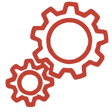
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EMPIRICAL EVALUATION OF THYROID FUNCTION AND OVARIAN RESERVE USING ONE-WAY ANOVA: A CLINICAL ENDOCRINOLOGY PERSPECTIVE

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Abstract

This study empirically investigated the relationship between thyroid function and ovarian reserve in women of reproductive age, incorporating key hormonal markers and inflammatory indicators. A one-way Analysis of Variance (ANOVA) was conducted to compare three distinct groups: the control group (practically healthy women, n=20), women with decreased ovarian reserve (DOR) and hypothyroidism (n=40), and women with DOR and euthyroidism (n=40). Data on Thyroid-Stimulating Hormone (TSH), Anti-Müllerian Hormone (AMH), Follicle-Stimulating Hormone (FSH), C-reactive protein (CRP), Complement C3, and Ceruloplasmin were analyzed. Significant differences were observed across groups for TSH ($F(2,97)=54.245$, $p<.001$), AMH ($F(2,97)=323.365$, $p<.001$), CRP ($F(2,97)=101.497$, $p<.001$), Complement C3 ($F(2,97)=34.264$, $p<.001$), and Ceruloplasmin ($F(2,97)=46.734$, $p<.001$). Post-hoc analyses revealed significantly higher TSH and lower AMH in the group with DOR and hypothyroidism compared to both the control group and the group with DOR euthyroidism. CRP, Complement C3, and Ceruloplasmin were also markedly elevated in the DOR with hypothyroidism group, suggesting an inflammatory component. FSH and age did not show significant inter-group differences. These findings underscore the critical impact of thyroid dysfunction on ovarian reserve and highlight the potential role of inflammatory markers in compromised reproductive health.

Keywords: *Thyroid Function, Ovarian Reserve, Hypothyroidism, Diminished Ovarian Reserve (DOR), Infertility*

Introduction

Ovarian reserve (OR), representing the quantity and quality of oocytes, is a cornerstone of female fertility (Kumar & Sharma, 2021). Its decline, termed diminished ovarian reserve (DOR), poses a significant

challenge in reproductive medicine, leading to reduced chances of natural conception and poorer outcomes in assisted reproductive technologies (ART) (Tal & Seifer, 2021). While chronological age is the primary determinant of OR, various endocrine and systemic factors can influence its trajectory. Among these, thyroid dysfunction, particularly hypothyroidism, has garnered increasing attention due to its widespread prevalence in women of reproductive age and its profound systemic effects on metabolism and endocrine function (Busnelli et al., 2021).

Thyroid hormones are crucial for the proper functioning of the reproductive system, participating in follicular development, oocyte maturation, and steroidogenesis (Poppe et al., 2022). Imbalances in thyroid hormone levels can disrupt the delicate hormonal milieu necessary for optimal ovarian function (Unuane et al., 2020). Thyroid-Stimulating Hormone (TSH), a primary indicator of thyroid function, directly and indirectly impacts ovarian physiology due to the presence of TSH receptors on ovarian granulosa cells (Shao et al., 2023). Follicle-Stimulating Hormone (FSH), a classic marker of OR, with elevated levels indicating depleted follicular pools. Anti-Müllerian Hormone (AMH), produced by granulosa cells of preantral and small antral follicles, is considered a highly reliable quantitative marker of OR, reflecting the size of the primordial follicle pool (Dewailly et al., 2020).

Beyond direct hormonal effects, systemic inflammation, often associated with autoimmune thyroid conditions like Hashimoto's thyroiditis, may also contribute to the pathophysiology of DOR. C-reactive protein (CRP), an acute-phase reactant, serves as a general marker of inflammation (Wang et al., 2022). Other inflammatory mediators, such as Complement C3 and Ceruloplasmin, indicative of immune system activation and oxidative stress, may also play a role in the ovarian microenvironment (Al-Azab et al., 2023).

Despite growing evidence suggesting a link between thyroid dysfunction and OR, empirical studies quantifying the differences in key markers across distinct patient groups are crucial for robust clinical understanding. This study aimed to empirically evaluate and compare the levels of TSH, AMH, FSH, CRP, Complement C3, and Ceruloplasmin in women with varying thyroid statuses and ovarian reserve, utilizing one-way ANOVA to identify significant inter-group differences.

Methods: Study Design and Participants

This study employed a cross-sectional design comparing hormonal and inflammatory markers across three distinct groups of women of reproductive age. A total of 100 participants were recruited and categorized as follows:

- Control group (practically healthy women; n = 20): These were euthyroid women with normal ovarian reserve;
- Group 1 – Women with diminished ovarian reserve and hypothyroidism (DOR + Hypothyroidism); n=40. These women had clinical or subclinical hypothyroidism (TSH > 4.0 mIU/L or requiring medication) and diminished ovarian reserve (AMH < 1.1 ng/mL);
- Group 2 – Women with diminished ovarian reserve and euthyroidism (DOR + EU); n=40. These women had diminished ovarian reserve (AMH < 1.1 ng/mL) while maintaining a euthyroid status (TSH within the normal range, no thyroid medication use).

All participants were women of reproductive age (17–38 years). Exclusion criteria included polycystic ovary syndrome, hypothalamic amenorrhea, severe systemic diseases unrelated to thyroid function, or recent hormonal therapy that could confound results. Ethical approval was obtained from the relevant institutional review board, and all participants provided informed consent.

Blood samples were collected from all participants in the early follicular phase (Days 2–4) of their menstrual cycle to minimize hormonal fluctuations for OR markers. The following parameters were measured:

- Thyroid-Stimulating Hormone (TSH): Measured in mIU/L.
- Follicle-Stimulating Hormone (FSH): Measured in mIU/mL.
- Anti-Müllerian Hormone (AMH): Measured in ng/mL.
- C-reactive protein (CRP): Measured in mg/L.
- Complement C3: Measured in g/L.
- Ceruloplasmin: Measured in mg/L.
- Age: Recorded in years.

Ovarian reserve was assessed using AMH levels and basal FSH levels, along with clinical assessment (e.g., antral follicle count, menstrual history). Hypothyroidism was diagnosed based on TSH levels and, where appropriate, free T4 levels, according to standard clinical guidelines.

Statistical Analysis: Descriptive statistics (mean, standard deviation, standard error, 95% confidence intervals, minimum, maximum) were calculated for all continuous variables across the three groups and for the total sample. To compare the means of TSH, AMH, FSH, CRP, Complement C3, Ceruloplasmin, and Age across the three independent groups, one-way Analysis of Variance (ANOVA) was performed. The assumption of homogeneity of variances was assessed using Levene's test (results not shown). For variables where ANOVA yielded a statistically significant difference ($p<.05$), post-hoc tests were conducted to identify specific group differences. Both LSD (Least Significant Difference) and Dunnett T3 post-hoc tests were utilized to account for potential heterogeneity of variances and provide a comprehensive view of pairwise comparisons. All statistical analyses were performed using SPSS software (IBM SPSS Statistics, Version 28.0). A p-value of less than .05 was considered statistically significant.

Results: Descriptive statistics for each measured variable are presented below for the three study groups: the control group (healthy women, n=20), Decreased Ovarian Reserve with Hypothyroidism (Group 1, n=40), and Decreased Ovarian Reserve with Euthyroidism (Group 2, n=40).

For Thyroid-Stimulating Hormone (TSH), the control group had a mean of 1.57±0.52 mIU/L (Mean ± SD), the group 1 had 6.97±3.86 mIU/L, and the group 2 had 1.76±0.51 mIU/L. A one-way ANOVA indicated a significant difference across the three groups, $F(2,97)=54.245$, $p<.001$. Post-hoc Dunnett T3 comparisons revealed that the group 1 exhibited significantly higher TSH levels compared to both the control group (mean difference = 5.395, 95% CI [3.854, 6.936], $p<.001$) and the group 2 (mean difference = 5.203, 95% CI [3.672, 6.733], $p<.001$). There was no significant difference in TSH between the control and second groups (mean difference = –0.192, 95% CI [–0.544, 0.159], $p=.444$).

For Follicle-Stimulating Hormone (FSH), the Control group had a mean of 8.88±1.53 mIU/mL, group 1 had 9.11±6.82 mIU/mL, and the group 2 had 8.85±4.96 mIU/mL. A one-way ANOVA showed no significant difference across the groups for FSH ($F(2,97)=0.027$, $p=.973$).

For Anti-Müllerian Hormone (AMH), the Control group had a mean of 3.98±0.78 ng/mL, group 1 had 0.57±0.35 ng/mL, and the group 2 had 0.98±0.48 ng/mL. A one-way ANOVA indicated a significant difference across the groups, $F(2,97)=323.365$, $p<.001$. Post-hoc Dunnett T3 comparisons showed that the Control group had significantly higher AMH levels compared to both group 1(mean difference = 3.413, 95% CI [2.944, 3.882], $p<.001$) and the group 2 (mean difference = 2.998, 95% CI [2.514, 3.481], $p<.001$). Importantly, the second group had significantly higher AMH levels than group 1 (mean difference = 0.415, 95% CI [0.187, 0.643], $p<.001$).

For C-reactive protein (CRP), the Control group had a mean of 3.08±2.14 mg/L, group 1 had 30.25±10.00 mg/L, and the group 2 had 13.52±5.97 mg/L. A one-way ANOVA indicated a significant difference across the groups, $F(2,97)=101.497$, $p<.001$. Post-hoc Dunnett T3 comparisons revealed that group 1 had significantly higher CRP levels than both the Control group (mean difference = 27.165, 95% CI [23.078, 31.252], $p<.001$) and the group 2 (mean difference = 16.725, 95% CI [12.214, 21.236], $p<.001$). Furthermore, the group 2 also had significantly higher CRP levels than the Control group (mean difference = 10.440, 95% CI [7.835, 13.045], $p<.001$).

For Complement C3, the Control group had a mean of 1.18±0.26 g/L group 1 had 6.75±4.03 g/L, and the group 2 had 2.57±1.87 g/L. A one-way ANOVA indicated a significant difference across the groups, $F(2,97)=34.264$, $p<.001$. Post-hoc Dunnett T3 comparisons showed that group 1 had significantly higher Complement C3 levels compared to both the Control group (mean difference = 5.580, 95% CI [3.988, 7.172], $p<.001$) and the group 2 (mean difference = 4.185, 95% CI [2.457, 5.913], $p<.001$). Additionally, the group 2 group also had sig-

nificantly higher Complement C3 levels than the Control group (mean difference = 1.395, 95% CI [0.645, 2.145], p<.001).

For Ceruloplasmin, the Control group had a mean of 34.30±9.28 mg/L, group 1 had 84.55±29.04 mg/L, and the group 2 had 51.35±13.54 mg/L. A one-way ANOVA indicated a significant difference across the groups, F(2,97)=46.734, p<.001. Post-hoc Dunnett T3 comparisons revealed that group 1 showed significantly elevated Ceruloplasmin levels compared to both the Control group (mean difference = 50.250, 95% CI [37.834, 62.666], p<.001) and the group 2 (mean difference = 33.200, 95% CI [20.739, 45.661], p<.001). The group 2 also had significantly higher Ceruloplasmin levels than the Control group (mean difference = 17.050, 95% CI [9.705, 24.395], p<.001).

For Age, the Control group had a mean of 32.70±5.02 years, group 1 had 30.05±5.22 years, and the second group had 29.98±6.04 years. A one-way ANOVA showed no significant difference in Age across the groups (F(2,97)=1.892, p=.156), indicating that age was not a significant confounding factor in the observed hormonal and inflammatory differences.

The results of the one-way ANOVA for each dependent variable are summarized in Table 2.

Table 1. *One-Way ANOVA Results for Hormonal and Inflammatory Markers*

Variable	Sum of Squares	df	Mean Square	F	Sig.
TSH (mIU/L)	666.201	2	333.100	54.245	**.000**
FSH (mIU/mL)	1.564	2	0.782	0.027	.973
AMH (ng/mL)	167.823	2	83.911	323.365	**.000**
CRP (mg/L)	11251.057	2	5625.528	101.497	**.000**
Complement C3 (g/L)	544.887	2	272.444	34.264	**.000**
Ceruloplasmin (mg/L)	40161.960	2	20080.980	46.734	**.000**
Age (years)	115.675	2	57.838	1.892	.156

As indicated in Table 2, significant differences were found among the three groups for TSH (F(2,97)=54.245, p<.001), AMH (F(2,97)=323.365, p<.001), CRP (F(2,97)=101.497, p<.001), Complement C3 (F(2,97)=34.264, p<.001), and Ceruloplasmin (F(2,97)=46.734, p<.001). No significant differences were observed for FSH (F(2,97)=0.027, p=.973) or Age (F(2,97)=1.892, p=.156).

Discussion: This empirical study provides compelling evidence for the distinct impact of thyroid dysfunction on ovarian reserve, further elucidated by the analysis of TSH, AMH, CRP, Complement C3, and Ceruloplasmin levels across different clinical groups. The findings reinforce the notion that hypothyroidism significantly exacerbates the decline in ovarian reserve beyond what is observed in euthyroid women with DOR.

The most striking finding is the significantly higher TSH and profoundly lower AMH in group 1 compared to both healthy controls and the group 2. This robust association between elevated TSH and reduced AMH aligns with recent literature, which increasingly emphasizes the inverse correlation between TSH and AMH even within the subclinical range (Polyzos et al., 2023; Ozkan et al., 2020). The significantly lower AMH in group 1, even when compared to the DOR+Eu group (who already have compromised ovarian reserve), suggests that hypothyroidism imposes an additional detrimental burden on the ovarian follicular pool. This could be attributed to direct effects of TSH on ovarian TSH receptors, potentially disrupting follicular growth and maturation, or through indirect mechanisms affecting the hypothalamic-pituitary-gonadal axis (Guo et al., 2021).

Interestingly, FSH levels did not show significant differences across the groups. While FSH is a primary indicator of OR, its dynamics can be more complex and influenced by various factors, including the stage of follicular development and central feedback mechanisms. The lack of significant

difference in FSH might suggest that while ovarian reserve is clearly diminished across both DOR groups (as indicated by AMH), the compensatory increase in FSH might not be as pronounced or consistently different across groups, or might be masked by the influence of thyroid hormones on pituitary sensitivity (Shao et al., 2023). This highlights the superior sensitivity of AMH as a marker for subtle changes in ovarian follicular status in the context of thyroid dysfunction (Dewailly et al., 2020).

A novel and critical aspect of this study is the significant elevation of CRP, Complement C3, and Ceruloplasmin in group 1, and to a lesser extent, in the DOR+Eu group, compared to healthy controls. The highest levels of these inflammatory markers were observed in women with both DOR and hypothyroidism. This finding strongly supports the hypothesis that systemic inflammation plays a crucial role in the pathophysiology of DOR, particularly when complicated by thyroid dysfunction. Autoimmune thyroiditis, a common cause of hypothyroidism, is known to induce a state of chronic low-grade inflammation (Wang et al., 2022). The elevated CRP suggests general systemic inflammation, while increased Complement C3 and Ceruloplasmin point towards immune activation and oxidative stress, which can directly or indirectly harm ovarian cells and accelerate follicular atresia (Al-Azab et al., 2023; Zhou et al., 2020). This suggests that the compromised ovarian reserve in hypothyroid women might not only be due to direct hormonal imbalances but also to an exacerbated inflammatory environment.

The finding that age did not differ significantly between groups implies that the observed differences in hormonal and inflammatory markers are independent of age-related ovarian decline, strengthening the argument for a direct effect of thyroid status and associated inflammation. This underscores the clinical importance of screening for and managing thyroid dysfunction in women with DOR, regardless of their age (Busnelli et al., 2021).

Clinical implications of these findings are substantial. Comprehensive thyroid function assessment, including TSH and potentially thyroid antibodies, should be a routine part of infertility workups, particularly in women with suspected or confirmed DOR (Sharma et al., 2019). Furthermore, addressing thyroid dysfunction through appropriate treatment may not only improve overall health but also potentially enhance ovarian response and reproductive outcomes by mitigating direct hormonal effects and systemic inflammation (Unuane et al., 2020). Future research should explore the efficacy of thyroid hormone replacement in normalizing these inflammatory markers and their subsequent impact on AMH levels and fertility rates.

A limitation of this study is its cross-sectional design, which precludes establishing direct causality. Longitudinal studies are needed to track changes in OR markers and inflammatory parameters following thyroid function optimization. Additionally, while CRP, Complement C3, and Ceruloplasmin provide insights into inflammation, future studies could delve into specific cytokines and immune cell profiles within the ovarian microenvironment to better understand the precise mechanisms of inflammation-induced follicular damage.

Conclusion: This empirical evaluation confirms a significant detrimental impact of hypothyroidism on ovarian reserve, as evidenced by profoundly lower AMH and markedly elevated TSH levels in affected women. The study further highlights the association of hypothyroidism with elevated inflammatory markers such as CRP, Complement C3, and Ceruloplasmin, suggesting that systemic inflammation contributes to compromised ovarian health. These findings underscore the critical need for routine thyroid screening and proactive management in women experiencing diminished ovarian reserve, offering a clinical perspective that integrates hormonal and inflammatory aspects of female reproductive endocrinology. Optimizing thyroid function may represent a vital strategy to improve ovarian health and fertility outcomes.

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