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## THE EFFECT OF HYPOTHYROIDISM ON THE OUTCOMES OF IN VITRO FERTILIZATION, DEPENDING ON AUTOIMMUNE STATUS

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### Abstract

**Introduction.** Subclinical hypothyroidism often goes undiagnosed due to the absence of pronounced clinical symptoms. However, even minimal thyroid hypofunction can have a negative impact on a woman's reproductive function, including ovarian reserve and the success of in vitro fertilization (IVF) programs.

**Materials and Methods:** The study included women with endocrine infertility against a background of hypothyroidism. The patients were divided into groups: Group 1-women with hypothyroidism of autoimmune etiology (n=39); Group 2-women with hypothyroidism without autoimmune thyroiditis (n=49). The control group consisted of women of reproductive age without thyroid dysfunction (n=20). To assess ovarian reserve, anti-Müllerian hormone (AMH) and inhibin B levels were measured on days 3–5 of the menstrual cycle, and antral follicle count was performed. Additionally, levels of TSH, free T4, antibodies to TPO and TG, and prolactin were assessed.

**Results:** Patients with hypothyroidism showed a statistically significant decrease in AMH and inhibin B levels compared with the control group ( $p < 0.05$ ). TSH levels were higher in the presence of autoimmune thyroiditis. In patients who became pregnant, inhibin B levels were statistically significantly higher ( $p < 0.001$ ). Conclusion. Hypothyroidism is associated with a decrease in ovarian reserve. Inhibin B is an informative prognostic marker of the effectiveness of IVF programs. The likelihood of pregnancy in patients with hypothyroidism is associated with higher levels of AMH and inhibin B.

**Keywords:** *hypothyroidism, autoimmune thyroiditis, infertility, ovarian reserve, AMH, inhibin B, TSH, IVF*

## Introduction

In modern reproductive medicine, significant attention is paid to assessing the impact of thyroid function on the effectiveness of assisted reproductive technologies. In clinical practice, when analyzing the outcomes of ART programs, indicators such as estradiol levels on the day of ovulation induction, the number of oocytes retrieved, their degree of maturity and fertilization potential, as well as implantation rates, clinical pregnancy rates, and live birth rates are considered. Nevertheless, research findings in this area remain inconsistent and do not allow for definitive conclusions (Unuane D., Velkeniers B., Bravenboer B., Drakopoulos P., Tournaye H., Parra J., De Brucker M., 2017).

Ovarian reserve plays a special role in predicting treatment efficacy. Anti-Müllerian hormone and inhibin B are most commonly used to assess it; however, their relationship with thyroid function has not been sufficiently studied. At the same time, there is evidence suggesting a possible role for thyroid hormones in regulating the processes that determine the quantitative and qualitative characteristics of oocytes, which in turn determines the subsequent outcomes of ART (Practice Committee of the American Society for Reproductive M., 2015).

Studies at the molecular and cellular levels have shown that thyroid hormone receptors are widely expressed in structures of the female reproductive system, including granulosa cells and oocytes. This indicates the direct involvement of thyroid hormones in the regulation of folliculogenesis. It has been established that changes in their concentration, even within the reference range, can influence the action of follicle-stimulating hormone, enhancing follicle growth and reducing the severity of apoptosis. In contrast, elevated levels of thyroid hormones may have an adverse effect, reducing aromatase activity in granulosa cells and disrupting the development of preantral follicles (Birjandi B., Ramezani Tehrani F., Amouzegar A., Tohidi M., Bidhendi Yarandi R., Azizi F., 2021; Korevaar T. I.M., Mínguez-Alarcón L., Messerlian C., de Poortere R. A., Williams P. L., Broeren M. A., Hauser R., Souter I. C., 2018).

The regulatory influence of thyroid hormones is also mediated through the

hypothalamic-pituitary-ovarian axis. They participate in the control of ovulation, ensure optimal conditions for implantation, and influence the early stages of embryonic development. Thyroid imbalance may be accompanied by a decrease in ovarian reserve, deterioration in oocyte quality, and a reduced likelihood of pregnancy (Korevaar T. I.M., Mínguez-Alarcón L., Messerlian C., de Poortere R. A., Williams P. L., Broeren M. A., Hauser R., Souter I. C., 2018; Weghofer, A., Barad, D.H., Darmon, S. *et al.*, 2016).

Clinical data indicate that subclinical hypothyroidism and autoimmune thyroid diseases are more commonly detected in women with infertility than in the general population. This confirms the significance of thyroid dysfunction as one of the factors affecting reproductive potential.

Thus, endocrine disorders associated with thyroid pathology play a significant role among the causes of female infertility and require further study from the perspectives of both clinical practice and the fundamental mechanisms regulating reproductive function.

However, the effect of hypothyroidism on markers of ovarian reserve (anti-Müllerian hormone (AMH) and inhibin B) has not been sufficiently studied.

The aim of the study was to investigate the effect of thyroid hormones on ovarian reserve and pregnancy outcomes in women with endocrine infertility due to hypothyroidism undergoing IVF treatment.

## Materials and Methods

The study included women with endocrine infertility due to hypothyroidism who were undergoing treatment using in vitro fertilization (IVF) programs. The patients were divided into two groups based on the presence of autoimmune thyroiditis. Group 1 consisted of women with autoimmune hypothyroidism (n=39), and Group 2 consisted of women with hypothyroidism without autoimmune thyroiditis (n=49). Within each group, subgroups were identified: those who achieved pregnancy (IVF+) and those who did not achieve pregnancy (IVF-). The control group consisted of women of reproductive age with tubal-peritoneal infertility without thyroid dysfunction (n=20).

Anti-Müllerian hormone (AMH), FSH concentration, and serum inhibin B levels

were measured on days 3–5 of the unstimulated menstrual cycle. The antral follicle count (AFC) was defined as the sum of antral follicles in both ovaries, measured by transvaginal ultrasound in the early follicular phase. Women with multifollicular ovaries, with an AFC >20 on either the left or right side, or with ovaries that were difficult to visualize, were excluded from the analysis.

To assess thyroid status, the patients' levels of thyroid-stimulating hormone (TSH), free T4, free T3, anti-TPO antibodies, and anti-thyroglobulin antibodies were measured. Statistical analysis of the results was performed using Student's t-test; differences were considered statistically significant at  $p < 0.05$ .

### Results

The age range of the patients was 20 to 45 years. The mean age of the patients was  $33.1 \pm 3.2$  years. Body mass index (BMI) was within the normal range at  $23.5 \pm 2.6$ . Analysis of the infertility pattern showed that primary infertility predominated in both groups of patients with hypothyroidism: 65.9% in Group 1 and 61.8% in Group 2. In the control

group, by contrast, all patients had secondary infertility, which was due to the specific characteristics of the control sample.

The average duration of infertility in patients with hypothyroidism was virtually the same in both groups and amounted to  $5.9 \pm 0.9$  and  $5.9 \pm 0.7$  years, respectively, whereas in the control group it was lower— $4.03 \pm 1.2$  years.

Analysis of clinical symptoms showed (Table 1) that in patients with hypothyroidism, regardless of the presence of an autoimmune component, complaints characteristic of thyroid insufficiency predominated. The most frequently reported symptoms were general weakness, dry skin, hair loss, and psychoemotional lability, reflecting the systemic effects of thyroid hormone deficiency.

An analysis of clinical symptoms revealed that, regardless of the presence of an autoimmune component, patients with hypothyroidism predominantly reported complaints characteristic of thyroid insufficiency: general weakness (73.9% and 83.8% in groups 1 and 2, respectively), dry skin and hair loss (69.6% and 75.7%, respectively), and psychoemotional lability (86.9% and 93.2%).

**Table 1.** Clinical symptoms in women with endocrine infertility due to hypothyroidism

	Group 1 (AIT+), n=39		Group 2 (AIT-), n=49	
	Abs	%	Abs	%
General weakness	28	71,8 ± 6,5	41	83,7 ± 4,2
Dry skin	27	69,2 ± 6,8	37	75,5 ± 5,0
Brittle nails	10	25,6 ± 6,5	11	22,9 ± 4,9
Hair loss	27	69,2 ± 6,8	37	75,5 ± 5,0
Drowsiness	7	17,9 ± 5,8	7	14,3 ± 4,1
Cold intolerance	10	25,6 ± 6,5	14	28,6 ± 5,2
Headache	12	30,7 ± 6,9	21	42,8 ± 5,7*
Psychoemotional lability	34	87,1 ± 5,0	46	93,8 ± 2,9*
Memory impairment	6	15,4 ± 5,3	8	16,3 ± 4,3
Constipation	9	23,1 ± 6,3	15	30,6 ± 5,3
Increased blood pressure	4	10,2 ± 4,6	5	10,2 ± 3,6
Edema	2	5,1 ± 3,6	4	8,1 ± 3,2
Weight gain	3	7,7 ± 4,2	4	8,1 ± 3,4

Note: \* – differences are statistically significant between the groups ( $p < 0.05$ )

A comparative analysis revealed that headaches were significantly more common in the group without AIT (43.2% vs. 32.6%;  $p < 0.05$ ) and psychoemotional lability (93.2%

vs. 86.9%;  $p < 0.05$ ) were significantly more common in the group without AIT, which may reflect a more pronounced influence of vascular and metabolic factors in this form of

hypothyroidism. No intergroup differences were found for the remaining symptoms – chilliness, constipation, sleep disturbances, and edema ( $p > 0.05$ ) – which indicates the similarity of the clinical picture of hypothyroidism regardless of its etiology and underscores the necessity of mandatory laboratory confirmation of the diagnosis.

It is known that in women, thyroid dysfunction is often associated with menstrual cycle disorders. Hypothyroidism affects the regulation of the hypothalamic-pituitary-ovarian axis and can lead to changes in the duration and nature of the menstrual cycle (MC).

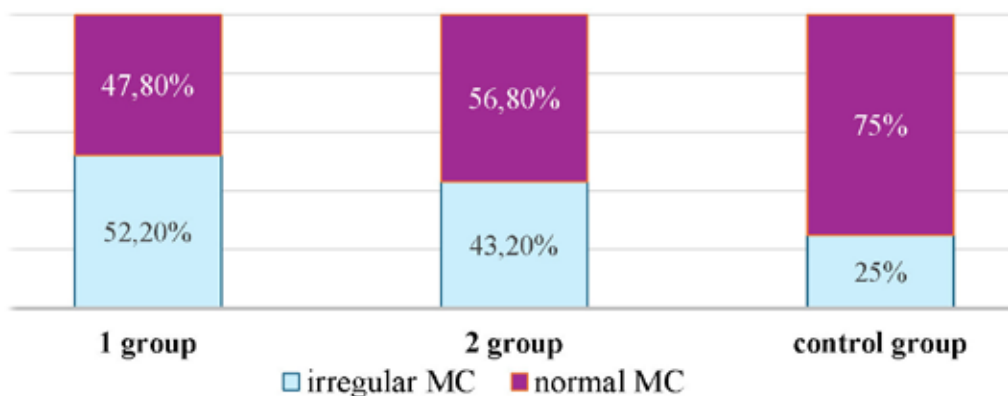
Analysis of menstrual function revealed statistically significant differences between groups. A normal menstrual cycle was observed significantly less frequently in patients in Group 1 (34.8%) compared with Group 2 (59.5%) and the control group (60%;  $p < 0.05$ ). The most pronounced differences were found regarding opsomenorrhea: in the AIT group, it was recorded in 47.8% of patients, which was 2.5 times higher than the rates in the second and control groups (18.9% and 15.0%, respectively;  $p < 0.01$ ). Amenorrhea was observed exclusively in patients in the first group (8.7%), indicating the most

pronounced disruption of hypothalamic-pituitary-ovarian regulation when hypothyroidism is combined with AIT. The overall frequency of menstrual cycle disorders was 52.0% in the first group, 43.0% in the second, and 25.0% in the control group, which confirms the pathogenetic role of thyroid dysfunction in menstrual cycle regulation and is consistent with the data of G. Krassas et al.

The identified changes suggest that thyroid dysfunction may have a significant impact on menstrual cycle regulation. In the group of patients with hypothyroidism against a background of AIT, a more pronounced tendency toward a prolonged menstrual cycle was observed, which may reflect disturbances in the hormonal regulation of the reproductive system compared to the group of women with hypothyroidism without AIT. Consequently, the presence of AIT in women with hypothyroidism can be considered an additional factor contributing to the development of anovulatory cycles and a decrease in reproductive potential.

To provide a clearer picture of the prevalence of menstrual cycle disorders among the patients surveyed, the overall incidence of menstrual dysfunction was analyzed (Fig. 1).

**Figure 1.** Prevalence of menstrual cycle disorders in women with endocrine infertility and hypothyroidism



It was found that menstrual cycle disorders were most frequently detected in patients in Group 1, in 52% of cases. In Group 2, this figure was 43.0%, and in the control group, 25.0%. The results indicate a higher frequency of menstrual dysfunction in women with hypothyroidism, especially in the presence of an autoimmune component.

Analysis of thyroid status showed that TSH levels in patients with hypothyroidism were significantly higher than those in the control group. Thus, in Group 1, the TSH level was  $6.1 \pm 0.8$  mIU/mL, which was nearly 2.5 times higher than in the control group ( $2.4 \pm 1.5$  mIU/mL;  $p < 0.01$ ). In Group 2, TSH levels also remained elevated ( $5.5 \pm 0.6$  mIU/mL), but were significantly

lower than in Group 1 ( $p < 0.05$ ), which may indicate more pronounced thyroid dysfunction in the presence of an autoimmune component (Table 2).

There was no statistically significant difference in free T4 levels between the study groups ( $p > 0.05$ ), indicating a predominance of subclinical hypothyroidism among the pa-

tients examined. The most pronounced differences were observed in the analysis of antithyroid antibodies. In Group 1, the level of anti-TPO antibodies was  $181.8 \pm 26.8$  IU/mL, which was more than 19 times higher than the values in Group 2 ( $9.5 \pm 1.1$  IU/mL) and the control group ( $10.7 \pm 3.7$  IU/mL) ( $p < 0.01$ ).

**Table 2.** Hormonal status indicators of women with endocrine infertility and hypothyroidism

Parameter	Group 1 (n=39)	Group 1 (n=39)	Group 1 (n=39)
TSH, mIU/mL	6.1±0.8	5.5±0.8	2.4±1.5*
T4, pmol/L	12.1±1.9	11.4±2.7	12.2±2.8
Anti-TPO, IU/mL	181.8±26.8	9.5±1.1**	10.7±3.7**
Anti-TG, IU/mL	186.2±27.5	27.2±3.2**	28.6±7.5**
Prolactin (PRL), ng/mL	59.9±8.8	51.5±6.0*	7.4±1.1**
AMH, ng/mL	1.9±0.3	1.8±0.2	3.3±1.6*

Note: \* – statistically significant differences between Group 1 and Group 2 ( $p < 0.05$ ); \*\* – statistically significant differences between Group 1 and the control group ( $p < 0.01$ )

A similar trend was observed for anti-thyroglobulin antibodies: in Group 1, their level reached  $186.2 \pm 27.5$  IU/mL, which was 6–7 times higher than in Groups 2 and the control group ( $p < 0.01$ ). The obtained data confirm the pronounced autoimmune nature of thyroid gland involvement in patients of the first group.

Analysis of prolactin levels showed that patients with hypothyroidism exhibited marked hyperprolactinemia. In Group 1, the prolactin level was  $59.9 \pm 8.8$  ng/mL, and in Group 2, it was  $51.5 \pm 6.0$  ng/mL, which was 7–8 times higher compared to the control group ( $7.4 \pm 1.1$  ng/mL;  $p < 0.01$ ). At the same time, prolactin levels were significantly higher in group 1 than in group 2 ( $p < 0.05$ ), which may reflect more pronounced neuroendocrine disturbances in the combination of hypothyroidism with autoimmune thyroiditis.

A comparison of ultrasound and laboratory data revealed certain patterns. Specifically, patients with pronounced structural changes in the thyroid gland (diffuse and nodular goiter) were more likely to have elevated levels of TSH and antithyroid antibodies, particularly in the group with autoimmune thyroiditis.

The correlation analysis revealed statistically significant associations between thyroid ultrasound characteristics and indicators of

thyroid status. It was found that thyroid volume positively correlated with TSH levels ( $r=0.3$ ;  $p < 0.05$ ), reflecting a tendency for the gland to enlarge as thyroid insufficiency progresses. However, this association was weak, indicating that the development of structural changes in the thyroid gland is multifactorial.

More pronounced correlations were found between thyroid volume and levels of antibodies to thyroid peroxidase ( $r = 0.4$ ;  $p < 0.01$ ), as well as antibodies to thyroglobulin ( $r = 0.35$ ;  $p < 0.05$ ), indicating a significant influence of autoimmune processes on the structural remodeling of thyroid tissue.

Analysis of individual ultrasound findings revealed that the presence of diffuse thyroid changes was significantly associated with elevated AT-TPO levels ( $r=0.45$ ;  $p < 0.01$ ), whereas nodular formations were more frequently detected with elevated AT-TG levels ( $r=0.32$ ;  $p < 0.05$ ). Heterogeneity of the thyroid gland structure also demonstrated a weak positive correlation with TSH levels ( $r=0.28$ ;  $p < 0.05$ ).

Thus, in patients with hypothyroidism, especially in the presence of autoimmune thyroiditis, more pronounced thyroid status abnormalities are detected, characterized by elevated TSH levels, a significant increase in antithyroid antibodies, and hyperprolac-

tinemia. The data obtained confirm that the autoimmune component is a key factor that exacerbates endocrine disorders and potentially affects reproductive function.

When assessing ovarian reserve based on anti-Müllerian hormone levels, a decrease was observed in women with hypothyroidism in both groups compared to the control group, indicating a reduction in ovarian reserve in this condition. At the same time, AMH levels were higher in patients who be-

came pregnant than in those who did not, although statistically significant differences were found only in the second group of patients ( $p < 0.05$ ).

Analysis of inhibin B levels on days 3–5 of the menstrual cycle revealed statistically significant differences between the IVF+ and IVF– subgroups. In patients who became pregnant, inhibin B levels were significantly higher compared to those who did not become pregnant ( $p < 0.001$ ).

**Table 3.** Ovarian reserve indicators and IVF outcomes depending on autoimmune status

	Group 1 (AIT+), n=39		Group 2 (AIT–), n=49		Control, n=20	P
	IVF+ (n=24)	IVF– (n=15)	IVF+ (n=38)	IVF– (n=11)		
TSH before treatment	7,5±1,6	7,3±2	4,9±0,8	4,8±1,3	2,1±0,6	>0,05
AMH	1,4±0,3	1,4±0,4	1,7±0,3	1,4±0,4	3,2±0,3	<0,05
Inhibin B	78±16,6	44,4±12,3	95,8±15,1	43,9±12,2	84,3±5,4	<0,001

Note: Data are presented as mean ± standard error of the mean (M±m). Abbreviations: AIT – autoimmune thyroiditis; IVF – in vitro fertilization; TSH – thyroid-stimulating hormone; AMH – anti-Müllerian hormone

Analysis of ovarian reserve indicators showed that baseline TSH levels did not have a statistically significant effect on pregnancy outcomes in IVF programs ( $p > 0.05$ ), despite higher values in patients with hypothyroidism compared to the control group (Table 2). This indicates that after correction of thyroid status, this parameter loses its independent prognostic value.

At the same time, ovarian reserve indicators demonstrated a significant association with IVF outcomes. The AMH level was significantly lower in patients with hypothyroidism compared to the control group ( $p < 0.05$ ), indicating a decrease in ovarian reserve in the presence of endocrine pathology.

Furthermore, it was found that in patients with autoimmune thyroiditis, inhibin B levels were statistically lower compared to patients without an autoimmune component, which may reflect a more pronounced suppression of ovarian reserve in autoimmune pathology.

Correlation analysis revealed a negative correlation between TSH levels and markers of ovarian reserve – inhibin B and AMH ( $r < 0$ ,  $p < 0.05$ )—suggesting a possible influence of thyroid dysfunction on reduced functional activity.

### Discussion

According to the study by Pirgon O. (Pirgon, O., Sivrice, C., Demirtas, H., & Dundar, B., 2016) and colleagues, patients with autoimmune thyroiditis (AIT) are at risk for developing premature ovarian failure, suggesting a possible association between autoimmune processes and impaired ovarian function.

The results of a large retrospective study by Polyzos N. P. (Nikolaos P. Polyzos, Evangelos Sakkas, Alberto Vaiarelli, Kris Poppe, Michel Camus, Herman Tournaye, 2015) and colleagues (“Thyroid autoimmunity, hypothyroidism and ovarian reserve: a cross-sectional study of 5,000 women based on age-specific AMH values”) demonstrated that in patients with a genetic predisposition to diminished ovarian reserve, the prevalence of hypothyroidism is higher compared to women with idiopathic diminished ovarian reserve, regardless of thyroid status. These data suggest the presence of common pathogenic mechanisms underlying both thyroid and ovarian dysfunction.

At the same time, studies by Saglam F. et al. and Ayesha et al. (Saglam, F., Onal, E. D., Ersoy, R., Koca, C., Ergin, M., Erel, O., & Cakir, B., 2015; Ayesha, Jha V., 2016) have

shown that diminished ovarian reserve is more strongly associated with hypothyroidism of autoimmune etiology, underscoring the significance of immune mechanisms in the pathogenesis of reproductive disorders.

According to Weghofer A. and colleagues (Weghofer, A., Barad, D.H., Darmon, S. *et al.*, 2016), the key role in the decline of ovarian reserve is played not so much by the autoimmune process itself as by the increase in thyroid-stimulating hormone (TSH) levels. In this regard, normalization of thyroid status, particularly achieving target TSH levels, may contribute to improved ovarian reserve parameters.

The results obtained in this study are consistent with the literature data and confirm the influence of thyroid hormones on ovarian function. Hypothyroidism is associated with a decrease in ovarian reserve markers, as evidenced by reduced levels of anti-Müllerian hormone (AMH) and inhibin B. These changes may be due to impaired folliculogenesis, reduced ovarian sensitivity to gonadotropic stimulation, and dysregulation of follicular growth and maturation mechanisms.

Inhibin B is of particular importance in assessing ovarian reserve, as it reflects the

functional activity of granulosa cells and the number of antral follicles. A decrease in its level may indicate early abnormalities in the ovarian follicular apparatus and serve as an additional marker of declining reproductive potential.

### Conclusions

Women with hypothyroidism exhibit a reduced ovarian reserve, as indicated by AMH and inhibin B levels, compared to healthy women. In women with AIT, pre-treatment TSH levels were statistically higher than in women with hypothyroidism of other etiologies. AMH levels were higher in patients who became pregnant; however, statistically significant differences were not observed in all groups. Inhibin B levels on days 3–5 of the menstrual cycle and after superovulation stimulation were significantly higher in patients who became pregnant ( $p < 0.001$ ). In patients with AIT, the pregnancy rate was significantly lower than in women without AIT. This may be related to the duration of hypothyroidism. This article did not account for the duration of hypothyroidism, which affects treatment efficacy and pregnancy rates.

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