의학박사 학위논문

Impact of presence of thyroid autoantibodies and serum TSH level in infertile women on clinical IVF outcomes

여성 난임 환자에서 갑상선자가항체 유무 및 혈청 갑상선자극호르몬 농도가 체외수정시술 결과에 미치는 영향

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서울대학교 대학원
의학과 산부인과학전공
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지도교수 김석현

이 논문을 의학박사 학위논문으로 제출함

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의학과 산부인과학전공

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문경용의 박사 학위논문을 인준함

2021 년 6월

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Abstract

Impact of presence of thyroid autoantibodies and serum TSH level in infertile women on clinical IVF outcomes

Kyoung Yong Moon
College of Medicine
The Department of Obstetrics & Gynecology
The Graduate School
Seoul National University

AIM: This study aimed to investigate the impact of thyroid autoantibodies and serum thyroid stimulating hormone (TSH) level in infertile women on the clinical and ongoing pregnancy after in vitro fertilization (IVF) treatment.

METHODS: This study included 260 Korean women who were scheduled for their first IVF cycle between March 2013 and February 2017. Just prior to ovarian stimulation for the first IVF cycle, serum levels of thyroid hormone, TSH, thyroid peroxidase antibody, and thyroglobulin antibody were measured. Clinical and ongoing pregnancy rates (PR) and

miscarriage rates after IVF were prospectively analyzed according to thyroid autoimmunity and serum TSH levels. The primary outcome was ongoing PR beyond 12 weeks of gestation.

RESULTS: The prevalence of positive thyroid autoantibodies was 15% overall (39/260). Embryo transfer was performed in 215 women (27 women with subclinical hypothyroidism and 188 euthyroid women). The clinical PR, ongoing PR, miscarriage rates were all similar between thyroid autoantibody−positive (n = 29) and negative women (n = 186). In women with subclinical hypothyroidism, ongoing PR was significantly lower than in the euthyroid group [22.2% (6/27) vs. 44.7% (84/188), p=0.045]. Clinical PR was also significantly lower [33.3% (9/27) vs. 53.7% (101/188), p=0.047], but miscarriage rate was not different. Among 215 women with subclinical hypothyroidism or euthyroid women, the group with serum TSH ≥ 3.4 μIU/mL showed a significantly lower ongoing PR than those with TSH < 3.4 μIU/mL [23.9% (11/46) vs. 46.7% (79/169), p= 0.005]. Clinical PR tended to be lower [39.1% (18/46) vs. 54.4%

(92/169), p=0.066], and miscarriage rate was significantly higher [38.9% (7/18) vs. 14.1% (13/92), p=0.020]. In multivariate logistic regression analysis, serum TSH levels ≥ 3.4 μIU/mL (OR 0.375, p=0.013) as well as old age (OR 0.893, p=0.007) were independent unfavorable predictors for ongoing PR and the number of good-quality embryos was an independent favorable predictor (OR 1.546, p=0.014).

CONCLUSION: Thyroid autoantibodies did not affect pregnancy or miscarriage after IVF, but infertile women with serum TSH level ≥ 3.4 μIU/mL demonstrated poor IVF outcomes.

Keywords: Thyroid stimulating hormone (TSH), thyroid autoantibodies, pregnancy, in vitro fertilization (IVF)
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Introduction

1. Study Background

Thyroid dysfunction may induce ovulatory disorders or infertility, and adverse obstetric and fetal outcomes including miscarriages (1-4). Thyroid dysfunction can also have detrimental effects during the process of implantation and early pregnancy; thus, it has been suggested that thyroid dysfunction could affect poor outcomes after in vitro fertilization and embryo transfer (IVF-ET) in infertile women.

Thyroid autoimmunity, defined as the presence of thyroid autoantibodies, such as thyroid peroxidase antibody and thyroglobulin antibody, is a common cause of hypothyroidism, but may also be present in women with normal thyroid function (5). Stagnaro-Green et al. (6) initially reported an association between thyroid autoimmunity and spontaneous miscarriage; thereafter, numerous studies and two meta-analyses supported this association (7, 8). Although the precise mechanism is not clear, direct actions of thyroid autoantibodies on the placenta, impeded adaptability of the

thyroid gland to the pregnancy state, and/or the co-presence of other autoimmune syndromes have been proposed (9).

Thyroid autoimmunity has been found in 2% to 30% of infertile women, although its prevalence differs according to the etiologies of infertility, study settings, types of antibodies, and ethnic and geographical origins of the study populations (10-16). It has been suggested that euthyroid infertile women with thyroid autoimmunity have poorer outcomes after IVF-ET. One meta-analysis showed higher miscarriage rate, but similar clinical pregnancy rate (PR) and live birth rate among women with thyroid autoimmunity compared to those without thyroid autoimmunity (9). Another recent meta-analysis found that women with thyroid autoimmunity had similar clinical PR, but higher miscarriage rate, and lower live birth rate (17).

Various criteria for serum thyroid stimulating hormone (TSH) level before IVF have been suggested for a successful clinical pregnancy; one study suggested serum TSH > 2.5 μIU/mL (18), while others suggested serum TSH > 4.2 μIU/mL or > 4.5 μIU/mL for poor clinical PR (19, 20). The latter two

studies showed an increased clinical PR after levothyroxine treatment in women with TSH > 4.2 μIU/mL or TSH > 4.5 μIU/mL.

However, no association between serum TSH levels before IVF and resultant pregnancy outcomes has been previously reported. Mintziori et al. reported no difference in live birth rate between women with TSH 0.5−2.5 μIU/mL vs. 2.6−4.5 μIU/mL (21). In a large retrospective cohort study including 1,055 first−cycle IVF cycles, no differences were found in clinical PR, delivery rate, or miscarriage rate when serum TSH 2.5 μIU/mL or 4.5 μIU/mL were used as cutoffs (22). Similarly, a recent retrospective study reported similar clinical PR among women with serum TSH 0.5−2.5 μIU/mL and 2.5−4.5 μIU/mL (23). Another recent retrospective study found similar clinical PR, live birth rate, and miscarriage rate with or without subclinical hypothyroidism (using a serum TSH threshold of 4.5 μIU/mL) (24).

The Practice Committee of the American Society for Reproductive Medicine recommended close observation or levothyroxine treatment, when serum TSH levels before IVF

are between 2.5 and 4.0 μIU/mL (25). The Committee also recommended levothyroxine treatment for women with positive thyroid autoantibodies before IVF.

The American Thyroid Association recommended that women with TSH > 2.5 μIU/mL undergoing IVF should be treated with levothyroxine (26), but they said there is insufficient evidence to determine levothyroxine treatment for thyroid peroxidase-positive euthyroid women.

Currently, there have been conflicting results on the role of thyroid autoantibodies and/or serum TSH levels on clinical pregnancy or miscarriage after IVF.

2. Purpose of Research

This prospective cohort study investigated whether thyroid autoantibodies or high serum TSH levels had a negative influence on clinical pregnancy or miscarriage after IVF-ET.

Material and methods

1. Study subjects

Two hundred and sixty infertile women aged 24−44 years who were undergoing their first IVF−ET cycle at three fertility clinics (Seoul National University Hospital/Seoul National University Bundang Hospital/Seoul Maria Fertility Hospital) were prospectively recruited between March 2013 and February 2017. This study was approved by the Institutional Review Board of three fertility clinics, and all participants gave written informed consent.

To control other confounding factors that may affect pregnancy rate, women with uterine factors such as uterine anomaly or synechia were excluded. Infertile couple with male factors were also excluded. Women with known thyroid disease, other endocrine/immunologic disorders, and obese women (body mass index ≥ 30 kg/m^2) were also excluded.

2. Blood test

Serum TSH, free thyroxine (T4), tri-iodothyronine (T3), thyroid peroxidase antibody, and thyroglobulin antibody were measured using electrochemiluminescence immunoassay (ECLIA) (Roche, E170, ELECSYS, Mannheim, Germany) with Elecsys and cobas immunoassay analyzers. The reference values were as follows: serum TSH 0.27-4.20 μIU/mL, free thyroxine 0.74-1.80 ng/dL, and T3 0.63-2.00 ng/mL. The reference values for thyroid peroxidase antibody and thyroglobulin antibody were < 34.0 IU/mL and < 115 IU/mL, respectively.

3. IVF-ET

IVF-ET was performed using the usual method. Flexible multiple-dose GnRH antagonist protocol or GnRH agonist long protocols were used for controlled ovarian stimulation according to the physician's preference. Follicular growth was monitored via transvaginal ultrasonography. When at

least two dominant follicles reached 18 mm or three follicles reached 17 mm in mean diameter, recombinant human chorionic gonadotropin (hCG) was administered to trigger final follicular maturation.

Oocyte retrieval was performed 35-36 hours after hCG trigger and luteal phase support was started. Oocytes were inseminated by conventional insemination (15 women), intracytoplasmic sperm injection (ICSI) (31 women), or combined (split) insemination (169 women), according to laboratory decision. Fertilization was assessed at 16-18 hours post-insemination. Embryos were transferred on day 2 (30 women), day 3 (159 women), day 4 (9 women), or day 5 (17 women). A good-quality embryo at the cleavage stage was defined as having at least three blastomeres on day 2, or six on day 3, with less than 20% cytoplasmic fragmentation. Morula stage at day 4 and blastocyst at day 5 were also classified as good-quality embryo.

Pregnancy was assessed by serum hCG measurement 14 days post-oocyte retrieval. If the hCG test was positive, luteal phase support was continued until 9-10 weeks of

gestation. Clinical pregnancy was defined as an intrauterine gestational sac with fetal heartbeat. Ongoing pregnancy was defined as pregnancy beyond 12 weeks of gestation. Miscarriage was defined as loss of pregnancy before 12 weeks of gestation (i.e. an early spontaneous abortion).

4. Statistical analysis

The sample size (n=260) was calculated at 80% power and 5% significance, assuming a 15% prevalence of thyroid autoantibodies and detecting a 20% difference in ongoing PR. The primary outcome was ongoing PR after IVF. Statistical analysis was carried out using Statistical Package for Social Sciences software version 24 (SPSS Inc., Chicago, Illinois) and SAS version 9.2 (SAS Institute, Cary, NC). Student's t-test, Mann-Whitney U test, chi-square test, and Fisher's exact test were used appropriately. Yates' continuity correction was used when necessary. The minimum p-value approach and two-fold cross validation were used to determine the optimal cut-off point for serum TSH levels. Univariate and multivariate logistic regression analyses were

performed to identify significant factors to influence ongoing pregnancy. The results were considered statistically significant at a p−value of < 0.05.

Results

1. Baseline characteristics of participants

The mean age of women was 34.9 ± 3.7 years (range: 24–44 years) and the mean body mass index was 22.1 ± 3.3 kg/m^2. The mean serum anti-müllerian hormone (AMH) level was 3.90 ± 3.47 ng/mL. There was no correlation between woman's age and serum TSH level (Pearson's correlation test) (Figure 1).

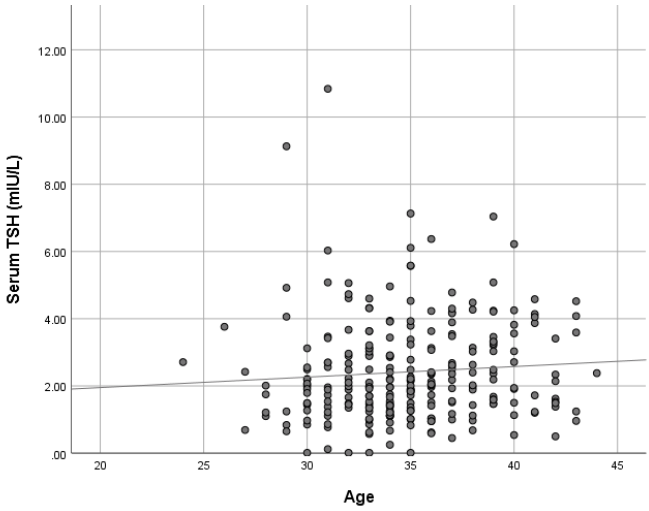

Figure 1. Serum TSH level and women's age (r=0.077, *p*=0.216)

2. Prevalence of abnormal thyroid function and thyroid autoantibodies

Overt hyperthyroidism was found in 5 women (1.9%) and subclinical hypothyroidism (TSH ≥ 4.2 μIU/mL, but normal free T4 and T3) was found in 31 women (11.9%). None of the women had overt hypothyroidism or subclinical hyperthyroidism. Two hundred and twenty-four women (86.2%) were euthyroid.

The overall prevalence of thyroid autoantibodies was 15.0% (39/260), 12.5% (28/224) in euthyroid women, 13.3% (34/255) in euthyroid women plus women with subclinical hypothyroidism, 19.4% (6/31) in women with subclinical hypothyroidism, and 100% (5/5) in women with overt hyperthyroidism.

Among 260 women, the age, body mass index, serum AMH level, and infertility factors between women with positive and negative thyroid autoantibodies were similar (Table 1).

Table 1. Baseline characteristics of patients according to thyroid autoantibody status

	Positive thyroid autoantibody (39 women)	Negative thyroid autoantibody (221 women)	*p*
Age of women (years)	35.2±3.6	34.9±3.7	0.562
BMI (kg/m^2)	22.3±3.3	22.1±3.2	0.655
Serum AMH level (ng/mL)	4.19±3.21	3.86±3.52	0.581
Indication of IVF			
Tubal	17 (43.6%)	51 (23.1%)	
Endometriosis	2 (5.1%)	13 (5.9%)	
Anovulatory	2 (5.1%)	20 (9.0%)	0.123
Decreased ovarian reserve	2 (5.1%)	28 (12.7%)	
Old age (≥40 years)	5 (12.8%)	24 (10.9%)	
Unexplained	11 (28.2%)	85 (38.5%)	
Status by thyroid function test			
Overt hyperthyroidism	5 (12.8%)	0	<0.001
Euthyroid	28 (71.8%)	196 (88.7%)	
Subclinical hypothyroidism	6 (15.4%)	25 (11.3%)	
Thyroid function tests			
Serum TSH level (μIU/mL)	2.76±1.55	2.36±1.50	0.124
Serum free T4 (ng/dL)	1.39±0.55	1.26±0.15	0.139
Serum T3 (ng/mL)	1.31±0.38	1.20±0.40	0.105
No. women with TPO antibody only	7		
No. women with TG antibody only	16		
No. women with both antibodies	16		
TPO antibody (IU/mL)	98.20±127.59	8.57±3.52	<0.001
TG antibody (IU/mL)	286.72±209.57	16.34±13.08	<0.001

BMI, body mass index; AMH, anti-müllerian hormone; IVF, in vitro fertilization; TSH, thyroid stimulating antibody; T3, tri-iodothyronine; TPO, thyroid peroxidase; TG, thyroglobulin.

Mean±SD (*p* value was obtained by Student' s t-test).

3. Overall IVF-ET outcomes

After recruiting 260 women, ovarian stimulation could not be started in 8 women due to loss during follow-up or spontaneous conception. Five women with overt hyperthyroidism were referred to an endocrinologist for anti-thyroid treatment and therefore, ovarian stimulation was not initiated. Ovarian stimulation was started but canceled in 7 women due to inadequate ovarian response.

Finally, oocyte retrieval was attempted in 240 women. However, oocyte was not obtained in 2 women, and fertilization failure occurred in 4 women. ET was canceled in 19 women for various reasons (ovarian hyperstimulation syndrome in 7 women, premature elevation of serum progesterone in 11 women, and systemic illness in one woman). Finally, ET was performed in 215 women.

4. Impact of thyroid autoantibodies on IVF pregnancy

Between women with positive and negative thyroid autoantibodies, the age, serum AMH levels, ovarian stimulation protocols, total gonadotropin doses, and the number of total, mature, and fertilized oocytes were all similar (Table 2). The number of total or good-quality embryos transferred were also similar. Clinical PR, ongoing PR, and miscarriage rate were all similar between women with positive and negative thyroid autoantibodies.

When a separately analysis of the 188 euthyroid women was carried out, clinical PR, ongoing PR, and miscarriage rate were similar between women with positive and negative thyroid autoimmunity (Table 3).

According to the presence of each thyroid autoantibody, clinical PR, ongoing PR, and miscarriage rate were also similar (data not shown).

Table 2. Stimulation outcomes and pregnancy results in 215 women with subclinical hypothyroidism or euthyroid women according to thyroid autoantibody status

	Positive thyroid autoantibody (29 women)	Negative thyroid autoantibody (186 women)	*p*
Status by thyroid function			
Euthyroid Subclinical hypothyroidism	23 (79.3%) 6 (20.7%)	165 (88.7%) 21 (11.3%)	0.222
Age of women (years)	35.2±3.6	34.7±3.6	0.451
Age of husband (years)	37.6±3.7	37.0±4.3	0.486
BMI (kg/m^2)	23.3±3.2	21.9±2.8	0.035
Serum AMH (ng/mL)	3.92±2.87	3.89±3.39	0.418
GnRH agonist long protocol GnRH antagonist protocol	18 (62.1%) 11 (37.9%)	102 (54.8%) 84 (45.2%)	0.466
Total gonadotropin dose (IU)	2,677±1,074	2,724±1,074	0.785
Stimulation outcome			
No. total oocytes	12.4±5.8	11.5±6.9	0.479
No. mature oocyte	9.7±5.1	8.3±5.2	0.179
No. fertilized oocyte	8.0±4.4	7.3±4.6	0.447
No. embryos transferred	2.0±0.4	2.1±0.5	0.586
No. good-quality embryos transferred	1.4±0.9	1.2±0.9	0.314
Pregnancy results			
Clinical pregnancy rate	48.3% (14/29)	51.6% (96/186)	0.738
Ongoing pregnancy rate	41.4% (12/29)	41.4% (77/186)	0.955
Miscarriage rate	14.3% (2/14)	19.8% (19/96)	1.000

BMI, body mass index; AMH, anti-müllerian hormone.

Mean±SD (*p* value was obtained by Mann-Whitney U test).

Table 3. Stimulation outcomes and pregnancy results in 188 euthyroid women according to thyroid autoantibody status

	Positive thyroid autoantibody (23 women)	Negative thyroid autoantibody (165 women)	*p*
Age of women (years)	35.2±3.8	34.7±3.6	0.467
Age of husband (years)	37.7±3.6	36.9±4.4	0.430
BMI (kg/m^2)	22.9±3.3	21.9±2.9	0.227
Serum AMH (ng/mL)	4.09±3.13	3.96±3.43	0.475
GnRH agonist long protocol	13 (56.5%)	91 (55.2%)	0.901
GnRH antagonist protocol	10 (43.5%)	74 (44.8%)	
Total gonadotropin dose (IU)	2,609±1,140	2,717±1,094	0.584
Stimulation outcome			
No. total oocytes	12.5±5.8	11.6±6.9	0.241
No. mature oocytes	9.8±4.8	8.3±5.2	0.105
No. fertilized oocytes	8.1±4.1	7.4±4.5	0.307
No. embryos transferred	2.0±0.4	2.1±0.5	0.663
No. good-quality embryos transferred	1.5±0.8	1.2±0.9	0.115
Pregnancy results			
Clinical pregnancy rate	52.2% (12/23)	53.9% (89/165)	0.874
Ongoing pregnancy rate	43.5% (10/23)	44.8% (74/165)	0.901
Miscarriage rate	16.7% (2/12)	16.9% (15/89)	1.000

BMI, body mass index; AMH, anti-müllerian hormone.

Mean±SD (*p* value was obtained by Mann-Whitney U test).

5. IVF outcomes in women with subclinical hypothyroidism and euthyroid women

Between women with subclinical hypothyroidism and euthyroid women, age, serum AMH level, number of total, mature, or fertilized oocytes, and the number of total or good-quality embryos transferred were all similar (Table 4). However, clinical PR and ongoing PR were significantly lower in women with subclinical hypothyroidism.

Table 4. Stimulation outcomes and pregnancy results between women with subclinical hypothyroidism (TSH ≥ 4.2μIU/mL) and euthyroid women

	Subclinical hypothyroidism (27 women)	Euthryoid (188 women)	*p*
Age of women (years)	35.4±3.7	34.7±3.6	0.404
Age of husband (years)	37.2±3.5	37.0±4.3	0.889
BMI (kg/m^2)	22.2± 2.6	22.0±2.9	0.658
Serum AMH (ng/mL)	3.36± 2.80	3.97±3.39	0.293
Positive thyroid autoantibody	6 (22.2%)	23 (12.2%)	0.222
GnRH agonist long protocol	16 (59.3%)	104 55.3%)	0.700
GnRH antagonist protocol	11 (40.7%)	84 (44.7%)	
Total gonadotropin dose (IU)	2,814±892	2,704±1,097	0.347
Stimulation outcome			
No. oocytes retrieved	10.6±6.1	11.7±6.8	0.445
No. mature oocytes	8.0±5.7	8.5±5.1	0.449
No. fertilized eggs	7.0±5.2	7.5±4.5	0.387
No. embryos transferred	2.1±0.5	2.1±0.5	0.951
No. good-quality embryos transferred	1.2±0.9	1.3±0.9	0.746
Pregnancy results			
Clinical pregnancy rate	33.3% (9/27)	53.7% (101/188)	0.047
Ongoing pregnancy rate	22.2% (6/27)	44.7% (84/188)	0.045
Miscarriage rate	33.3% (3/9)	16.8% (17/101)	0.360

BMI, body mass index; AMH, anti-müllerian hormone.

Mean±SD (*p* value was obtained by Mann-Whitney U test).

6. The cut-off value of serum TSH for prediction of ongoing pregnancy

An ROC curve analysis revealed that the cut-off value of serum TSH value was 3.4 μIU/mL to predict ongoing pregnancy in 215 women with subclinical hypothyroidism or euthyroid women. However, the area under the curve was 0.462 (95% CI 0.385-0.540), indicating it is not a good predictor (Figure 2).

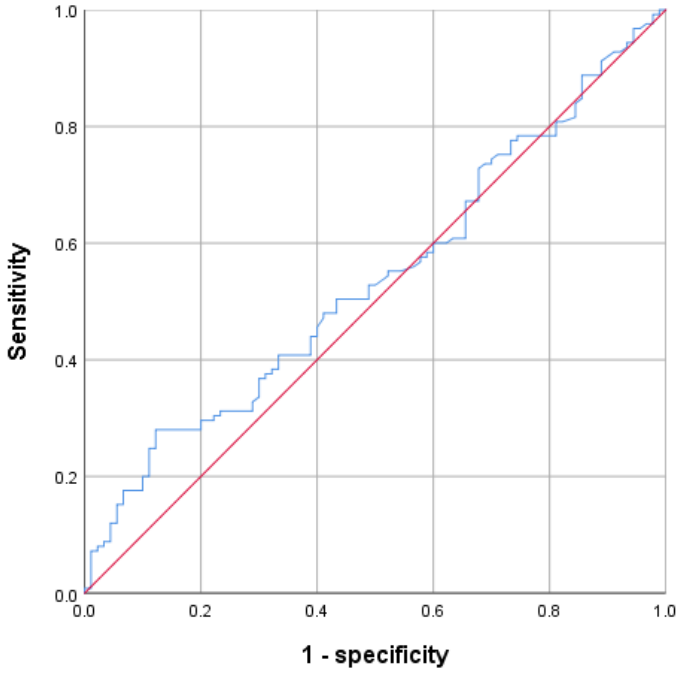

Figure 2. Receiver operating characteristics curve to predict ongoing pregnancy according to serum TSH level

When all of the women were divided into five groups according to the interval of serum TSH value, ongoing PR tended to decrease abruptly in the group with serum TSH ≥ 3.45 μIU/mL (Figure 3).

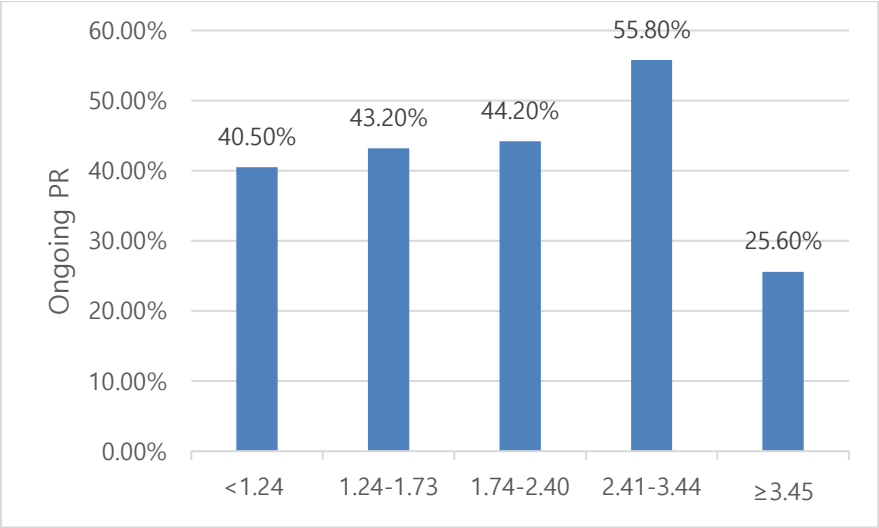

Figure 3. Ongoing pregnancy rate according to serum TSH levels (μIU/mL)

When the minimal p-value approach was applied, the difference in ongoing PR was most pronounced at the cut-off of serum TSH 3.4 μIU/mL (Table 5). The difference in ongoing PR was less prominent at serum TSH of 2.5 μIU/mL or 4.2 μIU/mL.

Table 5. Pregnancy results according to various cut-off values of serum TSH in 215 women with subclinical hypothyroidism or euthyroid women

Cut-off value of TSH	2.5 μIU/mL		3.4 μIU/mL		4.2 μIU/mL	
	<2.5 μIU/mL	≥2.5 μIU/mL	<3.4 μIU/mL	≥3.4 μIU/mL	<4.2 μIU/mL	≥4.2 μIU/mL
Number of women	136 (63.3%)	79 (36.7%)	169 (78.6%)	46 (21.4%)	188 (87.4%)	27 (12.6%)
Age of women	34.7±3.6	35.0±3.7	34.7±3.5	35.2±3.9	34.7±3.6	35.4±3.7
	p = 0.554		p = 0.398		p = 0.404	
No. good-quality embryos transferred	1.3±0.9	1.2±0.8	1.3±0.8	1.1±0.9	1.3±0.9	1.2±0.9
	p = 0.326		p = 0.110		p = 0.746	
Clinical pregnancy rate	51.5% (70/136)	50.6% (40/79)	54.4% (92/169)	39.1% (18/46)	53.7% (101/188)	33.3% (9/27)
	p = 0.906		p = 0.066		p = 0.047	
Ongoing pregnancy rate	44.1% (60/136)	38.0% (30/79)	46.7% (79/169)	23.9% (11/46)	44.7% (84/188)	22.2% (6/27)
	p = 0.775		p = 0.005		p = 0.045	
Miscarriage rate	14.3% (10/70)	25.0% (10/40)	14.1% (13/92)	38.9% (7/18)	16.8% (17/101)	33.3% (3/9)
	p = 0.161		p = 0.020		p = 0.360	

Mean±SD (p value was obtained by Mann-Whitney U test).

Table 6 shows stimulation outcomes and pregnancy results of women with serum TSH < 3.4 μIU/mL and ≥ 3.4 μIU/mL. The proportion of women with positive thyroid autoantibodies was significantly higher in women with serum TSH ≥ 3.4 μIU/mL. Ongoing PR was significantly lower and miscarriage rate was significantly higher in women with serum TSH ≥ 3.4 μIU/mL. Clinical PR tended to be lower in women with serum TSH ≥ 3.4 μIU/mL, but the difference was not significant.

Table 6. Stimulation outcomes and pregnancy results according to serum TSH threshold of 3.4 μIU/mL in 215 women with subclinical hypothyroidism or euthyroid women

	TSH < 3.4 μIU/mL (169 women)	TSH ≥ 3.4 μIU/mL (46 women)	*p*
Age of women (years)	34.7±3.5	35.2±3.9	0.398
Age of husband (years)	36.9±4.3	37.5±3.9	0.454
BMI (kg/m^2)	22.1±2.9	21.9±3.0	0.752
Serum AMH (ng/mL)	3.96±3.26	3.74±3.54	0.729
Positive thyroid autoantibody	17 (10.1%)	12 (26.1%)	0.005
GnRH agonist long protocol	94 (55.6%)	26 (56.5%)	0.913
GnRH antagonist protocol	75 (44.4%)	20 (43.5%)	
Total gonadotropin dose (IU)	2,715±1,096	2,726±990	0.951
Stimulation outcome			
No. oocytes retrieved	11.9±6.9	10.4±5.7	0.172
No. mature oocytes	8.7±5.2	7.7±5.2	0.257
No. fertilized eggs	7.6±4.6	6.6±4.5	0.184
No. embryos transferred	2.1±0.5	2.0±0.5	0.565
No. good-quality embryos transferred	1.3±0.8	1.1±0.9	0.110
Pregnancy results			
Clinical pregnancy rate	54.4% (92/169)	39.1% (18/46)	0.066
Ongoing pregnancy rate	46.7% (79/169)	23.9% (11/46)	0.005
Miscarriage rate	14.1% (13/92)	38.9% (7/18)	0.020

BMI, body mass index; AMH, anti-müllerian hormone.

Mean±SD (*p* value was obtained by Student's t-test).

7. Predictive factors influencing ongoing pregnancy

Univariate logistic regression analysis revealed that age, subclinical hypothyroidism, and serum TSH ≥ 3.4 μIU/mL were independently significant factors that negatively affected ongoing pregnancy (Table 7). In contrast, the number of good-quality embryos transferred was a significant factor that positively affected ongoing pregnancy. The presence of thyroid autoantibodies did not affect ongoing pregnancy.

Multivariate analysis found that both age of women and serum TSH ≥ 3.4 μIU/mL were significant factors that negatively affected ongoing pregnancy, and the number of good-quality embryos was still a significant factor that positively affected ongoing pregnancy.

Two-fold cross validation was conducted to confirm the generalization performance of these results, and the statistical significance was still preserved (Table 8).

Table 7. Univariate logistic regression analysis predicting ongoing pregnancy after IVF in 215 women with subclinical hypothyroidism or euthyroid women

Factors	Odds ratio	95% Confidence interval	*p*
Age of women (years)	0.891	0.822 – 0.965	0.004
Positive thyroid autoantibody	0.977	0.442 – 2.163	0.955
No. good–quality embryos transferred	1.599	1.144 – 2.234	0.006
Euthyroid	1	–	–
Subclinical hypothyroidism (serum TSH ≥ 4.2 μIU/mL)	0.354	0.137 – 0.916	0.032
Serum TSH < 3.4 μIU/mL	1	–	–
Serum TSH ≥ 3.4 μIU/mL	0.358	0.171 – 0.752	0.007

Table 8. Multivariate logistic regression analysis predicting ongoing pregnancy after IVF in 215 women with subclinical hypothyroidism or euthyroid women

Factor	Odds ratio	95% Confidence interval	*p*
Age of women (years)	0.893	0.822 – 0.970	0.007
No. good–quality embryos transferred	1.546	1.092 – 2.190	0.014
Serum TSH ≥ 3.4 μIU/mL	0.375	0.173 – 0.811	0.013
After two–fold cross validation			
Age of women (years)	0.894	0.823 – 0.970	0.007
No. good–quality embryos transferred	1.540	1.090 – 2.175	0.014
Serum TSH ≥ 3.4 μIU/mL	0.403	0.167 – 0.971	0.043

With the results of multivariate logistic regression, a graph was drawn for the adjusted probability of ongoing PR according to the serum TSH value using a generalized additive model (Figure 4).

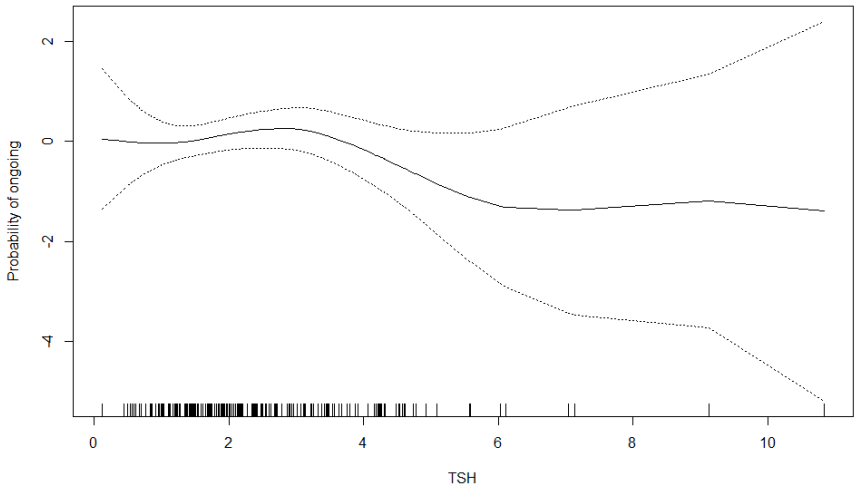

Figure 4. Adjusted probability of ongoing PR according to serum TSH levels using a generalized additive model.

Discussion

In this study, the presence of thyroid autoantibodies did not influence clinical IVF outcomes. However, the presence of subclinical hypothyroidism and serum TSH level (≥ 3.4 μIU/mL) before IVF negatively affected ongoing PR after IVF. There have been many debates about the appropriate TSH levels for the best IVF outcomes. In our study, ongoing PR was significantly different when the serum TSH threshold was 3.4 μIU/mL. As proposed in other studies, we also analyzed our data using a serum TSH threshold of 2.5 μIU/mL, however, ongoing PR, clinical PR, and miscarriage rate were not different.

The prevalence of thyroid autoantibodies in Korean infertile women undergoing IVF-ET was 15.0%, which was not much different from studies in other populations (27-29). There were no differences in the indications of IVF according to the presence or absence of thyroid autoantibodies. Researchers have proposed associations with thyroid autoimmunity and endometriosis (10, 12, 30), or PCOS (31), or premature ovarian failure (10). However, in this study, the associations

were not found. Such difference may be explained due to the different inclusion criteria.

One of the strengths of this study is that women were prospectively enrolled, and strict inclusion criteria were applied (i.e. first IVF cycle without male or uterine factors). Inclusion of the first IVF cycle is very important because it could exclude other factors contributing to repetitive implantation failure. Two recent meta-analyses that found an association of thyroid autoantibodies with poor IVF outcomes included a number of retrospective studies, and studies of inconsistent etiology of infertility and unknown IVF cycle numbers (9, 17). Most of the studies examining the relationship between serum TSH and IVF outcomes are also retrospective studies (18, 21-24).

Another strength of this study is that the measurements of thyroid function and thyroid autoantibodies were conducted just prior to ovarian stimulation (within two weeks' interval). Since intra-individual variation including seasonal variation in serum TSH exists (32), blood tests just before ovarian

stimulation would best reflect the effect of thyroid status on forthcoming ovarian stimulation and IVF-ET outcomes.

In this study, the practical threshold of serum TSH to lower ongoing pregnancy rate was established. If thyroid treatment is carried out for a woman with a TSH level lower than this threshold, it may be considered as an overtreatment treating one who does not need treatment. If we do not treat a woman with a serum TSH higher than this threshold, we may miss treatment of someone who actually requires treatment.

In the ROC curve predicting ongoing pregnancy according to serum TSH, the area under the curve was relatively low. Looking at the distribution of the data, when the serum TSH is less than 3.4 μIU/mL, the distribution is similar, therefore ongoing and non-ongoing cannot be clearly distinguished. Whereas when serum TSH is 3.4 μIU/mL or higher, it can be said that ongoing and non-ongoing can be distinguished well. However, in 78.6% (=169/215) of the total data (serum TSH < 3.4 μIU/mL), ongoing pregnancy cannot be distinguished from non-ongoing pregnancy. Therefore, the diagnostic

power of the whole data is weak, and the ROC area is considered to be low.

According to Figure 3 and logistic regression analysis, serum TSH value does not have an inverse linear relationship that progressively lowers the ongoing PR, but acts to lower the ongoing PR when it exceeds a certain threshold. In that sense, the analysis is valid for our purpose of finding the TSH cut-off value that decreases the ongoing pregnancy rate.

This study also has several limitations. The sample size was initially calculated based on the prevalence of thyroid autoantibodies, not serum TSH levels. In addition, during the study, 6 women with subclinical hypothyroidism (serum TSH ≥ 5.9 μIU/mL) were recommended to visit an endocrinologist for future management of their thyroid status. Unfortunately, it was unknown whether the women visited endocrinology clinic, and/or actually received any treatment.

In conclusion, the presence of thyroid autoantibodies per se did not affect IVF-ET outcomes. However, serum TSH ≥ 3.4 μIU/mL before IVF negatively affected ongoing PR after ET. According to our results, screening for thyroid antibodies may

be unnecessary in infertile women with normal thyroid function. It should be emphasized that levothyroxine therapy is strongly considered in women with TSH ≥ 3.4 μIU/mL before IVF to achieve better IVF-ET outcomes. However, further large scale prospective studies are warranted.

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국문 초록

목적: 본 연구에서 체외수정시술 전 혈청 갑상선자극호르몬 농도와 갑상선자가항체의 유무가 체외수정시술 결과에 미치는 영향을 분석하고자 하였다.

방법: 본 연구는 전향적 관찰 연구로 2013 년 3 월부터 2017 년 2 월까지 불임증으로 첫 번째 체외수정시술을 시행한 260 명의 여성들을 모집하였다. 과배란유도를 시작하기 직전 혈액 채취를 시행하여 갑상선자극호르몬 농도를 포함한 갑상선기능검사를 시행하고 thyroid peroxidase antibody, thyroglobulin antibody 를 분석하였다. 통상적인 방법으로 체외수정시술을 한 후 결과를 추적관찰하였다. 자가항체 양성인 군과 음성인 군 간에 체외수정시술 결과 임상적 임신율, 유산율, 진행임신율에 차이가 있는지 분석하였다. 또한 TSH 의 값에 따라 갑상선기능을 세분화하여 체외수정시술 결과에 미치는 영향도 분석하였다.

결과: 갑상선자가항체의 유병률은 15.0%(39/260)였다. 모두 215 명의 여성(27 명의 무증상갑상선기능저하증과 188 명의 정상

갑상선기능을 가진 여성)에게 배아이식을 시행하였고, 갑상선자가항체 양성인 29 명의 여성군과 음성인 186 명의 여성군에서 진행임신율, 임상적 임신율, 유산율에 유의한 차이가 없었다. 혈청 갑상선자극호르몬 농도가 4.2μIU/mL 이상을 무증상갑상선기능저하증으로 정의하였을 때, 갑상선기능이 정상인 군에 비해 진행임신율이 유의하게 낮았다 [22.2% (6/27) vs. 44.7% (84/188), p=0.045]. 임상적 임신율 또한 유의하게 낮았으며 [33.3% (9/27) vs. 53.7% (101/188), p=0.047], 유산율에는 차이가 없었다. 또한 215 명의 정상 갑상선 기능, 또는 무증상갑상선기능저하증 여성들 중 혈청 갑상선자극호르몬 농도가 3.4μIU/mL 이상인 여성들은 3.4μIU/mL 미만의 여성들에 비해 낮은 진행임신율 [23.9% (11/46) vs. 46.7% (79/169), p=0.005]을 보였다. 임상적 임신율은 통계적으로 유의하지 않았으나 낮은 경향 [39.1% (18/46) vs. 54.4% (92/169), p=0.066]을 보였으며, 유산율이 유의하게 높았다 [38.9% (7/18) vs. 14.1% (13/92), p=0.020]. 다변수 로지스틱회귀분석을 시행하였을 때, 혈청 갑상선자극호르몬 농도가 3.4μIU/mL 이상인 인자(OR 0.375, p=0.013), 고령(OR 0.893, p=0.007)이 진행임신율을 유의하게 낮추는 독립인자였으며, 양질의 배아 수(OR 1.546, p=0.014)는 진행임신율을 유의하게 높이는 독립인자였다

결론: 결론적으로 갑상선자가항체는 그 자체로는 체외수정시술 결과에 영향을 미치지 않으며, 3.4μIU/mL이상의 혈청 갑상선자극호르몬 농도는 체외수정시술 후 낮은 진행임신율의 독립적으로 유의한 인자였다.

주요어 : 갑상선자가항체, 갑상선자극호르몬, 임신, 체외수정
학 번 : 2014-31266

감사의 글

감사하게도 많은 분들께서 도움을 주셔서 이 연구를 완성할 수 있었습니다. 제가 생식내분비학 분야에서 더 깊은 공부를 하고 이 연구를 할 수 있도록 이끌어 주신 존경하는 김석현 교수님께 깊이 감사드립니다. 비단 이 연구 뿐 아니라, 제가 이 분야에서 평생 정진할 수 있도록 올바른 자세와 방향을 제시해주셨습니다.

또한, 작은 것 하나하나도 세심하게 살펴보고 열정적으로 조언해주신 지병철 교수님께 감사드립니다. 덕분에 어떤 어려운 과정에서도 망설임 없이 연구에 정진할 수 있었습니다. 연구의 설계 단계에서 연구 수행, 논문 완성까지 함께 해 주신 김지현, 김슬기, 주창우, 최승아 선생님, 감사합니다. 아울러 그동안 제가 산부인과학을 전공할 수 있게 도와주신 서울대학교 의과대학 산부인과학교실 모든 선생님들께 감사의 말씀 올립니다.

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