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THE ESTROUS CYCLE IN FEMALE RATS UNDER CONDITIONS OF EXPERIMENTAL HYPOTHYROIDIS

The article presents the results of an experimental study aimed at investigating changes in the estrous cycle of female rats following partial thyroidectomy. The goal of modeling hypothyroidism by removing half of the thyroid gland was to examine the mechanisms through which thyroid dysfunction affects the reproductive system. Following surgery, a statistically significant increase in thyroid-stimulating hormone (TSH) levels was observed in the animals, confirming the development of hypothyroidism.

An analysis of estrous cycle dynamics revealed significant alterations: the overall duration of the cycle was markedly prolonged due to an extended diestrus phase, indicating a delay or absence of ovulation. The frequency of estrus in hypothyroid females was significantly reduced, pointing to a decrease in reproductive activity. Persistent anovulatory cycles were identified, which are considered a prerequisite for infertility.

Possible pathophysiological mechanisms of this phenomenon are discussed, including the impact of reduced thyroid hormone levels on the hypothalamic–pituitary–gonadal axis, altered sensitivity of gonadotropin receptors, and disruption of sex hormone balance. Particular attention is given to the role of thyroid hormones in maintaining normal ovarian function and ensuring ovulatory processes. The findings have practical significance for understanding the pathogenesis of fertility disorders associated with hypothyroidism and may be applied to improve approaches to the diagnosis and treatment of endocrine-related infertility.

Thus, the study highlights the importance of regular monitoring of thyroid gland function in women of reproductive age to prevent potential reproductive complications.

Key words: *hypothyroidism, estrous cycle, thyroid-stimulating hormone, thyroid gland.*

Introduction. The thyroid gland plays a critical role not only in maintaining normal metabolism but also in supporting reproductive function. Disorders of this gland, such as autoimmune thyroiditis (Hashimoto's thyroiditis), subclinical and overt hypothyroidism, as well as thyrotoxicosis, are frequently associated with infertility and an increased risk of pregnancy complications. Thyroid hormone deficiency during gestation raises the likelihood of developing gestational hypertension, preeclampsia, preterm labor, and cognitive impairments in offspring (Zhou, Tao, Huang, Zhu, & Tao, 2017; Modi, & Garg, 2025; Dosiou, 2020; Egger, Conze, & Wehrend, 2024).

Thyroid dysfunction is one of the most common endocrine disorders in women of reproductive age, significantly affecting reproductive health. According to clinical and epidemiological studies, hypothyroidism occurs in 2–4% of women during their reproductive years. This condition may lead to anovulatory cycles, luteal phase defects, hyperprolactinemia, and general sex hormone imbalance, ultimately resulting in reduced fertility.

Undiagnosed and untreated thyroid dysfunction remains one of the leading causes of endocrine infertility. A large-scale study of 394 infertile women revealed elevated thyroid-stimulating hormone (TSH) levels above 4.2 μIU/mL in 23.9% of cases, indicating hypothyroidism. Another investigation identified thyroid dysfunction in 16% of infertile women, with overt hypothyroidism (9.6%) and subclinical hypothyroidism (4.0%) being the most prevalent forms, especially in cases of secondary infertility (over 21.8% of cases) (Verma, Sood, Juneja, & Kaur, 2012; Akande, Isah, Aliyu, & Adesiyun, 2022).

Experimental studies have demonstrated that thyroid hormones actively participate in the regulation of the hypothalamic–pituitary–gonadal axis by modulating gonadotropin secretion, ovarian steroidogenesis, and ovulatory processes. Specifically, reduced levels of triiodothyronine (T3) and thyroxine (T4) may decrease the synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as alter tissue sensitivity to gonadotropins (S. Wang, Pu, Chiang, Ho, & P. Wang, 1987; Chiao et al., 1999; Feldt-Rasmussen, Effraimidis, & Klose, 2021; Malova et al., 2021).

Menstrual cycle disturbances are among the most common reproductive dysfunctions in hypothyroidism (Güngör Semiz, & Hekimsoy, 2024). Hypothyroidism leads to hypomenstrual syndrome and such menstrual irregularities as opsoligomenorrhea, hyperpolymenorrhea, and less frequently, amenorrhea. Moreover, prolonged uncompensated hypothyroidism causes chronic anovulation and uterine bleeding. The pathogenesis, clinical course, diagnosis, and treatment of hypothyroidism remain incompletely understood. This is evidenced by frequent relapses, insufficient treatment efficacy, and resulting reproductive disorders.

Thus, the impact of hypothyroidism on menstrual and reproductive function remains insufficiently elucidated. A key factor is thyroid hormone deficiency, which plays a fundamental role in sustaining basal metabolism, tissue respiration, and the growth of most actively functioning cells and tissues, including those of the reproductive system. Diagnosis relies on TSH level assessment and confirmation via thyroxine concentration. Current guidelines emphasize the necessity of incorporating thyroid function testing, particularly TSH measurement, into the diagnostic protocols for women with reproductive disorders. This is especially pertinent in cases of unexplained infertility and frequent anovulatory cycles (Sheehan, 2016; Davis, & Phillippi, 2022; Akande, Isah, Aliyu, & Adesiyun, 2022).

Despite numerous clinical data, experimental models of hypothyroidism remain invaluable for detailed investigation of reproductive dysfunction mechanisms at molecular and physiological levels. Laboratory animal models, such as rats, allow the induction of hypothyroid states under controlled conditions and facilitate evaluation of their effects on estrous cycle dynamics, hormonal profiles, and the morphofunctional state of the reproductive system.

The objective of this study was to investigate the reproductive function of female rats under experimental hypothyroidism.

Materials and Methods. The experimental study was conducted at the Department of Human Anatomy and Physiology named after Professor Ya. R. Synelnikov at H. S. Skovoroda Kharkiv National Pedagogical University and at the Central Research Laboratory of Kharkiv National Medical University under a cooperation agreement (No. 75 /C 5-20/H, dated 2022).

The experiment was carried out on 27 sexually mature outbred female rats, whose estrous cycles remained regular for 14 consecutive days, with a cycle length not exceeding 5 days, which is typical for this species. The animals were housed in a vivarium under standard dietary conditions with free access to water. The rats were divided into two groups: a control group (5 animals) and an experimental group (21 animals). Hypothyroidism was induced in the experimental animals by surgical removal of one-half of the thyroid gland. The postoperative model of hypothyroidism is considered radical, more physiological, clinically relevant, and technically feasible (Stechenko et al., 2007). Considering that thyroid-stimulating hormone (TSH) serves as a marker of hypothyroidism (Surks et al., 2004), an enzyme-linked immunosorbent assay (ELISA) for TSH concentration was performed in control animals and in a subset of experimental animals using the TSH ELISA kit

(Germany). Blood samples for TSH assessment were collected post-mortem following euthanasia by intraperitoneal injection of a triple anesthetic dose of sodium ethaminal, in accordance with the euthanasia protocols outlined in the methodological guidelines of the Ministry of Health of Ukraine and the general ethical principles of animal experimentation approved by the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

In 12 females from the experimental group, the estrous cycle was monitored before and after the induction of hypothyroidism over a 14-day period via analysis of vaginal smears. Vaginal smear collection for the assessment of estrous cycle structure and duration was performed at the same time each day. After drying and staining the smears with methylene blue following standard methodology, the stages of the estrous cycle were determined under a light microscope: proestrus (follicular phase, follicle growth stage) is characterized by nucleated epithelial cells; estrus (ovulation) – clusters of numerous cornified squamous cells, visible macroscopically; metestrus (luteal phase) – a mixture of nucleated epithelial cells, squamous cells, and leukocytes; diestrus (quiescent period) – dominance of leukocytes covering the entire field of view.

All experimental procedures were performed in accordance with the "General Ethical Principles for Animal Experiments", approved by the First National Congress of Ukraine on Bioethics (Kyiv, 2001), the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) (European Convention…, 1986), and the Law of Ukraine of 21.02.2006 No. 3447-IV "On the Protection of Animals from Cruelty" (On the Protection of Animals…, 2006), with strict compliance with the Bioethics Committee of H. S. Skovoroda Kharkiv National Pedagogical University.

Statistical analysis was performed using the "Excel – 7" software (Microsoft Office, USA) with application of the Student's t-test.

Results and Discussion. At the first stage of the study, thyroid-stimulating hormone (TSH) concentration was measured in 5 control and 7 experimental female rats, in which half of the thyroid gland had been surgically removed. The results revealed that in the experimental group, TSH concentration significantly increased – nearly twofold – reaching 9.35 ng/mL compared to 4.57 ng/mL in the control group ($p < 0.05$), which confirmed the presence of hypothyroidism in these animals.

Further analysis of the estrous cycle in the experimental females demonstrated that induced hypothyroidism led to specific alterations in the cycle's structure and duration. In particular,

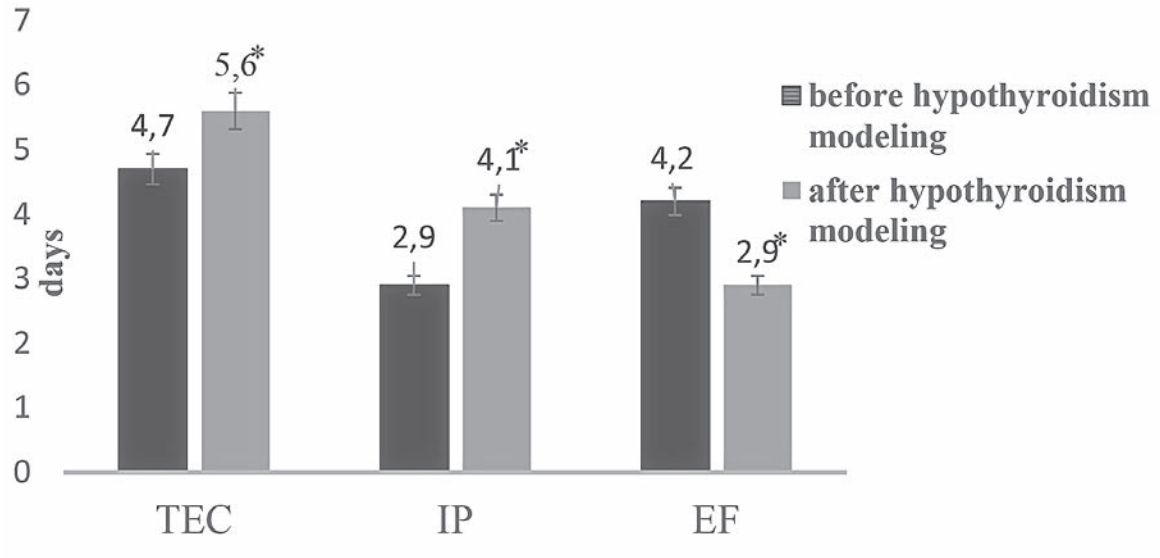

Fig. ***Estrous cycle in female rats.***
TEC – Total Estrous Cycle duration (days); IP – Interphase Period duration (days); EF – Estrus Frequency over 14 days of observation

Note: ** – statistically significant difference at $p < 0.05$ compared to baseline.*

following postoperative hypothyroidism, the total estrous cycle (TEC) duration significantly increased from 4.7 ± 0.89 to 5.6 ± 0.91 days ($p < 0.05$). This prolongation was mainly attributed to an extended interphase period (diestrus), which reached 3.6 ± 0.68 days compared to the baseline values ($p < 0.05$). Additionally, the frequency of estrus episodes over the 14-day observation period significantly decreased from 4.2 ± 0.49 to 2.9 ± 0.49 ($p < 0.05$) in comparison with the initial state (see Fig.).

The prolongation of the diestrus phase may be attributed to a number of mechanisms triggered by hypothyroidism. One such mechanism, discussed in the scientific literature, suggests that hypothyroidism disrupts the activity of hypothalamic biogenic amines, leading to a reduction in the secretion of gonadotropin-releasing hormone (GnRH or luteinizing hormone-releasing hormone), which in turn results in decreased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (B. Khabrat, A. Khabrat, Lytvak, & Lysenko, 2018; Wu, Zhao, Wang, Tang, & Liu, 2021).

It is also important to note that the interaction between reproductive and thyroid functions is regulated by common central mechanisms involving the tropic hormones of the pituitary gland – LH, FSH, thyroid-stimulating hormone (TSH), and prolactin – which are under the control of the hypothalamus and, to some extent, the cerebral cortex. Thyrotropin-releasing hormone (TRH) stimulates the secretion of both TSH and prolactin by the pituitary. Hypothyroidism leads to increased TRH secretion (via negative feedback), which may cause hyperprolactinemia. Elevated prolactin levels disrupt the pulsatile secretion of gonadotropins and contribute to decreased LH production and inhibition of ovulation (P. Goel, Kahkasha, Narang, Gupta, & K. Goel, 2015). Prolonged hypothyroidism is also known to induce the Van Wyk–Grumbach syndrome.

Another factor is the considerable similarity in the molecular structure of LH and TSH, which, as described in the literature, may lead to potential competitive binding of TSH to LH receptors on granulosa cells. This suggests that the reactive elevation of TSH levels in thyroidectomized females might competitively block LH receptors in ovarian follicles, thereby impairing ovulatory processes (Osuga, Toyoshima, Mitsuhashi, & Taketani, 1995).

Conclusions. In female rats with experimentally induced postoperative hypothyroidism, the overall pattern of the estrous cycle is altered – its total duration increases due to prolonged diestrus, while the frequency of estrus decreases.

The disruption of the estrous cycle caused by hypothyroidism may be attributed to shared central regulatory mechanisms of thyroid and reproductive function, as well as to the structural homology between gonadotropic hormones and TSH.

Thus, the study emphasizes the importance of regular monitoring of thyroid functional status in women of reproductive age to prevent potential reproductive complications.

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ЕСТРАЛЬНИЙ ЦИКЛ САМИЦЬ ЩУРІВ НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО ГІПОТИРЕОЗУ

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У статті представлені результати експериментального дослідження, спрямованого на вивчення змін естрального циклу у самок щурів після часткового видалення щитоподібної залози. Метою моделювання гіпотиреозу шляхом видалення ½ частини щитоподібної залози стало вивчення механізмів впливу тироїдної дисфункції на репродуктивну систему. Після оперативного втручання у тварин спостерігалося статистично значуще підвищення рівня тиреотропного гормону (ТТГ), що підтвердило розвиток гіпотиреозу.

Аналіз динаміки естрального циклу продемонстрував істотні зміни: спостерігалося значне подовження загальної тривалості циклу за рахунок пролонгації діеструсу, що свідчить про затримку або відсутність овуляції. Частота еструсу у самок із гіпотиреозом була суттєво зниженою, що вказує на зниження репродуктивної активності. Виявлено розвиток стійких ановуляторних циклів, які є передумовою безпліддя.

Обговорюються можливі патофізіологічні механізми даного явища, зокрема вплив зниженого рівня тироїдних гормонів на гіпоталамо-гіпофізарно-гонадну вісь, зміну чутливості гонадотропінових рецепторів і дисбаланс статевих гормонів. Окрему увагу приділено обговоренню ролі тиреоїдних гормонів у підтриманні нормальної функції яєчників та забезпеченні овуляторних процесів. Отримані результати мають практичне значення для розуміння па-

тогенезу порушень фертильності при гіпотиреозі, а також можуть бути використані для вдосконалення підходів до діагностики та лікування безпліддя, пов'язаного з ендокринними порушеннями.

Таким чином, дослідження підкреслює необхідність регулярного моніторингу функціонального стану щитоподібної залози у жінок репродуктивного віку для попередження можливих репродуктивних ускладнень.

Ключові слова: *гіпотиреоз, естральний цикл, тиреотропний гормон, щитоподібна залоза.*