The influence of thyroid autoimmunity on pregnancy outcome in infertile women: a prospective study

Yuko Inagaki¹, Ken Takeshima¹, Masahiro Nishi¹,², Hiroyuki Ariyasu¹, Asako Doi¹, Chiaki Kurimoto¹, Shinsuke Uraki¹, Shuhei Morita¹, Yasushi Furukawa¹, Hidefumi Inaba¹, Hiroshi Iwakura¹, Toshio Shimokawa³, Tomoko Utsunomiya⁴ and Takashi Akamizu¹

¹ First Department of Internal Medicine, Wakayama Medical University, Wakayama 641-8509, Japan
² Division of Clinical Nutrition and Metabolism, Wakayama Medical University, Wakayama 641-8509, Japan
³ Clinical Study Support Center, Wakayama Medical University, Wakayama 641-8509, Japan
⁴ Utsunomiya Ladies Clinic, Wakayama 640-8331, Japan

Abstract. Thyroid dysfunction and thyroid autoimmunity (TAI) have been reported to be linked to infertility, pregnancy loss and preterm birth. Infertile women undergoing assisted reproductive technology are recommended to maintain thyroid stimulating hormone (TSH) levels below 2.5 μIU/mL. It is unclear, however, whether levothyroxine (L-T4) treatment decreases the effects of TAI on fertility and pregnancy outcome in infertile women. We therefore aimed to clarify the influence of TAI on pregnancy undergoing L-T4 treatment for hypothyroidism. Prospectively recruited to this study were the 595 infertile women who visited the Utsunomiya Ladies Clinic between January 2013 and December 2015. Five patients with Graves’ disease were excluded. Clinical profiles of 590 women were as follows: proportion of SCH = 19.6%, thyroid peroxidase antibody (TPOAb) positivity = 10.4%, and thyroglobulin antibody (TgAb) positivity = 15.1%. Fertility was not affected by any thyroid-associated factors. Regarding pregnancy outcomes, TPOAb titers were significantly higher in women who had miscarriage than in those progressed to delivery (46.4 ± 114.1 vs. 18.9 ± 54.6 IU/mL, p = 0.039), notably in those undergoing intrauterine insemination (p = 0.046) and in vitro fertilization (p = 0.023). Multivariate logistic regression analysis revealed that higher age (odds ratio 26.4, p < 0.001) and higher TPOAb titer (odds ratio 11.8, p = 0.043) were risk factors for miscarriage. Higher TPOAb titer should be considered as one of the risk factors for miscarriage in infertile women, even if they have been treated with L-T4 for hypothyroidism.

Key words: Subclinical hypothyroidism, Thyroid autoimmunity, Pregnancy, Infertility, Assisted reproductive technology

WOMEN OF REPRODUCTIVE AGE are predisposed to have thyroid dysfunction and thyroid autoimmunity (TAI) [1]. Regarding the association between thyroid dysfunction and pregnancy, overt hypothyroidism (OH) is known to increase adverse events during pregnancy [2]. In addition, subclinical hypothyroidism (SCH) has reported association with infertility and increased pregnancy loss [3-5]. TAI is known to affect pregnancy. Women with positive anti-thyroid antibodies have higher rates of preterm birth and pregnancy loss than those without such antibodies [6-8].

A randomized controlled trial by Negro et al. (2010) first reported that levothyroxine (L-T4) intervention reduced pregnancy loss in TPOAb-positive women with SCH [4]. ATA guidelines (2011) therefore recommended L-T4 supplementation for SCH to maintain thyroid stimulating hormone (TSH) levels above 2.5 μIU/mL in the first trimester [9]. ATA guidelines (2017) recommended another TSH cut-off value of 4.0 μIU/mL or upper reference range minus 0.5 μIU/mL. The most appropriate TSH cut-off value is still debatable [10].

Among women with SCH who underwent assisted reproductive technology (ART) in previous reports, L-T4 replacement led to higher delivery rates and lower rates of miscarriage [11-14]. To achieve TSH levels below 2.5 μIU/mL, L-T4 supplementation is therefore recommended for women undergoing ART [10]. Insufficient evidence exists, however, to determine whether L-T4 treatment for infertile women with TSH levels between 2.5 and 5.0 μIU/mL could positively affect pregnancy outcome when undergoing ART [10]. Regarding the effects of TAI on fertility treatments, some articles report...
that fertilization, pregnancy rate and live birth rate are lower in TPOAb-positive infertile women undergoing ART, but others do not [15-18]. TAI is associated with hypothyroidism, so it is unclear whether TAI itself or the subsequent thyroid dysfunction adversely affects pregnancy. L-T4 treatment for euthyroid women with TAI undergoing ART is therefore widely debated [11, 14, 17, 19].

Here, to assess the effects of TAI on pregnancy in infertile women under conditions of minimal influence of thyroid dysfunction, we prospectively evaluated the infertile women who underwent treatment with L-T4 at our fertility clinic to achieve TSH levels below 2.5 μIU/mL.

Materials and Methods

Patients

Prospectively recruited to this study were the 595 women who had visited the Utsunomiya Ladies Clinic, Wakayama, Japan for fertility treatment between January 2013 and December 2015. (Fig. 1 details patient flow). Infertility was defined as failure to achieve pregnancy after 12 months or more of regular unprotected intercourse. Thyroid function and thyroid autoantibodies were measured on the first clinic visit. Women either positive for anti-thyroid antibodies or with thyroid dysfunction were referred to the Wakayama Medical University Hospital. Twenty of the patients had hyperthyroidism; five of whom were diagnosed with Graves’ disease and 15 were diagnosed with painless thyroiditis [20]. Patients with Graves’ disease were excluded from this study and were treated with anti-thyroid drugs. Patients with painless thyroiditis were not excluded from this study because their thyroid dysfunction improved within three months. There were no patients suggestive of hypothyroidism due to thyroid stimulation blocking antibody. Finally, 590 women were included in this study. No subjects had history of thyroid disease, thyroid surgery, or thyroid hormone replacement therapy before enrollment. The women with TSH levels above 2.5 μIU/mL had thyroid function remeasured. OH was defined as TSH levels above 10.0 μIU/mL or between 2.5 and 10.0 μIU/mL in conjunction with decreased free thyroxine (fT4) according to 2011 ATA guidelines [9]. SCH was also defined as TSH level between 2.5 and 10.0 μIU/mL, but with normal fT4 levels. All hypothyroid patients followed-up at the Wakayama Medical University Hospital were administered L-T4 to maintain TSH levels below 2.5 μIU/mL. The remaining patients whose thyroid function recovered spontaneously were suspected to have iodine overload due to iodine overconsumption or use of iodinated contrast agents for salpingography.

Thyroid function tests were regularly performed for

595 infertile women

5 patients with Graves’ disease excluded*

590 infertile women enrolled for analysis**

233 women did not become pregnant: NAT & TIM (n = 228), IUI (n = 4) / ART (n = 1)

357 pregnant women were analyzed: NAT & TIM (n = 133), IUI (n = 80) / ART (n = 139), Others (n = 5) ***

3 women excluded due to unknown pregnancy outcome

Pregnancy outcome evaluated on April 2018

46 women had a miscarriage

308 women had a live birth

Fertility status evaluated on June 2017

* Patients with painless thyroiditis were included because its improvement was confirmed within three months.
** Levothyroxine was administered for patients with subclinical hypothyroidism or overt hypothyroidism.
*** Others include pregnant women with unknown treatment.

NAT, natural conception; TIM, timing therapy; IUI, intrauterine insemination; ART, assisted reproductive technology.

Fig. 1 Flow chart of the study
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euthyroid women with positive anti-thyroid antibodies during pregnancy. Primary outcome was fertility status as evaluated in June 2017. Secondary outcome was pregnancy outcome as evaluated in April 2018, by which time all pregnant women were scheduled to have reached full gestation. Patient clinical profiles were extracted from paper and electronic medical records, they included age, smoking status, fertility treatment, comorbidities, L-T4 treatment, laboratory data, and pregnancy outcome. Fertility status and pregnancy outcomes were confirmed based on the information from the medical records.

Fertility treatment groups were classified into the following four groups: natural conception (NAT), timing therapy (TIM), intruterine insemination (IUI), and ART. NAT group was composed of women who became pregnant spontaneously before any fertility treatment or during temporary interruption of the treatment. Women in TIM group were advised about optimal timing of sexual intercourse by their clinician after screening for disease related to infertility. IUI is a method of introducing sperm into the uterine cavity. ART comprises technology for achieving fertility, including in vitro fertilization (IVF), splitting, intracytoplasmic sperm injection (ICSI), and frozen embryo transfer (FET) with IVF/splitting/ICSI. Treatments were intensified in this order (except FET) when infertile women could not become pregnant. IVF is a method of combining eggs and sperm in vitro. ICSI is an extension of IVF in which a single sperm is directly injected into eggs under a microscope. Splitting is a combined method of IVF and ICSI. The fertility treatments immediately before fertilization were those used in analyses. Women who were treated with any of the ART techniques were included in ART group. The cause of infertility was divided into female factors and male factors, which were extracted from medical records, and fertility treatments were determined. Written informed consent was obtained from all patients, and the study protocol was approved by the Wakayama Medical University Ethics Committee (Approval No. 1167) in accordance with the Declaration of Helsinki.

Thyroid function tests and thyroid autoantibodies

At the first visit to the Utsunomiya Ladies Clinic, serum TSH, fT4, and free triiodothyronine (fT3) levels were measured by chemiluminescent immunoassay (ECLusys, Roche Diagnostics, Tokyo, Japan). Reference ranges were defined as follows: TSH: 0.50–5.00 μIU/mL, fT4: 0.90–1.70 ng/dL, and fT3: 2.3–4.0 pg/mL. Thyroid stimulating hormone receptor antibody (TRAb) was determined by enzyme-linked immunosorbent assay (TRAb Cosmic III, Cosmic, Tokyo, Japan). TgAb and TPOAb were measured using an electrochemiluminescent immunoassay (ECLusys, Roche Diagnostics, Tokyo, Japan). Normal values were defined as follows: TRAb: <1.0 IU/L, TgAb: <28 IU/mL, and TPOAb: <16 IU/mL. Values of TPOAb <6, >600 and TgAb <11 were calculated as 6, 600, and 11, respectively. The inter-assay CVs were 2.90 for TSH, 4.10 for fT4, 3.49 for fT3, 2.53 for TgAb, and 10.66 for TPOAb. When thyroid function was remeasured at the Wakayama Medical University, serum TSH, fT4, and fT3 levels were measured by chemiluminescent immunoassay (ARCHITECT, Abbott Diagnostics, Tokyo, Japan). Reference ranges were defined as 0.35–4.94 μIU/mL, fT4: 0.70–1.48 ng/dL, and fT3: 1.71–3.71 pg/mL. The inter-assay CVs were 5.33 for TSH, 5.30 for fT4, and 2.69 for fT3.

Statistical analysis

Fisher’s exact test or chi-square test were used to assess data in the two-dimensional contingency tables. Student’s t-test or Mann-Whitney U test was used for comparisons between the two groups, where applicable. Kruskal-Wallis test was used to compare more than three groups. Bonferroni adjustment was performed when comparing more than three groups. Multivariate logistic regression analysis with stepwise variable selection method was performed to identify risk factors for miscarriage. Data for TgAb and TPOAb were analyzed with log-transformed values. Two-tailed p-values <0.05 were considered statistically significant (JMP 14, SAS Institute Inc., Cary, NC, USA). Data were presented as mean ± standard deviation (SD).

Results

Clinical profiles of infertile patients

Clinical profiles of the 590 infertile women are shown in Supplemental Table 1. Mean age was 32.2 ± 3.8 years at their first visit to the Utsunomiya Ladies Clinic before fertility treatment. History of smoking was reported by 7.1% of the women. Thyroid function tests revealed 19.6% of infertile women had SCH and only one patient had OH. TPOAb was positive in 10.4%, TgAb was positive in 15.1%, and 6.0% of women were positive for both antibodies.

Fertility status

Fertility status could be affected by fertility treatments including IUI and ART, so when analyzing fertility status we considered the women receiving such treatments separately from those in NAT and TIM groups. Comparisons of thyroid-associated parameters and changes to fertility status in NAT and TIM groups are shown in Table 1. Women in the non-pregnancy group were significantly older than women in the pregnant group (32.4 ± 3.9 vs. 31.1 ± 3.6, p = 0.003). Other possible factors
TPOAb and TgAb titer values were written as median [25–75%] because they were not normally distributed. TSH levels above 2.5 μIU/mL at their first visit. Thyroid function tests shown in this table are the original measurement at Utsunomiya Ladies Clinic before starting fertility treatments. TSH, thyroid stimulating hormone; TPOAb, anti-thyroid peroxidase antibody; TgAb, anti-thyroglobulin antibody; SCH, subclinical hypothyroidism; OH, overt hypothyroidism; L-T4, levothyroxine; NA, not applicable

Affecting pregnancy, which included smoking, TSH, the presence of SCH, TAI, and L-T4 treatment, were not significantly different between the groups. Fertility status was also evaluated in women with IUI and ART, but we consider the results to be limited because 98% of the women became pregnant.

Pregnancy outcomes

Pregnant women whose clinical course could be followed (n = 354) were divided into two groups: those who progressed to delivery (delivery group; n = 308, 87.0%) and those who had a miscarriage (miscarriage group; n = 46, 13.0%) (Table 2). All deliveries were live births. Age was significantly higher in the miscarriage group than in the delivery group; 34.3 ± 3.3 vs. 31.8 ± 3.8 years (p < 0.001). Smoking history, thyroid function tests, TgAb, and L-T4 treatment were not significantly different between the groups, but TPOAb titer was notably significantly higher in the miscarriage group than in the delivery group (46.4 ± 114.1 vs. 18.9 ± 54.6 IU/mL, p = 0.039). TPOAb positivity was higher in the miscarriage group than in the delivery group, but not significantly. Multivariate logistic regression analysis revealed that higher age (odds ratio 26.4, p < 0.001) and high TPOAb titer (odds ratio 11.8, p = 0.043) were independent risk factors for miscarriage (Table 3).

We further analyzed the association between TPOAb titer and fertility treatment (Fig. 2). TPOAb titer was the highest in the IUI group, but not significantly different from the other treatment groups. Among women treated with IUI, the miscarriage group had significantly higher TPOAb titer than the delivery group (126.4 ± 156.8 IU/mL vs. 19.9 ± 62.2 IU/mL, p = 0.046). TPOAb titers in women treated with ART, on the other hand, were not significantly different between miscarriage and delivery groups (24.9 ± 50.1 IU/mL vs. 23.7 ± 58.6 IU/mL, p = 0.969).

To clarify the influence of TPOAb titer on pregnancy

### Table 1 Comparison of thyroid associated parameters and fertility status in women with natural conception or timing therapy

| Smoking history [n (%)] | Mean ± SD (range) | n | Mean ± SD (range) | n | p-value |
|-------------------------|-------------------|---|-------------------|---|---------|
| SCH*                    | 22 (16.5%)        | 133 | 43 (18.9%)        | 228 | 0.580*  |
| OH*                     | 0 (0%)            | 133 | 0 (0%)           | 228 | NA      |

| Antibody positivity     | TPOAb+ [n (%)]    | 11 (8.3%) | 132 | 16 (7.0%) | 228 | 0.633*  |
|                         | TgAb+ [n (%)]     | 16 (12.1%) | 132 | 27 (11.8%) | 228 | 0.768*  |
|                         | TPOAb+ and TgAb+ [n (%)] | 5 (3.8%) | 132 | 8 (3.5%) | 228 | 0.880*  |
|                         | TPOAb+ or TgAb+ [n (%)] | 21 (15.9%) | 132 | 35 (15.4%) | 228 | 0.864*  |
|                         | TPOAb– and TgAb– [n (%)] | 110 (83.3%) | 132 | 193 (84.6%) | 228 | 0.864*  |

| L-T4 treatment         | L-T4 (μg/day)     | 45.8 ± 18.8 (25–75) | 6 | 53.1 ± 26.2 (25–100) | 12 | 0.554*  |

Values of <6, <11, and >600 were calculated as 6, 11, and 600, respectively.

* Diagnosis of SCH and OH was decided after remeasurement of thyroid function tests at Wakayama Medical University in women with TSH levels above 2.5 μIU/mL at their first visit.

** TPOAb and TgAb titer values were written as median [25–75%] because they were not normally distributed.

Thyroid function tests shown in this table are the original measurement at Utsunomiya Ladies Clinic before starting fertility treatments.

TSH, thyroid stimulating hormone; TPOAb, anti-thyroid peroxidase antibody; TgAb, anti-thyroglobulin antibody; SCH, subclinical hypothyroidism; OH, overt hypothyroidism; L-T4, levothyroxine; NA, not applicable.
outcome depending on the ART methods, the ART group was subdivided into seven groups despite the limited number of patients (Supplemental Table 2). Women who underwent IVF had higher TPOAb titers in the miscarriage group than those in the delivery group (p = 0.023), but in women treated with splitting or ICSI there was no clear association between TPOAb titer and pregnancy outcome.

**Comparison between euthyroid women without L-T4 treatment and hypothyroid women treated with L-T4**

Women with TSH levels above 2.5 μIU/mL or TAI at their first visit had serum TSH remeasured at their second visit, and were regularly followed-up before and after gestation. Thyroid-associated factors were compared between euthyroid women without L-T4 treatment (Eu_LT4(−), n = 445) and hypothyroid women treated with L-T4 (Hypo_LT4(+), n = 43) (Supplemental Table 3). Serum TSH levels in hypothyroid women were

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### Table 2  Clinical profile of pregnant women showing pregnancy outcomes

|                  | Delivery (n = 308, 87.0%) | Miscarriage (n = 46, 13.0%) | p-value |
|------------------|---------------------------|-----------------------------|---------|
|                  | Mean ± SD (range)         | Mean ± SD (range)           |         |
| Smoking history  |                           |                             |         |
| [n (%)]          | 19 (6.2%) (24–41)         | 3 (6.5%) (28–39)            | 0.777a  |
| Age (years)      | 31.8 ± 3.8 (24–41)        | 34.3 ± 3.3 (28–39)         | <0.001b |
| TSH (μIU/mL)     |                           |                             |         |
| First visit      | 2.09 ± 1.29 (0.01–9.20)   | 2.34 ± 1.70 (0.47–7.18)    | 0.243b  |
| Before gestation | 1.44 ± 0.94 (0.16–4.67)   | 1.36 ± 0.75 (0.56–2.94)    | 0.827b  |
| First trimester  | 1.26 ± 0.86 (0.07–5.52)   | 1.62 ± 0.95 (0.42–3.51)    | 0.279b  |
| Detailed TSH [n (%)] |                 |                             |         |
| TSH ≤ 0.5        | 8 (2.6%)                  | 2 (4.3%)                   | 46 NA    |
| 0.5 < TSH ≤ 2.5  | 216 (70.6%)               | 30 (65.2%)                 | 46 NA    |
| 2.5 ≤ TSH ≤ 5.0  | 75 (24.5%)                | 10 (21.7%)                 | 46 NA    |
| 5.0 < TSH ≤ 10.0 | 7 (2.3%)                  | 4 (8.7%)                   | 46 NA    |
| TSH > 10.0       | 0 (0.0%)                  | 0 (0.0%)                   | 46 NA    |
| Thyroid state [n (%)] |                 |                             |         |
| SCH*             | 61 (19.9%)                | 10 (21.7%)                 | 46 0.722a |
| OH*              | 1 (0.3%)                  | 0 (0.0%)                   | 46 NA    |
| Antibody positivity |                 |                             |         |
| TPOAb+ [n (%)]   | 36 (11.7%)                | 8 (17.8%)                  | 45 0.248a |
| TgAb+ [n (%)]    | 52 (16.9%)                | 10 (21.7%)                 | 45 0.414a |
| TPOAb+ or TgAb+ [n (%)] |             | 5 (11.1%)                  | 45 0.353a |
| TPOAb– and TgAb– [n (%)] |        | 12 (26.7%)                 | 45 0.436a |
| Antibody titers** |                 |                             |         |
| TPOAb (IU/mL)    | 6.0 [6.0–10.0] (6–461)    | 7.0 [6.0–11.0] (6–593)     | 45 0.039a |
| TgAb (IU/mL)     | 15.0 [11.0–20.0] (11–2,522) | 16.5 [12.3–24.0] (11–499) | 46 0.498a |
| L-T4 treatment   |                           |                             |         |
| L-T4 (+) [n (%)] | 36.0 (11.7%)              | 8 (17.4%)                  | 46 0.274a |
| L-T4 dose (μg/day) | 45.0 ± 19.9 (12.5–100)   | 42.2 ± 24.0 (12.5–75.0)    | 8 0.726b |

*P-values* were obtained using *a*Fisher’s exact test, *b*student’s *t*-test or *c*Mann-Whitney *U*-test, and *p-values* < 0.05 were accepted as significant (written in bold).

Values of <6 and <11 were calculated as 6 and 11, respectively.

* Diagnosis of SCH and OH was decided after remeasurement of thyroid function tests at Wakayama Medical University in women with TSH levels above 2.5 μIU/mL at their first visit.

** TPOAb and TgAb titer values were written as median [25–75%] because they were not normally distributed.

Thyroid function tests shown in this table are the original measurement at Utsunomiya Ladies Clinic before starting fertility treatments.

TSH, thyroid stimulating hormone; TPOAb, anti-thyroid peroxidase antibody; TgAb, anti-thyroglobulin antibody; SCH, subclinical hypothyroidism; OH, overt hypothyroidism; L-T4, levothyroxine; NA, not applicable

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higher than in euthyroid women on their first and second visits before L-T4 treatment. They decreased to the same levels, however, immediately before gestation due to appropriate treatment with L-T4.

When focusing on TAI, TPOAb titers (87.4 ± 163.7 vs. 13.5 ± 42.4 IU/mL, \( p < 0.001 \)) and TPOAb positivity (37.2% vs. 7.4%, \( p < 0.001 \)) were significantly higher in the hypothyroid group than in the euthyroid group, but TgAb positivity was not different between the groups. Women positive for both TPOAb and TgAb were also associated with hypothyroidism. Fertility status and pregnancy outcomes did not differ between euthyroid women without L-T4 treatment and hypothyroid women treated with L-T4 (Supplemental Table 3).

Underlying disease as possible causes of infertility

The underlying diseases causing infertility associated with sex factors are listed in Supplemental Table 4. Ovarian factors were the most common of the female factors, followed by uterine factors, tubal factors, autoimmune disorder, and endometriosis. More than half of the couples undergoing ICSI had male infertility factors. Detailed clinical information from the medical records revealed that most of the reasons for applying for ICSI were due to male factors.

Discussion

When we began this study in 2013, L-T4 treatment was recommended for only TPOAb-positive women with TSH levels above 2.5 μIU/mL in the first trimester according to 2011 ATA guidelines [9]. There was no evidence, however, to support L-T4 treatment for TPOAb-negative women with SCH. It was still unclear whether L-T4 treatment decreases the effects of TAI on fertility and pregnancy outcome in infertile women. We therefore evaluated the effects of TAI on pregnancy under treatment with L-T4 for infertile women with TSH levels

| Table 3 Predictive factors for miscarriage in pregnant women |
|---------------------------------------------------------------|
| Odds ratio* | β | 95% CI | p-value*** |
| Age** | 26.4 | 0.192 | 5.4 | 129.1 | <0.001 |
| TSH** | 1.6 | 0.049 | 0.2 | 14.9 | 0.695 |
| TPOAb titer** | 11.8 | 0.004 | 0.2 | 128.0 | 0.043 |
| TgAb titer** | 0.0 | -0.001 | 0.0 | 163.6 | 0.378 |
| Smoking | | | | | |
| smoking vs. non-smoking | 0.9 | 0.026 | 0.2 | 3.6 | 0.938 |
| L-T4 treatment treatment vs. no treatment | 0.9 | 0.042 | 0.3 | 2.8 | 0.883 |

Bold \( p \)-values indicate statistically significant difference (\( p < 0.05 \)).

TSH, thyroid stimulating hormone; TPOAb, anti-thyroid peroxidase antibody; TgAb, anti-thyroglobulin antibody; L-T4, levothyroxine; CI, confidence interval.

* Odds ratio is presented with the ratio of maximum and minimum odds.
 ** Covariates were used for age, TSH, TPOAb titer, and TgAb titer.
 *** Multivariate logistic regression analysis was performed to identify risk factor for miscarriage.

Fig. 2 Comparison of thyroid peroxidase antibody titers between delivery (white box) and miscarriage (gray box) group in each type of infertility treatment. Median and 25th and 75th percentiles of thyroid peroxidase antibody titers are shown as a box plot. Kruskal-Wallis test was used to compare TPOAb titers among four groups, followed by post-hoc testing using Mann-Whitney \( U \) tests with a Bonferroni adjustment. Mann-Whitney \( U \) tests were used to compare pregnancy outcome in each treatment group.

NAT, natural conception; TIM, timing therapy; IUI, intrauterine insemination; ART, assisted reproductive technology. Error bars represent standard error. *\( p \)-values < 0.05 are considered significant.
above 2.5 μIU/mL to minimize possible adverse events associated with thyroid dysfunction.

Recently, some benefits of L-T4 supplementation for SCH women with TSH levels above 2.5 μIU/mL have been reported; miscarriage and premature birth are reportedly decreased [21, 22], and others have reported reduction of miscarriage in infertile women with ART [14]. In 2017 ATA guidelines, L-T4 supplementation for women undergoing ART with TSH levels above 2.5 μIU/mL is recommended [10], but the risk factors of miscarriage under L-T4 treatment had not been explored. In our report, we revealed that higher TPOAb titer was one of the risk factors for miscarriage, although infertile women were treated with L-T4 to maintain TSH levels below 2.5 μIU/mL.

In the current study, 19.6% of all infertile women had SCH, a greater frequency than in the general female population [23]. We compared fertility and pregnancy outcomes between euthyroid women without L-T4 treatment and hypothyroid women treated with L-T4, but there was no significant difference between the groups. These results might indirectly show benefits of L-T4 treatment for infertile women with TSH levels above 2.5 μIU/mL. We speculate that appropriate treatment for infertile women with SCH alleviated the adverse effects on pregnancy, as previously reported [12, 14]. Randomized controlled trials are needed to directly clarify the effect of L-T4 treatment, but we did not perform such trials owing to consideration of ethical issues, such as the possible risk of miscarriage.

TPOAb and TgAb were measured to assess the complication of TAI. According to reports from Europe, TPOAb is slightly more prevalent than TgAb; approximately 4% and 3%, respectively [24, 25]. In our study, the prevalence of both antibodies was higher than those previously reported and TgAb was prevalent. This tendency is similar to a previous report of an Asian population [26], so we speculate that the difference could be attributed to an ethnic variance.

Various studies have reported adverse effects of TAI on pregnancy [25, 27-30]. These reports suggest that TAI has associations with pregnancy outcomes including premature birth and miscarriage, but there is limited evidence of association between TAI and fertility status. In the present study, we also found no association between TAI and fertility status in women whether they used ART or not. When focusing on pregnancy outcomes, TPOAb titers were significantly higher in the miscarriage group than in the delivery group. High TPOAb titers could therefore cause adverse effects towards maintaining pregnancy, despite appropriate treatment for hypothyroidism in infertile women. TPOAb positivity was higher in the miscarriage group than in the delivery group, but this difference was not significant. The comparatively low number of patients with TPOAb in the delivery group could be attributed to this non-significance. On the contrary, we found that TgAb was not associated with fertility or pregnancy outcomes.

When focusing on the result of comparison between euthyroid women and hypothyroid women in our study, TPOAb positivity had significant association with hypothyroidism, but TgAb positivity did not. TPOAb is therefore more likely to cause thyroid dysfunction than TgAb. Accordingly, we speculate that the thyroid of women with TPOAb, but not those with TgAb, inadequately responds to the demand for thyroid hormones due to dynamic changes occurring in the mother during pregnancy. Previous meta-analyses reported that L-T4 treatment improved pregnancy outcomes in ART-treated women with SCH and positivity for TPOAb [15, 31].

Three hypotheses are proposed regarding association between TAI and miscarriage [32]. First, women with TAI have an underlying condition with imbalance of autoimmunity, which may lead to rejection of the fetus [33]. Th1-oriented changes of innate immunity and activation of NK and NK-T cells in women with TAI are reported to be associated with a negative impact on pregnancy [34-36]. TPOAb is also detected in the fluid of the oocyte and correlated with serum antibody levels [37]. A second hypothesis is that the thyroids of pregnant women with TAI have difficulty in responding to the hormonal changes that occur in pregnancy [38-41]. A third hypothesis is an association between TAI and infertility; for example, women with TAI are more likely to be older because they have had difficulty over a long period in conceiving [42]. We supplied L-T4 to patients with hypothyroidism in our study, so the influence associated with the second hypothesis could be minimized. The pregnancy outcome therefore probably reflected the mechanism of the first hypothesis. The precise mechanism of TAI causing miscarriage is still unclear, however, because multiple factors are related.

Association between TAI and fertility treatment has been well evaluated in women undergoing ART [15, 31, 41]. Although limited evidence exists concerning TAI and infertility in women treated with ART, TAI has a reported association with impaired quality of fertilized eggs and/or a negative effect on ovarian responsiveness [43, 44]. According to our fertility treatment strategy, infertile women who cannot become pregnant spontaneously are treated with TIM or IUI, followed by treatment with IUI or ART. Women with more difficult conditions for conception therefore accumulate in the intensified treatment group. TPOAb titers in the miscarriage group tended to increase when the fertility methods were changed from TIM to IUI in our study, although not
significantly (Fig. 2). This trend suggests that pregnancy outcomes of women who need advanced fertility treatment are likely to be affected by TAI with high TPOAb titers. When applying the first hypothesis on TAI and miscarriage, it is possible that high TPOAb titer suggests a maternal condition with activated autoimmune environment, leading to the discontinuation of pregnancy.

Among women with IUI, TPOAb titers in the miscarriage group were significantly higher. As a fertility treatment, IUI is considered to be closer to natural sexual intercourse than ART. Since IUI theoretically exposes fertilized eggs to underlying autoimmune conditions in order to achieve gestation, we speculate that IUI is more likely to be affected by TAI than by ART. Only one previous report has evaluated the association between IUI and TAI [45]. It showed no relation between TPOAb positivity and pregnancy outcomes in women undergoing IUI, but TPOAb titers were not evaluated. Further investigation is needed to clarify this association.

We also confirmed that IVF-treated women whose pregnancy ended in miscarriage had higher rate of TPOAb titers than those who progressed to delivery, but by a small number of cases. We speculated that TPOAb titer could remain a predictive factor for miscarriage after L-T4 supplementation for hypothyroidism, but a larger number of patients is needed for conclusive results.

TAI has also been discussed in association with miscarriage in women treated with ICSI. Recent meta-analysis by Poppe (2018), however, showed that TAI is not a risk factor for miscarriage when infertile women were treated with ICSI [46]. They therefore proposed ICSI as their recommended ART technique for women with TAI. In the present study, TPOAb positivity and titers were not significantly different between delivery group and miscarriage group in women undergoing ICSI or FET/ICSI, in contrast with those treated with IUI or IVF. This result is compatible with the previous report [46].

The underlying causes of infertility are another possible factor that may affect pregnancy outcomes in women treated with ICSI. ICSI is a technique of injecting sperm into the cytoplasm of eggs under a microscope, the principle reason for its application is severe male disease related to infertility, such as asthenozoospermia or oligozoospermia. The effect of male factors on pregnancy could therefore overwhelm that of female factors including TAI.

As far as we know, only a single report by Chai et al. (2014) [47] has evaluated the association between titers of TPOAb and miscarriage rate in infertile women. TPOAb titers reportedly did not affect the live birth rate or the miscarriage rate in women who underwent IVF/ICSI cycle (splitting). In our study, women treated with splitting and ICSI also showed no significant difference of TPOAb titers between delivery group and miscarriage group, but IUI- and IVF-treated women showed higher TPOAb titers in the miscarriage group than in the delivery group. Further investigation is needed to evaluate the influence of TPOAb titers on delivery outcomes according to each treatment.

Our study has several limitations. The evaluation of fertility status in women undergoing ART could be limited because more than 95% of the women achieved gestation. Statistical power could be low when assessing the pregnancy outcomes of women undergoing ART due to subdivision into seven groups with a small number of patients. A larger number of patients is needed to draw stronger statistical results.

In conclusion, infertile women with high TPOAb titers are susceptible to miscarriage despite appropriate L-T4 treatment. TPOAb titers as well as TPOAb positivity should be considered when fertility treatments are administered. Further investigations with more patients are required to establish conclusive evidence.

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References

1. De Groot L, Abalovich M, Alexander EK, Amino N, Barbour L, et al. (2012) Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 97: 2543–2565.
2. Abalovich M, Gutierrez S, Alcaraz G, Maccallini G,
Garcia A, et al. (2002) Overt and subclinical hypothyroidism complicating pregnancy. *Thyroid* 12: 63–68.

3. Abalovich M, Mitelberg L, Allami C, Gutierrez S, Alcaraz G, et al. (2007) Subclinical hypothyroidism and thyroid autoimmunity in women with infertility. *Gynecol Endocrinol* 23: 279–283.

4. Negro R, Schwartz A, Gismondi R, Tinelli A, Mangieri T, et al. (2010) Increased pregnancy loss rate in thyroid antibody negative women with TSH levels between 2.5 and 5.0 in the first trimester of pregnancy. *J Clin Endocrinol Metab* 95: E44–E48.

5. Yoshioka W, Amino N, Ide A, Kang S, Kudo T, et al. (2015) Thyroxine treatment may be useful for subclinical hypothyroidism in patients with female infertility. *Endocr J* 62: 87–92.

6. Chen L, Hu R (2011) Thyroid autoimmunity and miscarriage: a meta-analysis. *Clin Endocrinol (Oxf)* 74: 513–519.

7. Thangaratnam S, Tan A, Knox E, Kilby MD, Franklyn J, et al. (2011) Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. *BMJ* 342: d2616.

8. Dhillon-Smith RK, Middleton LJ, Sunner KK, Cheed V, Baker K, et al. (2019) Levothyroxine treatment may be useful for thyroid peroxidase antibodies before conception. *N Engl J Med* 380: 1316–1325.

9. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, et al. (2011) American Thyroid Association Taskforce on Thyroid Disease During Pregnancy and Postpartum. 2011 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* 21: 1081–1125.

10. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, et al. (2017) 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. *Thyroid* 27: 315–389.

11. Negro R, Mangieri T, Coppola L, Presicce G, Casavola EC, et al. (2005) Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study. *Hum Reprod* 20: 1529–1533.

12. Rahman AHA, Abbasy HA, Abbasy AAE (2010) Improved in vitro fertilization outcomes after treatment of subclinical hypothyroidism in infertile women. *Endocr Pract* 16: 792–797.

13. Kim CH, Ahn JW, Kang SP, Kim SH, Chae HD, et al. (2011) Effect of levothyroxine treatment on in vitro fertilization and pregnancy outcome in infertile women with subclinical hypothyroidism undergoing in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril* 95: 1650–1654.

14. Rao M, Zeng Z, Zhou F, Wang H, Liu J, et al. (2019) Effect of levothyroxine supplementation on pregnancy loss and preterm birth in women with subclinical hypothyroidism and thyroid autoimmunity: a systematic review and meta-analysis. *Hum Reprod Update* 25: 344–361.

15. Toulis KA, Goulis DG, Venetis CA, Kolibianakis EM, Negro R, et al. (2010) Risk of spontaneous miscarriage in euthyroid women with thyroid autoimmunity undergoing IVF: a meta-analysis. *Eur J Endocrinol* 162: 643–652.

16. Tan S, Dieterle S, Pechlavanis S, Janssen OE, Fuhrer D (2014) Thyroid autoantibodies per se do not impair intracytoplasmic sperm injection outcome in euthyroid healthy women. *Eur J Endocrinol* 170: 495–500.

17. Łukaszuk K, Kunicki M, Kulwikowska P, Liss J, Pastuszek E, et al. (2015) The impact of the presence of antithyroid antibodies on pregnancy outcome following intracytoplasmic sperm injection-ICSI and embryo transfer in women with normal thyreotropine levels. *J Endocrinol Invest* 38: 1335–1343.

18. Busnelli A, Paffoni A, Fedele L, Somigliana E (2016) The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis. *Hum Reprod Update* 22: 775–790.

19. Wang H, Gao H, Chi H, Zeng L, Xiao W, et al. (2017) Effect of levothyroxine on miscarriage among women with normal thyroid function and thyroid autoimmunity undergoing *in vitro* fertilization and embryo transfer: a randomized clinical trial. *JAMA* 318: 2190–2198.

20. Takeshima K, Inaba H, Furukawa Y, Nishi M, Yamaoka H, et al. (2014) Elevated serum immunoglobulin G4 levels in patients with Graves’ disease and their clinical implications. *Thyroid* 24: 736–743.

21. Hernández M, López C, Soldevilla B, Ceeccarro L, Martínez-Barahona M, et al. (2018) Impact of TSH during the first trimester of pregnancy on obstetric and foetal complications: usefulness of 2.5 mIU/L cut-off value. *Clin Endocrinol (Oxf)* 88: 728–734.

22. Li J, Liu A, Liu H, Li C, Wang W, et al. (2019) Maternal TSH levels at first trimester and subsequent spontaneous miscarriage: a nested case-control study. *Endocr Connect* 8: 1288–1293.

23. Kasagi K, Takahashi N, Inoue G, Honda T, Kawachi Y, et al. (2009) Thyroid function in Japanese adults as assessed by a general health checkup system in relation with thyroid-related antibodies and other clinical parameters. *Thyroid* 19: 937–944.

24. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, et al. (2002) Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 87: 489–499.

25. Umuane D, Velkeniers B, Anckaert E, Schietecatte J, Tournaye H, et al. (2013) Thyroglobulin autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment? *Thyroid* 23: 1022–1028.

26. Chen CW, Huang YL, Huang RL, Tzeng CR, Chen CH (2017) Idiopathic low ovarian reserve is associated with more frequent positive thyroid peroxidase antibodies. *Thyroid* 27: 1194–1200.

27. Poppe K, Glinser D, Van Steirteghem A, Tournaye H,
Devroey P, et al. (2002) Thyroid dysfunction and autoimmunity in infertile women. Thyroid 12: 997–1001.

28. Van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JAM, et al. (2011) Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update 17: 605–619.

29. Karakosta P, Alegakis D, Georgiou V, Roumeliotaki T, Fthenou E, et al. (2012) Thyroid dysfunction and autoantibodies in early pregnancy are associated with increased risk of gestational diabetes and adverse birth outcomes. J Clin Endocrinol Metab 97: 4464–4472.

30. Bliddal S, Feldt-Rasmussen U, Rasmussen ÅK, Kolte AM, Hilsted LM, et al. (2019) Thyroid peroxidase antibodies and prospective live birth—a cohort study of women with recurrent pregnancy loss. Thyroid 29: 1465–1474.

31. Stewart-Akers AM, Krasnow JS, Brekosky J, DeLoia JA (1998) Endometrial leukocytes are altered numerically and functionally in women with implantation defects. Am J Reprod Immunol 39: 1–11.

32. Poppe K, Velkeniers B, Glinoer D (2007) Thyroid disease and female reproduction. Clin Endocrinol (Oxf) 66: 309–321.

33. Matalon ST, Blank M, Ornoy A, Shoenfeld Y (2001) The association between anti-thyroid antibodies and pregnancy loss. Am J Reprod Immunol 45: 72–77.

34. Miko E, Meggyes M, Doba K, Farkas N, Bogar B, et al. (2017) Characteristics of peripheral blood NK and NKT-like cells in euthyroid and subclinical hypothyroid women with thyroid autoimmunity experiencing reproductive failure. J Reprod Immunol 124: 62–70.

35. Kwak-Kim JY, Chung-Bang HS, Ng SC, Ntrivalas EI, Mangubat CP, et al. (2003) Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. Hum Reprod 18: 767–773.

36. Seshadri S, Sunkara SK (2014) Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. Hum Reprod Update 20: 429–438.

37. Monteleone P, Parrini D, Faviana P, Carletti E, Casarosa E, et al. (2011) Female infertility related to thyroid autoimmunity: the ovarian follicle hypothesis. Am J Reprod Immunol 66: 108–114.

38. Glinoer D, Riahi M, Grün JP, Kinthaert J (1994) Risk of subclinical hypothyroidism in pregnant women with asymptomatic autoimmune thyroid disorders. J Clin Endocrinol Metab 79: 197–204.

39. Poppe K, Glinoer D, Tourneay H, Devroey P, van Steirteghem A, et al. (2003) Assisted reproduction and thyroid autoimmunity: an unfortunate combination? J Clin Endocrinol Metab 88: 4149–4152.

40. Korevaar TI, Steegers EA, Pop VJ, Broeren MA, Chaker L, et al. (2017) Thyroid autoimmunity impairs the thyroidal response to human chorionic gonadotropin: two population-based prospective cohort studies. J Clin Endocrinol Metab 102: 69–77.

41. Hou Y, Liu A, Li J, Wang H, Yang Y, et al. (2019) Different thyroidal responses to human chorionic gonadotropin under different thyroid peroxidase antibody and/or thyroglobulin antibody positivity conditions during the first half of pregnancy. Thyroid 29: 577–585.

42. Velkeniers B, Van Meerhaeghe A, Poppe K, Unuane D, Tourneay H, et al. (2013) Levothyroxine treatment and pregnancy outcome in women with subclinical hypothyroidism undergoing assisted reproduction technologies: systematic review and meta-analysis of RCTs. Hum Reprod Update 19: 251–258.

43. Magri F, Capelli V, Gaiti M, Brambilla E, Montesion L, et al. (2013) Impaired outcome of controlled ovarian hyperstimulation in women with thyroid autoimmune disease. Thyroid 23: 1312–1318.

44. Andrisani A, Sabbadin C, Marin L, Ragazzi E, Dessaule F, et al. (2018) The influence of thyroid autoimmunity on embryo quality in women undergoing assisted reproductive technology. Gynecol Endocrinol 34: 752–755.

45. Unuane D, Velkeniers B, Bravenboer B, Drakopoulos P, Tourneay H, et al. (2017) Impact of thyroid autoimmunity in euthyroid women on live birth rate after IUI. Hum Reprod 32: 915–922.

46. Poppe K, Autin C, Veltri F, Kleyne N, Grabczan L, et al. (2018) Thyroid autoimmunity and intracytoplasmic sperm injection outcome: a systematic review and meta-analysis. J Clin Endocrinol Metab 103: 1755–1766.

47. Chai J, Yeung WY, Lee CY, Li HW, Ho PC, et al. (2014) Live birth rates following in vitro fertilization in women with thyroid autoimmunity and/or subclinical hypothyroidism. Clin Endocrinol (Oxf) 80: 122–127.