Thyroid function during controlled ovarian hyperstimulation as part of in vitro fertilization

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Objective: To determine the exact nature and timing of alterations in thyroid function throughout controlled ovarian hyperstimulation (COH).
Design: Prospective cohort study.
Setting: University fertility clinic.
Patient(s): Fifty-seven women undergoing COH as part of planned in vitro fertilization.
Intervention(s): None.
Main Outcome Measure(s): Timing and magnitude of change in serum thyroid hormones, including TSH, total and free T_4, E_2, and thyroxine-binding globulin (TBG), measured at six time points from before stimulation to 2 weeks after serum pregnancy test.
Result(s): Geometric mean serum TSH increased during stimulation, peaking 1 week after hCG administration compared with baseline (2.44 vs. 1.42 mIU/L), as did free T_4 (1.52 vs. 1.38 ng/dL) and TBG (32.86 vs. 21.52 μg/mL). Estradiol levels increased, peaking at hCG administration (1743.21 vs. 71.37 pg/mL). Of 50 women with baseline TSH $\leq$2.5 mIU/L, 22 (44.0%) had a subsequent rise in TSH to $>$2.5 during or after COH. The pattern of change over time in TSH concentrations was significantly influenced by baseline hypothyroidism and whether pregnancy was achieved.
Conclusion(s): COH led to significant elevations in TSH, often above pregnancy appropriate targets. These findings were particularly evident in women with preexisting hypothyroidism and may have important clinical implications for screening and thyroid hormone supplementation. (Fertil Steril® 2012;97:585–91. ©2012 by American Society for Reproductive Medicine.)
Key Words: Thyroid function, in vitro fertilization, ovarian hyperstimulation, hypothyroidism, infertility

Infertility affects 7.4% of women aged 15–44 years in the U.S. (1). Women who achieve pregnancy after assisted reproductive technologies (ART) are exposed to a supraphysiologic hormone environment. Although pregnancy is known to cause perturbations in thyroid hormone metabolism, the high E_2 levels caused by controlled ovarian hyperstimulation (COH) may also affect thyroid function. Evidence suggests that COH as part of in vitro fertilization (IVF) increases serum TSH concentration, but associations with free thyroxine (T_4) are unclear (2–4).

Determining how thyroid function fluctuates during ART is important because adverse pregnancy outcomes, including miscarriage, preterm delivery, and decreased developmental scores in children have been associated with inadequately or untreated maternal hypothyroidism (5–9). Although increased levothyroxine (LT_4) requirements during pregnancy have been well documented (10–12), the timing of initiation or adjustment of LT_4 therapy in women undergoing ART has not. It is important to recognize that thyroid function tests are not routinely ordered during IVF, even in women with treated hypothyroidism. The interplay of these hormonal changes with antithyroid antibodies is an important area of investigation. Hypothyroidism and antithyroid antibodies may be associated with differences in patterns of change in thyroid function during COH. Although antithyroid antibodies have also been associated with an increased risk of miscarriage (6), their clinical relevance remains unclear. It is not known whether antithyroid antibodies are associated with mild thyroid failure during COH which may contribute to pregnancy failure.

The goal of the present prospective cohort study was to characterize alterations in thyroid function associated

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with COH and early gestation during IVF. We hypothesized that hormonal changes during COH, specifically supraphysiologic elevations in E_2, alter thyroid hormone metabolism, resulting in significant increases in TSH and decreases in free T_4 concentration. Furthermore, we hypothesized that thyroid disease and thyroid autoimmunity would accentuate changes in thyroid function associated with ART.

MATERIALS AND METHODS

Subjects were recruited for this Institutional Review Board–approved study from March 2006 to December 2007 at the Hospital of the University of Pennsylvania. Participants were >22 years old, scheduled for COH in preparation for IVF with luteal-phase leuprolide and recombinant human FSH (rhFSH; long protocol), rhFSH and GnRH antagonist (antagonist protocol), or rhFSH and low-dose leuprolide (microdose flare protocol).

At enrollment, study participants completed a standardized questionnaire and were classified as euthyroid or hypothyroid treated. Euthyroid was defined as having no history of hypothyroidism and normal TSH before IVF (within the normal assay range for the patient's clinical laboratory, obtained before study enrollment). Hypothyroid treated was defined as having a history of hypothyroidism and receiving LT_4 therapy. Etiology and current LT_4 dosing were obtained from clinic records for all hypothyroid-treated participants. Additionally, all participants with preexisting or newly diagnosed hypothyroidism were receiving a stable LT_4 dose for ≥6 weeks with a TSH concentration within the normal assay range for their clinical laboratory. Classification of antithyroid peroxidase antibodies (TPOAb) status was based on analysis of banked study serum samples.

During COH, participants were monitored closely with transvaginal ultrasound and serum hormone levels. When a lead follicle ≥18 mm in size was identified, intramuscular hCG was administered with oocyte retrieval 36 hours later. Three to 5 days after retrieval, embryo transfer (ET) was performed, and participants returned 14 days later for measuring serum hCG levels. Pregnancy was defined as positive serum hCG, and participants were followed to determine pregnancy outcome.

For study purposes, venous blood samples were collected at six time points: before stimulation (time 1), 4–7 days after initiating stimulation (time 2), at hCG administration (time 3), 1 week later (time 4), at serum pregnancy test (time 5), and 2 weeks after pregnancy test (time 6). Only participants with three or more study visits were included. Samples were processed and serum stored at −80°C for batched analysis.

Laboratory Analysis

Hormones assayed at the Clinical Translational Research Center included TSH, free T_4, E_2, thyroxine-binding globulin (TBG), and TPOAb. Total T_4 was measured at all time periods in participants with sufficient serum. Thyroid hormone assays were measured with the Immulite analyzer, a fully automated solid-phase third-generation immunoassay analyzer with a chemiluminescent detection system (Diagnostic Products Corp.). The E_2 radioimmunoassay was performed using Coat-A-Count Kits (Diagnostic Products Corp.).

The TSH assay had a range of 0.25–75 mIU/L with intra-assay coefficient of variation (CV) of 7.5%. Euthyroid reference values for this assay are 0.4–4.0 mIU/L. The free T_4 assay had a range of 0.3–6.0 ng/dL with intra-assay CV of 6.3%. The total T_4 assay range was 1.00–24 μg/mL with intra-assay CV of 8.4%. The TBG assay range was 3.5–80 μg/mL with intra-assay CV of 9.3%. The TPOAb assay range was 5.0–1,000 IU/mL with intra-assay CV of 6.5%. Positive TPOAb status was >35 IU/mL. The E_2 assay range was 20–1,800 pg/mL with intra-assay CV of 7.5%.

Statistical Analysis

A priori sample size calculations determined that 44 study participants were needed. Sample size calculation used a one-sample test of proportions assuming that 10% of the sample would have an elevation in TSH above the normal range during COH (i.e., consistent with hypothyroidism [>4.0 mIU/L]) compared with the expected rise in an untreated population (2%). Other assumptions included alpha of .05 and power of 80%. Given up to six measurements per participant, analysis of repeated hormone measurements over time would have even greater power.

General linear mixed effects models with robust variance estimates (GEE) were used to compare log-transformed hormone levels at various times during COH to baseline (13). This method accounts for correlation due to repeated measures within participants. We explored the effect on hormone parameters of hypothyroidism, TPOAb status, and pregnancy outcome. We considered P values of ≤.01 to be statistically significant to account for multiple comparisons. Analyses were performed using Stata 11.2 (Statacorp).

RESULTS

Ninety-two women were enrolled and 57 women included in the final analysis. Thirty-five women were excluded because they completed fewer than three study visits (n = 33), voluntarily withdrew for personal reasons (n = 1), or had untreated hypothyroidism diagnosed after study results were obtained (n = 1). Forty-two of 57 women (73.7%) completed all prepregnancy study visits.

Study participants were 36.4 ± 4.8 (mean ± SD) years old (range 26.3–47.5) and had a body mass index (BMI) of 25.3 ± 5.8 kg/m^2 (range 17.0–43.4). Of the 57 participants, 45 (78.9%) were white and none smoked. Thirty-one of 57 participants (54.4%) reported a previous pregnancy and 11/57 (19.3%) had a child. Twenty of 57 (35.1%) reported a previous miscarriage. In those reporting miscarriage, the mean (±SD) number of miscarriages was 1.75 ± 1.7.

Etiology of infertility was abstracted from medical charts. Two of 57 participants (3.5%) had evidence of anovulation; 10/57 (17.5%) had tubal factor infertility, defined as tubal disease on imaging or laparoscopy; 3/57 (5.3%) had surgical evidence of endometriosis; 10/57 (17.5%) had male factor infertility with reduced sperm concentration (<20 million/mL) on semen analysis; 21/57 (36.8%) had unexplained infertility, defined as patent fallopian tubes, normal sperm concentration, and regular menstrual cycles; and 11/57 (19.3%) had multiple factors for infertility. Long stimulation protocol

was used for 44/57 participants (77.2%), short protocol for 10/57 (17.5%), and microdose flare for 3/57 (5.3%).

Overall, 9/57 participants (15.8%) were classified as hypothyroid-treated. The etiology of hypothyroidism for all was Hashimoto's disease. Forty-eight participants were euthyroid, and only 3/48 (6.3%) were euthyroid-TPOAb positive. This was not sufficient for independent group analysis.

Three participants did not have an ET, and five underwent intrauterine insemination. Of the women who underwent ET, 19/49 (38.8%) became pregnant. Seventeen pregnancies resulted in live births and two resulted in miscarriages (at 7 and at 8 weeks of gestation). Both miscarriages occurred in euthyroid–TPOAb negative participants. Chromosomal analysis on chorionic villi detected 47XX, +9 in one case; the second case did not yield sufficient cells for analysis.

Seven of 57 participants (12.3%) had an initial TSH level >2.5 mIU/L. Of these seven, five were euthyroid–TPOAb negative, one was euthyroid–TPOAb positive, and one was hypothyroid-treated. Five of these participants became pregnant (four euthyroid–TPOAb negative, one hypothyroid-treated), and all had live births. Of the remaining 50 participants with an initial TSH ≤2.5 mIU/L, 22 (44.0%) had a subsequent rise in TSH to >2.5 mIU/L during or after COH and 6/22 (27.3%) subsequently became pregnant, all resulting in live births. Pregnancy rates were similar among those with and without an elevated TSH at any time period (P=.454).

Geometric mean serum hormone levels over time from GEE models are presented in Table 1. All hormone levels changed significantly over time (P<.001 for each). Inclusion of BMI and age in regression models did not significantly change hormone estimates. Compared with baseline (time 1), serum E_2 concentrations peaked on the day of hCG administration (time 3) and levels of TSH, free T_4, and TBG peaked 1 week later (time 4). Total T_4 levels were significantly elevated at all time periods compared with baseline and peaked at time 4.

Additional analyses were performed to examine patterns of thyroid parameters in euthyroid–TPOAb negative participants (n = 45) and hypothyroid-treated participants (n = 9). Although TSH did not differ between groups overall (P=.234), the interaction between thyroid disease and time was statistically significant (P<.001), indicating that the patterns of TSH over time differed between those with and without thyroid disease (Fig. 1). When levels were compared between groups at each time, TSH was significantly higher in hypothyroid-treated participants only at time 4 (P<.001).

Compared with euthyroid-TPOAb negative participants, hypothyroid-treated women had uniformly higher levels of free T_4 (P=.001), but the pattern of change over time was no different (P=.325; Fig. 1). Overall levels of total T_4 were significantly higher in hypothyroid-treated participants (P=.006), but patterns of change did not meet our criteria for statistically significant effect modification (P=.070; data not shown). Similarly, individual pairwise comparisons between groups at each time point did not meet our criteria for statistical significance. Overall levels of E_2 (P=.941) and TBG (P=.908) were not different between groups (Fig. 2). The interaction between time and thyroid disease was not significant for E_2 (P=.537) or TBG (P=.249).

Analyses to assess changes in thyroid function in euthyroid participants, with and without TPO antibodies, were not informative, owing to insufficient euthyroid–TPOAb positive participants (n = 3).

Changes in TSH and free T_4 levels were then compared among the 19 participants achieving pregnancy and the 38 who did not (Fig. 3). For TSH, the interaction between time and pregnancy was significant (P=.006). TSH rose until time 4 in both groups, but in those who achieved pregnancy, TSH remained elevated through time 6 compared with nonpregnant participants, whose levels fell after time 4. Although not reaching our criteria for statistical significance, TSH appeared elevated at times 5 (P=.032) and 6 (P=.042) compared with nonpregnant participants. For free T_4 (data not shown), the interaction between time and pregnancy was not significant (P=.369) and overall levels no different (P=.512). Comparisons between groups at individual time periods were not significant.

Among the 19 participants who achieved pregnancy, two were hypothyroid-treated (Fig. 3). Both participants had an increase in LT_4 dose between times 5 and 6. The interaction of TSH over time was significantly different in those with hypothyroidism (P<.001). The increase in TSH was significantly higher at time 5 (P=.004), before LT_4 dose increase, and appeared higher at time 4 but without reaching significance (P=.029).

TABLE 1

Serum hormone levels by time period.

Time	n	TSH (mIU/L)	Free T_4 (ng/dL)	E_2 (pg/mL)	TBG (μg/mL)	Total T_4 (μg/mL)
1	56	1.42 (1.25–1.60)	1.38 (1.32–1.44)	71.37 (59.04–86.26)	21.52 (20.21–22.91)	8.16 (7.71–8.64)
2	57	1.74 (1.50–2.02)[a]	1.41 (1.35–1.48)	163.33 (132.85–200.79)[b]	20.74 (18.94–22.71)	8.61 (8.15–9.09)[a]
3	55	1.89 (1.64–2.17)[b]	1.32 (1.26–1.37)	1,743.21 (1,459.22–2,082.46)[b]	25.51 (23.31–27.92)[b]	8.82 (8.27–9.40)[b]
4	42	2.44 (2.07–2.87)[b]	1.52 (1.45–1.59)[b]	743.78 (573.34–964.89)[b]	32.86 (29.71–36.33)[b]	11.28 (10.58–12.03)[b]
5	43	2.02 (1.68–2.44)[b]	1.46 (1.38–1.54)[a]	136.34 (72.91–254.93)	31.03 (27.40–35.13)[b]	10.73 (10.03–11.47)[b]
6	30	1.28 (0.97–1.69)	1.48 (1.39–1.57)	331.06 (195.84–559.63)[b]	29.03 (24.75–34.06)[b]	10.16 (9.22–11.19)[b]

Note: Data presented are geometric means with 95% confidence intervals. Tests of significance reflect pairwise comparisons with time 1. For total T_4, n = 40, 41, 41, 41, 33, and 27 for time periods 1–6, respectively. Time points: 1) before stimulation; 2) 4–7 days after initiating stimulation; 3) at hCG administration; 4) 1 week after hCG; 5) at pregnancy test; and 6) 2 weeks after pregnancy test. To convert free T_4 to pmol/L, multiply ng/dL values by 12.87; to convert E_2 to pmol/L, multiple pg/mL values by 3.67; to convert TBG and total T_4 to nmol/L, multiply μg/mL values by 17.09.

[a] P≤.01.

[b] P≤.001.

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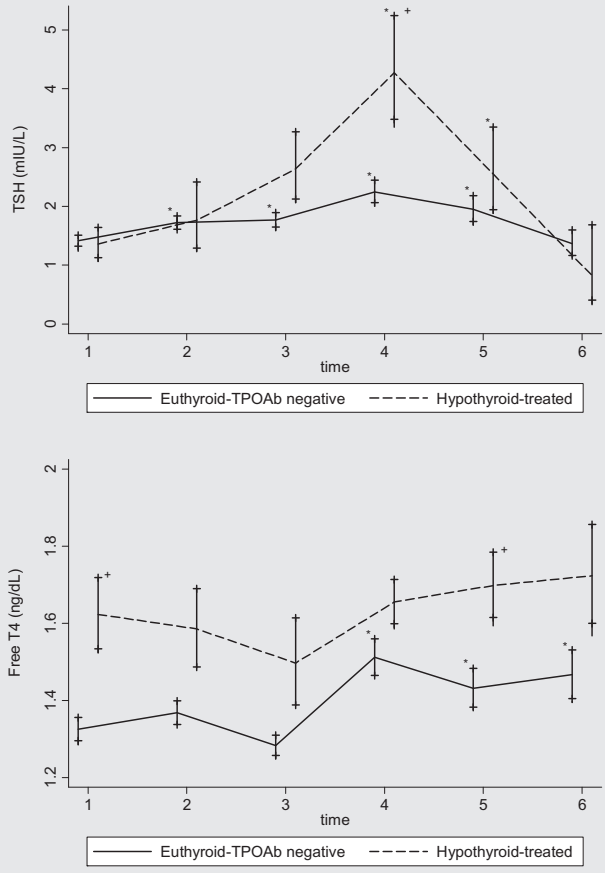
FIGURE 1



(Top) TSH over time by hypothyroid status. (Bottom) Free T_4 over time by hypothyroid status. Geometric mean values with standard error bars shown. For both: euthyroid–TPOAb negative (n = 45) and hypothyroid-treated (n = 9). *$P \leq .01$ for within-group comparisons at each time point compared with time 1. †$P \leq .01$ for between-group comparisons at each time point. Time points: 1) before stimulation; 2) 4–7 days after initiating stimulation; 3) at hCG administration; 4) 1 week after hCG; 5) at pregnancy test; and 6) 2 weeks after pregnancy test.

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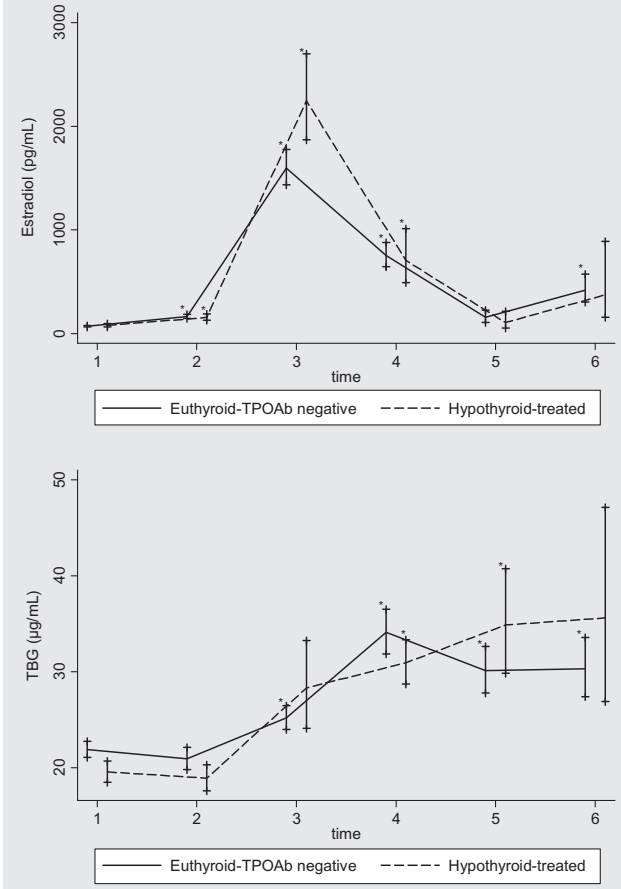
FIGURE 2



(Top) Estradiol over time by hypothyroid status. (Bottom) TBG over time by hypothyroid status. Geometric mean values with standard error bars shown. For both: euthyroid-TPOAb negative (n = 45) and hypothyroid-treated (n = 9). *$P \leq .01$ for within-group comparisons at each time point compared with time 1. †$P \leq .01$ for between-group comparisons at each time point. Time points: 1) before stimulation; 2) 4–7 days after initiating stimulation; 3) at hCG administration; 4) 1 week after hCG; 5) at pregnancy test; and 6) 2 weeks after pregnancy test.

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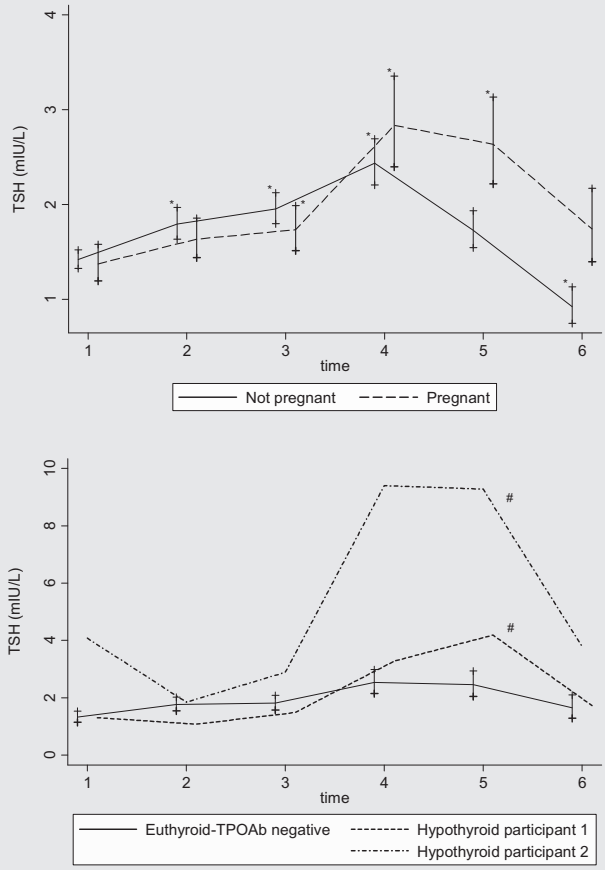
DISCUSSION

The goal of this study was to characterize alterations in thyroid function in women pursuing pregnancy with ART. We hypothesized that supraphysiologic estrogen levels associated with COH stress the hypothalamic-pituitary-thyroid axis, altering thyroid homeostasis. Characterization of thyroid function in ART is important because untreated and undertreated hypothyroidism can lead to adverse pregnancy outcomes and may affect both the immediate health of the developing fetus and childhood development (5–9). In the present study, we assessed thyroid function longitudinally at six time points, from before stimulation to early pregnancy.

We found that thyroid parameters vary considerably during COH. After rapid increases in E_2, which peaks at hCG administration, TSH increases, peaking 1 week later, before declining to near baseline levels. This increase in TSH occurs simultaneously with significant increases in free and total T_4. In response to elevated E_2 levels, TBG increases remaining elevated into the first trimester. It may be that increased TBG at time 3 transiently lowered free T_4 between times 3 and 4, resulting in a surge in TSH secretion and increased free T_4 concentrations at time 4.

Our results have considerable overlap with, but important differences from, other prospective studies of thyroid function in ART. In one study of 65 women, a decrease in free T_4 was noted at oocyte retrieval compared with baseline, whereas TSH, TBG, and total T_4 increased. Although time points in that study differed from ours, we noted similar changes in TSH, TBG, and total T_4, but not free T_4 (3). Two studies by Poppe et al. prospectively examined thyroid function in women after COH. The first measured TSH and free T_4 every 20 days in 35 women who became pregnant after COH. That study found significant elevations of both TSH and free T_4 at 20 days after ovulation induction compared with baseline

FIGURE 3



(Top) TSH over time by pregnancy (pregnant [n = 19] vs. not pregnant [n = 38]). *$P \leq .01$ for within-group comparisons at each time point compared with time 1. †$P \leq .01$ for between-group comparisons at each time period. (Bottom) TSH over time in participants that became pregnant (16 euthyroid–TPOAb-negative participants and two hypothyroid participants). Both hypothyroid participants had an increase in LT_4 dose between times 5 and 6 (#). Geometric mean values with standard error bars shown. Time points: 1) before stimulation; 2) 4–7 days after initiating stimulation; 3) at hCG administration; 4) 1 week after hCG; 5) at pregnancy test; and 6) 2 weeks after pregnancy test.

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(2). The second followed 77 euthyroid women undergoing COH, assessing TSH and free T_4 at baseline and 2, 4, and 6 weeks after ET (4). Although that study did not assess thyroid parameters around ET, the authors noted significant elevations in TSH and free T_4 2 weeks after ET compared with baseline. We noted a similar rise in TSH approximately 2 weeks after ET (time 5) and a significant rise in free T_4 1 week after hCG administration (time 4) and when the pregnancy test was checked (time 5).

Although results for TSH appear to be consistent, the change in free T_4 during COH remains unclear. This may be due to differences in free T_4 assays. Equilibrium dialysis is the criterion standard to measure free T_4, not the automated analog free T_4 method designated in our protocol and other publications. The analog free T_4 method can be sensitive to abnormal binding-protein states, such as the COH estrogen-induced 50% increase in serum TBG levels, and may report falsely higher serum free T_4 values (14). Therefore, compared with baseline, the analog-assayed stable free T_4 values in hypothyroid-treated women at the time of peak TBG concentration may mask a true physiologic decrease in serum free T_4.

We also noted greater perturbations in thyroid parameters, particularly TSH, among hypothyroid-treated participants. This difference was greatest 1 week following hCG administration, shortly after ET. Although baseline free and total T_4 were higher in participants with disease, likely owing to baseline LT_4 supplementation, these levels gradually decreased during stimulation before responding to increasing TSH levels. The increases in TBG were no different between groups. However, in our population it appears that euthyroid–TPOAb negative participants were better able to respond to increased TBG, as reflected by relatively stable TSH levels during stimulation, whereas hypothyroid-treated participants could not, and TSH increased. These changes in thyroid parameters in women with and without hypothyroidism during COH, especially 1 week after ovulation induction, have not been previously reported in prospective studies.

Rising levels of TBG, in response to elevated E_2 during COH, decrease unbound serum T_4 and T_3. In participants with hypothyroidism, this contributes to the increased LT_4 requirement during pregnancy (15), which may occur as early as the fifth week of gestation, a critical period for fetal development. LT_4 requirements may be even greater in women who undergo ART.

The effect of TPOAbs on thyroid function and pregnancy outcome in the ART population remains unclear. Poppe et al. reported higher TSH and lower free T_4 curves in those with thyroid autoimmunity (defined as the presence of TPOAbs >100 kU/L) undergoing ART. Although changes were significant overall, between-group comparison of measures at baseline and at 20 days after ovulation induction were not statistically significant (2). Diverse patient populations, stimulation protocols, timing of measurements, and individual assays/cutoffs for TPOAb positivity may account for conflicting findings. In the present study, because of a limited number of euthyroid–TPOAb positive participants, we were unable to make comparisons of thyroid parameters in this subgroup.

At the time of the pregnancy test we detected elevations in TSH but not in free T_4 among pregnant participants. However, the elevations did not reach statistical significance. Furthermore, two hypothyroid-treated participants in our study became pregnant. Their pattern of TSH over time differed from euthyroid–TPOAb negative participants, appearing higher 1 week after hCG administration and at the pregnancy test. However, owing to the limited number of hypothyroid patients achieving pregnancy, these elevations were not statistically significant. Thyroid supplementation was increased in both participants, which explains the subsequent decrease in TSH at time 6. Had the study population been larger with more hypothyroid participants, significant differences may have been detected.

Additionally, all but two participants who achieved pregnancy had a live birth, limiting the ability to compare thyroid parameters between participants with different pregnancy outcomes. However, thyroid function among the ART

population appears to be no different among those with an ongoing clinical pregnancy versus miscarriage (4).

Substantial controversy surrounds thyroid disease screening in the general population attempting conception (16, 17). The American College of Obstetricians and Gynecologists and the Endocrine Society recommend targeted screening (18, 19), even though such screening may miss women with subclinical hypothyroidism (defined as elevated serum TSH with normal free T_4) and autoimmune thyroid disease (defined as positive TPOAbs) (20, 21). Although the cost-effectiveness of such recommendations is not clear, available evidence suggests some economic savings for universal screening in the general population (22, 23).

Regarding the infertile population, obtaining a serum TSH in women with a diagnosis of infertility or recurrent miscarriage is currently recommended (19). However, the TSH cutoff at which to initiate treatment in nonpregnant women is debatable, because high-quality evidence is lacking. It must be recognized that lowering the cutoff to initiate treatment would result in a dramatic increase of patients receiving LT_4 supplementation. In the present study, we found that 44% of participants with an initial TSH $\leq$2.5 mIU/L at baseline had a subsequent rise in TSH to >2.5 mIU/L during COH. Had we monitored thyroid function in "real time," we might have supplemented these patients. Nonetheless, our pregnancy outcomes were reassuring.

The increased requirement for thyroid hormone supplementation during pregnancy in women with primary hypothyroidism is well documented (10, 11, 15). However, it is unclear if thyroid supplementation improves pregnancy outcomes in women with subclinical hypothyroidism and/or TPOAbs (24, 25). One randomized study of LT_4 supplementation in TPOAb-positive women during ART showed that supplementation did not affect pregnancy rates (24). Although TPOAb-positive participants had a higher rate of miscarriage overall, LT_4 treatment did not decrease miscarriages. Another study randomizing ART patients with subclinical hypothyroidism to LT_4 treatment found clinical pregnancy rates to be no different between groups (25). However, that study reported lower rates of miscarriage in the group receiving LT_4. False negative results in pregnancy rates cannot be excluded, because those studies were underpowered. Overall, data are limited and controversy remains whether supplementation among those without overt hypothyroidism affects clinical pregnancy outcomes.

We believe that our study has several strengths. To our knowledge, it is the most comprehensive examination of thyroid function during and after COH. We captured multiple time points, starting early in stimulation, in an attempt to detect changes in thyroid function as the hormonal environment rapidly changes. We decided not to restrict our study population based on the presence of antibodies or preexisting thyroid disease, because we wanted to characterize thyroid function in a typical population of IVF patients. Without restrictions, our results are likely generalizable to other patients pursuing IVF.

Several limitations of the present study must be also recognized. First, to determine the true effect of COH on thyroid function, an optimal study would compare thyroid changes in women undergoing IVF with women attempting pregnancy without assistance. Nonetheless, we were able to demonstrate considerable fluctuation in thyroid parameters during ART. These findings should be validated through comparison with unexposed women to determine if the changes are unique to COH. In addition, the small sample size included few hypothyroid or euthyroid–TPOAb positive women. Therefore, it is possible that real differences in thyroid parameters were not detected. Finally, our results may not necessarily apply to all women, because our population was principally white, had a normal BMI, and did not smoke.

In conclusion, we have demonstrated that COH leads to considerable perturbations in thyroid parameters, particularly in participants with preexisting hypothyroidism. Significant increases in TSH may occur before the results of serum pregnancy tests are known. Among some women who achieved pregnancy, serum TSH concentrations rose above pregnancy-appropriate targets and these elevations may have clinically significant consequences. More data are needed to determine whether hypothyroid women who undergo COH should be monitored and supplemented differently from the general population. Larger long-term prospective studies assessing thyroid parameters and childhood outcomes in women undergoing ART would add clarity to this important topic.

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REFERENCES

1. Chandra A, Martinez GM, Mosher WD, Abma JC, Jones J. Fertility, family planning, and reproductive health of U.S. women: data from the 2002 National Survey of Family Growth. Vital Health Stat 23 2005:1–160.
2. Poppe K, Glinoer D, Tournaye H, Schiettecatte J, Devroey P, van Steirteghem A, et al. Impact of ovarian hyperstimulation on thyroid function in women with and without thyroid autoimmunity. J Clin Endocrinol Metab 2004;89:3808–12.
3. Muller AF, Verhoeff A, Mantel MJ, de Jong FH, Berghout A. Decrease of free thyroxine levels after controlled ovarian hyperstimulation. J Clin Endocrinol Metab 2000;85:545–8.
4. Poppe K, Glinoer D, Tournaye H, Schiettecatte J, Haentjens P, Velkeniers B. Thyroid function after assisted reproductive technology in women free of thyroid disease. Fertil Steril 2005;83:1753–7.
5. Kutteh WH, Yetman DL, Carr AC, Beck LA, Scott RT Jr. Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction. Fertil Steril 1999;71:843–8.
6. Poppe K, Glinoer D, Tournaye H, Devroey P, van Steirteghem A, Kaufman L, et al. Assisted reproduction and thyroid autoimmunity: an unfortunate combination? J Clin Endocrinol Metab 2003;88:4149–52.
7. Poppe K, Glinoer D. Thyroid autoimmunity and hypothyroidism before and during pregnancy. Hum Reprod Update 2003;9:149–61.
8. Abalovich M, Gutierrez S, Alcaraz G, Maccallini G, Garcia A, Levalle O. Overt and subclinical hypothyroidism complicating pregnancy. Thyroid 2002;12:63–8.
9. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999;341:549–55.
10. Mandel SJ, Larsen PR, Seely EW, Brent GA. Increased need for thyroxine during pregnancy in women with primary hypothyroidism. N Engl J Med 1990;323:91–6.
11. Abalovich M, Alcaraz G, Kleiman-Rubinsztein J, Pavlove MM, Cornelio C, Levalle O, et al. The relationship of preconception thyrotropin levels to

requirements for increasing the levothyroxine dose during pregnancy in women with primary hypothyroidism. Thyroid 2010;20:1175–8.
12. Loh JA, Wartofsky L, Jonklaas J, Burman KD. The magnitude of increased levothyroxine requirements in hypothyroid pregnant women depends upon the etiology of the hypothyroidism. Thyroid 2009;19: 269–75.
13. Liang KY, Zeger SL. Longitudinal data-analysis using generalized linear-models. Biometrika 1986;73:13–22.
14. Stockigt JR. Free thyroid hormone measurement. A critical appraisal. Endocrinol Metab Clin North Am 2001;30:265–89.
15. Alexander EK, Marqusee E, Lawrence J, Jarolim P, Fischer GA, Larsen PR. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. N Engl J Med 2004;351: 241–9.
16. Burman KD. Controversies surrounding pregnancy, maternal thyroid status, and fetal outcome. Thyroid 2009;19:323–6.
17. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA 2004;291:228–38.
18. ACOG. Committee opinion no. 381: subclinical hypothyroidism in pregnancy. Obstet Gynecol 2007;110:959–60.
19. Abalovich M, Amino N, Barbour LA, Cobin RH, De Groot LJ, Glinoer D, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society Clinical Practice guideline. J Clin Endocrinol Metab 2007;92:S1–47.
20. Vaidya B, Anthony S, Bilous M, Shields B, Drury J, Hutchison S, et al. Detection of thyroid dysfunction in early pregnancy: universal screening or targeted high-risk case finding? J Clin Endocrinol Metab 2007;92:203–7.
21. Negro R, Schwartz A, Gismondi R, Tinelli A, Mangieri T, Stagnaro-Green A. Universal screening versus case finding for detection and treatment of thyroid hormonal dysfunction during pregnancy. J Clin Endocrinol Metab 2010;95:1699–707.
22. Thung SF, Funai EF, Grobman WA. The cost-effectiveness of universal screening in pregnancy for subclinical hypothyroidism. Am J Obstet Gynecol 2009;200:267.e1–7.
23. Dosiou C, Sanders GD, Araki SS, Crapo LM. Screening pregnant women for autoimmune thyroid disease: a cost-effectiveness analysis. Eur J Endocrinol 2008;158:841–51.
24. Negro R, Mangieri T, Coppola L, Presicce G, Casavola EC, Gismondi R, et al. Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study. Hum Reprod 2005;20:1529–33.
25. Kim CH, Ahn JW, Kang SP, Kim SH, Chae HD, Kang BM. Effect of levothyroxine treatment on in vitro fertilization and pregnancy outcome in infertile women with subclinical hypothyroidism undergoing in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2011;95:1650–4.