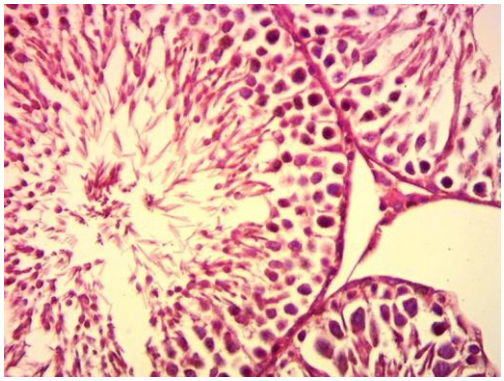

Fig. 5: Testis of control rat. Showing normal structures of Seminiferous tubules (St), Germ cells (Gs), Sertoli cells (Sc) and Leydig cells (Lc). Stain (H&E) 400X.

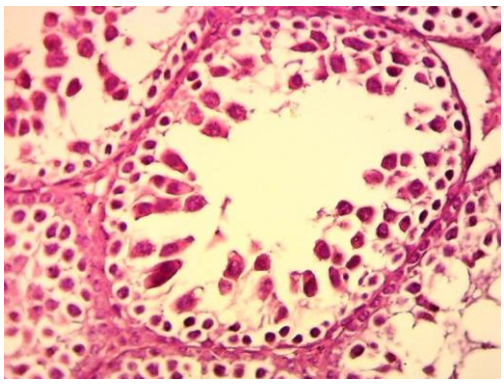

Fig. 6: Testis of G group rat. Showing clear disarrangement of Sertoli cells (Sc), absence of spermatogonia (empty lumen of seminiferous tubule) with decreasing interstitial tissue (Lc). Stain (H&E) 400X.

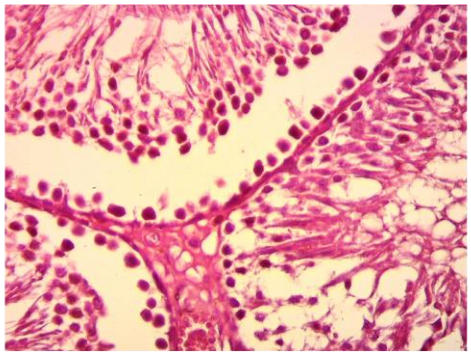

Fig. 7: Testis of L group rat. Showing thin lining epithelial with disturbance allocation of Sertoli cells (Sc), little formation of spermatogonia and interstitial tissue (Lc). Stain (H&E) 400X.

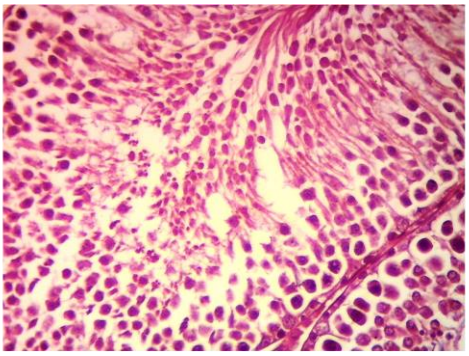

Fig. 8: Testis of G+L group rat. Showing normal seminiferous tubule (St) with normal spermatogenesis. Stain (H&E) 400X.

Discussion

The results of the present study in **Table 2** indicate that there was a significant increase ($P \leq 0.05$) in serum concentration of TSH and a significant decrease ($P \leq 0.05$) in the thyroid hormones (T_4 and T_3) concentrations of male offspring (60 days old) of hypothyroid dam during gestation. These results are in consistent with [22] who found that plasma total T_4 and T_3 were significantly lower and plasma TSH significantly higher at 20-day post-partum in offspring of PTU- treated rats during pregnancy. In addition to histological studies of thyroid gland **Figure 2** which showed a histopathological change in thyroid tissue that confirm the occurrence of hypothyroidism. These results revealed that hypothyroidism was effectively induced in offspring by maternal hypothyroidism during gestation period. Maternal hypothyroidism which induced in dam by PTU and this was agreed with [23] who found a reduction in circulating T_4 and T_3 concentrations and marked increase in the levels of TSH in rats treated with PTU compared to the euthyroid rats are sensitive indicators for hypothyroidism. In accordance with the present study, [24] demonstrated that the cross section of male offspring testis at 60 days born to dams treated with PTU during pregnancy showed disruption of the spermatogenesis and germ cell arrangement associated with a reduction in serum T_4 and T_3 compared with the control group. However, TSH secreted by the thyrotrope cells of the anterior pituitary, plays an essential role in control of the thyroid axis and serves as the most useful physiologic marker of thyroid hormone action [25]. TSH secretion is regulated by the hypothalamus via TRH; receptors in the hypothalamus control levels of circulating T_4, when these levels are low, receptors signal the hypothalamus to release thyroid releasing hormone, as T_4 levels increase, TSH secretion declines in a process called negative feedback control of TSH secretion [26]. However, thyroid hormones in the rat are found in the embryonic and fetal tissue before the onset of fetal thyroid function which occurs on day 18 of gestation, the T_4 and T_3 available to embryos and fetuses are of maternal origin, at term 17.5% of fetal extrathyroidal T_4 is still of maternal origin [27]. So, decrease of thyroid hormones in dams due to hypothyroidism maybe lead to induced a hypothyroidism in their offspring during gestation and this is not caused by PTU because it was believed to have less potential for fetal/neonatal hypothyroidism, to cross the placenta or to enter breast milk [28].

The present study also indicates that in male offspring born from dams with hypothyroidism during lactation showed a significant increase ($P \leq 0.05$) in serum concentration of TSH and a significant decrease ($P \leq 0.05$) in the serum concentration of T_4 and reduction in the T_3 concentration but not a significant as well as the result of histopathological changes as seen in **Figure 3** indicate to hypothyroidism but lesser degree compared with **G** group. On the other hand, the results of the **G+L** group of offspring male which their mother was hypothyroid along the period of gestation and lactation indicate that there was no significant difference ($P \leq 0.05$) in TSH and T_3 even though there was increase and decrease in TSH and T_3 respectively, but not to a significant extent. Where there was

a significant decrease (P≤0.05) in the T_4. This may indicate to mild hypothyroidism. This result is supported by histological findings of thyroid in **G+L** group rats in **Figure 4** which shows less changes than other groups. These results in line with [29] who showed that the offspring (their mother were administered 5 ppm and 15 ppm PTU in the drinking water from gestational day 6 till PN 28) displayed hypothyroidism, where TSH levels were increased significantly (P<0.05) in 15 ppm and 5 ppm PTU-treatment offspring compared with controls and 15 ppm groups had significantly lower serum free (F) T_3 and FT_4 than the controls on PN 14, PN 21 and PN 28 (P<0.05), while on PN 42, the concentrations of serum FT_3, FT_4 and TSH were restored. As well as, [30] indicated that female rats administered PTU in drinking water (0.1% w/v) from birth to lactation day (LD) 30, showed a highly significant reduction in serum T_4 and T_3 levels and remarkable elevation (P<0.01) in serum concentration of TSH level in both dams and their offspring at LDs 20 and 30 compared with the control group. However, gestation and lactation period were the longest period than other periods. So, offspring of the **G+L** group maybe adapt to exposure to maternal hypothyroidism and return to euthyroid state. The histopathological findings of thyroid and testis in **Figure 4** and **Figure 8** respectively that show normal structures especially in testis are referred clearly to normal state.

The results of the present study represented in the **Table 2** indicate that there was a significant decrease (P≤0.05) in serum concentration of T in **G** group and it was decrease in another group but not statistically significant. On the other hand, the serum concentration of LH was a significant decrease (P≤0.05) in all three group **G**, **L**, and **G+L** compared with **control** group. There was a notable increase in the FSH serum concentration in all treated group especially in **G** group compared with **control** group but not statistically significant. However, these results revealed that the **G** group was the most affected among other groups and the histopathological findings of the testes in the present study supported these results, where there was nearly disappearance of interstitial tissue in testis and this may be attributed to decrease T level as showed in **Figure 6** and **Figure 7**. Because, the interstitial cells (Leydig cells) secrete the male hormone T when stimulated by the LH [31]. In line with the present observations, the previous study indicates that the treatment of rats with the PTU from late pregnancy to weaning age leads to testicular hypertrophy, increased number of Sertoli cells and the normal number of Leydig cells in adult life [32]. The decrease in T and gonadotropins may reflect delay in the puberty of male rat of 60-day age. In rats, the peri-pubertal period has been suggested to subsume the interval from about PN day 30-40 in females and PN day 35-55 in males [33]. And the normal testis development is under the regulation of LH and FSH secreted by pituitary gland [34]. Which are both decreased in the results of the present study. Marty et. al., [35] showed that weanling CD rats administered PTU by gavage on postnatal day (21 to 51) resulted in delayed growth and preputial separation; decreased thyroid, testis and epididymis weights, decreased serum levels of T_4, DHT, and testosterone increased TSH levels, and altered thyroid histology indicative of increased TSH secretion. Low T_3, high TSH caused by hypothyroidism can work directly on Leydig cells to suppress steroidogenesis [36]. In consistent with our results, previous studies were observed that hypothyroidism associated with a significant reduction in gonadotropins, testosterone levels, dehydroepiandrosterone (DHEA), DHEA-sulfate, and pregnenolone sulfate [37,38,39]. However, the significant decrease in serum T_3 concentration as shown in the **Table 1** may contributed in histopathological changes in testes because it is well known that T_3 regulates the maturation and growth of testis, controlling Sertoli cells and Leydig cells proliferation and differentiation during testicular development in rats and other mammal species [40,41]. So, the changes in thyroid hormone levels during early period of testes development affect testicular maturity and reproduction [1].

Conclusions

Based on the results of the present study it may be concluded the following: the different periods of maternal hypothyroidism have impact on pituitary-thyroid axis and pituitary- gonadal axis of male offspring at puberty. On the basis of hormonal and histopathological changes the **G** group of male offspring rats was the most affected than **L** and **G+L** groups in which their mothers were had a hypothyroid state during gestation the shortest periods than other two periods. Unanticipated result was that the lowest effective period on offspring male rats was the gestation + lactation period, even though it was the longest period than other periods where the dams where kept on hypothyroid state during gestation and lactation.

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Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. MS Wagner, SM Wajner and AL Maia. The role of thyroid hormone in testicular development and function. The Journal of endocrinology. 2008; 199, 351–365.
2. W Ray Clarke, and C John Watkinson. Scott-Brown's Otorhinolaryngology and Head and Neck Surgery, 8th ed. CRC Press, 2018.
3. J Bernal. Thyroid hormone receptors in brain development and function. Nature clinical practice. Endocrinology & metabolism. 2007; 3, 249–259.
4. M Ghanbari and A Ghasemi. Maternal hypothyroidism: An overview of current experimental models. Life sciences. 2017; 187, 1–8.
5. G Morreale de Escobar, R Pastor, MJ Obregon, and F Escobar del Rey. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after onset of fetal thyroid function. Endocrinology. 1985; 117, 1890-1900.
6. J Patel, K Landers, H Li, RH Mortimer, and K Richard. Delivery of maternal thyroid hormones to the fetus. Trends in endocrinology and metabolism. 2011; 22, 164-70.
7. MB Hapon, M Simoncini, G Via and GA Jahn. Effect of hypothyroidism on hormone profiles in virgin, pregnant and lactating rats, and on lactation. Reproduction. 2003; 126, 371–382.
8. KS Rakesh and VS Nagesh. Hypothyroidism in pregnancy. Indian journal of endocrinology and metabolism. 2012; 16(3): 364–370.
9. S Bhanja and S Jena. Modulation of antioxidant enzyme expression by PTU-induced hypothyroidism in cerebral cortex of postnatal rat brain. Neurochemical research. 2013; 38 (1) 42-49.
10. WA Hassan, MS Aly, T Abdel Rahman, and AS Shahat. Impact of experimental hypothyroidism on monoamines level in discrete brain regions and other peripheral tissues of young and adult male rats. International journal of developmental neuroscience. 2013; 31 (4), 225–233.
11. O E Janssen, N Mehlmauer, and S Hahn. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. European journal of endocrinology. 2004; 150 (3), 363-69.
12. TIM Korevaar, M Medici, and T J Visser. Thyroid disease in pregnancy: new insights in diagnosis and clinical management. Nature reviews. Endocrinology. 2017;13(10), 610–622.
13. F Arfuso, F Fazio, M Levanti, M Rizzo, S Di Pietro, and E Giudice. Lipid and lipoprotein profile changes in dairy cows in response to late pregnancy and the early postpartum period. Archives Animal Breeding. 2016; 59, 429–34.
14. RG Ahmed. 2009, Effects of experimentally-induced hypothyroidism and hyperthyroidism in pregnant albino rats on the development of their offspring. Ph. D. Dissertation. University of Beni-Suef. Egypt.
15. E Auso, R Lavado-Autric, E Cuevas, F Escobar del Rey, G Morreale de Escobar, PA Berbel. Moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology. 2004;145, 4037-4047.
16. AL Fowden and AJ Forhead. Endocrine interactions in the control of fetal growth. In: J Bhatia, Z Bhutta and SC Kahlan. (Eds.). Maternal and Child Nutrition. Nutrition Workshop Series. vol 74. Nestle Freiburg, Germany, Karger, 2013, p. 91–102.
17. NH Hillman, SG Kallaour and A Jobe. Physiology of transition from intrauterine to extrauterine life. Clinics in perinatology. 2012; 39 (4), 769–783.
18. AN Sferruzzi-Perri, OR Vaughan, AJ Forhead and AL Fowden. Hormonal and nutritional drivers of intrauterine growth. Current opinion in clinical nutrition and metabolic care. 2013; 16 (3), 298–309.

19. S Francavilla, G Cordeschi, G Properzi, L Di Cicco, EA Jannini, S Palmero, E Fugassa, B Loras, and M D'Armiento. Effect of thyroid hormone on the pre- and post-natal development of the rat testis. The Journal of endocrinology. 1991; 129 (1):35-42.
20. S Rajender, MG Monica, and L Walter. Thyroid, spermatogenesis, and male infertility. Frontiers in bioscience. 2011; 843-855.
21. A Hernandez. Thyroid Hormone Deiodination and Action in the Gonads. Current opinion in endocrine and metabolic research. 2018; 2: 18-23.
22. A Abd El-Wanees Al-Awdan, E Saleh Idrees, A Saadia Shalaby, M Essam Mehlab, M Samia Mannawy. Prenatal and Postnatal Effects of Hypothyroidism and Thyroxin Replacement on the Development of Rat Testis. Journal of American Science. 2014;10,9.
23. CM Lopez-Fontana, G Pennacchio, LE Zyla, J Toneatto, FA Bruna, N Ortiz, PL Sassi, FE Santiano, S Garcia, CV Sasso, EO Pietrobon, GA Jahn, V Pistone Creydt, M Soaje, and RW Caron. Effects of hypothyroidism on the mesenteric and omental adipose tissue in rats. Molecular and Cellular Endocrinology. 2019; 490, 88–99.
24. AB Yildirim, S Ozdamar and E Kaymak. The Effects of Maternal Hypothyroidism on the Immunoreactivity of Cytochrome P450 Aromatase in the Postnatal Rat Testicles. Acta endocrinologica (Bucharest, Romania: 2005). 2017; 13 (1): 7-16.
25. J Jameson Larry. Harrison's Endocrinology, 3rd ed. McGraw-Hill Education, LLC, 2013, p. 63.
26. M Jahangir. Anatomy and physiology for health professionals, 3rd ed. Jones & Bartlett Learning. 2020, p. 401.
27. F Delange and P Keith West. Micronutrient Deficiencies in the First Months of Life. Karger Medical and Scientific Publishers, 2003, p. 96.
28. P Carl Weiner and M Clifford. Drugs for Pregnant and Lactating Women, 3rd ed. Elsevier Inc, 2018.
29. D Jing, L Wanyang, W Yi, H Yi, X Qi and C Jie. Developmental iodine deficiency resulting in hypothyroidism reduces hippocampal ERK1/2 and CREB in lactational and adolescent rats. BMC neuroscience. 2009;10,149, doi:10.1186/1471-2202-10-149.
30. RG Ahmed and AW El-Gareib. Lactating PTU exposure in neonatal cerebellum: II- Alters thyroid-axis and prooxidant- antioxidant interactions. European Journal of Biology and Medical science Research. 2014; 2(1), 1-24.
31. DF Anna and M Christianne. Anatomy and physiology of farm animals, 8th ed. John Wiley & Sons, Inc, 2018.
32. K Kobayashi, H Kubota, R Hojo and M Miyagawa. Dose-dependent effects of perinatal hypothyroidism on postnatal testicular development in rat offspring. The Journal of Toxicological Sciences. 2014; 39, 867–874.
33. SR Ojeda and MK Skinner. Puberty in the rat. In: E Knobil and JD Neill (Eds.). Physiology of Reproduction. Elsevier Academic Press, St. Louis, MO, 2006, p. 2061–2126.
34. HE Virtanen, J Toppari. Embryology and physiology of testicular development and descent. Pediatric endocrinology reviews. 2014; 11, 2, 206–13.
35. MS Marty, JW Crissman, and EW Carney. Evaluation of the male pubertal assay's ability to detect thyroid inhibitors and dopaminergic agents. *Toxicological Sciences*. 2001; 60 (1), 63–76.
36. S Zahringer, A Tomova, K von Werder, G Brabant, P Kumanov, and J Schopohl. The influence of hyperthyroidism on the hypothalamic-pituitary-gonadal axis. Experimental and clinical endocrinology & diabetes. 2000;108,282-9.
37. RR Maran, K Ravichandran, J Arunakaran and MM Aruldhas. Impact of neonatal hypothyroidism on Leydig cell number, plasma, and testicular interstitial fluid sex steroids concentration. Endocrine research. 2001; 27,119–141.
38. JN Rao, JY Liang, P Chakraborti, and P Feng. Effect of thyroid hormone on the development and gene expression of hormone receptors in rat testes in vivo. Journal of endocrinological investigation. 2003; 26, 435–443.
39. GE Krassas and N Pontikides. Male reproductive function in relation with thyroid alterations. Best Practice & Research: Clinical Endocrinology & Metabolism. 2004; 18(2), 183-95.
40. DR Holsberger and PS Cooke. Understanding the role of thyroid hormone in Sertoli cell development: a mechanistic hypothesis. Cell and Tissue Research. 2005; 322, 133–140.

41. SM Mendis-Handagama and HB Siril Ariyaratne. Leydig cells, thyroid hormones and steroidogenesis. Indian Journal of Experimental Biology. 2005; 43, 939–962.