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Original Article

Ovarian Reserve and Anti-Thyroid Peroxidase Antibodies in Euthyroid Infertile Women: A Cross-Sectional Study

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Abstract

Objective: This study investigated the association between Anti-TPO levels and ovarian reserve markers, including anti-Müllerian hormone (AMH) and antral follicle count (AFC), in euthyroid infertile women.

Materials and methods: In this cross-sectional analytical study, 231 euthyroid infertile women were enrolled and classified as Anti-TPO positive (n = 92) or Anti-TPO negative (n = 139). Serum levels of AMH, FSH, LH, TSH, prolactin, and Anti-TPO were measured, and AFC was assessed by transvaginal ultrasonography. Because hormonal variables were not normally distributed, logarithmic transformation was applied prior to parametric analyses. Data were analyzed using independent t-tests, Pearson correlation, and multivariate regression ($\alpha = 0.05$).

Results: No significant differences were observed between groups in log-transformed AMH, AFC, FSH, or LH levels ($p > 0.05$). Age was negatively associated with log-AMH. Subgroup analyses demonstrated that in Anti-TPO–positive women, higher Anti-TPO levels were associated with lower log-AMH and higher log-FSH. Multivariate regression identified age as the only independent predictor of log-AMH levels ($\beta = -0.337$, $p < 0.001$).

Conclusion: Ovarian reserve decline appears primarily age-related; however, thyroid autoimmunity may have a potential modulatory role in ovarian function. Consideration of thyroid autoantibody status may be clinically relevant in infertility evaluation.

Keywords: Infertility; Thyroid Disorders; Anti-Thyroid Antibodies; Ovarian Reserve; Anti-Müllerian Hormone

Introduction

Anti-Müllerian hormone (AMH) secreted by granulosa cells of growing pre-antral and early antral follicles plays a pivotal role in regulating follicular development (1). The AMH is recognized as a reliable quantitative marker for assessing ovarian reserve (2), and its serum concentration after ovarian stimulation is significantly correlated with the number of retrieved oocytes (3).

Ovarian reserve, defined as the functional potential of the ovary, reflects both the quantity and quality of the remaining oocytes. Diminished ovarian reserve is one of the major contributing factors to

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female infertility (4). Female infertility is a critical issue in reproductive health, exerting a profound impact on global fertility rates (5). In the United States, it is estimated that 7% to 15.5% of women of reproductive age are affected by infertility (6).

Globally, the burden of female infertility has intensified between 1990 and 2021, with an 84.44% increase in prevalence and an 84.43% rise in disability-adjusted life years (DALYs). Considerable variations have also been reported across different regions and countries (5). In Iran, the overall prevalence of infertility has been reported to be 13.2% (7).

Several factors have been identified as contributors to diminished ovarian reserve, including pelvic radiotherapy, prior chemotherapy, smoking, genetic causes, and aneuploidy. However, the underlying cause remains unknown in 70-90% of cases (4). Thyroid hormones regulate metabolism in virtually all tissues of the human body, and adequate thyroid hormone availability is crucial for normal female reproduction (8). Thyroid dysfunction can lead to menstrual irregularities and infertility (9). Thyroid peroxidase (TPO) is a membrane-bound enzyme with low glycosylation that plays a key role in thyroid hormone synthesis, and anti-TPO antibodies (TPOAb) can inhibit its enzymatic activity (10, 11). TPOAb positivity is among the most common biomarkers for autoimmune thyroid diseases (AITD) (12). The global prevalence of thyroid autoantibody positivity has been reported to be approximately 20% (13).

Although previous studies have investigated the prevalence of thyroid autoimmunity in infertile women, substantial differences in study designs and participant characteristics have led to inconsistent findings (13). Several studies have reported conflicting results regarding the association between anti-TPO antibodies and ovarian reserve. A meta-analysis published in 2021 indicated that thyroid autoimmunity (TAI) and hypothyroidism may potentially affect ovarian reserve (14). Chen et al. reported a higher prevalence of TPOAb positivity among infertile Chinese women with low AMH levels (4). However, two recent studies found no significant association between ovarian reserve, thyroid hormones, and thyroid autoimmunity (15, 16). Moreover, a recent meta-analysis indicated that AMH levels were not significantly correlated with TgAb levels, although the authors emphasized the need for further studies to clarify these associations across different reproductive stages and metabolic profiles (17).

Given these conflicting results, it remains unclear whether thyroid function, thyroid autoantibodies, or both impact ovarian reserve. Therefore, the present study was designed to investigate the association between ovarian reserve (assessed by AMH and AFC) and levels of Anti-TPO antibodies in euthyroid infertile women.

Materials and methods

Study Design and Population: This cross-sectional analytical study was conducted on euthyroid infertile women who attended the infertility clinic of Ardabil University of Medical Sciences from May 2023 to May 2024. The study aimed to investigate the association between thyroid autoimmunity, as indicated by anti-thyroid peroxidase antibody (Anti-TPO Ab) status, and ovarian reserve parameters in infertile women with normal thyroid function. Inclusion criteria were: age between 18 and 40 years, documented infertility within the previous year, normal serum thyroid-stimulating hormone (TSH) levels, and normal prolactin concentrations.

Exclusion criteria included a history of thyroidectomy, radioactive iodine therapy, or use of thyroid-related medications (levothyroxine, methimazole, or propylthiouracil); previous cervical or ovarian surgery; history of radiotherapy, chemotherapy, or malignancy; presence of autoimmune disorders; chronic liver or kidney disease; and diagnosis of polycystic ovary syndrome (PCOS).

Sample Size and Sampling Method: All eligible euthyroid infertile women who had undergone Anti-TPO Ab testing during the study period were consecutively recruited. A total of 231 participants were enrolled, including 92 women with positive Anti-TPO Ab and 139 women with negative Anti-TPO Ab results.

Data Collection and Laboratory Assessment: Venous blood samples were obtained to measure serum levels of Anti-TPO antibodies, AMH (ng/mL), follicle-stimulating hormone (FSH, IU/L), luteinizing hormone (LH, IU/L), TSH (mIU/L), and prolactin using standardized laboratory methods according to the manufacturer's instructions.

Transvaginal ultrasonography was performed on days 2–3 of the menstrual cycle to assess antral follicle count (AFC). Follicles measuring 2–10 mm were counted in both ovaries, and the total AFC was

calculated as the sum of follicles from both ovaries.

Based on Anti-TPO Ab status, participants were categorized into Anti-TPO–positive and Anti-TPO-negative groups, and demographic, hormonal, and ovarian reserve parameters were compared between the two groups.

Statistical Analysis: Statistical analyses were performed using SPSS version 26. The normality of continuous variables was assessed using the Kolmogorov–Smirnov test and visual inspection of histograms. Due to skewed distribution, AMH, basal FSH, basal LH, and AFC were log10-transformed prior to inferential statistical analyses to approximate normality. Group comparisons were conducted using independent sample t-tests based on log-transformed values where appropriate. Pearson correlation coefficients were calculated using log-transformed hormonal variables. Multiple linear regression analysis was performed to identify independent predictors of log-transformed AMH levels. Standardized beta coefficients (β) were reported. A p-value < 0.05 was considered statistically significant. For clarity, descriptive statistics of AMH are presented in their original clinical units (ng/mL), while inferential analyses were performed on log-transformed data.

Ethical Considerations: The study was approved by the Ethics Committee of Ardabil University of Medical Sciences (IR.ARUMS.MEDICINE.1402.017). Informed consent was obtained from all participants prior to inclusion in the study. All patient information was de-identified to ensure confidentiality. All procedures were conducted in accordance with the ethical standards of the committee and in full compliance with the Declaration of Helsinki.

Results

The mean age in the Anti-TPO positive and negative groups was 32.46 ± 6.20 and 32.23 ± 5.83 years, respectively. The mean of BMI was 27.26 ± 5.34 kg/m² in the positive group and 28.98 ± 23.62 kg/m² in the negative group. No statistically significant differences were observed between the two groups in terms of age, BMI, number of pregnancies, number of deliveries, or number of miscarriages (Table 1).

Comparison of fertility-related indices between Anti-TPO positive and negative groups revealed no statistically significant differences (p > 0.05).

Mean AMH levels (reported in original units) were slightly higher in the Anti-TPO positive group compared to the negative group (3.41 ± 3.36 vs. 2.94 ± 3.07 ng/mL); however, this difference was not statistically significant (p = 0.284).

Because AMH, basal FSH, basal LH, and AFC demonstrated non-normal distribution, statistical comparisons were performed using their log10-transformed values. No significant differences were observed between groups in log-transformed AMH, FSH, LH, or AFC levels. The results show that only TSH levels differ significantly between the groups (P=0.013), with higher TSH levels observed in the TPO-positive group (2.30 vs. 1.98).

These findings indicate that Anti-TPO positivity was not independently associated with significant alterations in ovarian reserve markers (Table 2).

Correlation matrix analysis demonstrated that Anti-TPO levels showed a weak positive correlation with age (r = 0.151, p < 0.05). No significant correlations were observed between Anti-TPO and log-transformed AMH or AFC in the overall sample. In the Anti-TPO negative group, Anti-TPO levels were positively correlated with log-transformed AMH (r = 0.303, p < 0.001) and log-transformed AFC (r = 0.202, p = 0.017).

In the Anti-TPO positive group, Anti-TPO levels were negatively correlated with log-transformed AMH (r = -0.239, p = 0.022) and positively correlated with log-transformed FSH (r = 0.297, p = 0.004). These correlations were observed within the log-transformed scale and should be interpreted accordingly (Table 3).

In multivariate linear regression analysis with log-transformed AMH as the dependent variable, age was identified as the only significant independent predictor (β = -0.337, p < 0.001).

Table 1: Demographic characteristics of participants in the study groups

Parameter	Negative TPO Group (n=139)	Positive TPO Group (n=92)	P-value
Age (years)	32.23±5.83	32.46±6.21	0.506
Body mass index (kg/m²)	27.56±6.03	27.54±4.48	0.911
Number of Living Children	0.19±0.41	0.26±0.55	0.017
Gravidity (Number of Pregnancies)	0.64±1.13	0.80±1.30	0.113
Parity (Number of Deliveries)	0.20±0.45	0.25±0.55	0.144
Number of Miscarriages	0.42±0.96	0.51±1.05	0.230

TPO: Thyroid peroxidase

Table 2: Comparison of laboratory parameters in the study groups

Parameter	Negative TPO Group (n=139)	Positive TPO Group (n=92)	P-value
AMH (ng/mL)	2.94±3.07	3.41±3.36	0.284
TSH	1.98±0.93	2.30±0.97	0.013
log10 FSH (IU/L)	1.29±6.83	0.87±2.46	0.569
log10 LH (IU/L)	0.87±3.22	0.94±4.25	0.883
log10 AFC	0.84±0.83	0.86±0.86	0.807

AMH: Anti-Müllerian Hormone; AFC: Antral Follicle Count

Table 3: Correlation of Anti-TPO with ovarian reserve markers, age, and BMI stratified by study groups

	Total partcipants	TPO Negative group	TPO Positive group
AMH	-0.109	0.303**	-0.239*
FSH	0.058	-0.034	0.297**
LH	0.110	0.017	0.161
AFC	-0.029	0.202*	-0.065
BMI	-0.036	-0.031	-0.096
Age	0.151*	-0.041	0.242*

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

BMI and Anti-TPO levels were not significantly associated with log-transformed AMH ($p > 0.05$). Thus, increasing age was independently associated with lower log-transformed AMH levels, whereas Anti-TPO status did not independently predict ovarian reserve after adjustment (Table 4).

Table 4: Multivariate regression analysis of AMH levels with age, BMI, and Anti-TPO

Model		Standardized Beta	t	p-value
1	Age	-0.337	-5.439	0.001
	BMI	0.094	1.519	0.130
2	Age	-0.329	-5.240	0.001
	BMI	0.092	1.486	0.139
	Anti_TPO	-0.056	-0.894	0.372

Discussion

Thyroid dysfunction is a key factor affecting female infertility, exerting direct effects on oocytes and indirect effects through elevated prolactin secretion and disruption of GnRH function, thereby impacting the reproductive system (18). Another proposed mechanism involves the potential role of Anti-TPO antibodies in ovarian dysfunction. Given the importance of elucidating the mechanisms through which thyroid disorders influence infertility, the present study was designed to investigate the association between ovarian reserve and Anti-TPO levels in euthyroid infertile women.

Our findings demonstrated that although AMH levels and AFC were slightly higher in the Anti-TPO positive group compared to the negative group, these differences were not statistically significant. The study identified age as the strongest predictor of declining AMH, while BMI and Anti-TPO levels were not significant predictors in the regression model. However, subgroup correlation analyses revealed distinct patterns: in the TPO-negative group, Anti-TPO showed a weak positive correlation with AMH and AFC, whereas in the TPO-positive group, Anti-TPO was associated with decreased AMH and increased FSH, highlighting that serologic status may critically influence the impact of thyroid autoantibodies on ovarian reserve.

Overall, these results emphasize the central role of age in ovarian reserve decline while also underscoring the potential influence of thyroid autoimmunity, particularly in Anti-TPO positive women, on diminished ovarian function and hypothalamic-pituitary-ovarian axis dysregulation.

Consistent with our findings, Notaro et al. reported no significant differences in AMH levels and AFC between Anti-TPO positive and negative groups (19). Similarly, Silva et al. observed comparable results (20). Polyzos et al. also found no significant differences in Anti-TPO levels among groups stratified by low, medium, or high ovarian reserve (21). In the study by Koevaar et al., no significant association was observed between AFC and Anti-TPO antibodies; however, they reported a significant correlation between AFC and Anti-Tg positivity (8). Notaro et al. also noted significant differences in AFC when comparing Anti-Tg positive and negative individuals (19). In contrast, Safarian et al. reported that both AMH and AFC were significantly higher in the Anti-TPO negative group compared to the positive group, which differs from the findings of the present study (22).

A review of previous studies reveals substantial heterogeneity regarding the diagnostic criteria for thyroid autoimmunity (positivity for one or both antibodies) and the markers used to assess ovarian

reserve. This variability likely contributes to the inconsistent findings across studies. Another factor that may explain discrepancies between our results and some previous studies, including Safarian et al. (22), is the age of the participants.

In the present study, AMH levels showed a negative correlation with Anti-TPO in the antibody-positive group, whereas a positive correlation was observed in the Anti-TPO negative group. Consistent with our findings, similar studies demonstrated that AMH levels are reduced in women with thyroid autoantibody positivity (14, 22, 23). Similarly, a study in China reported that higher Anti-TPO levels were associated with lower AMH and antral follicle count (AFC) (24). Furthermore, Qin et al. identified TPOAb concentration as a significant factor influencing adverse reproductive outcomes (25). Also, a significant correlation was seen between serum TSH and the number of oocytes retrieved (26).

AMH exerts a physiological inhibitory role in the initiation of primordial follicle growth, thereby preventing premature depletion of the ovarian reserve. Moreover, by reducing follicular sensitivity to FSH, AMH suppresses FSH-dependent follicular development, as evidenced by the reduced preantral follicle growth in the presence of AMH (27, 28).

In our cohort, AMH levels were negatively correlated with age in both Anti-TPO positive and negative groups, indicating a significant decline in AMH with increasing age. Notably, the correlation coefficient was higher in the Anti-TPO positive group. Age-related decline in ovarian reserve is well-established; Almong et al. reported an inverse linear association between increasing age and declining AMH among infertile women aged 30-50 years (29). In a study by Silva et al., multivariate analysis showed that age significantly predicted AMH and AFC inversely, whereas BMI and thyroid autoantibody positivity did not predict ovarian reserve, aligning with our current findings (20).

In the present study, TSH levels were significantly higher in the Anti-TPO positive group compared to the negative group. FSH is a key parameter used alongside AMH and AFC to estimate ovarian reserve, and previous studies have shown that FSH levels are negatively correlated with the number of oocytes retrieved, fertilized oocytes, and developing follicles (30). Women with elevated baseline FSH respond well to ovarian stimulation, producing a sufficient number of oocytes, thus maintaining comparable chances of conception to age-matched controls (31).

This study was conducted as a cross-sectional analytical investigation at a single infertility center in Ardabil, which inherently limits the generalizability of the findings. Additionally, due to the sample size constraints, matching between the two groups regarding age, reproductive history, assisted reproductive interventions, and underlying comorbidities was not performed, which may have introduced potential bias.

Conclusion

The findings of this study indicate that Anti-TPO positivity alone was not associated with significant changes in reproductive parameters including AMH, AFC, FSH, or LH. However, correlation patterns differed substantially between the positive and negative groups. In Anti-TPO positive women, this autoantibody was associated with decreased AMH and increased FSH, suggesting a potential role of thyroid autoimmunity in reducing ovarian reserve and disrupting the hypothalamic-pituitary-ovarian axis. Conversely, in the Anti-TPO negative group, weak but positive correlations between Anti-TPO and AMH and AFC were observed. Furthermore, multivariate regression analysis identified age as the sole independent and significant predictor of AMH, whereas BMI and Anti-TPO were not significant in the final model. Overall, these results underscore the primary role of advancing age in ovarian reserve decline, while highlighting the potential modulatory effect of Anti-TPO status on ovarian function in women with thyroid autoimmunity.

Conflict of Interests

Authors declare no conflict of interests.

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