A relook at the relevance of thyroid stimulating hormone and thyroid autoimmunity for pregnancy outcomes: Analyses of randomized control trials data from Pregnancy in Polycystic Ovary Syndrome and Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation

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Objective: We examined if thyroid autoimmunity is relevant to the relationship between maternal thyroid stimulating hormone (TSH) levels and pregnancy outcomes.
Design: Retrospective cohort analysis of data from 2 randomized controlled trials (RCTs).
Subjects: Participants of the Pregnancy in Polycystic Ovary Syndrome (PPCOS II, n = 746) and the Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS, n = 832 with unexplained infertility) RCTs.
Exposure: Pre-RCT intervention levels of TSH at threshold of ≥2.0 mU/L and thyroid peroxidase antibody (TPO-Ab) at titer threshold of ≥30 U/mL.
Main Outcome Measures: Live birth (primary outcome), pregnancy loss, and preterm birth (secondary outcomes). Generalized linear model (GLM) analyses examined the relationship between exposure to TSH and TPO-Ab at specified thresholds with the specified outcomes; covariates adjusted for included age, body mass index, race, ethnicity, education, smoking, duration of infertility, PCOS (vs. unexplained infertility), and randomized intervention arm in the respective RCTs.
Results: On adjusted analyses, live birth was significantly reduced in the exposed population (those with TSH ≥2.0 mU/L and TPO-Ab ≥30 U/mL, n = 117/1,578, 7.4%, adjusted risk ratio [ARR]: 0.55; 95% CI: 0.35–0.87) compared with the unexposed (those with TSH <2.0 mU/L and TPO-Ab <30 U/mL, n = 865/1,578, 54.8%). Furthermore, the risk of pregnancy loss and of early preterm birth (<32 weeks) was significantly higher in the exposed compared with the unexposed (ARR for pregnancy loss was 1.66; 95% CI: 1.14–2.42, and ARR for early preterm birth was 4.82, 95% CI: 1.53–15.19).
Conclusion: In women with TPO-Ab titers ≥30 U/mL, pregnancy outcomes may be compromised at TSH threshold of ≥2 mU/L. These findings of an interaction between TSH and TPO for pregnancy outcomes merit further investigation in prospective studies.

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El resumen está disponible en Español al final del artículo.

Key Words: Thyroid autoimmunity, TSH, adverse pregnancy outcomes, PCOS, unexplained infertility

Overt hypothyroidism increases the risk of pregnancy complications, including pregnancy loss, preterm birth, and low birth weight (1), and occurs in approximately 0.5% of pregnancies (2). Subclinical abnormalities of thyroid function and serologic evidence of thyroid autoimmunity are more prevalent than overt thyroid dysfunction (2). Subclinical hypothyroidism (SCH) has been associated with pregnancy loss and preterm birth, albeit inconsistently (3–11). Furthermore, in euthyroid women, serologic evidence of thyroid autoimmunity independent of overt thyroid dysfunction has also been associated with pregnancy loss (4, 12–15) and with preterm birth (13, 16, 17), but again inconsistently (8).

A diagnosis of SCH is based on thyroid stimulating hormone (TSH) level that is above the upper limit of normal range for assay with serum-free thyroxine (T4) concentration that is within the normal range. Despite a recognition of SCH as an entity that is distinct from overt hypothyroidism, agreement is lacking regarding a single threshold level of TSH that would reflect SCH for the general population. Until recently, for reproductive-age women planning to pursue fertility, it has been common clinical practice to consider empiric initiation of thyroid supplementation at TSH level >2.5 mU/L despite inconsistent evidence to support the benefit of such an intervention (18–20). This doctrine of initiating thyroid supplement at TSH level >2.5 mU/L in fertility seeking women underwent stringent scrutiny by a taskforce of experts and led to an update to the Practice Committee Opinion of the American Society for Reproductive Medicine on the topic with explicit recommendations that diagnosis of SCH in nonpregnant pregnancy seeking population should only be made based on laboratory-specific cutoffs for TSH levels (21). This updated document represents a consensus based on a critical review of the available evidence for defining criteria for SCH in fertility seeking populations. However, despite this consensus, the statements "Although there are limited high-quality data available, consistent trends in the literature allow for the guidelines set forth in this document" and "Additional clinical trials evaluating the screening and treatment of SCH in infertile patients and in patients receiving IVF therapies could result in a change in recommendations" reflect not just the limitations of existing data, but also convey a recognition that the status quo on the topic remains susceptible to being reshaped by future studies (21).

Given both discrepancies as well as trends in existing literature regarding the relationship between prepregnancy TSH levels and thyroid autoimmunity with pregnancy outcomes, we theorized an interaction between thyroid autoimmunity and thyroid function in their relationship to adverse pregnancy outcomes. Pursuing a hypothesis that TSH should be interpreted in the context of coexisting thyroid autoimmunity when examining what constitutes optimal thyroid function in fertility seeking women, we conducted a retrospective cohort analysis on data from 2 multicenter, double-blinded, randomized control trials undertaken in 2 well-characterized populations of otherwise healthy infertile women for whom TSH levels and titers for thyroid peroxidase antibody (TPO-Ab) were available to concomitantly examine a relevance of thyroid function (as reflected by TSH level) and thyroid autoimmunity for pregnancy outcomes. The Pregnancy in Polycystic Ovary Syndrome trial (PPCOS II) enrolled infertile women who met the Rotterdam criteria for polycystic ovary syndrome (PCOS) (22) and compared effectiveness of clomiphene citrate (CC) vs. letrozole (LET) in achieving successful conception and live birth (LB). The Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) clinical trial in a population of women with unexplained infertility compared the effectiveness of three different treatment modalities (CC vs. LET vs. gonadotropins) in achieving successful conception and LB (23). We investigated the association of TSH at varying threshold levels in relation to TPO positivity to determine if we can improve the identification of women at risk of adverse pregnancy outcomes by combining the two thyroid indices (TSH above a threshold level and TPO positivity) compared with using each thyroid index individually.

MATERIALS AND METHODS

This study was approved by the Reproductive Medical Network Steering Committee. Deidentified data (demographic information, participant data, clinical trial data including study drug assignment and outcomes, as well as data for TSH levels and TPO-Ab titers) were made available for analyses. Based on the use of deidentified existing data, this study was deemed exempt from review by the central institutional review board and by the Yale School of Medicine Institutional Review Board.

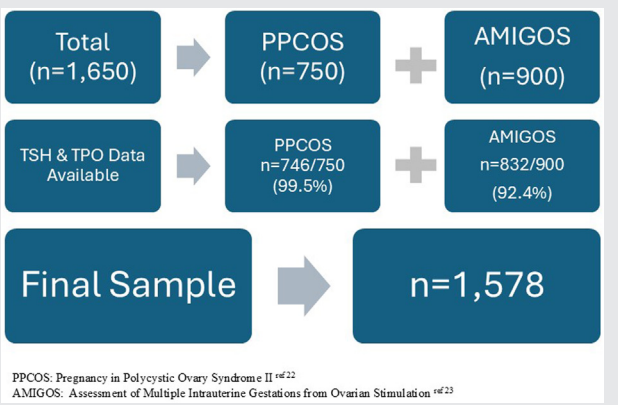
Study population

Details about the methods, design, and participant recruitment for the two multicenter prospective, double-blind RCTs, the PPCOS II (clinicaltrials.gov NCT00719186) (22) and the AMIGOS (clinicaltrials.gov NCT01044862) (23) have been extensively published, and are briefly reviewed here. Pregnancy in Polycystic Ovary Syndrome II (n = 750) compared the effectiveness of 2 commonly used ovulation induction strategies (CC vs. letrozole) vs. placebo for the treatment of ovulatory infertility in reproductive-age women with PCOS. Assessment of Multiple Intrauterine Gestations

from Ovarian Stimulation (n = 900) compared the effectiveness of aromatase inhibitor vs. CC vs. gonadotropins in women undergoing intrauterine insemination.

Data on TSH levels and TPO-Ab titers were available for 746 of 750 (99.5%) participants in PPCOS II and 832 of 900 (92.4%) in the AMIGOS; a total of 1,578 participant data were available for this study. The assay methodologies for TSH and TPO-Ab have been previously detailed (14). Briefly, the assays were performed on blood samples that were collected before initiation of trial-related intervention and had been stored at −80°C. The assays were performed centrally at the Ligand Assay Core laboratory at the University of Virginia (Immulite 2000 system, Siemens) in batch (14). The intraassay and interassay CV's for TSH were 3.6% and 4.7%, respectively; interassay CV for TPO-Ab at three specific titer thresholds were 10% at 20 U/mL and 5% at both 70 U/mL and 200 U/mL.

FIGURE 1



Population sample.

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Data analysis

Pregnancy was defined as a rising serum level of β unit of human chorionic gonadotropin (β-hCG) on 2 consecutive tests (AMIGOS) or a serum β-hCG level of >10 IU/mL (PPCOS II). Clinical pregnancy was defined based on a transvaginal ultrasound at 6–9 weeks gestation, demonstrating an intrauterine pregnancy with the presence of fetal cardiac activity. All ongoing pregnancies were followed by medical record review until completion, either as miscarriage or delivery. Pregnancy loss was defined as any loss at or before 20 weeks of pregnancy, including biochemical losses; pregnancy loss was further categorized as early (1st trimester, before 13 weeks) or late (between 13 and 20 weeks) for subanalyses. LB was defined as the delivery of a live-born infant after 20 weeks of gestation. Based on gestational age at delivery, births were categorized as preterm if gestation at delivery was <37 weeks; preterm birth was further categorized as early (<32 weeks) and late (between ≥32 weeks and <37 weeks) preterm birth for secondary analyses. Births at ≥37 weeks of gestation were defined as term birth.

LB was deemed as the primary outcome of interest; pregnancy loss and preterm birth were examined as secondary outcomes of interest. Thyroid stimulating hormone and TPO-Ab were examined *together* in relation to the outcomes at varying thresholds specified further in statistical methods section.

Statistical methods

Participants with missing data for TSH or TPO-Ab (0.5% in PPCOS II and 7.6% in the AMIGOS) were excluded from the analyses (Fig. 1).

Data distributions for TSH levels and TPO-Ab titers are shown in Supplementary Figures 1 and 2. Visual examination of scatterplots for TSH levels (Supplementary Fig. 1, available online) and TPO-Ab titers (Supplementary Fig. 2, available online) were used to determine cutoff points for TSH at a threshold of ≥2.0 mU/L. Given evidence relating thyroid autoimmunity with risk for miscarriage risk (4, 5), locally estimated scatterplot smoothing (LOWESS) plots (24) examined for relationship between TPO-Ab titers and pregnancy loss; a linearity in this relationship was evident at TPO-Ab level of ≥30 U/mL (Supplementary Fig. 3, available online) and hence this threshold was used for primary analyses.

For the primary analyses, the population was categorized into 3 groups (A, B, and C) based on the specified cutoffs for TSH and TPO-Ab. Group A (exposed, n = 117) comprised those with TSH ≥2 mU/L and TPO-Ab titer ≥30 U/mL; group B comprised women with TSH <2 mU/L and TPO-Ab titer <30 U/mL (unexposed, n = 596). The remaining population included those with TSH ≥2 mU/L but TPO-Ab level <30 U/mL, or those with TSH <2 mU/L and TPO-Ab level ≥30 U/mL comprised group C (others, n = 865).

Demographics including age, race, ethnicity, duration of infertility, smoking status, and education level, as well as clinical characteristics including infertility diagnosis (PCOS vs. unexplained infertility), body mass index (BMI), blood pressure, and antimüllerian hormone levels were examined in relation to TSH and TPO-Ab at specified cutoff levels.

Continuous data were examined using correlation analyses (Pearson's for Gaussian and Spearman for skewed data), student's *t*-test or ANOVA (Gaussian distribution), or Mann- Whitney *U* or Kruskal-Wallis Rank Sum tests (skewed continuous data). Categorical data were examined using χ^2, Fisher's exact, and Kruskal-Wallis Rank Sum tests.

Multivariable generalized linear model (GLM) analyses examined the association of exposure (TSH ≥2 mU/L and TPO-Ab ≥30 U/mL) with the specified outcomes. Because of the small number of occurrences of early preterm birth (<32 weeks), a propensity score adjustment was used to ensure the validity of the statistical model (25). The covariate selection for inclusion in the multivariable models was based on biological plausibility and $P<.20$ for association with the specified outcomes on univariate analyses. Covariates included in the final models were age, BMI, duration of infertility, race, ethnicity, education, smoking, PCOS diagnosis (vs. unexplained infertility), and RCT assigned intervention arm.

Two sets of sensitivity analyses were conducted to further gain clarity on the relationship between thyroid

autoimmunity, TSH level, and pregnancy outcomes. The first set of sensitivity analyses examined if thyroid autoimmunity (TPO-Ab titers ≥30 IU/mL) independent of TSH held any relevance for the specified outcomes and to contrast if and how the observed magnitude of association differed when thyroid autoimmunity was considered with concomitant TSH at ≥2.0 mU/L. A second set of sensitivity analyses examined TSH at a higher threshold value of ≥2.5 mU/L in conjunction with TPO-Ab ≥30 U/mL in relation to the specified outcomes; our a priori assumption was that the directionality of associations will hold, and the magnitude of associations will amplify, albeit at the cost of loss of statistical significance because of smaller sample size (Supplementary Fig. 1).

Results for continuous data are presented as mean ± standard deviation or median (interquartile range [IQR]), and categorical data are presented as numbers (percentage). The magnitude of associations is reported as risk ratio (RR) or adjusted RR (ARR) and 95% confidence interval (95% CI). Stata 13.0 (College Station, TX) and SAS 9.4 (Raleigh, NC) were used for analyses. A two-tailed *P* value <.05 was considered statistically significant for the univariate analyses. Given multiple outcomes of interest (LB, pregnancy loss, preterm birth – early and late), *P*<.012 was deemed as threshold for statistical significance for multivariable GLM analyses (0.05/4) to account for multiplicity of examined outcomes.

RESULTS

Complete demographic data and TSH and TPO-Ab data were available for 99.5% of the PPCOS II (746 of 750) and 92.4% of the AMIGOS (832 of 900) populations for a combined total of 1,578 women (Fig. 1).

Table 1 provides an overview of demographic and participant characteristics based on the specified exposure (TSH and TPO-Ab thresholds). Group A includes those with TSH ≥2 mU/L and TPO-Ab titer ≥30 U/mL (exposed). Group B includes those with TSH<2 mU/L and TPO-Ab titer<30 U/mL (unexposed). Group C includes the remaining population with variable levels of TSH and TPO who do not meet specifications for classification under groups A and B (other).

Populations across groups of exposure were comparable in age and ovarian reserve but differed by RCT assignment, race, BMI, and smoking status (Table 1). The exposed were significantly more likely to have unexplained infertility and

TABLE 1

Demographic and clinical characteristics of participants in the analytic cohorts for which data on TSH and TPO-Ab levels were available.

Participant characteristics	Exposed[a] TSH ≥2 mU/L and TPOs ≥30 U/mL n=117 (7.4%)	Unexposed[b] TSH <2 mU/L and TPOs <30 U/mL n=865 (54.8%)	Other[c] Variable TSH/TPO N=596 (37.8%)	*P* value
Clinical trial, n (%)				.045[e]
PPCOS, n (%)	43 (36.8)	409 (47.3)	294 (49.3)	
AMIGOS, n (%)	74 (63.2)	456 (52.7)	302 (50.7)	
Age, (y)	31.05 ± 4.172	30.47 ± 4.57	30.89 ± 4.61	.296
AMH, ng/mL	3.0 (1.5–7.2)	3.5 (1.8–6.4)	3.3 (1.6–6.5)	.836
Race, n (%)				<.001[e]
White	91 (78)	479 (71.7)	433 (72.5)	
Black	5 (4.3)	55 (8.2)	44 (7.4)	
Asian	5 (4.3)	33 (4.9)	27 (4.5)	
Other race[d]	0 (0)	22 (3.3)	18 (3.0)	
Ethnicity, n (%)				.267
Hispanic	16 (13.7)	127 (14.7)	79 (11.8)	
Non-Hispanic	101 (86.3)	738 (85.3)	589 (88.2)	
Education level, n (%)				.337
High school or less	12 (10.3)	137 (15.8)	91 (15.2)	
College graduate or some college	87 (74.4)	567 (65.5)	382 (64.0)	
Graduate degree	18 (15.4)	161 (18.6)	124 (20.8)	
Infertility duration (mo)	38.6 ± 32.0	37.5 ± 31.3	38.9 ± 33.6	.742
Smoking status, n (%)				.031[e]
Current	6 (5.1)	109 (12.6)	60 (10.0)	
Quit	28 (23.9)	235 (27.2)	161 (27.0)	
Never	83 (70.9)	521 (60.2)	375 (63)	
BMI (kg/m^2)	30.7 ± 8.8	30.0 ± 8.1	31.8 ± 9.9	.048[e]
Blood Pressure (mm Hg)				
Systolic	116.45 ± 12.9	118.1 ± 13.1	117.2 ± 12.6	.275
Diastolic	75.84 ± 9.5	75.8 ± 9.7	75.62 ± 9.5	.918
TSH mU/L	3.3 ± 1.3	1.3 ± 0.4	2.6 ± 1.0	<.001[e]
Anti-TPO U/mL	195 (52–523)	10 (10–15)	12.5 (10–22)	<.001[e]

AMH = antimüllerian hormone; AMIGOS = Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation; BMI = body mass index; PPCOS = Pregnancy in Polycystic Ovary Syndrome; TPO-Ab = thyroid peroxidase antibody; TSH = thyroid stimulating hormone.
[a] Exposed—those with TSH value ≥2 mU/L and with anti-TPO titer ≥30 U/mL.
[b] Unexposed—those with TSH value <2 mU/L and with anti-TPO <30 U/mL or negative.
[c] Population excluded from multivariable analyses that did not meet specifications for exposed or unexposed.
[d] Includes American Indian, Native Hawaiian, and more than one race.
[e] Statistically significant.

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belong to white race, and significantly less likely to have PCOS, be of black race, and be current smokers ($P<.05$ for each of the specified associations).

Table 2 presents results of univariate analyses examining associations of predetermined exposure with the specified outcomes. The likelihood of attainment of LB differed significantly between the three groups ($P=.014$). Infertile women in the exposed group (group A) were 36% less likely to achieve clinical pregnancy ($P<.05$) and 47% less likely to achieve LB ($P<.05$) compared with the referent population (comprised of groups B and C); RR and 95% CI for clinical pregnancy and LB for the exposed group were 0.64 (0.43–0.95) and 0.53 (0.33–0.84), respectively, Table 2. Furthermore, the risk of pregnancy loss differed significantly between the groups ($P=.021$); those in the exposed group had nearly twice the risk of pregnancy loss compared with the referent population (RR: 1.92; 95% CI: 1.2–2.89; $P=0.002$).

Results of multivariable GLM analyses are presented in Table 3. After adjusting for the specified covariates, those in the exposed group were significantly less likely to achieve LB (primary outcome, ARR: 0.55; 95% CI: 0.35–0.87) and were significantly more likely to experience pregnancy loss (secondary outcome, ARR: 1.66; 95% CI: 1.14–2.4). The overall risk for preterm birth (<37 weeks of gestation at delivery) was higher in the exposed compared with the referent population; the difference was not of statistical significance ($P=.178$); however, the risk of early preterm birth (<32 weeks of gestation at delivery) was nearly 5 times higher in the exposed than in the referent population (ARR: 4.82; 95% CI: 1.53–15.19).

Sensitivity analyses

The likelihood of attaining LB was significantly lesser in those with evidence of isolated thyroid autoimmunity (TPO-Ab ≥30 U/mL) after adjusting for TSH in addition to the earlier specified covariates vs. those in whom TPO-Ab was either undetectable or titers were <30U/mL (RR: 0.70; 95% CI: 0.52–

TABLE 2

Clinical trial outcomes of the analytic cohorts for which data on TSH and TPO-Ab levels were available.

Clinical trial outcomes	Exposed[a] TSH ≥2 mU/L and TPOs ≥30 U/mL n = 117 (7.4%)	Unexposed[b] TSH < 2 mU/L and TPOs < 30 U/mL n = 865 (54.8%)		Other[c] Variable TSH/TPO N = 596 (37.8%)	*P* value
Achieved pregnancy[d]					.266[e]
Yes n (%)	34 (29.0)	317 (36.6)		217 (36.4)	
No n (%)	83 (71.0)	548 (63.4)		379 (63.6)	
Relative risk of pregnancy	*0.79 (0.59–1.06)*		*Reference*		*.125*
Clinical pregnancy[f]					.061[e]
Yes n (%)	22 (19.0)	253 (29.0)		166 (28.0)	
No n (%)	95 (81.0)	612 (71.0)		430 (72.0)	
Relative risk of clinical pregnancy	0.64 (0.43–0.95)		*Reference*		*.027*[h]
Pregnancy loss[g]					.021[e,h]
Yes n (%)	7 (32)	29 (11.5)		21 (12.6)	
No n (%)	15 (68)	224 (88.5)		145 (87.4)	
Relative risk of pregnancy loss	1.91 (1.27–2.89)		*Reference*		*.002*[h]
Live birth					.014[e,h]
Yes n (%)	16 (13.7)	225 (26.0)		145 (24.3)	
No n (%)	101 (86.3)	640 (74.0)		451 (75.7)	
Relative risk of live birth	*0.53 (0.33–0.84)*		*Reference*		*.007*[h]
Preterm birth					.153[e]
Early preterm birth (<32 wk gestation) n (%)	3 (18.7)	7 (3.1)		6 (4.1)	
Relative risk of early preterm birth	*6.00 (1.71–21.02)*		*Referent*		*.005*[h]
Late preterm birth (≥32 and <37 wk gestation) n (%)	5 (31.3)	41 (18.3)		20 (13.6)	
Relative risk of late preterm birth	*1.71 (0.78–3.71)*		*Referent*		*.718*

Note: Participant information is presented based on exposure groups: Group A includes those with TSH ≥2 mU/L and TPO-Ab titer ≥30 U/mL (exposed). Group B includes those with TSH <2 mU/L and TPO-Ab titer <30 U/mL (unexposed). Group C includes the remaining population with variable levels of TSH and TPO who do not meet specifications for classification under groups A and B (other). Data are presented as numbers (%), and magnitude of association is presented as relative risk (95% confidence interval) as calculated by GLM analysis.
TPO = thyroid peroxidase; TSH = thyroid stimulating hormone.
[a] Exposed—those with TSH value ≥2 mU/L and with anti-TPO titer ≥30U/mL.
[b] Unexposed—those with TSH value <2 mU/L and with anti-TPO <30 U/mL or negative.
[c] Population excluded from multivariable analyses that did not meet specifications for exposed or unexposed.
[d] Positive pregnancy test.
[e] *P* value for group difference (Kruskal-Wallis Rank Sum test).
[f] Ultrasound evidence of intrauterine pregnancy with positive fetal cardiac activity.
[g] Pregnancy loss before 20 weeks gestation (includes biochemical and clinical losses).
[h] Statistically significant.

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TABLE 3

Results of multivariable GLM analyses examining relevance of minimal elevation in TSH level in conjunction with evidence of thyroid autoimmunity for primary (live birth) and secondary (pregnancy loss, preterm birth) outcomes in the exposed (group A, TSH ≥2 mU/L and TPO-Ab titer ≥30 U/mL) vs. the remainder of population (Groups B and C).

Outcomes	Risk ratio (95% CI)	*P* value
Live birth	0.55 (0.35–0.87)	.011[c]
Pregnancy loss[a]	1.66 (1.14–2.42)	.008[c]
Early preterm birth (< 32 wk)[b]	1.76 (0.82–3.79)	.145
Late preterm birth (≥32 and < 37wk)[b]	4.82 (1.53–15.19)	.007[c]

Note: Covariates adjusted for included: age, BMI, race, duration of infertility, education, smoking history, PCOS (vs. unexplained infertility), and clinical trial intervention assignment. Magnitude of association is presented as risk ratio (95% confidence interval).
Sample size of population deemed as exposed (TSH ≥2.0 mU/L and TPO-Ab ≥30 U/mL): n = 117.
[a] Includes biochemical and clinical losses up to 20 weeks gestation.
[b] Propensity score analysis used because of small sample size of outcome.
[c] Statistical significance for sensitivity analysis was set at two-tailed *P* value of 0.012, accounting for multiple comparisons (0.05/4).

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0.94); the magnitude of this difference however was further exaggerated when TSH ≥2 mU/L was considered in conjunction with evidence of autoimmunity (ARR for LB 0.54; 95% CI: 0.34–0.86, Supplementary Table 1, available online).

Not only did the directionality of the previously observed relationships between mild elevations in TSH in conjunction with thyroid autoimmunity with the specified outcomes persist at the higher TSH cutoff value of ≥2.5 mU/L, but an exaggeration in the magnitude of relationships was evident (Supplementary Table 2, available online).

Given that mitigation of risk of adverse pregnancy outcomes has been suggested with thyroid hormone supplementation, use of thyroid medication was also examined. Information on medication use was available in baseline data for both cohorts. Of the eligible population sample (those with TSH and TPO-Ab results), use of thyroid supplements (yes/no) was identifiable in a subset (797 of 1,578, 50%, Table 1). Participants in the exposed group (group A) were nearly 4 times more likely to be using thyroid supplement (OR: 3.97; 95% CI: 2.16–7.32).

DISCUSSION

In this secondary analysis of the PPCOS II and AMIGOS clinical trials, women with the combined thyroid function indices of TPO-Ab titer ≥30 U/mL and TSH level ≥2 mU/L had lower rates of LB and higher rates of adverse pregnancy outcomes of pregnancy loss and early preterm birth. Although evidence of thyroid autoimmunity alone was also associated with lesser likelihood of LB and a trend for higher risk of pregnancy loss and preterm birth (Supplementary Table 1), the magnitude of associations for the observed relationships was more robust when elevated TPO-Ab titers ≥30 U/mL were considered together with TSH of ≥2 mU/L, a level that is well within the normal TSH assay range.

The choice of chosen thresholds for TSH levels and TPO-Ab titers was based on distribution of the available data in the 2 RCT population cohorts. Interestingly, the proportion of women with both TPO-Ab levels ≥30 U/mL and TSH levels of ≥2 mU/L was greater in the unexplained infertility population compared with the PCOS study population. The prevalence of thyroid autoimmunity previously has been found to be greater among women with PCOS (26–28) and with unexplained infertility (29) than control group women. However, the previous studies have not compared thyroid autoimmunity between women with the 2 infertility diagnoses.

Prior studies investigating associations between thyroid hypofunction, both preconception and during pregnancy, with adverse pregnancy outcomes have had inconsistent results. Similar to our approach, Chen et al. (30) had previously reported on preconception TSH levels and their relationship with pregnancy outcomes in a large population sample (n=184,611) of women; the investigators identified significantly greater occurrence of spontaneous miscarriages, preterm births and operative delivery in women with preconception TSH level of ≥2.5 mU/L (n=51,379) compared with those with preconception TSH <2.5 mU/L (n=133,232). Not only do our findings corroborate those reported by Chen et al. (30) but we observed similar associations of TSH at a lower threshold (≥2.0 mU/L) than previously reported in women with concomitant evidence of thyroid autoimmunity. Korevaar et al. (2) proposed that the frequently used threshold of TSH of 2.5 mU/L in pregnant women without thyroid immunity during the first trimester is too low to define thyroid dysfunction. However, our data suggest that in women with evidence of thyroid autoimmunity, those with a TSH level as low as 2.0 mU/L or higher may be at an increased risk of adverse pregnancy outcomes. Our observation may be explained by the notion that TPO-Ab positivity and TSH levels seem to synergistically contribute to the risk of compromised pregnancy outcomes. In support of this, the presence of both SCH and TPO-Ab positivity in pregnant women is associated with a higher risk of pregnancy complications as compared with either thyroid test abnormality alone (4, 31).

Thyroid autoimmunity, in particular TPO-Ab positivity, has been linked with the severity of thyroid dysfunction (32). During pregnancy, the presence of thyroid autoimmunity itself is suggested to reflect thyroid dysfunction, even if TSH remains within the normal range (32, 33). In line with these findings, we observed higher TSH levels in those with TPO-Ab titers ≥30 U/mL than those with TPO-Ab titers that were undetectable or <30 U/mL (2.41 ± 1.41 vs. 1.88 ± 0.97, $P<.001$). Whether preconception TPO-Ab positivity together with a TSH level of ≥2.0 mIU/L might herald impending inability of the thyroid gland to adapt to increased demands during pregnancy or whether other direct or indirect mechanisms contribute to the observed adverse pregnancy outcomes remains to be investigated. In light of the lack of consistent benefit from levothyroxine treatment in improving pregnancy outcomes in women with mild thyroid dysfunction or isolated thyroid autoimmunity (18–20), it is plausible that the downstream effects of TPO-Ab involve pathways that are distinct from those mediated via thyroid hormone signaling. This hypothesis would explain the null findings from thyroid supplementation in SCH clinical trials. Proposed mechanisms

for how TPO-Ab may cause pregnancy loss include observations of abnormalities in B-cell activation and endometrial T-cell function (34). Alternative endocrine underpinnings have also been proposed. Toulis et al. (12) suggested that TPO-Ab inhibits luteinizing hormone and hCG receptors in the corpus luteum because of receptor cross-reactivity, resulting in reduced progesterone secretion and subsequent pregnancy loss. However, luteal insufficiency is an unlikely mechanism for the adverse outcomes observed in this study because ovulation was induced with fertility medications in both PPCOS II and in AMIGOS (oral in PPCOS II and oral agents or injectable gonadotropins in AMIGOS).

Strengths of the present study include the methodological approach that took into consideration the strengths and limitations of the available data for this retrospective cohort study. Our population of well-characterized infertile women with widespread geographic representation across the United States and a wide reproductive-age range (18–40 years) makes our results more generalizable. The hormonal assay for both PPCOS II and AMIGOS cohorts was analyzed in the centralized laboratory, allowing direct comparisons of thyroid function tests between the groups. Lastly, the use of several sensitivity analyses to test the robustness of the associations assessed the relevance of different cutoff levels for TSH and TPO-Ab.

Despite the strengths, we acknowledge several limitations. This is a secondary analysis of data from two RCTs that were specifically designed to address fertility interventions. Untreated overt thyroid dysfunction was an exclusion criterion, and <5% of participants had TSH levels ≥4 mU/L. Although TSH was measured at baseline in both the RCTs, TPO-Abs were assayed at a later time (14). Treatment with thyroxine for SCH was relatively common in clinical practice at the time the 2 RCTs were conducted. Because risk mitigation for adverse pregnancy outcomes has previously been suggested with thyroid hormone supplementation (35), the greater use of thyroid supplements by the exposed population would, if at all, have biased our findings toward null, adding credence to our hypothesis. Free thyroxine levels and antithyroglobulin antibodies (TgAb) were not assessed in either RCT; we are therefore unable to analyze a relationship between isolated hypothyroxinemia (defined as normal TSH but low free thyroxine level) or isolated TgAb elevation with the specified pregnancy outcomes. Generalizability of our findings is limited because the 2 cohorts (PPCOS II and AMIGOS) are select populations of women with 2 distinct infertility diagnoses (PCOS and unexplained infertility). Furthermore, given that adverse pregnancy outcome was not the primary objective of the 2 RCTs, the parent study designs may have failed to consider conditions that could account for the observed associations, and residual confounding cannot be ruled out. Our group has previously queried this relationship between thyroid indices and pregnancy outcomes in the same cohort as our current work. However, it is critical that we clarify how our current approach differs from our prior work in which we had individually examined TPO-Ab titer at a threshold of ≥ 35 U/mL and TSH at threshold of ≥ 2.5 mU/L in relation to pregnancy outcomes (14). Some salient differences between our prior and our presented work are: 1) we had previously not considered the possibility of an interaction between TSH and TPO-Abs for the examined outcomes; 2) we had previously examined a single TSH cutoff threshold (>2.5 mU/L) based on the 2012 guidelines of the Endocrine Society (36). In our current exploration, distribution and range of TSH and TPO-Ab data available for the 2 RCT populations were scrutinized with attention to power consideration when arriving at respective threshold values. In light of a skewed distribution of the TPO-Ab data (Supplementary Fig. 1), by reassigning the cutoff threshold for TPO-Ab at 30 U/mL (present study) compared with 35 U/mL (prior work), we have improved the study power for the question being asked as the population of interest (women with TPO-Ab titers ≥ 30 U/mL) increased by n = 33 (from 185 in our prior study to 218 in our current work). Thus, rather than being duplicative, our current work serves not only toward broadening our perspective but also for generating new hypotheses. Lastly, our inability to tease out iatrogenic contribution to the observed preterm births in the 2 cohorts can also be considered as a study limitation.

We wish to underscore that our findings not be interpreted as a justification for initiating thyroid supplements at TSH levels ≥2 mU/L in women with evidence of thyroid autoimmunity. Rather, our belief is that these findings will guide future studies aimed at examining plausible causative mechanisms. It is possible that pathways other than downstream to thyroid receptor are relevant to the observed reproductive detriment, thereby explaining the inconsistencies in data from thyroid hormone supplemental trials.

CONCLUSIONS

In conclusion, findings from this study suggest that a composite of TSH and TPO-Ab assessment may offer an improved ability to identify women at risk for adverse pregnancy outcomes. We observed that when the TSH level was ≥ 2 mU /L and the TPO-Ab titer was ≥ 30 U/mL, there were fewer LBs, more pregnancy losses, and more early preterm births. These findings should be considered hypothesis-generating and highlight a need for appropriately designed clinical and basic studies to examine pathophysiological mechanisms that may underlie the observed relationships.

CRediT Authorship Contribution Statement

Satu Kuokkanen: Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation. **Aimee Seungdamrong:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Nanette Santoro:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Harry Lieman:** Writing – review & editing, Conceptualization. **Fangbai Sun:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Robert Wild:** Writing – review & editing, Methodology. **Heping Zhang:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Lubna Pal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project

administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Interests

N.S. is a consultant to Ansh Laboratories, and scientific advisory board member for Amazon (Project Ember) and Astellas. LP is a consultant to Win Fertility and advisory board member for Flo Health. S.K. has nothing to disclose. A.S. has nothing to disclose. H.L. has nothing to disclose. F.S. has nothing to disclose. R.W. has nothing to disclose. H.Z. has nothing to disclose.

SUPPLEMENTAL MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fertnstert.2024.12.005.

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Una nueva mirada a la relevancia de la hormona estimulante del tiroides (TSH) y la autoinmunidad tiroidea en el embarazo: Análisis de los datos aleatorizados de control del embarazo en Síndrome de Ovarios Poliquístico y Evaluación de gestaciones intrauterinas múltiples por estimulación ovárica

Objetivo: Evaluar si la autoinmunidad tiroidea es relevante para la relación entre los niveles maternos de la hormona estimulante del tiroides (TSH) y los resultados del embarazo.

Diseño: Análisis de cohortes retrospectivo de los datos de 2 ensayos controlados aleatorizados (ECA).

Sujetos: Participantes en los ECA "Embarazo en el síndrome de ovario poliquístico (PPCOS II, n =746) y Evaluación de gestaciones intrauterinas múltiples a partir de la estimulación ovárica (AMIGOS, n = 832 con infertilidad inexplicada).

Exposición: Niveles de TSH previos a la intervención del ECA en el umbral ≥2,0 mU/L y títulos de anticuerpos de peroxidasa tiroidea (TPO-Ab) ≥ 30 U/Ml

Principales Medidas de Desenlace: Nacidos vivos (resultado primario), abortos y parto prematuro (resultados secundarios). Los análisis del modelo lineal generalizado (GLM) examinaron la relación entre la exposición a TSH y TPO-Ab en los umbrales específicos con los resultados especificados; las covariables ajustadas incluían la edad, el índice de masa corporal, la raza, el origen étnico, la educación, el tabaquismo, la duración de la infertilidad, el SOP (frente a la infertilidad inexplicada) y el brazo de intervención aleatorizado en los RCT respectivos.

Resultados: En los análisis ajustados, los nacidos vivos se redujeron significativamente en la población expuesta (aquellos con TSH ≥2,0 mU/L y TPO-Ab ≥30 U/mL, n= 117/1.578, 7,4%, cociente de riesgos ajustado [ARR]: 0,55; IC 95%: 0,35-0,87) en comparación con los no expuestos (aquellos con TSH <2,0 mU/L y TPO-Ab <30 U/mL, n= 865/1.578, 54,8%). Además, el riesgo de pérdida del embarazo y de parto prematuro temprano (<32 semanas) fue significativamente mayor en las expuestas en comparación con las no expuestas (la RRA de pérdida de embarazo fue de 1,66; IC 95%: 1,14-2,42, y la RRA de parto prematuro precoz fue de 4,82; IC 95%: 1,53-15,19).

Conclusión: En las mujeres con títulos de TPO-Ab ≥30 U/mL, los resultados del embarazo pueden verse comprometidos con un umbral de TSH de ≥2mU/L. Estos hallazgos de una interacción entre TSH y TPO para los resultados del embarazo merecen una mayor investigación en estudios prospectivos.