Subclinical hypothyroidism and thyroid autoimmunity are not associated with fecundity, pregnancy loss or live birth

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Context: Prior studies examining associations between subclinical hypothyroidism (SCH) and anti-thyroid antibodies with early pregnancy loss and live birth indicate mixed results and time to pregnancy (TTP) has not been studied in this patient population.

Objective: To examine associations of pre-pregnancy TSH concentrations and thyroid autoimmunity with TTP, pregnancy loss, and live birth among women with proven fecundity and a history of pregnancy loss.

Design: Prospective cohort study from a large, randomized controlled trial.

Setting: Four medical centers in the United States.

Patients or Other Participants: Healthy women, ages 18–40, who were actively attempting to conceive and had one or two prior pregnancy losses and no history of infertility.

Intervention: None.

Main Outcome Measure: TTP, pregnancy loss and live birth.

Results: Women with TSH $\geq$ 2.5 did not have an increased risk of pregnancy loss (risk ratio [RR] 1.07, 95% confidence interval [CI] 0.81, 1.41) or a decrease in live birth rate (RR 0.97, 95% CI 0.88, 1.07) or TTP (fecundability odds ratio [FOR] 1.09, 95% CI 0.90, 1.31) compared to women with TSH $<$2.5 mIU/L after adjustment for age and body mass index. Similar findings were observed for women with thyroid autoimmunity and after additional adjustment for treatment assignment.

Conclusions: Among healthy fecund women with a history pregnancy loss, TSH levels $\geq$2.5, or the presence of anti-thyroid antibodies, were not associated with fecundity, pregnancy loss or live birth. Thus, women with SCH or thyroid autoimmunity can be reassured that their chances of conceiving and achieving a live birth are likely unaffected by marginal thyroid dysfunction.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received January 7, 2016. Accepted March 23, 2016.

Abbreviations:

Appropriate thyroid function is essential to establishing and maintaining pregnancy. Indeed, hyperthyroidism has been shown to be associated with infertility, pregnancy loss and other adverse obstetrical and fetal outcomes (1–3). Conversely, overt hypothyroidism also adversely influences the female reproductive system (4), likely through decreased feedback to the hypothalamic-pituitary axis and disturbance of GnRH pulsatility and subsequent ovulation (5), and has been associated with menstrual irregularity and with decreased fecundity (6).

The effects of subclinical hypothyroidism on reproduction are less understood, and available evidence evaluating certain definitions of subclinical hypothyroidism, ie, moderately elevated TSH levels of $>$ 2.5 mIU/L or thyroid autoimmunity, and pregnancy loss is mixed (7–9). These studies are also limited in that enrollment typically occurred in the late first trimester or early second trimester, limiting the ability to assess associations with preconception TSH levels, as well as associations with early pregnancy losses (10). Also, studies of subclinical hypothyroidism have often been limited to women with a prior diagnosis of infertility. Lastly, time to pregnancy in women with subclinical hypothyroidism has not been studied. Yet, understanding the relationship between subclinical hypothyroidism and thyroid autoimmunity and pregnancy outcomes is critical to establish whether clinical intervention should be explored to improve reproductive outcomes in this population. Thus, our objective was to examine the association of prepregnancy TSH concentrations and thyroid autoimmunity with time to pregnancy, pregnancy loss, and live birth among women with proven fecundity and a history of prior pregnancy loss.

Materials and Methods

This study is a prospective cohort study from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, which was a multicenter, double-blind, block-randomized, placebo-controlled trial that assessed the effect of low dose aspirin (LDA) on pregnancy loss in women with a history of one or two pregnancy losses (11). A total of 1228 women with a history of pregnancy loss who were trying to becoming pregnant were recruited at four U.S. clinical sites from 2007–2011. The detailed study design and methods have been described previously (11); methods relevant to the present analysis are included here. Institutional Review Board authorization at the data coordinating center and at all clinical centers was obtained and served as the IRB designated by the National Institutes of Health under a reliance agreement. All participants provided written informed consent. The Data Safety and Monitoring Board (DSMB) ensured patient safety. The trial was registered with ClinicalTrials.gov, NCT00467363.

Study Design and Population

Participants were women aged 18–40 years, with regular menstrual cycles (21–42 days in length), attempting pregnancy with a history of one or two documented pregnancy losses. None had a known history of infertility, pelvic inflammatory disease (PID), tubal occlusion, endometriosis, anovulation, uterine abnormality, or polycystic ovarian syndrome. Also, women with major medical disorders, including conditions with indication for aspirin therapy and thyroid dysfunction were excluded.

Participants were followed until completion of six cycles attempting pregnancy and also throughout pregnancy if they conceived. Follow-up was discontinued if women did not become pregnant after 6 cycles or after experiencing a second periconception loss during the study. Fertility monitors were utilized to assist with scheduling study visits in the preconception phase and timing intercourse (Clearblue Easy Fertility Monitor; Inverness Medical).

Thyroid Function Assessment

Preconception TSH, fT4, thyroid globulin antibody (anti-TG), and thyroid peroxidase antibody (anti-TPO) levels were measured in serum collected at the randomization visit (ie, prior to receiving LDA or placebo treatment) and stored at –80°C after collection. TSH was measured in serum using the TSH reagent sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN). The reference range was 0.4 to 5 mIU/L and the interassay coefficient of variation (CV) was 2.1% at 1.596 mIU/L and 2.9% at 9.037 mIU/L. fT4 was measured using a fT4 competitive immunoassay (Roche Diagnostics, Indianapolis, IN) and the reference range was 0.7 to 1.85 ng/dL. The interassay CV was 4.3% at 97.54 nmol/L and at 140.28 nmol/L. Anti-TG was measured using a TSH sandwich immunoassay method whereas anti-TPO was measured using an anti-TPO competitive immunoassay method (both Roche Diagnostics, Indianapolis, IN). The interassay CV for anti-TG was 7.2% at 91.4 IU/mL and 6.7% at 171 IU/mL. Results for anti-TG were considered positive if they were $\geq$ 115 IU/mL. The interassay CV for anti-TPO was 17% at 31.5 IU/mL and 11.9% at 76.13 IU/mL, and results were considered positive if anti-TPO was $\geq$ 35 IU/mL.

Outcome Measures

The primary outcomes were time to hCG pregnancy (in cycles), pregnancy losses (both biochemical and clinical) and live birth. Clinically recognized pregnancy losses were defined as a pregnancy loss detected by the woman or her primary care provider after clinical recognition of pregnancy by early ultrasound at approximately 6.5 weeks of gestation (eg, gestation sac, clinical documentation of fetal heart tones, or a later stage confirmation of pregnancy).

Biochemical pregnancies were identified in two ways. First, a positive urine pregnancy test at the clinical site (Quidel Quickvue, Quidel Corporation, San Diego, CA, sensitive to 25 mIU/ml hCG) followed by the absence of signs of clinical pregnancy, with or without missed menses. Second, we also identified additional early pregnancy losses from batched augmented urine hCG testing that was performed later in the laboratory on the last 10 days of each woman's first and second cycle of study participation (using daily first-morning urine collected at home) and on spot urine samples collected at all postcycle visits. Free beta hCG was measured in these urine samples to enable more sensitive detection of very early pregnancy than possible with conventional

urine pregnancy testing. Two laboratory assays for free beta hCG (catalogue no. 4221–16, Diagnostic Automation Inc., Calabasas, CA, USA; catalogue no. RIS0011R, BioVendor, Asheville, NC, USA) were sequentially employed to determine, first, "potentially positive" values (n = 164), out of which 21 were verified as positive tests for early hCG detected pregnancy.

Statistical Analysis

Among 1228 participants in the original study, all participants whose fT4 fell outside of normal parameters (0.7 – 1.85 ng/dL), which are specific to the laboratory analyzing fT4, or whose fT4 was not recorded were excluded (n = 35). Traditionally, SCH has been defined as a TSH level exceeding the upper limit of normal range (4.5 – 5.0 mIU/L) in the setting of a normal fT4 level (4). The National Academy of Clinical Biochemistry (NACB) noted that most patients (95%) who did not have symptoms of thyroid dysfunction had a TSH of 2.5 mIU/L or below (12). They further suggested that the upper limit of normal for TSH is skewed to the right and should subsequently be set at 2.5 mIU/L (12). Thus, we categorized participants into two groups based on TSH level: TSH < 2.5 or ≥ 2.5 mIU/L. Additionally, women were evaluated based on presence or absence of anti-TG and anti-TPO antibodies (positive = anti-TG $\geq$ 115 IU/mL or anti-TPO $\geq$ 35 IU/mL; negative = anti-TG $<$ 115 IU/mL and anti-TPO $<$ 35 IU/mL).

Characteristics between women in these groups were compared using Fisher's exact test and t-tests as appropriate. We additionally evaluated descriptive statistics of the distribution of TSH in our study population. Risk ratios (RR) and 95% confidence intervals (CIs) for live birth and pregnancy loss were estimated using generalized linear models adjusted for age and body mass index (BMI) given strong associations with age and TSH levels and pregnancy outcomes, as well as existing evidence that there is an association between elevated TSH and elevated BMI, even among euthyroid people (13–15). Models compare women with TSH < 2.5 vs ≥ 2.5 mIU/L, as well as by presence or absence of anti-TG and anti-TPO antibodies. We further stratified these results (elevated TSH and thyroid autoimmunity) by the number of prior pregnancy losses and by women with vs without prior live births. Finally, we restricted the analysis to only women with positive thyroid antibodies and stratified based on their TSH level. Cox proportional hazard regression models were used to assess fecundability odds ratios (FOR), adjusting for age and BMI and accounting for both left truncation (cycles trying to become pregnant prior to study entry) and right censoring (that women were only followed for up to 6 cycles while trying to become pregnant) to evaluate associations between TSH and thyroid autoimmunity and time to pregnancy. Results were additionally compared to models that also adjusted for age, BMI, and treatment assignment.

As pregnancy loss is conditional upon becoming pregnant, we next restricted our analysis to women with any hCG detected pregnancy, and utilized inverse probability weights to control for potential selection bias introduced by restricting the analytical cohort to women who achieved pregnancy during the 6 cycles of follow-up. Weights were based on factors associated with becoming pregnant, including maternal age, parity, marital status, number of prior losses, and treatment assignment since low dose aspirin was observed to improve pregnancy rates within certain women in this cohort (16). Weighted log-binomial regression was used to estimate RRs and 95% CIs. We also performed a sensitivity analysis to evaluate the robustness of our findings using various cut-point for TSH. All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC).

Results

The overall mean TSH level of women in this cohort was 2.1 ± standard deviation (SD) 1.3 (2.fifth percentile, 0.56; 97.fifth percentile, 5.10), and the mean fT4 level was 1.1 ± 0.1 (2.fifth percentile, 0.88; 97.fifth percentile, 1.45 [see histogram in Supplemental Figure 1 for the distribution of TSH in this population]. Women with TSH < 2.5 mIU/L were similar to those with a TSH ≥ 2.5 mIU/L in age, race, education level, household income level, alcohol and tobacco consumption, and the time from last loss to randomization. However, participants with a TSH $\geq$ 2.5 mIU/L had a higher mean BMI (27.4 ± 7.3 vs 25.9 ± 6.2, $P = .001$) and were more often nulliparous (52.1% vs 44.3%, $P = .01$) compared to TSH < 2.5 mIU/L [Table 1]. Women positive for antithyroid antibodies were similar with regard to age, BMI, household income, smoking, alcohol consumption, prior number of live births and prior number of previous pregnancies compared with women without thyroid antibodies [Table 1]. However, women with thyroid antibodies were more often Caucasian compared to women without thyroid antibodies ($P = .04$).

A total of 566 women with TSH < 2.5 mIU/L became pregnant (566/884, 64.0%) vs 205 (205/303, 67.7%) with ≥ 2.5 mIU/L ($P = .25$). Overall, participants with TSH of ≥ 2.5 mIU/L did not have higher risk of any pregnancy loss (RR 1.15, 95% CI 0.86, 1.54) nor were they less likely to have a live birth (RR 1.01, 95% CI 0.89, 1.14), even after adjusting for age and BMI, compared with TSH < 2.5 mIU/L [Table 2]. Adjusting for treatment assignment did not change these results. Similar results were noted when the analysis was limited to women who became pregnant during the study (pregnancy loss RR 1.07, 95% CI 0.81, 1.41; live birth RR 0.97, 95% CI 0.88, 1.07 comparing women with TSH ≥ 2.5 mIU/L relative to those with TSH < 2.5 mIU/L) [Table 2]. In an attempt to identify a cut-point in which TSH affected pregnancy loss, we additionally examined both TSH tertiles, and continuous TSH, but found similar results [Supplemental Table 1[rsqb].

There were a total of 900 (900/1054, 85.4%) women without antithyroid antibodies and 154 (154/1054, 14.6%) with antithyroid antibodies. A total of 650 (650/900, 72.2%) women without antithyroid antibodies became pregnant vs 114 (114/154, 74.0%) with antithyroid antibodies ($P = .64$). The prevalence of anti-TPO and anti-TG in our study is consistent with prior reports of reproductive age women (14.6% of our study population

Table 1. Demographics and baseline characteristics by TSH levels and presence of anti-thyroid antibodies among women with normal fT4 (0.7 to 1.85 ng/dL) in the EAGeR trial

Characteristics N (%)	Total	TSH mIU/liter fT4 0.7 – 1.85			Anti-thyroid antibodies (presence of anti-TPO or anti-TG antibodies)		
	n = 1193	<2.5 *n* = 884	≥2.5 *n* = 303	P value	Negative *n* = 1018	Positive *n* = 168	P value
TSH: Mean ± SD	2.1 ± 1.3	1.5 ± 0.5	3.6 ± 1.5	<0.001	1.9 ± 0.9	2.9 ± 2.2	<0.001
fT4: Mean ± SD	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	<0.001	1.1 (0.1)	1.1 (0.2)	0.292
Age, y: Mean ± SD	28.7 ± 4.8	28.8 ± 4.8	28.5 ± 4.7	0.37	28.7 ± 4.8	28.8 ± 4.7	0.86
BMI, kg/m^2: Mean ± SD	26.3 ± 6.5	25.9 ± 6.2	27.4 ± 7.3	0.001	26.1 ± 6.3	27.1 ± 7.4	0.08
Race							
White	1126 (94.9)	835 (94.5)	291 (96)	0.28	962 (94.5)	165 (98.2)	0.04
Others	61 (5.1)	49 (5.5)	12 (4)		56 (5.5)	3 (1.8)	
Education*							
≤ High School	160 (13.5)	122 (13.8)	38 (12.5)	0.58	144 (14.2)	16 (9.5)	0.10
> High School	1026 (86.5)	761 (86.2)	265 (87.5)		873 (85.8)	152 (90.5)	
Household income (annual)							
≥ $100 000	474 (40)	348 (39.4)	126 (41.6)	0.77	398 (39.1)	72 (42.9)	0.30
$75 000-$99 999	144 (12.1)	110 (12.5)	34 (11.2)		124 (12.2)	21 (12.5)	
$40 000-$74 999	175 (14.8)	136 (15.4)	39 (12.9)		155 (15.2)	21 (12.5)	
$20 000-$39 999	302 (25.5)	221 (25)	81 (26.7)		267 (26.3)	36 (21.4)	
≤ $19 999	91 (7.7)	68 (7.7)	23 (7.6)		73 (7.2)	18 (10.7)	
Employed*							
Yes	868 (75.7)	632 (74.4)	236 (79.7)	0.06	741 (75.5)	125 (76.2)	0.85
No	278 (24.3)	218 (25.6)	60 (20.3)		240 (24.5)	39 (23.8)	
Time from last loss to randomization (months)							
≤ 4 Months	629 (53.8)	460 (52.8)	169 (56.9)	0.49	544 (54.2)	87 (53)	0.31
5–8 Months	214 (18.3)	163 (18.7)	51 (17.2)		179 (17.8)	35 (21.3)	
9–12 Months	96 (8.2)	70 (8)	26 (8.8)		80 (8)	17 (10.4)	
>12 Months	230 (19.7)	179 (20.5)	51 (17.2)		201 (20)	25 (15.2)	
Number of previous pregnancies, not including losses*							
0	506 (42.6)	363 (41.1)	143 (47.2)	0.12	429 (42.1)	76 (45.2)	0.76
1	421 (35.5)	315 (35.6)	106 (35)		364 (35.8)	57 (33.9)	
2	239 (20.1)	191 (21.6)	48 (15.8)		208 (20.4)	31 (18.5)	
3	21 (1.8)	15 (1.7)	6 (2)		17 (1.7)	4 (2.4)	
Number of previous live births*							
0	550 (46.3)	392 (44.3)	158 (52.1)	0.01	463 (45.5)	86 (51.2)	0.36
1	430 (36.2)	321 (36.3)	109 (36)		374 (36.7)	57 (33.9)	
2	207 (17.4)	171 (19.3)	36 (11.9)		181 (17.8)	25 (14.9)	
Smoking in past year							
Never	1033 (87.8)	764 (87.1)	269 (89.7)	0.46	880 (87)	153 (92.7)	0.08
<6 times/week	83 (7.1)	64 (7.3)	19 (6.3)		76 (7.5)	5 (3)	
Daily	61 (5.2)	49 (5.6)	12 (4)		55 (5.4)	7 (4.2)	
Alcohol consumption in past year							
Often	26 (2.2)	16 (1.8)	10 (3.3)	0.13	21 (2.1)	5 (3)	0.22
Sometimes	369 (31.5)	285 (32.7)	84 (28)		325 (32.3)	43 (25.9)	
Never	777 (66.3)	571 (65.5)	206 (68.7)		659 (65.6)	118 (71.1)	

BMI, body mass index; fT4, free thyroxine; TSH, thyroid stimulating hormone.

Values are Mean ± SD or n (%) as indicated.

*Data on covariates were missing for education (*n* = 1), income (*n* = 1), employment (*n* = 41), time from last loss to randomization (*n* = 18), smoking (*n* = 10), and alcohol (*n* = 15).

compared to 10%–13% for anti-TPO and 9%–14% for anti-TG reported) (17). Overall, women with antithyroid antibodies did not have a higher risk of any pregnancy loss (RR 0.90, 95% CI 0.61, 1.33) and did not have a lower live birth rate (RR 1.04, 95% CI 0.90, 1.20), after adjusting for age and BMI, compared to women without antibodies. After additional adjustment for treatment assignment, no differences were noted between the two groups. Similar results were noted among women who became pregnant during this study for both pregnancy loss (RR 0.85, 95% CI 0.59, 1.22) and live birth (RR 1.04, 95% CI 0.94, 1.16) [Table 2].

Furthermore, among women with only one previous loss, those with TSH ≥ 2.5 mIU/L were not at higher risk

Table 2. Association between TSH level and anti-thyroid antibody positivity with live birth and pregnancy loss among women with normal fT4 (0.7 to 1.85 ng/dL) in the EAGeR Trial

		TSH		Anti-thyroid antibodies	
	Model[a]	Overall	Among Pregnancies[b]	Overall	Among Pregnancies
		RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Live birth	Unadjusted	0.99 (0.87,1.12)	0.97 (0.88,1.07)	1.05 (0.91,1.22)	1.03 (0.93,1.15)
	Adjusted Model 1	1 (0.88,1.13)	0.97 (0.88,1.07)	1.03 (0.89,1.19)	1.04 (0.94,1.16)
	Adjusted Model 2	1 (0.88,1.13)	0.97 (0.87,1.06)	1.03 (0.89,1.19)	1.05 (0.94,1.17)
Any pregnancy loss	Unadjusted	1.11 (0.83,1.48)	1.09 (0.83,1.44)	0.92 (0.63,1.35)	0.9 (0.63,1.3)
	Adjusted Model 1	1.12 (0.84,1.49)	1.07 (0.81,1.41)	0.91 (0.62,1.33)	0.85 (0.59,1.22)
	Adjusted Model 2	1.12 (0.84,1.49)	1.07 (0.81,1.41)	0.91 (0.62,1.33)	0.85 (0.59,1.22)
Clinical loss	Unadjusted	1.29 (0.91,1.82)	1.27 (0.91,1.77)	0.9 (0.56,1.44)	0.88 (0.55,1.4)
	Adjusted Model 1	1.31 (0.93,1.85)	1.26 (0.91,1.77)	0.89 (0.56,1.43)	0.83 (0.52,1.33)
	Adjusted Model 2	1.31 (0.93,1.85)	1.26 (0.9,1.76)	0.89 (0.56,1.43)	0.84 (0.53,1.33)

CI, confidence interval; fT4, free thyroxine; RR, risk ratio; TSH, thyroid stimulating hormone.

[a]Model 1 adjusts for age and body mass index, with the TSH models comparing TSH ≥2.5 *vs.* <2.5 mIU/liter, and anti-thyroid antibody models comparing those with positive antibodies *vs.* negative. Adjusted model 2 adjusts for age, body mass index, and treatment assignment (low dose aspirin *vs.* placebo).

[b]Models restricted to women who achieved an hCG pregnancy, with inverse probability weights used control for potential selection bias introduced by restricting to women who achieved pregnancy. Weights were based on factors associated with becoming pregnancy, including age, parity, marital status, number of prior losses and treatment assignment. Weighted log-binomial regression was used to estimate risk ratios and 95% confidence intervals.

of having any pregnancy loss (RR 1.13; 95% CI 0.80, 1.6) compared with TSH < 2.5 mIU/L [Table 3], even when analysis was further limited to those with no previous live birth (RR 0.94; 95% CI 0.63, 1.39). There was also no difference in risk of pregnancy loss by thyroid status when examining women with a history of two pregnancy losses with or without a prior live birth [Table 3]. Similar results were seen for thyroid autoimmunity among women with one or two prior pregnancy losses, regardless of prior live birth history [data not shown].

Also, among women with thyroid autoimmunity, we found no difference in women with TSH ≥ 2.5 mIU/L compared to < 2.5 mIU/L for risk of pregnancy loss (RR 0.82; 95% CI 0.41, 1.65) or live birth rate (RR 1.02; 95% CI 0.84, 1.25) [Table 3].

Lastly, women with a TSH ≥ 2.5 mIU/L did not have a longer time to conceive vs women whose TSH was < 2.5 mIU/L (FOR 1.09, 95% CI 0.90, 1.31) [Table 4]. Similarly, women with thyroid autoimmunity did not have a significant delay in pregnancy compared to those without (FOR 1.11; 95% CI 0.88, 1.40) [Table 4].

Table 3. Association between TSH level and live birth and pregnancy loss among women with normal fT4 (0.7 to 1.85 ng/dL), within women with one prior loss, two prior losses, or positive anti-thyroid antibody status in the EAGeR Trial

		TSH ≥2.5 *vs.* TSH <2.5 Among Pregnancies[b] RR (95% CI)		
	Model	History of 1 previous loss *n* = 508	History of 2 previous losses *n* = 267	Positive anti-thyroid antibodies *n* = 114
Live birth	Unadjusted	0.96 (0.85,1.07)	1.00 (0.84,1.19)	1.03 (0.84,1.25)
	[a]Adjusted	0.95 (0.85,1.06)	1.03 (0.86,1.24)	1.02 (0.84,1.25)
Any pregnancy loss	Unadjusted	1.16 (0.82,1.63)	0.99 (0.63,1.56)	0.91 (0.46,1.81)
	Adjusted	1.13 (0.80,1.59)	0.96 (0.61,1.52)	0.82 (0.41,1.65)
Clinical loss	Unadjusted	1.33 (0.87,2.02)	1.18 (0.68,2.05)	1.10 (0.46,2.61)
	Adjusted	1.32 (0.87,2.01)	1.18 (0.68,2.05)	1.03 (0.43,2.46)

CI, confidence interval; fT4, free thyroxine; RR, risk ratio; TSH, thyroid stimulating hormone.

[a]Models adjusted for age and body mass index.

[b]Models restricted to women who achieved an hCG pregnancy, with inverse probability weights used control for potential lselection bias introduced by restricting to women who achieved pregnancy. Weights were based on factors associated with becoming pregnancy, including age, parity, marital status, number of prior losses and treatment assignment. Weighted log-binomial regression was used to estimate risk ratios and 95% confidence intervals.

Table 4. Time to hCG pregnancy by TSH and antibody status among women with normal fT4 (0.7 to 1.85 ng/dL) in the EAGeR Trial

	TSH fT4 0.7 – 1.85		Anti-thyroid antibodies	
Outcomes: N (%)	**<2.5**	**≥ 2.5**	**Negative**	**Positive**
Total N	884 (74.5)	303 (25.5)	1018 (85.8)	168 (14.2)
TTP				
1 month	204 (23.1)	56 (18.5)	218 (21.4)	44 (26.2)
2 months	137 (15.5)	54 (17.8)	163 (16.0)	30 (17.9)
3 months	84 (9.5)	44 (14.5)	115 (11.3)	12 (7.1)
4 months	60 (6.8)	28 (9.2)	76 (7.5)	12 (7.1)
5 months	47 (5.3)	12 (4.0)	50 (4.9)	9 (5.4)
6 months	34 (3.8)	11 (3.6)	37 (3.6)	8 (4.8)
>6 months	318 (36.0)	98 (32.3)	359 (35.3)	53 (31.5)
Unadjusted FOR (95% CI)	Reference	1.06 (0.88, 1.28)	Reference	1.10 (0.87, 1.39)
Adjusted FOR Model 1 (95% CI) [a]	Reference	1.09 (0.90, 1.31)	Reference	1.12 (0.89, 1.41)
Adjusted FOR Model 2 (95% CI) [b]	Reference	1.08 (0.90, 1.31)	Reference	1.13 (0.89, 1.42)

CI, confidence interval; FOR, fecundability odds ratio; fT4, free thyroxine; TSH, thyroid stimulating hormone; TTP, time to pregnancy.

[a]Models adjusted for age and body mass index.

[b]Models adjusted for age, body mass index and treatment assignment (low dose aspirin *vs.* placebo)

Discussion

We found no association between preconception subclinical hypothyroidism and time to pregnancy, pregnancy loss and live birth among healthy fecund women attempting to conceive. Additionally, no relationships between thyroid autoimmunity and pregnancy loss or live birth were observed. Thus, women with a history of pregnancy loss having subclinical hypothyroidism or thyroid autoimmunity can be reassured that their chances of conceiving and achieving a live birth are likely unaffected by marginal thyroid dysfunction.

Previous studies have reported conflicting results regarding whether women with subclinical hypothyroidism have a higher likelihood of pregnancy loss or lower likelihood of live birth. Our findings were consistent with studies reporting no association between subclinical hypothyroidism and pregnancy loss, including a study of women enrolled between 10 and 13 weeks gestation in the First and Second Trimester Evaluation of Risk (FASTER) Trial (10). Of note, such studies that enrolled during pregnancy were unable to assess time to pregnancy or early pregnancy loss. Also, our results were also consistent with two retrospective studies which examined pregnancy loss and live birth in relation to subclinical hypothyroidism specifically in an infertile population (7, 18). Of note, our results were not limited to an infertile population, making these results more generalizable.

In contrast, a few studies observed higher rates of pregnancy loss among women with subclinical hypothyroidism (19, 20). A large prospective cohort study of 2497 women in Amsterdam found that women without overt thyroid dysfunction had higher odds of pregnancy loss as their TSH increased, though in this study pregnant women were enrolled after 11 weeks gestation (average 13 weeks) and all women in the study had a TSH < 2.5 mIU/L (19). Additionally, there were only 27 total pregnancy losses in this cohort. Thus, the incidence of miscarriage reported is very low and most early losses were unobserved. Similarly, a large prospective study (n = 4123) reported that the rate of spontaneous pregnancy loss was significantly lower among pregnant women who were thyroid antibody negative and TSH levels of < 2.5 compared to those with TSH level of 2.5 – 5 mIU/L (3.6% vs 6.1%, $P = .006$) (20). Again, in this study the enrollment occurred during the first trimester (average of 8.9 weeks), and loss rates were much lower than expected. Our ability to recruit preconception and capture early losses are strong improvements over previous studies. In addition, it is well known that TSH levels change markedly during the first trimester (21), and as such the timing of the measurement during pregnancy could have an important influence on the interpretation of these previous findings.

Previous studies of thyroid autoantibodies and pregnancy loss have also produced mixed results. Several studies yielded conclusions which support the results of this study. One prospective study evaluated thyroid autoimmunity and pregnancy outcome among 870 nonpregnant women with a history of recurrent pregnancy loss and found no associations between thyroid antibodies and live birth rates (22). Similarly, the rate of autoimmunity was

found to be comparable between women with a history of recurrent pregnancy loss and fertile controls (n = 149) (23). Conversely, women with thyroid autoimmunity (presence of anti-TPO) were reported to have higher risk of miscarriage, although only 3 of 29 women with anti-TPO had a pregnancy loss and the overall loss rates were lower than in the general population (2% overall and 10.3% among women with anti-TPO) (24). Of note, prior studies report clinical pregnancy loss rate from 8%–20%, but as high as 26%–31% when including very early pregnancy losses (25–27). As with studies discussed above, this study was unable to assess early loss and, consequently, the population had a very low loss rate. Another study observed that women with either isolated thyroid autoimmunity or autoimmunity in combination with TSH levels above 2.5 were at an increased risk of pregnancy loss compared to euthyroid women (28). However, importantly, when compared to euthyroid women, this association only held for women with a TSH value $\geq$ 5.22 mIU/L, but did not hold true for women with a TSH between 2.5 and 5.22 mIU/L, which agrees with the present findings. Overall, the evaluation of prepregnancy autoimmunity status and including observation of very early pregnancy losses are important strengths of our study compared with prior research.

In addition to prospective data collection including early pregnancy with prepregnancy thyroid function testing, our study also prospectively followed women who became pregnant throughout their pregnancies, providing the ability to comment on live birth outcomes as well. Moreover, our population has proven fecundity, as all participants had a history of either 1 or 2 prior pregnancy losses, differentiating these data from those of infertile populations and providing more generalizable results. Additionally, there were a large number of participants in this study allowing for adequate numbers of loss events among groups, and we were also able to evaluate associations with both anti-TPO and anti-TG. However, we were limited in our ability to evaluate associations among women with thyroid autoimmunity and TSH $\geq$ 5 as there were only 25 women in this group.

In conclusion, we did not observe differences in time to pregnancy, pregnancy loss or live birth when comparing women with subclinical hypothyroidism to those with normal TSH among women with a history of prior pregnancy loss. Currently, the American Congress of Obstetricians and Gynecologists (ACOG) does not recommend screening of TSH in healthy, low risk, asymptomatic patients and our data support maintaining this guideline. Additionally, the cohort of patients in this study with thyroid antibodies did not have a higher risk of pregnancy loss compared to women without thyroid antibodies. These findings can be reassuring to both clinicians and women with subclinical hypothyroidism or thyroid autoimmunity.

Acknowledgments

This research was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland (Contract Nos. HHSN267200603423, HHSN267200603424, HHSN267200603426). We would like to especially thank the EAGeR study participants for their extraordinary commitment to the study, all of the EAGeR investigators and staff who devoted their time and energy to the success of this trial, and the Data Safety and Monitoring Board members for ongoing oversight, constant support, and advice throughout the trial.

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This work was supported by Funding: This research was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD (Contract Nos. HHSN267200603423, HHSN267200603424, HHSN267200603426).

Disclosure Statement: The authors have nothing to disclose.

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