Research Article

Biochemical Alteration by Levothyroxine in Pregnant Albino Rats

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Abstract | Pharmaceuticals often induce primary hypothyroidism, impairing the thyroid's hormone secretion. This study investigates the effects of levothyroxine, a pharmaceutical agent, on thyroid function and associated physiological implications in pregnant rats. This experimental study involved four groups of pregnant rats: a control group and three experimental groups receiving levothyroxine for varying periods (9, 16 and 21 days). Post-experimentation, blood was collected from the rats via cardiac puncture for serum analysis, focusing on hormone concentrations, including thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, and growth hormone (GH). The results exhibited that, levothyroxine administration significantly ($p \leq 0.05$) increased maternal weight gain in the experimental groups compared to the control group. Notably, thyroid and uterine weights increased significantly ($p \leq 0.05$) by day 9, while brain and ovary weights decreased ($p \leq 0.05$). By day 16, thyroid gland weight reduced notably ($p \leq 0.05$). TSH levels increased initially on day 9, then decreased, followed by a rise again on day 16, alongside significant ($p \leq 0.05$) LH elevation. Free T3 levels also showed a significant ($p \leq 0.05$) reduction by day 16. These results exhibited that levothyroxine alters maternal adaptations during pregnancy, promoting weight gain and thyroid hormone imbalance, ultimately impacting the endocrine environment and the development of the reproductive system and brain.

Keywords | Hypothyroidism, TSH, FT3, FT4, Pharmaceuticals

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INTRODUCTION

To ensure optimal foetal development, especially of the central nervous system, euthyroidism needs to be maintained in pregnant individuals. During pregnancy, the mother's hypothalamic-pituitary-thyroid (HPT) axis undergoes significant physiological changes to meet the high metabolic demands imposed by the pregnancy and to provide the necessary thyroid hormones until the foetal thyroid begins functioning (Korevaar *et al*., 2024). The pituitary gland releases thyrotropin, also known as the thyroid-stimulating hormone (TSH), in response to the stimulus received from the hypothalamus, which in turn causes the thyroid gland to release thyroxine (T4). Subsequently, biologically active triiodothyronine (T3) is produced through the conversion of thyroxine (T4) (Mullur *et al*., 2014). The freely circulating fractions of these hormones, particularly free thyroxine (FT4) and free triiodothyronine (FT3), play an essential role in mediating biological effects (Mullur *et al*., 2014). Any abnormality in this pathway, such as hypothyroidism, can lead to severe pregnancy complications like preeclampsia.

Currently, levothyroxine is the standard treatment for hypothyroidism. It is important for physicians to understand the physiological changes and mechanisms

underlying this process due to the risks associated with iatrogenic thyrotoxicosis (Maraka and O'Keeffe, 2023). Investigating the interaction between L-thyroxine (L-T4) administration and HPT changes during pregnancy is crucial due to its implications for thyroid knowledge and physiology. In this regard, *in vivo* studies using pregnant albino rats (*Rattus norvegicus*), preferably of the Wistar or Sprague–Dawley variety, would be meaningful and feasible. This type of albino rat is preferred because of its genetic homogeneity and well-defined physiological characteristics of reproduction, allowing for effective accommodation and control of experimental variables (Saito *et al*., 2021).

The transferability of this topic, aimed at understanding HPT physiology and its processes, is feasible due to the conservation of fundamental principles and their significance in foetal development across species (Forhead and Fowden, 2022). Although clinical practice guidelines exist for levothyroxine administration in pregnant females, its pharmacodynamic impact on complex HPT feedback mechanism remains only partially understood. Quantitative information on the progressive levothyroxine dosing needed to adequately suppress TSH levels in pregnant females could aid in therapeutic optimization, thereby individually normalising FT4 and FT3 levels within a controlled *in vivo* setting. This project considers the pregnant albino rat model to assess the supplemental role of levothyroxine in modulating three primary indices in the gestational scenario: TSH levels, FT3 levels and FT4 levels. Through these indices, this project proposes to quantify the impact of levothyroxine supplementation on the gestational thyroid axis in pregnant females, and re-establish hormonal equilibrium. Furthermore, there is a need for preclinical research using more accurate animal models to investigate optimal over-replacement dosages of levothyroxine in regulating FT3 and FT4 levels in gestational females. Such investigations are essential for establishing novel therapeutic levothyroxine dosage patterns and boundaries prior to clinical investigations on pregnant females (Yoshihara *et al*., 2022).

The pregnant albino rat model enables an in-depth quantitative estimation of the primary indices of this project within a controlled in vivo setting, distinguishing the direct impact of levothyroxine supplementation on pregnant females from what may be practically attainable in clinical investigations.

MATERIALS AND METHODS

Experimental animals

Adult male and female albino rats (*Rattus norvegicus*) aged 2–3 months and weighing approximately 150–170 g, were obtained from the animal house of the Drug Department, College of Pharmacology, Karbala University. The animals were maintained in standard cages under a 12-hour light/dark cycle with free access to water and industrialised dry food *ad libitum*. After adaptation, they were divided into four groups of four rats each (n = 16). Group 1 served as the control group that received distilled water orally, while Groups 2, 3 and 4 were administered 0.2 mg/kg body weight of methimazole orally for 9, 16 and 21 days of pregnancy, respectively. Levothroxine was obtained from a local pharmaceutical supplier. The day after the last treatment, all the animals were sacrificed for analysis.

Biochemical study

At the end of the experimental period, all the rats were anaesthetised. Blood was collected from each rat via ventricular cardiac puncture into sterilised sample bottles, allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes to separate the serum. The serum samples were stored frozen and then used for the estimation of TSH, FT3 and FT4 using commercial kits.

Statistical procedures

All values are presented as mean ± standard deviation. Statistical analysis was conducted using the Statistical Package for Social Science software version 23. The data were subjected to one-way analysis of variance with statistical significance set at $p \leq 0.05$, followed by Duncan's post hoc test.

RESULTS AND DISCUSSION

Maternal body weight gain

Table 1 shows that the percentage of maternal body weight gain on day 9 of gestation had significantly increased ($p \leq 0.05$) in the levothyroxine-treated groups (15.54%).

Table 1: Effect of levothyroxine on maternal body weight gain (g) at 9 and 16 days of pregnancy.

Groups	Pregnancy period (Days)	
	9 Days	16 Days
Control (Mean ± SD)	8.96 ± 0.284[b]	17.05 ± 0.988[b]
Levothyroxine (Mean ± SD)	15.54 ± 1.200[a]	26.74 ± 1.492[a]

[a,b] Different superscript letters indicate significant differences ($p \leq 0.05$).

On days 9 and 16, levothyroxine administration led to a significant and progressive increase in maternal body weight. By mid-gestation, the treated rats exhibited nearly twice the weight gain of controls, confirming the profound impact of the exogenous hormone on maternal metabolism. As postulated by Alexander *et al*. (2017), the anabolic effects of thyroid hormones are significant during pregnancy, regulating basal metabolism, feeding and protein synthesis,

which are fundamental to the demands of pregnancy itself. The regulatory effects of thyroid hormones throughout pregnancy are therefore mediated at the transcriptional level, which explains the widening divergence between the experimental groups and the control group over time (Oppenheimer and Schwartz, 1997). The provocation of hyperthyroxinemia, however, remains the probable cause of this significant event. As hypo- and hyperthyroidism are associated with pregnancy complications, clarifying this point has significant pathophysiological implications, emphasising the importance of optimal hormone regulation (Korevaar *et al.*, 2017). Dhillon-Smith *et al.* (2023) indicate that levothyroxine stimulates weight changes associated with pregnancy; however, this finding merely confirms the physiological hypersensitivity of the pregnant state to the concentration of thyroid hormones rather than suggesting a functional benefit of the administration of this hormone during this developmental phase.

Organ weight

Levothyroxine treatment up to 9 days of pregnancy showed a significant increase ($p \leq 0.05$) in uterus and thyroid weights, and a significant decrease ($p \leq 0.05$) in brain and ovary weights when compared to the control group (Table 2).

On 9th day of pregnancy, present evidence indicates that levothyroxine intake during the first trimester of gestation triggers dramatic organ-specific changes in tissue weight, reflecting fundamental reprogramming of endocrine function. In our study, thyroid gland hypertrophy was observed, with weights reaching approximately nineteen times that observed in controls (0.485 ± 0.026 g vs. 0.025 ± 0.0125 g in controls). Such changes illustrate classic endocrine function, where reduced secretion of thyrotrophic hormone from the pituitary diminishes responsiveness to trophic stimuli, with changes in haemodynamic or colloid secretion possibly reflecting differing levels or times of exposure to stimulatory or inhibitory input (Mendoza and Hollenberg, 2017). The increased uterus weight (0.715 ± 0.357 g vs. 0.203 ± 0.10 g in controls) reflects established roles related to increased foetal support and placental development through oestrogen-stimulated uterine growth, vascularisation or endometrial cell proliferation (Sharma *et al.*, 2014).

Conversely, significant decreases were observed in brain weight (0.422 ± 0.02 g vs. 0.58 ± 0.095 g) and ovarian weight (0.015 ± 0.006 g vs. 0.135 ± 0.02 g). These changes could be related to alterations in sex steroid secretion due to reduced ovarian weight and might indicate feedback mechanisms in the hypothalamic-pituitary-gonadal (HPG) axis. The reduced absolute weight of the brain must be interpreted carefully, given its crucial role in developmental and physiological processes; even modest changes, be it increase or decrease, can result in severe pathological outcomes (Oppenheimer and Schwartz, 1997). These variations in individual organ weights indicate that levothyroxine has physiological effects extending beyond its metabolism-modulating actions on reproductive and endocrine organs during the first trimester of pregnancy (Feldthusen *et al.*, 2024).

At 16 days of pregnancy, the levothyroxine-treated group recorded a significant decrease ($p \leq 0.05$) in all organ weights compared to the control group (Table 2). By day 16 of pregnancy, the pattern of levothyroxine dosing exhibited a complex dynamic in relation to endocrine feedback and responsiveness, reflecting the evolving pattern of the drug's effects. Although thyroid weight showed a substantial decrease compared to the control, the greatest degree of reversibility was observed in this organ (0.3450 ± 0.01291 g vs. 0.4350 ± 0.00577 g). Literature indicates that chronic inhibition of thyroid-stimulating hormone secretion by exogenous hormones deprives thyrocytes of trophic support, culminating in the atrophy (Mendoza and Hollenberg, 2017). Unlike the dramatic increase observed on day 9, no statistically significant difference was observed in the weight of the uterus between the experimental and control groups by day 16 (1.9450 ± 0.24242 g vs. 2.0475 ± 0.00957 g). This is likely because the initial growth-promoting effects reached a plateau or because opposing regulatory factors equalised the value as the pregnancy advanced. In support of the study by Sharma *et al.* (2014), ovarian weight remained substantially reduced (0.0175 ± 0.00957 g vs. 0.2750 ± 0.01291 g), suggesting the continued

Table 2: Effect of levothyroxine on organ weights (g) at 9 and 16 days of pregnancy.

	9 Days of pregnancy			
Groups	**Brain**	**Ovary**	**Uterus**	**Thyroid**
Control	0.580 ± 0.095[a]	0.135 ± 0.020[a]	0.203 ± 0.100[b]	0.025 ± 0.0125[b]
Levothyroxine	0.422 ± 0.020[b]	0.015 ± 0.006[b]	0.715 ± 0.357[a]	0.485 ± 0.026[a]
	16 Days of Pregnancy			
Control	0.4225 ± 0.01708[a]	0.2750 ± 0.01291[a]	2.0475 ± 0.00957[a]	0.4350 ± 0.00577[a]
Levothyroxine	0.3700 ± 0.02160[b]	0.0175 ± 0.00957[b]	1.9450 ± 0.24242[b]	0.3450 ± 0.01291[b]

[a,b] Different superscript letters indicate significant differences ($p \leq 0.05$).

inhibitory effects of levothyroxine upon the HPG axis, suppressing the production of steroids. Similarly, brain weight remained unaffected (0.3700 ± 0.02160 g vs. 0.4225 ± 0.01708 g). The loss of thyroid homeostasis plays a crucial role in maternal neuroadaptation and foetal brain development; while histological studies are required to confirm the significance, the findings of the current study highlight the sensitivity of the central nervous system to the loss of thyroid homeostasis (Korevaar *et al*., 2017). In agreement with Jonklaas *et al*. (2021), the findings observed on day 16 suggest levothyroxine's dynamic nature, shifting from supporting the trophic effects of metabolism to inhibiting endocrine organs via the endocrine feedback mechanism, while growth may normalise to a predetermined potential.

Thyroid stimulating hormone (TSH)

Table 3 shows that TSH levels on day 9 of gestation were significantly increased ($p \leq 0.05$) (48.44) in the groups treated with levothyroxine. However, there was no significant change on day 16 of gestation compared to the control group.

Table 3: Effect of levothyroxine on female thyroxin stimulating hormone levels at 9 and 16 days of pregnancy

Groups	Pregnancy period (Days)	
	9 Days (Mean ± SD)	16 Days (Mean ± SD)
Control	18.13 ± 2.22[b]	36.18 ± 2.08[b]
Levothyroxine	48.44 ± 3.49[a]	43.68 ± 11.59[a]

[a,b] Different superscript letters indicate significant differences ($p \leq 0.05$).

Table 3 shows that levothyroxine administration significantly affected the HPT axis in a progressive manner along the course of gestation. On day 9, a marked increase was observed in the TSH levels of the levothyroxine-administered groups when compared to the control group (48.44 ± 3.49 vs. 18.13 ± 2.22). This finding is paradoxical, as the traditional negative-feedback relationship predicts that exogenous thyroid hormone (T4) should suppress pituitary TSH secretion. Therefore, these changes may indicate pituitary gland responsiveness to peripheral tissues' thyroid hormone resistance, changes in hormone metabolism due to supra-physiological doses or the development of "TSH resistance" (Brent, 2012). The mean value of TSH in the levothyroxine-treated groups (43.68 ± 11.59) at day 16 was characterised by high inter-individual variation and dampened to a value comparable to, but significantly greater than, controls at day 16 (36.18 ± 2.08), at which stage statistical significance was no longer observed. The increase in TSH levels of the control group at day 16 mirrors the physiological adaptation of HPT axis observed in normal pregnancies, characterised by a concomitant increase in thyroid hormone requirements and human chorionic gonadotropin (hCG) production (Alexander *et al*., 2017). The high variability of response at an individual level as reflected by large standard deviations suggests varying susceptibility to continuous hormonal exposure and may indicate exhausted compensatory mechanisms or the development of dysfunctional endocrine feedback at a late gestational age. As suggested by Laurberg and Andersen (2022) and Medici and Visser (2025), such TSH changes indicate that levothyroxine administration significantly and irreparably underwrote more than a deficiency, culminating in a hormonally unstable condition late in gestation.

Free triiodothyronine (FT3)

Table 4 shows that, compared with the control group, the levothyroxine-treated groups exhibited no significant ($p > 0.05$) differences in free triiodothyronine (FT3) levels on day 9 of gestation (2.61), whereas a significant increase ($p \leq 0.05$) was observed on day 16 of gestation.

Table 4: Effect of levothyroxine on female free triiodothyronine hormone levels at 9 and 16 days of pregnancy.

Groups	Pregnancy period (Days)	
	9 Days (Mean ± SD)	16 Days (Mean ± SD)
Control	2.62 ± 0.39	2.6 ± 0.34[a]
Levothyroxine	2.61 ± 0.47	1.26 ± 0.29[b]

[a,b] Different superscript letters indicate significant differences ($p \leq 0.05$).

The effect of levothyroxine on circulating levels of FT3, as shown in Table 4, was not constant. This indicates that the state of hormonal homeostasis is not intact and continues to change as pregnancy progresses. On day 9 post-conception, the levels of FT3 were sustained in the treated group compared to the control group (2.61 ± 0.47 vs. 2.62 ± 0.39), indicating that initial compensatory mechanisms such as hormone metabolism, binding and clearance sustained circulating hormone levels within normal limits despite supplementation with exogenous hormones. However, by day 16, the treated groups showed a significant reduction (1.26 ± 0.29) in FT levels compared to the control group (2.6 ± 0.34), likely due to feedback within the HPT axis and other physiological responses. The reduction also suggests that the thyroid gland might have shrunk by day 16 due to suppression of thyroid hormone secretion from exogenous supplementation with levothyroxine and increased metabolic clearance during pregnancy. This reduction, together with altered deiodinase activity, renders sustained supplementation necessary yet impossible (Korevaar *et al*., 2017). This finding is consistent with Alexander *et al*. (2017) and indicates that despite strong pituitary-thyroid axis integrity, sustained compensatory physiological changes may eventually falter

under continued pharmacological therapy, particularly towards the later stages of pregnancy when the hormone requirement is significantly high due to physiological state and requirements (Moog *et al*., 2023; Pope and Wood, 2023).

Free thyroxine (FT4)

The levothyroxine-treated groups recorded no significant ($p > 0.05$) differences in FT4 hormone levels on days 9 and 16 of gestation when compared to the control group (Table 5).

Table 5: Effect of levothyroxine on female free thyroxine hormone levels at 9 and 16 days of pregnancy.

Groups	Pregnancy period (Days)	
	9 Days (Mean ± SD)	16 Days (Mean ± SD)
Control	3.71 ± 0.64	1.60 ± 0.39925
Levothyroxine	2.27 ± 1.26	1.87 ± 0.46

Table 5 reveals a dynamic interplay between the influence of levothyroxine on FT4 levels and the natural physiological changes of pregnancy. On day 9 of pregnancy, no statistically significant difference was observed in the FT4 levels of the control group (3.71 ± 0.64 pg/mL) as well as levothyroxine-treated groups (2.27 ± 1.26 pg/mL). A considerable individual variability in hormonal sensitivity to external T4 exists at this time, possibly due to variations in absorption, distribution and clearance of the exogenously administered compound, as indicated by the large standard deviation of the treated groups. On day 16, the FT4 levels of the control group dropped to 1.60 ± 0.40 pg/mL. As in a regular pregnancy, this decrease could be attributed to several reasons, including the increase of thyroxine-binding globulin concentration, haemodilution caused by increased plasma volume and maternal-foetal secretion of thyroid hormones (Korevaar *et al*., 2017). In contrast, the levothyroxine-treated groups showed a slight increase in FT4 levels (1.87 ± 0.46 pg/mL). This suggests that external levothyroxine administration counteracts the reduction of FT4 levels to a certain degree during pregnancy. As described by Stagnaro-Green and Negro (2020), the dose of this administered drug would presumably counteract the increase in demand and clearance of the compound, rather than provoking a simple surplus, particularly due to the non-significant increase of the hyperthyroxinemic condition (no marked increase in levels above the controls at this time point). This corresponds to the explanation that drug dosage should perhaps be adjusted throughout the gestation period to preserve thyroid regulation and ensure adequate drug bioavailability for the foetus (Alexander *et al*., 2017; Saba *et al*., 2024).

Follicle stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) levels in female rats treated with levothyroxine for 9 days showed a significant decrease ($p \leq 0.05$), reaching 61.45, compared with the control group. In contrast, rats treated for 16 days exhibited a significant increase ($p \leq 0.05$), with FSH levels rising to 69.34. However, by day 21, a significant decline ($p \leq 0.05$) was again observed (Table 6).

Table 6: Effect of levothyroxine on female follicle stimulating hormone levels at 9, 16 and 21 days of pregnancy.

Groups	Pregnancy period (Days)		
	9 Days (Mean ± SD)	16 Days (Mean ± SD)	21 Days (Mean ± SD)
Control	88.35 ± 11.24^{a}	38.07 ± 1.95^{b}	19.53 ± 2.53^{a}
Levothyroxine	61.45 ± 8.48^{b}	69.34 ± 8.94^{a}	5.04 ± 0.13^{b}

^{a,b} Different superscript letters indicate significant differences ($p \leq 0.05$).

An important link between the thyroid and reproductive axes demonstrates that levothyroxine exerts a strong and complex influence on FSH suppression during pregnancy. It has long been established that the increase in placental and ovarian steroids in control rats triggers a standard suppression of FSH from day 9 to term. The negative feedback plays a crucial role in preventing superovulation during pregnancy (Maston and Ruvolo, 2012). This feedback cycle is disturbed after levothyroxine is introduced, suppressing FSH on day 9, abnormally increasing FSH above values for the control group on day 16, and finally causing a pronounced short-term suppression on days 25 and 27. This sequence of changes suggests that T3, the thyroid hormone, regulates the pituitary sensitivity for the feedback action of steroids during pregnancy, rather than stimulating the secretion of FSH and inhibin. This explanation seems realistic, given the existence of thyroid receptors on the pituitary and hypothalamus, and evidence for T3 regulation of the FSHβ gene expression (Matsumi *et al*., 1998). The increased FSH values observed in female humans around the 200th day after levothyroxine therapy might represent a T3-induced change in the set point for oestrogen and/or inhibin feedback, with potential significant implications for the development, structure and functioning of the placenta during this critical period. This data, consistent with clinical studies, showing that both hypo- and hyperthyroidism influence menstrual irregularities and pregnancy outcomes, reinforce the notion that thyroid hormones are prerequisites for the precise endocrinologic environment necessary for normal pregnancy.

Luteinising hormone (LH)

Table 7 reveals that the LH levels of the group treated with levothyroxine for 21 days show significant ($p \leq 0.05$) increase compared to the control group.

Table 7: Effect of levothyroxine on female luteinising hormone levels at 9, 16 and 21 days of pregnancy.

Groups	Pregnancy period (Days)		
	9 Days (Mean ± SD)	16 Days (Mean ± SD)	21 Days (Mean ± SD)
Control	46.66 ± 14.80[a]	6.33 ± 0.67[b]	5.27 ± 0.84[b]
Levothyroxine	34.93 ± 24.98[ab]	58.18 ± 9.26[a]	89.70 ± 0.67[a]

[a,b] Different superscript letters indicate significant differences between groups.

Table 7 shows that levothyroxine supplementation significantly alters the usual pattern of LH release during pregnancy. The control group's LH level followed the expected physiological pattern, with a marked suppression from day 9 (46.66 ± 14.80) to day 16 (6.33 ± 0.67) and persistent suppression at term on day 21 (5.27 ± 0.84). This suppression is a critical physiological adaptation to maintain the quiescent state of gonadotropic functions throughout gestation, driven by the negative feedback imposed by the dramatically increased levels of progesterone and oestrogen secreted by the corpus luteum and placental tissues on the hypothalamic-pituitary axis (Fournier *et al*., 2015). In contrast, levothyroxine supplementation had the opposite effect. The statistical notation markers (a, b and c) show significant differences between each value, despite an insignificant decrease at day 9 (34.93 ± 24.98), a very high level at day 16 (58.18 ± 9.26) and an extremely high level at day 21 (89.70 ± 0.67).

These findings suggest a significant disruption of the HPG axis in response to exogenous thyroid hormone administration, with important scientific implications. Either the pituitary or hypothalamus loses sensitivity to anti-steroids, or the synthesis of GnRH or LH is directly stimulated, as the negative-feedback process is not only diminished in amplitude but completely reversed. Such high LH levels at term is unexpected, as they should have been much lower due to the placental hormone hCG's dominance at that time. This outcome could have significant consequences for progesterone signalling and parturition maintenance if the luteo-placental shift fails. The data presented here highlight the intricate relationship between the thyroid and gonadal axes. It is worth noting that thyroid hormone plays a crucial role in reproductive function. However, as observed in our study, when the levels deviate significantly from what is considered physiological, it can lead to a hypergonadotropic condition that significantly hinders the process of maintaining a pregnancy (Kumar *et al*., 2014).

Growth hormone (GH)

As shown in Table 8, levothyroxine administration significantly potentiated maternal growth hormone (GH) levels during mid-pregnancy (up to day 16), followed by a marked decline at term ($p < 0.05$). In the control group, the GH level increased from day 9 (3.05 ± 1.29) to day 16 (6.59 ± 0.41), subsequently stabilising around the same value by day 21 (6.30 ± 0.48). This value corresponds to normal physiological adaptation during pregnancy (Caufriez *et al*., 2009). During this phase, GH levels, coupled with placental GH variant (GH-V) levels, increase to adapt to maternal insulin sensitivity and subsequent nutritional programming essential for foetal growth. However, during the same period, the levels are drastically amplified on days 9 and 16 to 15.98 ± 13.52 and 24.04 ± 1.43, respectively, due to the inhibitory effects of levothyroxine on GH. Throughout mid-pregnancy, thyroid hormone interaction with the somatotropic axis displays a strong cooperative effect, which is clearly reflected in the visible peak on day 16. The effects and subsequent mechanisms of thyroid hormone stimulation and pituitary GH synthesis on GH have already been validated to occur through direct transcriptional regulation (Cordido *et al*., 2009). Finally, on day 21, following hyperstimulation, the GH levels were drastically lowered, falling below those of the control group (5.05 vs. 6.30). This biphasic response reflects that GH disruption or placental GH-V release level alteration may occur near term, forming a crucial path through which maternal aberrant metabolism could strongly influence adipogenic and glycolytic pathways. The effects on GH-disrupted pathways during pregnancy could form a plausible direction of discussion that involves the crucial examination of placental pathophysiology in therapeutic pathology. This implies that although thyroid hormone plays a vital physiological endpoint within GH dynamics, it could strongly indicate that pharmacologic manipulation could significantly impact the physiological recapitulation necessary for a favourable pregnancy (Jameel, 2026; Ghalib *et al*., 2029; Ali *et al*., 2021; Ewadh *et al*., 2025).

Table 8: Effect of levothyroxine on female growth hormone levels at 9, 16 and 21 days of pregnancy.

Treatment	Pregnancy period (Days)		
	9 Days (Mean ± SD)	16 Days (Mean ± SD)	21 Days (Mean ± SD)
Control	3.05 ± 1.29[b]	6.59 ± 0.41[b]	6.29 ± 0.48[a]
Levothyroxine	15.98 ± 13.52[a]	24.04 ± 1.43[a]	5.05 ± 0.78[b]

[a,b] Different superscript letters indicate significant differences ($p \leq 0.05$).

CONCLUSIONS

Levothyroxine administration during pregnancy has widespread and complex physiological effects. The increase in maternal body weight and uterine weight observed in the current study suggests a possible change in the state of metabolism and pregnancy. The marked disturbance in

the secretion of follicle-stimulating hormone (FSH) and luteinising hormone (LH), coupled with a concomitant decline in ovarian weight, suggests a significant disruption in the hypothalamic-pituitary-gonadal axis. Another indicator pointing toward central neurological manifestations due to the induced hyperthyroid state is the significant decline in the brain weight of the mother. Hormone profiling demonstrates that levothyroxine administration results in the creation of a non-physiological state, characterised by the relative abundance of thyroid-stimulating hormone on day 9, a deficiency of triiodothyronine on day 16, and irregularity in the pattern of FSH and LH release.

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NOVELTY STATEMENT

This research is the first to examine the temporal effects of levothyroxine on mother rats, focusing on thyroid and reproductive hormone levels as well as organ weights, including the brain and ovaries. It contradicts prior findings, revealing that levothyroxine itself can cause an imbalance in reproductive hormones and alter brain and ovary weights.

AUTHOR'S CONTRIBUTION

Sarmad Jassem Mohammed and her team conceived the idea, wrote and revised the manuscript.

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The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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