

# The effect of thyroid autoantibody on assisted reproduction technology outcome in euthyroid women: One center experience

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**Abstract:** Thyroid dysfunction is an important factor to cause failure in assisted reproduction technology (ART) procedures. In this study, we recorded the serum level of thyroid autoantibody to figure out its relationship with the ART outcome. The results showed that the serum concentrations of TSH had a statistically significant increase between the basal level and the levels at time of serum pregnancy test both in women with and without thyroid autoantibody ( $p=0.002$  and  $p=0.019$ , respectively). Additionally, the TSH level increased significantly in thyroid autoantibody-positive group than those in thyroid autoantibody-negative group during controlled ovarian hyper stimulation (COH) process ( $p=0.006$ ). The risk of preterm delivery was lower in thyroid autoantibody-negative group. In sum, the present study provided evidence of an association between thyroid autoantibody and preterm delivery in euthyroid women.

**Keywords:** Thyroid stimulating hormone (TSH), thyroid auto-antibodies, controlled ovarian hyper stimulation, assisted reproductive, technology.

## INTRODUCTION

Thyroid disease is one of the most common endocrine disorders of reproductive-aged women and the thyroid stimulating hormone (TSH) levels are a sensitive marker for thyroid gland dysfunction (Mebis and Van den Berghe, 2011). The thyroid gland dysfunction may cause impaired ovulation (Bussen *et al.*, 2000), lower clinical pregnancy rates (Goldsmith *et al.*, 1952), higher miscarriage rates (Kilic *et al.*, 2008) and poor obstetrical outcomes (Abalovich *et al.*, 2002; Baker *et al.*, 2006). The National Center on Birth Defects and Developmental Disability of the Centers for Disease Control and Prevention (CDC) and the American Thyroid Association recommended that the treatment with levothyroxine should be initiated if the serum TSH  $> 2.5$  mIU/L is confirmed before pregnancy (De Groot *et al.*, 2012; Stagnaro-Green *et al.*, 2011).

The thyroid autoantibody may cause infertility and pregnancy loss and lead to the failure of assisted reproductive techniques (ART) (Kilic *et al.*, 2008; Matalon *et al.*, 2011; Poppe *et al.*, 2007; Bellver *et al.*, 2008). The controlled ovarian hyperstimulation (COH) is an integral part of many ART procedures. During the COH, the estradiol (E2) levels would rise and reach the peak during the second trimester of pregnancy (Poppe *et al.*, 2011; Muller *et al.*, 2000). Although there were some evidences that COH could impair thyroid function (Poppe *et al.*, 2004; Poppe *et al.*, 2008), the relevant pathophysiology had not been fully elucidated. Some researchers found the serum TSH exceeded the threshold of 2.5mIU/L during the COH (Gracia *et al.*, 2012; Benaglia *et al.*, 2014). Moreover, the women with thyroid

autoimmunity might have higher TSH and lower free thyroxin (FT4) curves undergoing ART (Poppe *et al.*, 2008). However, other studies identified that the TSH and FT4 concentrations did not change after the COH (Kim *et al.*, 2011; Monteleone *et al.*, 2011; Reh *et al.*, 2011). In this study, the change of thyroid function in women with and without thyroid autoantibodies during the COH and the effect of thyroid autoantibodies on pregnancy outcome would be analyzed.

## MATERIALS AND METHODS

### Subjects

A prospective study was performed in the reproduction center of Yantai Yuhuangding Hospital in China between October 2013 and June 2014. Patients aged 38 years or less who underwent their first in-vitro fertilization (IVF) cycle were eligible to participate in this study. All patients underwent screening for TSH, free triiodothyronine (FT3), free thyroxin (FT4), anti-thyroglobulin antibody (TG-Ab), and anti-thyropoxidase antibody (A-TPO) before the COH. They fulfilled the following criteria: (i) both ovaries present; (ii) Day 3 Follicle-stimulating hormone (FSH)  $<10$  IU/l, oestradiol  $<80$ pg/ml and prolactin in the normal range before ovarian stimulation; (iii) presence of a normal uterine cavity; (iv) normal TSH concentration or euthyroid as determined by the investigator, and the normal range of our institution's laboratory was 0.27-4.2mIU/L. (v) no current or past diseases affecting the administration of gonadotrophin. The women who had recurrent spontaneous abortion were excluded. (vi) the etiology of infertility was in tubal factor. All the details were mentioned in our previous manuscript (Zhang *et al.*, 2013). Informed written consents were obtained from all the patients.

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### Treatment protocol

All patients underwent standard pituitary down-regulation protocol with GnRHa (triptorelin, Diphereline; Ipsen Pharma Biotech, France) 0.05 mg between the day 5-7 after ovulation or on the day 21 of the oral contraceptive cycles. During the COH, these participants were monitored closely with transvaginal ultrasound and serum hormone levels. Fourteen days later, complete pituitary desensitization was confirmed by the detection of serum oestradiol concentrations  $<50$  pg/ml, LH  $<3$  IU/l, no follicles  $>10$  mm in diameter and endometrial thickness  $<7$  mm by ultrasound examination. Gonadotrophin was administered with recombinant FSH (Gonal F; Merck Serono, Switzerland) 150-300 IU/day. When a lead follicle  $\geq 18$  mm in size was identified, the intramuscular hCG was administered (250 $\mu$ g, Ovidrel; Merck Serono, Switzerland). Transvaginal oocyte retrieval was performed 34–36 h after the administration of HCG. Embryo transfers were performed 3 days after oocyte retrieval. Progesterone vaginal capsules (Besins, Iscovesco, France) were administered 600mg/day as luteal support from the day of the oocyte retrieval. All participants returned 14 days later for measuring serum hCG levels. Pregnancy was defined as positive serum hCG. Clinical pregnancy was defined as the presence of a gestational sac confirmed 5 weeks after embryo transfer by ultrasonography and preterm delivery was defined as less than 34 weeks.

Blood was drawn at three time points: Before the stimulation, at the hCG administration and at the serum pregnancy test. Samples were processed and serum stored at  $-80^{\circ}\text{C}$  for analysis. Once all subjects completed their treatment cycles, samples were thawed and analyzed for TSH, FT4 and thyroid antibodies. The Elecsys 2010 (Cobas Integra 400 Plus, Roche, Germany) was used to assay the TSH, FT4 and thyroid antibodies during and after the ART treatment. The range of normal for TSH is 0.27–4.2mIU/L. Patients with anti-thyroglobulin (anti-Tg) antibodies of  $>115$  IU/mL or anti-thyroperoxidase (Anti-TPO) antibodies of  $>34$  IU/mL were considered “antibody-positive” at our centre.

### Ethical approval

The study was approved by the Ethic and Research Committees of Yuhuangding Hospital, Qingdao University and was conducted in accordance with the Declaration of Helsinki Principles. informed consent was obtained for all participating patients.

### STATISTICAL ANALYSIS

Data were statistically described in terms of means  $\pm$  SD and independent-samples t-test and Fisher’s exact test were used. The  $p$ -values of  $<0.05$  were considered statistically significant. All statistical analyses were performed with Statistical Package for Social Sciences version 23.0 (SPSS, Chicago, IL, USA).

## RESULTS

### Baseline characteristics

Baseline characteristics were shown in table 1. Before COH the mean TSH of patients with TAI was  $2.11\pm 1.13$  mIU/L. For patients without TAI, the mean TSH at that time was compared to a mean of  $1.94\pm 1.22$  mIU/L and there was no significant differences between the two groups ( $p>0.05$ ). Moreover, there was no difference between the two groups in the age ( $32.37\pm 3.85$  vs  $31.82\pm 3.39$  year,  $p>0.05$ ), FSH ( $6.49\pm 1.67$  vs  $6.07\pm 1.51$  U/L,  $p>0.05$ ), BMI ( $24.91\pm 3.44$  vs  $23.21\pm 2.69$  kg/  $\text{m}^2$ ,  $p>0.05$ ), FT4 ( $5.01\pm 0.67$  vs  $4.86\pm 0.71$  pmol/L,  $p>0.05$ ) and FT3 ( $16.65\pm 2.21$  vs  $17.10\pm 2.43$  pmol/L,  $p>0.05$ ) levels at baseline.

### COH and Thyroid function

The change of thyroid function in women with and without TAI during COH has been shown in table 2. We found TSH levels increased during COH, and this change was significant at the serum pregnancy time. And those who had TAI had a larger degree of change in their TSH ( $2.11\pm 1.13$  mIU/L vs  $4.04\pm 1.43$  mIU/L,  $p=0.002$ ) than those without TAI ( $1.94\pm 1.22$  mIU/L vs  $2.35\pm 1.32$  mIU/L,  $p=0.019$ ), which had a significant difference ( $4.04\pm 1.43$  mIU/L vs  $2.35\pm 1.32$  mIU/L,  $p=0.006$ ). After COH, we also found FT4 decreased slightly, but they had no statistical difference no matter whether they had TAI or not. The FT3 did not changed obviously in the two groups.

### Outcome of ART

Finally, the thyroid auto antibodies did not influence the pregnancy rate and the early miscarriage rate. However, the preterm delivery rate was significantly higher in the TAI group compared with the control group ( $p=0.03$ ),

## DISCUSSION

Hypothyroidism was associated with various obstetrical complications such as spontaneous abortion, preeclampsia and growth restriction. Many women with sub-clinical hypothyroidism might be at risk to become hypothyroid during treatment and might benefit from replacement therapy. Thyroid autoimmunity (TAI) was common in the women of reproductive age and the presence of thyroid auto antibodies increased the risk of developing sub-clinical hypothyroidism during pregnancy (Muller *et al.*, 2000; Vanderpump *et al.*, 1995). Therefore, identification of thyroid dysfunction during COH was important because untreated and under treated hypothyroidism could lead to adverse pregnancy outcomes.

Many studies focused on the influences of COH on thyroid function in women found that serum TSH could increase significantly on the day of pregnancy test after COH (Muller *et al.*, 2000) and reach a peak 1 week after

**Table 1:** Characteristics of patients with and without TAI (The *p*-values of <0.05 were considered statistically significant.)

	Patients with TAI ( <i>n</i> = 42)	Patients without TAI ( <i>n</i> = 109)	<i>p</i> -value
Age(year)	32.37±3.85	31.82±3.39	>0.05
FSH (U/L)	6.49±1.67	6.07±1.51	>0.05
TSH(mIU/L)	2.11±1.13	1.94±1.22	>0.05
BMI(kg/ m <sup>2</sup> )	24.91±3.44	23.21±2.69	>0.05
FT3(pmol/L)	5.01±0.67	4.86±0.71	>0.05
FT4(pmol/L)	16.65±2.21	17.10±2.43	>0.05

**Table 2:** The TSH and FT levels in two groups during COH (-1:Before the stimulation; -2: at the hCG administration; -3:at the serum pregnancy test. The *p*-values of <0.05 were considered statistically significant.)

	Patients with TAI ( <i>n</i> = 42 )	Patients without TAI ( <i>n</i> = 109)	<i>p</i> -value
TSH-1(mIU/L)	2.11±1.13	1.94±1.22	>0.05
TSH-2(mIU/L)	2.42±1.21	2.11±1.27	>0.05
TSH-3(mIU/L)	4.04±1.43	2.35±1.32	0.006
FT4-1(pmol/L,)	16.65±2.21	17.10±2.43	>0.05
FT4-2(pmol/L,)	15.71±2.01	15.40±2.26	>0.05
FT4-3(pmol/L,)	15.34±1.98	15.21±2.03	>0.05
FT3-1(pmol/L,)	5.01±0.67	4.86±0.71	>0.05
FT3-2(pmol/L,)	5.15±0.72	4.98±0.69	>0.05
FT3-3(pmol/L,)	4.95±0.64	4.80±0.65	>0.05

**Table 3:** The outcome of assisted reproduction technology in two groups (The *p*-values of <0.05 were considered statistically significant.)

	Patients with TAI ( <i>n</i> = 42 )	Patients without TAI ( <i>n</i> = 109 )	<i>p</i> -value
Clinical pregnancy rate	44.8%(18/42)	46.8%(51/109)	>0.05
Early miscarriage rate	16.7%(3/18)	11.8%(6/51)	>0.05
Preterm delivery rate	26.7% (4/15)	4.4%(4/45)	0.03

Values are mean ± SD or % (*n* / total).

HCG trigger (Gracia *et al.*, 2012). Besides, FT4 would decrease around the time of conception and increase within 1 month after COH (Poppe *et al.*, 2008; Poppe *et al.*, 2005; Poppe *et al.*, 2008). The aim of this study was to find the change of thyroid function in women with and without thyroid auto antibodies during COH. Our results validated that the COH could increase the TSH, especially in the patients with TAI (Muller *et al.*, 2000; Reinblatt *et al.*, 2013). The pathophysiology of thyroid function impairment by COH has not been fully elucidated. The COH leads to hyperestrogenism, which increases thyroxin-binding globulin (TBG) levels and therefore causes a drop in free thyroxine (FT4). At the same time, HCG is also hypothesized to be involved, as its receptor shares structural homologies with the TSH receptor (Mebis and Van den Berghe, 2011). We found the TSH levels increased slightly at the HCG administration day (at maximal E<sub>2</sub> levels). Other researchers observed that the TSH level increased significantly at 2 weeks post-embryo transfer in the patients with ovarian hyperstimulation syndrome (extremely high E<sub>2</sub> levels) after COH (Poppe *et al.*, 2011). Therefore, we suggested that the change in TSH might be occurring at least 2 weeks

and the effect depended on the time.. The presence of thyroid auto antibodies might increase the risk of developing sub-clinical hypothyroidism during COH and added additional burden to the hypothalamic-pituitary-thyroid axis, resulting in a change of thyroid homeostasis. However, we did not find clinically meaningful changes in FT4, which was consistent with the previous study (Monteleone *et al.*, 2011). Maybe there were some other complex mechanisms in regulating thyroid function, which needed to be investigated in the future. Afterwards, we found that the thyroid autoantibodies did not influence the pregnant rate and the early miscarriage rate and the preterm delivery rate was significantly higher in the TAI group. However, a previous meta-analysis revealed that TAI would determine an increased risk of miscarriage and a decreased chance of live birth (Busnelli *et al.*, 2016). And treatment with levothyroxine could decrease the risk of preterm delivery in women who were for TPOAb-positive (Nazarpour *et al.*, 2017). All the results indicated that TAI could influence the preterm delivery and more clinical trials were needed in the future.

## CONCLUSION

We found that COH led to a significant rise in TSH, especially in women with TAI and TAI might cause a risk of preterm delivery. We might consider levothyroxine before COH in women with TAI and “borderline” high TSH. We also suggested that thyroid function should be detected at the pregnancy test day to identify those patients requiring replacement especially in patients with TAI.

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