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Thyroglobulin Autoantibodies: Is There Any Added Value in the Detection of Thyroid Autoimmunity in Women Consulting for Fertility Treatment?

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Background: Thyroid autoimmunity (TAI) is frequent in infertile women, but to what extent thyroglobulin autoantibodies (Tg-Abs) contribute to TAI is unclear in the literature. The aims of the present study were to determine the prevalence of TAI in women consulting for fertility problems and to investigate the impact of isolated Tg-Abs, isolated thyroid peroxidase autoantibodies (TPO-Abs), and the presence of both autoantibody types on thyroid function. Furthermore, thyroid function was compared between women with and without TAI and between infertile and fertile women.

Methods: A cross-sectional data analysis nested within an ongoing prospective cohort study was performed in order to determine the prevalence of TAI in unselected women consulting our tertiary referral center for reproductive medicine (CRM). The women underwent a determination of serum thyrotropin (TSH), free thyroxine (FT4), TPO-Abs, and Tg-Abs. The cause of infertility, age, body-mass index (BMI), and smoking habits were recorded.

Results: The prevalence of TAI was 16% (163/992). In 8% of cases, both types of autoantibodies were present, in 5% isolated positive Tg-Abs were found, and 4% had isolated positive TPO-Abs ($p = 0.025$ and $p = 0.003$ respectively). The prevalence of TAI was significantly higher in infertile women as compared to that in fertile controls (19% vs. 13%; $p = 0.047$). The median serum TSH level was significantly higher in the women with TAI and with isolated positive Tg-Abs compared to that in women without TAI (1.83 [1.44] and 1.90 [0.85] vs. 1.47 [0.94] mIU/L; $p < 0.001$ respectively). The median FT4, age, BMI, and smoking habits were comparable between the study groups.

Conclusions: The prevalence of TAI was higher in infertile women as compared to fertile women consulting our CRM. Five percent of the women had isolated positive Tg-Abs and a significantly higher serum TSH compared to that in women without TAI.

Introduction

Thyroid autoimmunity (TAI) is common in women of reproductive age, and the presence of thyroid autoantibodies increases the risk of developing (sub)clinical hypothyroidism during pregnancy (1,2). Infertility, particularly women with polycystic ovary syndrome and idiopathic infertility, has been associated with an increased prevalence of TAI (3,4). Furthermore, the presence of TAI has been associated with an increased risk of a first trimester miscarriage and premature delivery both in spontaneous pregnancies and pregnancies after assisted reproductive technology (ART) (5,6).

Increased levels of thyroid peroxidase autoantibodies (TPO-Abs) have been defined as the most sensitive marker of TAI, and are associated with hypo- or hyperthyroidism, which is not the case for the presence of thyroglobulin autoantibodies (Tg-Abs). In the 1988–1994 National Health and Nutrition Examination Study (NHANES III) performed in more than 13,000 individuals without thyroid disease or any treatment that could have influenced thyroid function, TPO-Abs, Tg-Abs, and both TPO-Abs and Tg-Abs were detected in 4.4%, 3.4%, and 6.9% of the subjects respectively. However, a closer look at these data reveals that in women between 30 and 40 years of age, the presence of Tg-Abs seems to be slightly predominant, and this finding was confirmed in a Danish study in women of reproductive age (7,8).

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Most studies investigating the prevalence of TAI in infertile women or in relation to pregnancy outcomes identified TAI associated with the presence of TPO-Abs only and not with that of Tg-Abs (3,9,10). Nonetheless, in one recent study, the presence of isolated positive Tg-Abs was associated with recurrent miscarriages (11).

The main objectives of our study were therefore to investigate the prevalence of TAI as measured by the presence of Tg-Abs and TPO-Abs in a prospective cohort of women consulting at our center for reproductive medicine (CRM), and to determine the number of women with isolated positive Tg-Abs. The prevalence of TAI in infertile women was also compared with that in fertile women. Finally, the presence of thyroid antibodies was correlated with thyroid function parameters, the types of infertility, age, body-mass index (BMI), and smoking habits.

Patients and Methods

Overall study design

The current cross-sectional data analysis is nested within an ongoing prospective cohort study, conducted in collaboration with the CRM of the University Hospital of Brussels (Belgium) and approved by the institutional review board. Nine hundred ninety-two women consulting at the CRM over a period of 6 months in 2011 were systematically screened for thyroid dysfunction (serum thyrotropin [TSH] and free thyroxine [FT4]) and for the presence of TAI by means of TPO-Abs and Tg-Abs.

The diagnosis of an underlying thyroid disease was further evaluated based on the personal history of goiter, known thyroid diseases, and/or prior use of thyroid medication. Hereby, 16 women were excluded from further thyroid function assessment. Thyroid function was determined in two groups of women (with and without TAI) as well as in subgroups of TAI-positive women with isolated positive Tg-Abs or TPO-Abs, and in women positive for both autoantibodies. The prevalence of increased and decreased serum TSH, using reference values of a normal nonpregnant population (respectively >4.2 mIU/L and <0.27 mIU/L), was compared between the different groups. The analyses were extended using the TSH cut-off values for first trimester pregnant women (<2.5 mIU/L). The prevalence of women with TSH between 0.27 mIU/L and <2.5 mIU/L and with TSH between >2.5 mIU/L and <4.2m IU/L was estimated in the different groups.

The patients were referred to the CRM by their gynecologist. A complete gynecological work-up was performed to identify the causes of infertility, including medical history, gynecological examination, transvaginal ultrasonography, hormonal profile, screening for infectious disease, and, when indicated, hystero-salpingography and/or laparoscopy. Accordingly, patients were classified into one of the following diagnostic categories: endometriosis, tubal disease, or ovulation disorders (OD) as causes of female infertility. Abnormal semen analysis led to the diagnosis of male infertility, after excluding all the identifiable female causes of infertility. In the case of a normal spermatogram and in the absence of female infertility, the couple was considered to have idiopathic infertility. Some women consulted the CRM for alternative reasons besides infertility (such as egg cell donation, single-parent request, same-sex couples, among others). Together with women in whom the male partner was the cause of the infertility, they were used as a fertile control group. For the comparison of the prevalence of TAI between infertile women and the fertile control group, women lost for follow-up were excluded ($n = 67$). Important confounding demographic characteristics such as age, smoking habit, and BMI were documented for all patients.

Serum assay

All provisions were implemented by the laboratory of hormonology and tumor markers of our institution. Serum TSH, FT4, Tg-Abs, and TPO-Abs were measured using the Elecsys electrochemiluminescence immunoassays on a Cobas 6000 immunoanalyzer (Roche Diagnostics, Mannheim, Germany). The reference values were 0.27–4.2 mIU/L for TSH, 9.3–17.0 ng/L (12–23.2 pmol/L; 1 ng/L = 1.29 pmol/L) for FT4, <115 IU/mL for Tg-Abs, and <34 kIU/L for TPO-Abs.

The Elecsys TPO-Ab assay was performed on samples from 208 healthy test subjects in three clinical centers in Austria and Germany, and in 95% of the population, values were below 34 IU/L. The Elecsys Tg-Ab assay cut-off was determined in five clinical centers covering a total of 392 healthy subjects. The threshold value of 115 IU/mL corresponds to the 95th percentile.

The within-run and the total imprecision CVs were ≤3% and <7.5% for TSH, ≤2% and <5% for FT4, <5% and <6.5% for Tg-Abs, and <6.5% and <10% for TPO-Abs. There is no information available on the epitope patterns of Tg-Abs detected by the widely used commercial Tg-Ab immunoassay used in the current study (Roche Diagnostics).

Statistical analysis

Categorical data are presented as number of cases and/or percentages. Continuous data are presented as mean and standard deviation (SD) or median and interquartile range (IQR).

Differences between groups were analyzed by chi-square or Fisher's exact tests for categorical data and by Mann–Whitney *U*-test for continuous data. Correlations between continuous variables were quantified using Spearman's rho correlation coefficient.

The impact of cause of infertility and potential confounding effects of age (years), BMI (kg/m^2), and smoking status (smoking/nonsmoking) on TAI positivity status were explored by fitting logistic regression models. Likewise, potential confounding effects of age (years), BMI (kg/m^2), and smoking status (smoking/nonsmoking) on TSH were explored by fitting multivariable regression models on log-transformed TSH values.

All statistical tests were considered significant whenever $p < 0.05$. Computational procedures were performed using Microsoft Office Excel 2003 and IBM SPSS Statistics version 20.

Results

Thyroid autoimmunity

Figure 1 shows the prevalence of TAI in all screened women. Before the exclusion of 16 women treated with levothyroxine (LT4) and 67 women lost for infertility work-up, the prevalence of women with both positive TPO-Abs and Tg-Abs was significantly higher compared to that of women with

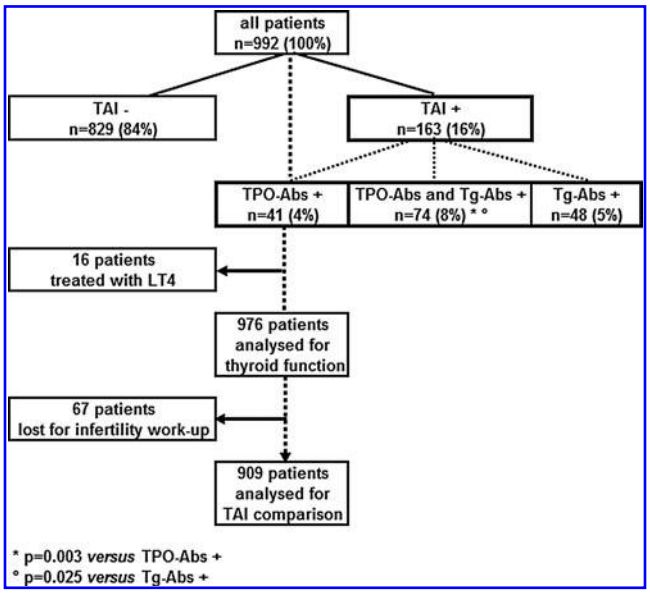

FIG. 1. Prevalence of thyroid autoimmunity (TAI) in all patients screened, and flowchart of further analyses of thyroid function and infertility work-up. TPO-Abs, thyroid peroxidase autoantibodies; Tg-Abs, thyroglobulin autoantibodies.

isolated positive Tg-Abs or TPO-Abs (8% vs. 5% and 4%; $p=0.025$ and $p=0.003$ respectively). The prevalence of women with isolated positive Tg-Abs was comparable to that in women with only TPO-Abs (5% vs. 4%; not significant).

In women with TAI, Tg-Abs levels were categorized as follows: 46% had titers between 115 and 200 kIU/L, 18% between 201 and 300 kIU/L, 16% between 301 and 400 kIU/L, and 20% >401 kIU/L. Concerning TPO-Ab levels, 25% of the women had titers between 34 and 100 kIU/L, 25% between 101 and 200 kIU/L, 25% between 201 and 300 kIU/L, and 25% >301 kIU/L. In both patients with positive Tg-Abs and positive TPO-Abs, the median TSH values were comparable between the different categories of thyroid autoantibodies (data not shown).

Table 1 shows the prevalence of TAI in the fertile controls (bottom row) and according to cause of infertility. Women treated with LT4 ($n=16$) and women lost for infertility work-up ($n=67$) were excluded from this analysis. Female causes (including ovarian dysfunction, tubal disorders, and endometriosis) accounted for 39% of all studied patients. In 11% of the women, no cause was determined after full work-up (i.e., idiopathic infertility). Women without infertility problems (male infertility), together with women consulting the CRM for other than infertility reasons (same-sex couples, egg cell donation, preimplantation genetic diagnosis, etc.) accounted for 50% of the cohort and were used as fertile controls. The prevalence of TAI was significantly higher in the group of women with identifiable female causes of infertility, compared to that in the fertile controls (19% vs. 13%; $p=0.047$). Logistic regression analyses confirmed that this finding was similar when simultaneously adjusting for age, BMI, and smoking status ($p=0.016$). The prevalence of TAI in the idiopathic group was comparable to that in the fertile controls (14% vs. 13%).

Thyroid function

Table 2 shows thyroid function and demographic characteristics of all women after excluding 16 women under LT4 treatment and after stratification according to the TAI status (826 women with TAI and 150 without TAI). Median [IQR] serum TSH was significantly higher in women with TAI, compared to that in women without TAI (1.83 [1.44] mIU/L vs. 1.47 [0.94] mIU/L; $p<0.001$). Serum TSH was also significantly higher in women with isolated positive Tg-Abs and in patients positive for both Tg-Abs and TPO-Abs (1.90 [0.85] mIU/L and 1.86 [1.59] mIU/L; $p<0.001$ and $p=0.012$, respectively). In the women with isolated positive TPO-Abs, serum TSH was comparable to that in women without TAI (1.38 [1.56] mIU/L vs. 1.47 [0.94] mIU/L; not significant). Serum FT4 levels were comparable in all groups. Demographic data, age, BMI, and smoking habits were comparable between the different groups, as shown in Table 2. When comparing women with infertility with fertile controls, BMI and smoking status did not differ significantly ($p=0.53$ and 0.14, respectively). Fertile control women were 2.5 years younger on average ($p<0.001$).

Serum TSH levels were positively and significantly correlated with TPO-Ab levels but not with Tg-Ab levels (Spearman's rho = 0.09, $p=0.008$ and Spearman's rho = 0.04, $p=0.22$, respectively). The magnitude of these correlation coefficients was low.

Multivariable analyses confirmed that previous findings were similar when simultaneously adjusting for age, BMI, and smoking status (data not shown).

Table 1. Prevalence of Thyroid Autoimmunity in the Different Causes of Infertility and in Fertile Controls

Cause of infertility	*All patients (n = 909)*	*TAI− (n = 768)*	*TAI+ (n = 141)*	*TAI+, TPO-Abs+, Tg-Abs+ (n = 61)*	*TAI+, isolated TPO-Abs+ (n = 36)*	*TAI+, isolated Tg-Abs+ (n = 44)*
Ovarian	230 (25)	189 (82)	41 (18)	17 (42)	10 (24)	14 (34)
Tubal	61 (7)	47 (77)	14 (23)	5 (36)	3 (21)	6 (43)
Endometriosis	65 (7)	52 (80)	13 (20)	10 (77)	2 (15)	1 (8)
Female infertility[a]	356 (39)	288 (81)	68 (19)*	32 (9)	15 (4)	21 (6)
Idiopathic	95 (11)	82 (86)	13 (14)	2 (3)	6 (6)	5 (5)
Fertile controls[b]	458 (50)	398 (87)	60 (13)	27 (6)	15 (3)	18 (4)

Data values are presented as number (percentage).
[a]Group pooling the identifiable causes of female infertility.
[b]Group pooling the alternative reasons (egg cell donation, single-parent request, *etc.*) and the male infertility cause.
*$p=0.047$ versus fertile controls.
TAI, thyroid autoimmunity; TPO-Abs, thyroid peroxidase autoantibodies; Tg-Abs, thyroglobulin autoantibodies.

Table 2. Thyroid Function and Demographic Characteristics According to Thyroid Autoimmunity Status

	All patients (n = *976*)	*TAI−* (n = *826*)	*TAI+* (n = *150*)	*TAI+, TPO-Abs+, Tg-Abs+* (n = *63*)	*TAI+, isolated TPO-Abs+* (n = *40*)	*TAI+, isolated Tg-Abs+* (n = *47*)
TSH (mlU/L)	1.49 [1.02]	1.47 [0.94]	1.83 [1.44][a]	1.86 [1.59][b]	1.38 [1.56]	1.90 [0.85][a]
FT4 (ng/L)	11.8 [1.9]	11.8 [1.9]	11.7 [2.2]	11.8 [2.1]	11.5 [2.7]	11.8 [2.1]
Age (years)	32.5 ± 5.6	32.6 ± 5.6	32.4 ± 5.5	31.8 ± 5.0	34.1 ± 6.1	31.7 ± 5.4
BMI (kg/m^2)	24.6 ± 5.2	24.6 ± 5.3	24.4 ± 5.0	23.4 ± 5.0	24.5 ± 5.0	25.6 ± 4.8
Smokers (%)	21	22	15	22	9	12

Data values are presented as median [IQR], mean ± standard deviation, or percentage.
[a] $p < 0.001$ vs. TAI.
[b] $p = 0.012$ vs. TAI.
TSH, thyrotropin; FT4, free thyroxine; BMI, body-mass index; IQR, interquartile range.

Interestingly, the effects of age, BMI, and smoking status on log-transformed TSH values were not statistically significant (with p values of 0.17, 0.12, and 0.65, respectively).

Table 3 shows the prevalence of women with and without TAI according to categorical serum TSH groups. Women with TAI had a significantly higher proportion of high normal TSH levels (2.5–4.2 mIU/L) and increased serum TSH levels (>4.2 mIU/L) compared to the fraction in women without TAI (21% and 8% vs. 12% and 1%; $p = 0.017$ and $p < 0.001$, respectively). In the subgroup, with women with isolated positive Tg-Abs or TPO-Abs, no statistically significant higher prevalence of one of these TSH subgroups was present compared to women without TAI.

Table 4 shows thyroid function in the fertile controls (bottom two rows) and according to cause of infertility. Median serum TSH and FT4 levels were comparable between all study groups.

Discussion

In the present study, 5% of an unselected group of women consulting for infertility and/or other reproductive procedures had isolated positive Tg-Abs, associated with a significantly higher median serum TSH compared to TAI-negative women and a higher proportion of women with TSH values >2.5 mUI/L, considered to be the ideal cut-off for first pregnancy trimester, that should not be spanned before pregnancy (3). Thyroid dysfunction and/or the presence of TAI have both been associated with worse pregnancy outcomes, including miscarriage, recurrent miscarriage, preterm delivery, low birth weight, and postpartum thyroiditis in both spontaneous and assisted pregnancies (3,5,12). Ovarian stimulation used in the preparation of an ART procedure is known to increase the need of thyroid hormones in women with TAI and those with hypothyroidism (13–16). Moreover, intervention trials with LT4 in pregnant women with TAI have documented a relative risk reduction in the miscarriage rate and premature delivery, comparable to the risk observed in women without TAI, but the data are not consistent (17).

In most studies addressing thyroid disorders and its effect on reproduction, the definition of TAI was based primarily on the presence of TPO-Abs, without taking into account the presence of Tg-Abs. A possible explanation for this might be that most authors consider Tg-Abs to be less sensitive in the detection of TAI compared to TPO-Abs, data supported by large epidemiological studies in the general population such as the NHANES study and a Dutch study that documented a higher prevalence of TPO-Abs compared to that of Tg-Abs (13% and 24% vs. 11.5% and 7%, respectively) (7,18). The additional cost of Tg-Abs measurement may further impede their determination as markers of the presence of TAI in the patient population of reproductive age. Yet, data such as a Danish study in which women between 25 and 30 years were included found 14% Tg-Abs versus 12% TPO-Abs in this young female population—in line with the present findings (19).

This discrepancy in the prevalence of Tg-Abs among different studies may be related to the iodine content of Tg, with a lower prevalence of Tg-Abs in iodine deficient regions (20). We did not determine urinary iodine in our study, but a recent survey showed that Belgian women in the reproductive age group still have relative iodine deficiency, despite bread fortification with iodized salt. Therefore, the hypothesis linking iodine and Tg-Abs does not support the higher prevalence of Tg-Abs in our study (21).

Table 3. Prevalence of Women With and Without Thyroid Autoimmunity According to Categorical Serum Thyrotropin Groups

Serum TSH	*All patients* (n = *976*)	*TAI−* (n = *826*)	*TAI+* (n = *150*)	*TAI+, TPO-Abs+, Tg-Abs+* (n = *63*)	*TAI+, isolated TPO-Abs+* (n = *40*)	*TAI+, isolated Tg-Abs+* (n = *47*)
TSH <0.27 mIU/L	1	1	2	3	3	0
TSH 0.27–2.5 mIU/L	83	86	69	62	70	77
TSH 2.5–4.2 mIU/L	14	12	21[a]	27[a]	18	17
TSH >4.2 mIU/L	2	1	8[b]	8	10	6

Data values are presented as percentages.
[a] $p = 0.01$ vs. TAI.
[b] $p < 0.001$ vs. TAI.

Table 4. Thyroid Function in the Different Causes of Infertility and in Fertile Controls

Cause of infertility	*All patients* (n = *909*)	*TAI−* (n = *768*)	*TAI+* (n = *141*)	*TAI+, TPO-Abs+, Tg-Abs+* (n = *61*)	*TAI+, isolated TPO-Abs+* (n = *36*)	*TAI+, isolated Tg-Abs+* (n = *44*)
Ovarian						
TSH (mlU/L)	1.51 [4.73]	1.49 [4.73]	1.80 [3.88]	1.63 [2.78]	1.48 [3.17]	1.91 [3.42]
FT4 (ng/L)	11.6 [9.8]	11.6 [9.8]	11.6 [5.9]	12.9 [5.9]	11.2 [3.6]	11.6 [4.0]
Tubal						
TSH (mlU/L)	1.40 [27.1]	1.23 [4.34]	2.17 [27.01]	3.01 [27.09]	1.28 [4.19]	1.87 [1.55]
FT4 (ng/L)	11.6 [9.9]	12.0 [6.7]	10.8 [9.9]	10.5 [9.9]	10.8 [2.5]	10.9 [4.4]
Endometriosis						
TSH (mlU/L)	1.43 [3.94]	1.43 [3.60]	1.20 [3.47]	1.53 [3.47]	1.97 [1.62]	0.66 [0.00]
FT4 (ng/L)	11.8 [13.8]	11.8 [6.0]	12.5 [12.8]	11.7 [12.8]	13.2 [4.0]	16.9 [0.0]
Female infertility[a]						
TSH (mlU/L)	1.50 [27.36]	1.45 [4.73]	1.83 [27.36]	1.88 [27.36]	1.38 [4.44]	1.90 [3.77]
FT4 (ng/L)	11.7 [16.8]	11.8 [9.8]	11.5 [16.8]	12.3 [16.8]	11.3 [4.5]	11.5 [8.3]
Idiopathic						
TSH (mlU/L)	1.57 [7.0]	1.46 [5.23]	1.82 [7.01]	3.52 [7.01]	1.33 [1.82]	2.05 [1.60]
FT4 (ng/L)	12.0 [13.9]	11.8 [9.8]	12.8 [12.4]	18.0 [10.3]	13.5 [2.1]	11.4 [2.1]
Fertile controls[b]						
TSH (mlU/L)	1.52 [63.17]	1.49 [9.45]	1.87 [63.17]	1.86 [6.47]	1.92 [63.17]	1.75 [3.27]
FT4 (ng/L)	11.8 [13.8]	11.8 [8.9]	11.8 [13.8]	11.6 [8.1]	11.4 [13.8]	12.5 [5.5]

Data values are presented as median [IQR].
[a]Group pooling the identifiable causes of female infertility.
[b]Group pooling the alternative reasons (egg cell donation, single-parent request, *etc.*) and the male infertility cause.

The NHANES study and the study by Strieder *et al.* reported a positive correlation between TPO-Abs levels and thyroid function (7,18). Titers of TPO-Abs are related to the degree of lymphocytic infiltration of the thyroid gland, potentially explaining the correlation between TPO-Abs and thyroid function in those studies (22). Although we observed a significant positive (yet weak) correlation between serum TSH levels and TPO-Abs levels (but not with Tg-Abs levels), serum TSH levels were not significantly higher in women with isolated TPO-Abs compared to women without TAI. A thorough medical history was performed in all patients, and excluded the possibility of treatment leading to lower TSH values in patients with TPO-Abs as a confounding factor for this finding.

However, women with isolated positive Tg-Abs and those testing positive for both types of autoantibodies had significantly higher serum TSH levels compared to those in women without TAI. This observation may predispose them to the development of (sub)clinical hypothyroidism during ovarian stimulation, interfering with normal ovarian function, pregnancy rate after ART, and subsequent pregnancy outcomes (14,16,23). The prevalence of women with a cut-off value for TSH >2.5 mUI/L was indeed higher in patients with isolated positivity for Tg-Abs and in the group with both positive Tg-Abs and TPO-Abs compared to that in women without TAI. Michalakis *et al.* also detected a high prevalence (23%) of serum TSH levels between 2.5 and 4.2 mIU/L in women undergoing ART. However, the authors did not investigate the prevalence of TAI in their cohort (24). Moreover, the presence of Tg-Abs may be involved in miscarriage independently from their effect on thyroid hormone function (11,25). The association between Tg-Abs and serum TSH levels did not result from differences in age, BMI, and smoking habits according to our multivariable analysis.

It remains to be proven whether the presence of Tg-Abs has a negative impact on pregnancy outcomes using a prospective cohort design and also whether intervention with LT4 in these patients might change the most important outcome—live birth. In the study by Kim *et al.*, the prevalence of TPO-Abs was 80% in the treated group with subclinical hypothyroidism, and 20% had Tg-Abs. The pregnancy outcomes were significantly better in women receiving LT4 compared to those in the placebo group, and this was independent of the type of Abs. The intervention groups were small, however, with only 32 patients included (26). The most recent study also took into account the presence of Tg-Abs, and showed a higher prevalence of recurrent miscarriage in women with Tg-Abs compared to those with only TPO-Abs, and a direct pathogenic role of Tg-Abs was postulated by some authors (11). Studies looking at epitope recognition of Tg-Abs are scarce. A recent publication using a panel of Tg monoclonal autoantibodies showed that epitope patterns of Tg-Abs might be used as a prediction marker of the progression in patients with Hashimoto's thyroiditis (27). Distinct commercial Tg-Ab assays used in different studies might detect Tg-Abs with other epitope patterns. There is no information available on the epitope patterns of Tg-Abs detected by the widely used commercial Tg-Ab immunoassay used in the current study (Roche Diagnostics), nor is this information available for many other commonly used Tg-Ab assays. Moreover, the techniques to detect epitope specificities used in the study by Liu *et al.* are not available in a routine clinical laboratory. Therefore, based on the current study, we cannot answer whether the presence of Tg-Abs has any pathogenic significance. In the near future, pregnancy outcomes of these women will be available, and this may shed light on this issue. In the present study, we document a high prevalence of TAI (19%) in women with female causes of infertility (endometriosis, tubal disorders, and ovulatory disorders)—significantly higher compared to that in the fertile controls. These data confirm and extend the results of a previous study in which we compared the presence of TAI between fertile and infertile women (28). The reason for the increased prevalence of TAI in infertile

women remains largely speculative. It is known that endometriosis is associated with (non)organ specific autoimmune diseases, and that in women with polycystic ovary syndrome (PCOS), a higher ratio of estrogens over progesterone may be an explanation for the higher level of autoimmunity associated with endometriosis (29,30). Other reasons that could explain an increased prevalence of TAI in women with PCOS are polymorphisms in the thyroid adenoma associated protein (THADA) and the presence of thyroid autoantibodies in ovarian follicles (23,31). Thyroid function, as reflected by TSH levels, has also been associated with insulin resistance in women with PCOS, independently of age and BMI (32). Our multivariable analysis did not support the independent effect of BMI on thyroid function in a selected population of women consulting for infertility and/or an ART procedure. As to why women with tubal disorders have a high prevalence of TAI remains also speculative, but many of these patients have had infectious diseases (such as tuberculosis and/or Chlamydia trachomatis), a potential trigger for the development of (thyroid) autoimmunity (33). Furthermore in women with TAI and infertility, an impaired cellular and humeral immune response has been documented (34,35). In contrast to the results of a recent meta-analysis, indicating that women with idiopathic infertility have a high prevalence of TAI, the women in our study with idiopathic infertility had a prevalence of TAI comparable to that of the fertile controls. A referral bias cannot be excluded because our CRM is a tertiary center and the prevalence of idiopathic infertility is rather low. Smoking has been inversely correlated with the presence of Tg-Abs, but the reasons for this observation have not yet been established (18). Cessation of smoking has also been associated with the development of TAI, but we were unable to obtain this particular information from our present cohort (36,37). Although women with isolated positive Tg-Abs and isolated positive TPO-Abs seemed to smoke less, there was no significant difference in smoking habits compared with the TAI negative group.

The determination of TAI by measuring Tg-Abs and TPO-Abs may be proposed as a standard procedure in the work-up of infertile couples, since it may ultimately lead to the detection of women at risk for developing an elevated serum TSH level before and during an ART procedure and subsequently during pregnancy (38). Presence of these autoantibodies may alternatively be linked to negative pregnancy outcomes, independent of thyroid function. According to the recent Endocrine Society guidelines on thyroid screening during pregnancy, infertility is one of the risk factors that should be taken into account when opting for targeted screening, and this study adds evidence in favor of screening for autoimmunity by adding the presence of Tg-Abs to that of TPO-Abs (39). However, until persuasive arguments such as an altered pregnancy outcome are available, the additional cost of Tg-Abs may further impede their determination as a marker of TAI.

In conclusion, TAI is more frequent in infertile women compared to fertile controls. Five percent of women consulting at our CRM had isolated positive Tg-Abs and a higher serum TSH compared to women without TAI. These TAI-positive patients would have been missed by the measurement of TPO-Abs only. Further prospective studies in women with Tg-Abs evaluating the evolution of their thyroid function during pregnancy and subsequent pregnancy outcomes are, however, still warranted.

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