Introduction

Primary ovarian insufficiency (POI) is defined as primary hypogonadism in women aged below 40 years. POI is clinically characterized by amenorrhea, low estrogen levels, and elevated follicle-stimulating hormone (FSH) levels in the menopausal range; therefore, it is one of the causes of intractable infertility. The incidence of POI is approximately 1 in 100 women of reproductive age [1].

There are some known causes of ovarian failure, such as chromosomal defects and iatrogenic factors. Autoimmune diseases such as myasthenia gravis, adrenal autoimmunity, and thyroid autoimmunity are strongly associated with and considered possible causes of POI [2]. In addition, the prevalence of clinical autoimmune disease in patients with POI was found to be 40–55 % [3, 4].

Thyroid disorders are the most common autoimmune disorders associated with POI. According to previous reports, thyroid autoimmune disorders are detected in 12–33 % of patients with POI [3, 4].

Clinical POI presents in several stages. Overt POI presents as elevated serum FSH levels with amenorrhea and reduced fertility. Prior to the development of overt POI, patients present with pre-POI statuses such as occult POI (reduced fertility with normal menstrual cycle and FSH levels) and biochemical POI (reduced fertility and elevated FSH levels with normal menstrual cycle) [5]. Patients with POI usually visit the hospital when they develop amenorrhea or irregular menses. However, fertility cannot be easily recovered after patients have developed overt POI. POI results from the premature exhaustion of the follicle pool. Therefore, it is important to

Thyroid Autoantibodies do not Impair the Ovarian Reserve in Euthyroid Infertile Women: A Cross-Sectional Study

ABSTRACT

Patients with primary ovarian insufficiency (POI) have a high prevalence of thyroid autoimmune disorders. However, the extent of the contribution of thyroid autoantibodies or elevated thyroid-stimulating hormone (TSH) levels to decreased ovarian reserve is unclear. Therefore, we evaluated the serum levels of anti-Müllerian hormone (AMH) and thyroid autoantibodies [antithyroperoxidase antibody (TPOAb), and antithyroglobulin antibody (TgAb)] in euthyroid infertile women. One hundred and fifty-three women with normal menstrual cycles were recruited for this retrospective study. Serum levels of AMH were compared between patients with positive and negative thyroid autoantibodies. The correlation between serum levels of AMH and each thyroid autoantibody was also evaluated. Participants were observed to be either TPOAb or TgAb positive (n = 27), only TPOAb positive (n = 8), only TgAb positive (n = 7), TPOAb and TgAb positive (double positive; n = 12), and TPOAb and TgAb negative (double negative; n = 126). No significant differences were found in serum AMH levels between the TPOAb- or TgAb-positive women and the antibody-double negative women. Serum AMH levels did not show a significant correlation with the concentration of TgAb or TPOAb. On the other hand, serum AMH levels negatively correlated with TSH levels in patients who were either positive for TPOAb or TgAb. Thyroid autoantibodies are not likely to influence ovarian reserve in euthyroid women whose TSH levels fall within the normal range although elevated TSH levels may be involved in the decline of serum AMH levels.
diagnose POI in patients with occult POI who present with a gradually diminishing follicle pool and normal serum FSH levels [6]. Anti-Müllerian hormone (AMH), a member of the transforming growth factor-β superfamily, is produced by granulosa cells of preantral and early antral follicles and its production decreases with advancing age [7]. Serum AMH levels are indicative of the number of follicles in the follicle pool and have been established as reliable markers for the ovarian reserve in the field of assisted reproductive technology [8, 9]. It has been demonstrated that serum AMH levels closely correlate with both the antral follicle count obtained by ultrasonography and the number of oocytes retrieved during in vitro fertilization treatment [10, 11]. AMH has gained widespread popularity because of its several advantages, including sensitivity to the changes associated with advancing age and intra- and inter-cycle consistencies. Therefore, AMH may be used as a marker to diagnose poor pregnancy prospects in women aged above 38 years [12]. In addition, many researchers have started determining serum AMH levels to evaluate ovarian damages, such as those caused by surgical excision of the ovary and chemotherapy [13–15].

In a previous prospective study, the number of women with normal AMH levels was significantly lower in the pre-POI group (women with mildly elevated FSH levels who did not fulfill the POI criteria) than in the control group (normo-ovulatory women) (33% vs. 98%) [16]. Therefore, infertile patients, with normal menses, who have POI-related autoimmune diseases (thyroid autoantibodies) may have decreased ovarian reserves. AMH can potentially be used to assess the depletion of the ovarian reserve and predict the progression of POI in these patients.

In this study, we assessed the presence of thyroid autoantibodies [antithyoperoxidase antibody (TPOAb), and antithyroglobulin antibody (TgAb)] in infertile patients with normal menstrual cycles to investigate the correlation between serum AMH levels and thyroid autoantibodies.

Materials and Methods
This study was approved by the ethical committee of the Nagoya University Graduate School of Medicine and is retrospectively registered as UMIN000026923. Informed consent was obtained from all patients.

Patients
Patients who were referred to the fertility clinic of the Nagoya University Hospital for the first time, between January 2008 and May 2016, were recruited for the study. Women, aged <40 years and with regular menstrual cycles (25–35 days), were included. The exclusion criteria were as follows: diagnosis of polycystic ovary syndrome according to the 2003 criteria of the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine [17], hyperthyroidism, hypothyroidism, and history of adnexal surgery.

Hormonal measurements
Blood samples were obtained on the third or fourth day of the menstrual cycles of the patients. Serum was separated from whole blood, transferred to sterile polypropylene tubes, and stored at –80 °C until the assays were performed. The serum AMH concentrations were measured using an enzyme immunoassay kit, according to the manufacturer’s instructions (AMH GenII ELISA, Beckman Coulter Inc., Brea, CA, USA) [18]. The absorbance measurements were performed using a microplate reader (Elx808TM Absorbance Microplate Reader; BioTek, Winooski, VT, USA). The serum thyroid-stimulating hormone (TSH), TPOAb, and TgAb concentrations were measured using ARCHITECT® TSH (Abbot Japan Co., Ltd, Tokyo, Japan), Elecsys Anti-Tg, and Elecsys Anti-TPO (Roche Diagnostics, Mannheim, Germany), respectively, and serum FSH concentrations were measured using ARCHITECT® FSH (Abbot Japan Co., Ltd). Serum TPOAb levels >16 IU/ml and TgAb levels >30 IU/ml were considered positive.

Statistical analyses
Data were analyzed using the GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). Student’s t-test, Mann–Whitney U-test, or Fisher exact test were used to compare patient characteristics, including levels of hormones and antibodies, among the antibody-negative, TPOAb-positive, and TgAb-positive groups. The Mann–Whitney U-test was applied, instead of the Student’s t-test, when the variables were not normally distributed. Spearman coefficients were used to analyze the associations between serum thyroid autoantibody and AMH levels. A p-value of <0.05 was considered statistically significant.

Results
A total of 153 women were recruited for this retrospective-cohort study. The participants in this cohort were observed to be either TPOAb or TgAb positive (n = 27), only TPOAb positive (n = 8), only TgAb positive (n = 7), TPOAb and TgAb positive (double positive; n = 12), and TPOAb and TgAb negative (double negative; n = 126). The clinical characteristics and the results of the hormonal measurements are presented in ▶ Table 1. There was no significant difference in the age of the patients in the three groups. Although serum AMH levels tended to be a little bit lower in women who were TPOAb positive, no significant differences were found either between Tg-positive and Tg-negative groups or between TPO-positive and TPO-negative groups (▶ Fig. 1). There were no significant differences in the serum TSH levels between women who were antibody-double negative and antibody positive.

We then analyzed the correlation between serum AMH and patient age, serum TSH levels, and thyroid antibody levels (▶ Fig. 2). Serum AMH levels showed negative correlation with patient age (r = –0.117, p = 0.0086). There were no significant correlations between serum AMH and TSH levels (r = –0.00354, p = 0.664), TgAb levels (r = 0.1022, p = 0.2087), or TPOAb levels (r = 0.0544, p = 0.504). Additionally, we analyzed the correlation between serum AMH and TSH levels in patients who were either TPOAb positive or TgAb positive. Serum AMH levels negatively correlated with TSH levels (Spearman r = –0.398, p = 0.0399). On the other hand, there were no significant correlations between serum AMH and TPOAb levels (Spearman r = –0.0192, p = 0.9369) or TgAb levels (Spearman r = –0.2035, p = 0.4034) in patients with thyroid autoantibodies (▶ Fig. 3).
The effects of the autoimmune system on female reproduction have been demonstrated in many studies, and thyroid autoimmunity is the most common endocrine autoimmune disorder in women of reproductive age. Chronic autoimmune thyroiditis (Hashimoto’s thyroiditis) is known to occur in 3.5 per 1000 women, which is a 4.4-fold higher incidence than the incidence in men [19]. TgAb and TPOAb have been demonstrated as markers of chronic autoimmune thyroiditis considering that their production occurs as a secondary phenomenon after thyroid damage. However, many individuals have these autoantibodies in subclinical situations or disease-free states. According to the United States National Health and Nutrition Examination Survey, TPOAb and TgAb were detected in 14.6% and 13.8% of disease-free women, respectively [20].

It has been demonstrated that thyroid autoimmune disorders are often diagnosed in patients with POI [3, 4]. In fact, it has been recently reported that the frequency of having TPOAb was 24.1% in patients with POI [21]. Thyroid dysfunction and/or thyroid autoimmunity could influence the ovarian reserve and ovarian senescence. Magri et al. reported that women with autoimmune thyroid diseases tended to show poor response to controlled ovarian hyperstimulation [22]. Therefore, to prevent ovarian insufficiency-realted thyroid autoimmunity, it should be specified how the ovarian reserve is impaired in this condition. However, it is still unclear how autoimmune hypothyroidism can impair ovarian function and ovarian reserve. Either or both autoantibodies and/or elevated TSH have been considered to possibly affect ovarian function. TSH possesses a conformational similarity to FSH, such as a common α subunit. Nonetheless, such effects of TSH in ovarian function in autoimmune hypothyroidism have rarely been explored in conjunction with ovarian reserve. Meanwhile, it was reported that thyroid autoantibodies exist in follicular fluid depending on their serum concentration levels [23]. Although intrafollicular thyroid autoantibodies could exert some in-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics.</th>
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<tr>
<td>Variable</td>
<td>Double negative</td>
</tr>
<tr>
<td>(n = 145)</td>
<td>(n = 28)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.89 (32.28, 38.83)</td>
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<tr>
<td>AMH (ng/ml)</td>
<td>1.78 (1.08, 3.26)</td>
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<tr>
<td>FSH (miU/ml)</td>
<td>5.70 (4.70, 6.90)</td>
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<tr>
<td>TSH (IU/ml)</td>
<td>1.37 (0.93, 1.96)</td>
</tr>
<tr>
<td>TPOAb (IU/ml)</td>
<td>8.0 (6.0, 10.0)</td>
</tr>
<tr>
<td>TgAb (IU/ml)</td>
<td>12.0 (11.0, 15.0)</td>
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TPOAb: Antithyroperoxidase antibody; TgAb: Antithyroglobulin antibody; AMH: Anti-Müllerian hormone; FSH: Follicle-stimulating hormone; TSH: Thyroid-stimulating hormone; Values are indicated as median (25th, 75th percentile); * p < 0.0001; compared to double negative using Mann–Whitney U-test.

![Image](https://example.com/image_url)

**Fig. 1** Serum anti-Müllerian hormone (AMH) levels (a) between antithyroglobulin antibody (TgAb)-positive and TgAb-negative women; (b) antithyroperoxidase antibody (TPOAb)-positive and TPOAb-negative women. Bar: Median; p: Mann–Whitney U-test.

**Discussion**

The effects of the autoimmune system on female reproduction have been demonstrated in many studies, and thyroid autoimmunity is the most common endocrine autoimmune disorder in women of reproductive age. Chronic autoimmune thyroiditis (Hashimoto’s thyroiditis) is known to occur in 3.5 per 1000 women, which is a 4.4-fold higher incidence than the incidence in men [19]. TgAb and TPOAb have been demonstrated as markers of chronic autoimmune thyroiditis considering that their production occurs as a secondary phenomenon after thyroid damage. However, many individuals have these autoantibodies in subclinical situations or disease-free states. According to the United States National Health and Nutrition Examination Survey, TPOAb and TgAb were detected in 14.6% and 13.8% of disease-free women, respectively [20].

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fluence on folliculogenesis, how they affect oocytes, granulosa cells, or stromal cells in the ovary is still unclear.

Recently, serum AMH has been drawing attention as a reliable marker for ovarian reserve. Several researchers have analyzed the correlation of ovarian reserve, assessed by serum AMH levels and TSH concentrations and/or thyroid autoantibodies, in autoimmune hypothyroidism. Polyzos et al. analyzed the association between serum AMH levels, TSH levels, and thyroid autoantibodies, and demonstrated that TPOAb or TSH levels did not affect serum AMH levels [24]. However, this study lacked detailed information on the treatment history of patients with hypothyroidism. Similarly, Waghfoer et al. demonstrated that there was no significant difference in serum AMH levels between the low-normal TSH (<2.5 μIU/ml) group and normal-high TSH (>2.5 μIU/ml) group [25].

On the contrary, Saglam et al. reported lower serum AMH levels in patients with autoimmune thyroid disease than in controls [26]. However, TSH was not the factor influencing AMH levels in multivariate analysis. The serum AMH levels in this study were relatively lower than those of previous reports. According to Thomas et al., the mean serum AMH levels of 35-year old women is around 2.5 ng/ml [27]. On the other hand, the AMH levels of the cohort in this study was 1.16 ng/ml in the patients (mean age: 35 years old) and 1.28 ng/ml in the controls (mean age: 35.4 years old). Therefore, it is still difficult to reach a firm conclusion. Kuroda et al. demonstrated that serum AMH levels showed an inverse correlation with serum TSH levels [28]. However, they did not include thyroid autoantibodies in their analysis. Women with higher TSH levels were likely to be positive for thyroid autoantibodies. In this situa-

![Fig. 2](image1.png)

**Fig. 2** Correlation of serum anti-Müllerian hormone (AMH) levels with (a) patient age (r = -0.212), and levels of (b) TSH (r = -0.0354), (c) antithyroglobulin antibody (TgAb, r = 0.209), and (d) antithyroperoxidase antibody (TPOAb, r = 0.504). r: Spearman correlation coefficient.

![Fig. 3](image2.png)

**Fig. 3** Correlation of serum anti-Müllerian hormone (AMH) levels with (a) TSH (r = 0.0399), (b) antithyroglobulin antibody (TgAb, r = 0.403), and (c) antithyroperoxidase antibody (TPOAb, r = 0.936) in patients with either TgAb or TPOAb. r: Spearman correlation coefficient.
tion, we cannot conclude whether higher TSH levels or positive thyroid autoantibodies are more implicated in the decline of ovarian reserve. It has recently been reported that women with TSH < 3 μIU/ml showed higher serum AMH levels after adjustment for thyroid autoimmunity [29]. The limitation of our study is the small number of subjects used. Nevertheless, we recruited women without overt hypothyroidism and most of them showed TSH levels within the normal range.

When we compared the serum AMH levels between thyroid antibody positive and thyroid antibody negative women, we did not observe any significant decline in serum AMH levels. However, in women with thyroid autoantibodies, serum AMH levels negatively correlated with serum TSH levels. On the other hand, we did not observe any significant correlation between AMH levels and TPOAb levels or TgAb levels. Taken together, compared to the presence of thyroid autoantibodies, higher TSH levels in women with hypothyroidism have an influence on ovarian reserve.

Recently, an interesting finding regarding TSH and ovarian reserve has been reported. Erol et al. showed that serum AMH levels were significantly higher in adolescents with Hashimoto’s thyroiditis than in age- and BMI-matched healthy controls [30]. As mentioned above, TSH possesses a conformational similarity to FSH. Therefore, it may be hypothesized that elevated TSH stimulates folliculogenesis in relatively younger patients with hypothyroidism and upregulates serum AMH levels. This could be followed by depletion of follicles in the ovary, resulting in the decline of ovarian reserve. Further studies, including animal studies and longitudinal clinical studies, will be required to test this hypothesis.

In conclusion, the presence of thyroid autoantibodies is not in itself a risk factor for the development of POI in euthyroid women whose TSH levels are controlled to fall within the normal range. However, elevated TSH may be involved in the decline of serum AMH levels in adult women with hypothyroidism.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


