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REPRODUCTIVE PHYSIOLOGY AND DISEASE

# Elevated serum thyroid-stimulating hormone is associated with decreased anti-Müllerian hormone in infertile women of reproductive age

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**Abstract**
*Purpose* Thyroid dysfunction and autoimmune thyroiditis are associated with fertility in women of reproductive age. Anti-Müllerian hormone (AMH), a known biomarker of ovarian function, may be affected by impaired thyroid function; however, the relationship between AMH and thyroid hormone has not been elucidated.
*Methods* In this case–control study, to identify the impact of thyroid hormone on ovarian reserve, we recruited 67 consecutive Japanese infertile patients and 27 normal fertile women aged 30–39 years without impact factors on thyroid and ovarian functions between 2012 and 2013. We assessed patient age, BMI and AMH, prolactin, TSH and FT4 levels of all study participations as independent variables. To evaluate the relationship between AMH and thyroid hormone, we matched patients by age and body mass index as confounding factors using 1:1 matching for statistical analysis of healthy fertile women and infertile patients and obtained 23 pairs. Then, independent variables were subjected to multiple regression analysis.
*Results* Multiple regression analysis showed that both thyroid-stimulating hormone (TSH) levels and patient age were negatively correlated with AMH levels in infertile patients (patient age and TSH: standardized partial regression coefficient (β), −0.534 and −0.361; $p$=0.003 and 0.036, respectively), but not in normal fertile women.
*Conclusions* AMH levels were inversely correlated with TSH levels in infertile women of reproductive age.

**Keywords** Infertility · Hypothyroidism · Anti-Müllerian hormone · Ovarian reserve · Retrospective study

Keiji Kuroda and Toyoyoshi Uchida contributed equally to this work.

*Capsule*: Serum anti-Müllerian hormone levels were inversely correlated with thyroid-stimulating hormone levels in infertile women of reproductive age, but not in normal fertile women.

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## Introduction

Thyroid dysfunction and autoimmune thyroiditis are known adverse risk factors for pregnancy as well as fertility, regardless of the presence of disease, in women of reproductive age. In particular, hypothyroid women are at an increased risk of menstrual disorders and infertility because of altered peripheral estrogen metabolism, hyperprolactinaemia and abnormal release of gonadotropin-releasing hormone [1]. The prevalence of subclinical hypothyroidism, characterized by aberrant high serum thyroid-stimulating hormone (TSH) levels with normal free thyroxine (FT4) levels, in infertile women is reported to be approximately 20 % and it is a primary cause of subfertility [2, 3]. Indeed, average TSH levels in infertile women were reportedly higher than those in normal fertile women [4, 5]. And elevated serum TSH levels were associated with diminished ovarian reserve in infertile patients [6].

Although levothyroxine replacement therapy for subclinical hypothyroidism in infertile patients remains debatable, thyroxine supplementation may improve fertility, in particular, implantation and miscarriage rates, leading to successful pregnancy [7, 8]. These data suggest that hypothyroidism is strongly correlated with infertility.

Female fecundity decreases with increasing age, primarily because of decreased ovarian function. Anti-Müllerian hormone (AMH) is produced by granulosa cells of early developing follicles. Ovarian research after oophorectomy showed that serum AMH levels were closely correlated with the number of primordial follicles; therefore, AMH is a suitable biomarker of ovarian age in women of reproductive age [9].

Expectedly, ovarian function may be affected by impaired thyroid function, although this association has not been elucidated. In this study, we evaluated the relationship between thyroid function and AMH levels by comparing them in infertile patients and healthy fertile women.

## Materials and methods

Between December 2012 and December 2013, 251 consecutive Japanese women who visited the Fertility Outpatient Clinic at the Department of Obstetrics and Gynaecology of Juntendo University Hospital (Tokyo, Japan) and were diagnosed as infertile according to the diagnostic criteria shown below were recruited for participation in this study. We also recruited 27 consecutive normal fertile women aged 30–39 years who visited our clinic for screening of uterine cancer from July to December 2013. They recently had normal deliveries and had no history of treatment for infertility or thyroid disorders. We measured thyroid-related hormone and serum AMH levels in the infertile and fertile women. We excluded the patients with factors that adversely impact thyroid hormone and ovarian function. In the patients with polycystic ovary syndrome (PCOS), increased production of AMH from granulosa cells is attributed to high antral follicle numbers [10]. And ovarian reserve is adversely affected in the patients with ovarian insufficiency and failure, ovarian tumor including endometriosis [11, 12], ovarian surgery [11, 13, 14], smoking [15, 16] and aging [17, 18]. As regarding thyroid, we included newly diagnosed thyroid abnormality, but excluded the patients being treated for thyroid dysfunction. Out of 184 excluded patients, the impact factors were PCOS ($n$=50), primary ovarian insufficiency and (premature) ovarian failure ($n$=24), treated thyroid dysfunction ($n$=12), endometriosis ($n$=53), ovarian tumor ($n$=21), post-ovarian surgery ($n$=25), history of smoking ($n$=17) and ≥40 years ($n$=66). Sixty-four patients fulfilled 2–4 exclusion criteria (Table 1). Informed consent was obtained from all study participants and the study protocol was approved by the ethics committee of Juntendo University.

**Table 1** Exclusion criteria

| The reasons of exclusion | $n$ |
|---|---|
| Polycystic ovary syndrome | 50 |
| (Premature) ovarian dysfunction | 24 |
| Treated thyroid dysfunction | 12 |
| Endometriosis | 53 |
| Ovarian tumor | 21 |
| Post-ovarian surgery | 25 |
| Smoking | 17 |
| 40 years old or more | 66 |
| Total | 184 |
| (2–4 exclusion factors 64) | |

In patient selection, we excluded 184 patients with factors that adversely impact thyroid hormone and ovarian function including polycystic ovary syndrome, ovarian insufficiency and failure, ovarian tumor including endometriosis, ovarian surgery, smoking, aging and treated thyroid dysfunction

Infertility is defined as the failure to achieve clinical pregnancy after 12 months or more. Serum levels of luteinizing hormone, follicle stimulating hormone, estradiol, progesterone, prolactin and testosterone were analysed at 2–5 days of the menstrual cycle to screen for infertile patients.

Blood samples were collected from all study participants, and serum FT4, TSH and prolactin levels were measured using a commercial electrochemiluminescence immunoassay (Roche Diagnostics, Tokyo, Japan; reference range: FT4, 1.00–1.70 ng/dL; TSH, 0.56–4.30 μIU/mL; prolactin, 4.91–29.32 ng/mL). Serum AMH levels were measured using the AMH GenII enzyme-linked immunosorbent assay kit (functional sensitivity: <0.16 ng/mL; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan).

To investigate the relationship between thyroid function and serum AMH levels, we matched patients by age and body mass index (BMI) as confounding factors using 1:1 matching for statistical analysis of healthy fertile women and infertile patients [19]. Infertile patients were matched with healthy fertile women by age±1 year and by BMI±1.0 kg/m$^2$.

Results are presented as means and standard deviations (SDs) or medians and interquartile ranges and were compared using Student's $t$-test or the Mann–Whitney $U$-test, as appropriate. Independent variables such as patient age, BMI and AMH, prolactin, TSH and FT4 levels, which strongly correlate with infertility, were estimated by stepwise logistic regression analysis. Independent variables with a probability ($p$) value of <0.10 in the univariate regression analysis were entered into a multiple regression analysis model. The assumption of multicollinearity among selected independent variables was assessed by each correlation coefficient. Finally, independent variables that were strongly correlated with an infertility-related variable were estimated by multiple regression analyses. A $p$-value of <0.05 was considered

**Table 2** Baseline characteristics of women with normal fertility and infertility

| | Pre-matching | | | Post-matching | | |
|---|---|---|---|---|---|---|
| | Fertility | Infertility | *p* | Fertility | infertility | *p* |
| Patients number | 27 | 67 | | 23 | 23 | |
| Age (yrs) | 34.0±3.5 | 35.0±3.2 | 0.116 | 33.7±3.5 | 33.7±3.4 | 0.966 |
| BMI (kg/m$^2$) | 20.4±2.0 | 20.2±2.2 | 0.634 | 20.4±2.0 | 20.5±2.0 | 0.942 |
| AMH (ng/ml) | 4.49±2.00 | 2.60±2.00 | <0.001 | 4.36±2.07 | 2.55±1.86 | 0.003 |
| TSH (μU/ml) | 1.55±0.82 | 2.14±1.28 | 0.009 | 1.52±0.86 | 2.01±1.09 | 0.096 |
| FT4 (ng/dl) | 1.20±0.18 | 1.23±0.26 | 0.519 | 1.20±0.18 | 1.18±0.12 | 0.567 |
| Prolactin (ng/ml) * | 10.6 (7.6–17.2) | 14.5 (10.6–17.0) | 0.266 | 10.6 (7.6–17.2) | 14.8 (10.4–16.4) | 0.384 |

* Prolactin data are expressed as median (interquartile range), while other data are expressed as mean±SD

*BMI* body mass index, *AMH* anti-Müllerian hormone, *TSH* thyroid stimulating hormone, *FT4* free thyroxine

statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL, USA).

## Results

### Baseline characteristics of infertile patients

We finally enrolled 67 infertile patients and 27 healthy fertile women aged <40 years. As shown in Fig. 1, serum TSH levels in the infertile patients were inversely correlated with AMH levels. To compare the clinical characteristics of infertile patients with those of healthy fertile women, we used 1:1 matching (normal fertility:infertility) of age and BMI because of the influence of age on AMH levels. Consequently, 23 infertile patients were extracted by 1:1 matching (Table 1). In post-matched clinical characteristics, AMH levels were significantly lower in infertile patients than in healthy fertile women, whereas TSH and FT4 levels were comparable. This statistical difference in AMH levels between infertile patients and healthy fertile women was also shown in total cohort of healthy fertile and infertile women.

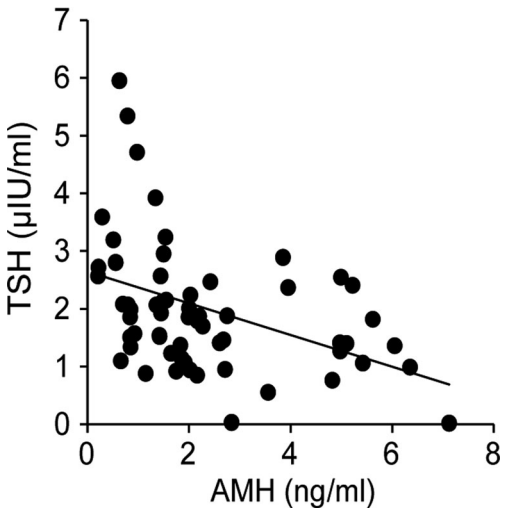


**Fig. 1** Inverse correlation between serum thyroid stimulating hormone (*TSH*) and anti-Müllerian hormone (*AMH*) levels in infertile patients aged <40 years Analysis of data of 67 infertile patients aged <40 years showed inverse correlation between serum TSH and AMH levels

### Analysis of factors related to infertility

We used multivariate logistic regression model to evaluate independent variables strongly correlated with infertility in post-matched patients and included the following covariates in the model: patient age, BMI and AMH, TSH, FT4 and prolactin levels. Stepwise logistic regression analysis showed that AMH levels strongly correlated with infertility [hazard ratio (HR), 0.622; 95 % confidence interval (CI), 0.432–0.895, $p$=0.011). This result was comparable with the statistical result in total cohort of infertile patients (HR, 0.650; 95 % CI, 0.493–0.858, $p$=0.002). Next, we evaluated covariates (including prolactin) that were strongly correlated with AMH levels in 23 post-matched infertile patients using multiple regression analysis. Two covariates showed a $p$-value of <0.10 in univariate regression analysis: TSH and patient age

**Table 3** Multiple regression analysis for AMH (Stepwise method)

| Independent variable | Standardized partial regression coefficient (β) | *p* |
|---|---|---|
| Total cohort of infertile women | | |
| Age | −0.293 | 0.01 |
| TSH | −0.358 | 0.002 |
| Post-matched infertile women | | |
| Age | −0.534 | 0.003 |
| TSH | −0.361 | 0.036 |

*AMH* anti-Müllerian hormone, *TSH* thyroid stimulating hormone, *CI* confidence interval

($p$=0.002 for both). Multiple regression analyses showed that both TSH levels and patient age significantly impacted AMH levels in infertile patients (TSH levels and age: standardized partial regression coefficient (β), −0.361 and −0.534; $p$= 0.036 and 0.003, respectively) (Table 2). This result was also comparable with the statistical results of 67 total cohort of infertile patients (TSH levels and age: standardized partial regression coefficient (β), −0.358 and −0.293; $p$=0.002 and 0.010, respectively). However, single regression analysis of AMH levels in post-matched healthy fertile women showed that both TSH levels and patient age, as well as BMI, FT4 and prolactin levels, were not correlated with AMH levels ($p$= 1.000, 0.476, 0.758, 0.470 and 0.654, respectively).

## Discussion

The results of this study showed that serum AMH levels in infertile patients, but not healthy fertile women, were inversely correlated with patient age and TSH levels (Fig. 1), and patient age and TSH levels were shown to impact AMH levels in infertile patients (Table 3). AMH levels were significantly lower in infertile patients than in healthy fertile women ($p$<0.001, Table 2); therefore, it was reasonable that a decrease in AMH levels was strongly correlated with infertility, in accordance with the results of a previous study [9]. These data revealed an inverse correlation between TSH and AMH levels in infertile women with decreased ovarian function without other factors affecting thyroid and ovarian function. Indeed, in a previous study, TSH was identified as a significant predictor of fertilization failure in women undergoing in vitro fertilization [20]. Therefore, TSH may be a factor influencing ovarian reserve in infertile patients. Furthermore, thyroid hormone plays an important role for implantation as well as embryo and placenta development during early pregnancy [21].

Given that subclinical hypothyroid women are at an increased risk of infertility, elevated TSH levels may have deleterious effects on ovarian function. Michalakis et al. also reported clinical data in high prevalence of diminished ovarian reserve in the infertile patients with elevated serum TSH levels [6]. An in vivo study using propylthiouracil-induced hypothyroid rats revealed that hypothyroidism may impact ovarian folliculogenesis, granulosa cell differentiation and steroidogenesis without affecting proliferation [22]. Thyroid hormone plays an important role in follicular development [23, 24]. In addition, TSH directly suppressed follicle development in a concentration-dependent manner [25]. However, TSH levels in infertile patients in this study were almost within the normal range; therefore, ovarian function of infertile patients is somehow more susceptible to TSH compared with that of healthy fertile women.

On the other hand, Gerhard et al. suggested that secretion of TSH and prolactin, which are stimulated by thyrotropin-releasing hormone, was significantly higher in infertile patients than in healthy fertile women [4, 5]. However, in this study, prolactin levels were almost within the normal range in infertile patients, primarily because TSH levels were in the normal range, and were less strongly correlated with AMH levels in single regression analysis ($p$=0.623). These data suggested that ovarian function in infertile patients is directly related to TSH levels.

This study has some limitations. First, it was a single-center observational study. Second, we were unable to obtain data on autoimmune thyroid antibodies (thyroglobulin antibody and thyroid peroxidase antibody) and the estimated dietary iodine consumption for use as covariates in multivariate logistic regression analysis.

In conclusions, we showed an inverse relationship between serum TSH levels, which were within the normal range, and AMH levels in infertile women of reproductive age. These findings may influence current infertility treatment strategies. However, a prospective study of levothyroxine therapy for patients with decreased AMH and increased TSH levels is warranted in the future.

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