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Impact of Thyroid Antibodies on In Vitro Fertilization/Intracytoplasmic Sperm Injection Outcomes

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ABSTRACT

Background: Thyroid autoimmunity (TAI) and the success of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have been linked in a number of studies; however, the findings are still debatable.

Objective: The aim of the current study is to examine whether the presence of thyroid antibodies affects IVF/ICSI results, such as the number of retrieved oocytes, embryo quality, clinical pregnancy rate, and miscarriage rate. Furthermore, subgroup analysis was performed to examine the relationship between the kinds and titers of thyroid antibodies and pregnancy outcomes during IVF/ICSI.

Patients and methods: A retrospective cohort study was conducted on 80 infertile women who were nominated for IVF/ICSI; 40 cases with negative and another 40 with positive anti-thyroid antibodies. We compared between both groups regarding clinical/chemical pregnancy rate, doses/duration of induction of ovulation, number of collected oocytes, fertilization rate, quality of transferred embryos and miscarriage rate. **Results:** Among enrolled cases; 21 (52.5%) had positive anti-TG, 28 (70.0%) had positive anti-TPO and cases that had both anti-TG and TPO were 9 (22.5%), while those who had either anti-TG or TPO were 31 (77.5%). There were no significant differences between study groups regarding embryo quality, clinical/biochemical pregnancy rates, embryo cleavage, induction duration, endometrial thickness and number of fertilized/M2 oocytes or transferred embryos. However, the number of used induction ampoules and abortion rate were statistically significant increased among pregnant women.

Conclusion: Infertile women with TAI treated with ICSI had no increased risk of poor IVF/ICSI outcomes except significant increased risk for a first-trimester miscarriage compared with women without TAI.

Keywords: Thyroid Autoimmunity, In Vitro Fertilization, Intracytoplasmic Sperm Injection.

INTRODUCTION

Thyroid autoimmunity (TAI), which is recognized by the presence of thyroid antibodies, is the most common autoimmune disorder in females of reproductive age [(1)]. There is mounting evidence that successful pregnancies following intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF) treatments are correlated with thyroid antibodies [(2)].

TAI, which is characterized by the presence of anti-thyroid peroxidase (anti-TPO) and/or anti-thyroglobulin (anti-TG) antibodies, is seen in 5-20% of women of reproductive age, according to prior study [(3)]. Thyroglobulin is a chemical that thyroid cells create in order to synthesize and store thyroid hormones (TG). The enzyme thyroid peroxidase (TPO) aids in iodinating surfactant protein to create thyroid hormones a tyrosine residue [(4)]. TAI illnesses usually go undetected because they may exist for years without evident thyroid impairment [(5)].

Therefore, compared to the general population, TAI is more prevalent in women who frequent fertility clinics [(6)]. The link between TAI and infertility is still mostly hypothetical, though [(7)].

Positive anti-TPO has been linked to adverse effects on spermatogenesis, fertilization rates (FRs), embryo quality, and folliculogenesis, according to a previous study that looked into the effects of anti-TPO on reproductive biology [(8)].

The aim of the current study is to examine whether the presence of thyroid antibodies affects IVF/ICSI results, such as the number of retrieved oocytes, embryo quality, clinical pregnancy rate, and miscarriage rate. Furthermore, subgroup analysis was performed to examine the relationship between the kinds and titers of thyroid antibodies and pregnancy outcomes during IVF/ICSI.

PATIENTS AND METHODS

A retrospective cohort study was carried out on 80 infertile women nominated for IVF/ICSI attended assisted reproductive technology unit of Ain Shams Maternity Hospital and another private IVF center during 2020.

Inclusion criteria:

Women aged between 20 and 40 years old with normal TSH level (0.45–4.5 iu /ml) suffered from infertility due to either tubal factor, polycystic ovarian syndrome, male factor, endometriosis (stage 1 or 2) or unexplained cause with positive thyroid autoimmunity status were enrolled in the study. Thyroid autoimmunity was defined as positive when anti-TPO and/or anti-TG levels exceeded the upper limit of the reference range (TPO antibodies 35 IU/ml, TG antibodies 40 IU/ml).

Exclusion criteria:

Women with other autoimmune diseases as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) and positive anticardiolipin, lupus anticoagulant, anti-nuclear antibodies or rheumatoid factor, hyperprolactinemia, severe liver/renal dysfunction or endometriosis (stage 3 or 4) were excluded from the study.

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Study procedure:

Assisted Reproduction Unit's computerized database was used in this retrospective cohort study.

All participants gave their written consent before being screened and enrolled. Participants gave their consent to participate in the study, and their confidentiality had been upheld. After the research ethical committee gave its approval, benefits of involvement in the study were explained to each participant. All women who had IVF/ICSI cycles had their thyroid function, including anti-thyroid peroxidase antibodies (anti-TPO), anti-thyroglobulin antibodies (anti-TG), and thyroid stimulating hormone, checked. Each of them was requested by the referring physician prior to ovarian stimulation.

Induction of ovulation:

All patients had the same baseline hormonal profile on the third day of their spontaneous menstrual cycle (FSH, LH, E2, TSH and prolactin).

Additionally, a 5–9 MHZ transvaginal probe was used for transvaginal ultrasound TVUS. Any patient with uterine anomalies was disqualified.

A long GnRH agonist protocol was used to stimulate the ovaries, beginning in the midluteal phase with daily SC injections of triptoreline acetate. Then, on day 3 of the subsequent cycle, daily HMG injections (Menogon 75 lU/amp or Merional 75 IU/amp) were started to stimulate the ovaries.

Gonadotropins were first prescribed based on the subjects' age and body weight, and then the dose was changed in response to the ovarian response as determined by transvaginal folliculometry, which began on the sixth day of the cycle. When the leading follicle reaches a diameter of 16 millimeters, daily TVUS is performed until the largest follicle reaches a diameter of >18 millimeters, depending on the ovarian response. To induce ovulation, human chorionic gonadotropin (hCG) (Choriomon 10,000 lU/amp.) was administered.

Ovum pick up:

The transducer was linked to the U/S system between thirty-four and thirty-six hours following the hCG injection. The guidance beam's direction was examined. The TV transducer's front and back ends were connected by a fixation ring to aspiration equipment, which defined the direction of puncture that corresponded to the U/S image's guiding beam. Use of test tubes was made to evaluate the aspiration. The vision in both planes allowed for the identification of the uterus, both ovaries, and iliac arteries. The distance between the ovary and the top pole of the vagina was carefully measured (care had been taken to avoid intestinal or vascular interposition). The distance between the upper vaginal pole and the follicle's center was measured to determine the closest accessible follicle's depth. The center of the follicle was struck by the needle with force.

IVF- ICSI:

The direct penetration method was used for ICSI on metaphase II oocytes, and 16 to 19 hours after ICSI, fertilization outcomes were evaluated. Two pronuclei and/or a second polar body were indicators that fertilization was normal. The separation from the zona and collapse of the cytoplasmic contents were indicators of oocyte degeneration. The absence of pronuclei was used to define unsuccessful fertilization.

Embryo transfer:

The same gynecologist used a cook catheter to transfer the embryos on the third or fifth day after insemination, about 1-1.5 cm away from the fundus. The largest number of embryos transplanted was 3 on day 3 and 2 on day 5.

After transferring embryos, a blood hCG test was performed 12 days later, then it was repeated after 48 hours. Six weeks later, an ultrasound was performed.

Ethical consent:

An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 28 for Windows was used to code, process, and analyse the obtained data (IBM SPSS Inc, Chicago, IL, USA). Using the Shapiro Walk test, the distribution of the data was examined for normality. Frequencies and relative percentages were used to depict qualitative data. To determine differences between two or more sets of qualitative variables, Chi-square test (X^2) was used. Quantitative information was presented as mean and standard deviation (SD). Two independent groups of normally distributed variables were compared using the independent samples t-test (parametric data). P value ≤ 0.05 was considered significant.

RESULTS

We recruited 40 cases with negative anti-thyroid antibodies and another 40 cases with positive anti-thyroid antibodies, amongst them 21 (52.5%) had positive anti-TG, 28 (70.0%) had positive anti-TPO, cases that had both anti-TG and TPO were 9 (22.5%), while those who had either anti-TG or TPO were 31 (77.5%). No statistical significant differences according to presence of anti-thyroid antibodies regarding age, duration of infertility, cause of infertility, Number of previous trials or basal FSH, AMH, TSH (**Table 1**).

Table (1): Comparison of the demographic characteristics of the study population.

Variables		Anti-thyroid antibodies		P-value*
		Positive (Total=40)	Negative (Total =40)	
Demographic characteristics				
Age (years)		33.6 ± 4.8	32.9 ± 5.6	0.524
BMI		26.0 ± 6.71	25.8 ± 4.7	0.25
Duration of infertility (years)		6.3 ± 1.4	6.6 ± 1.5	0.674
Cause of infertility (n, %)	**Male**	11 (27.5%)	18 (45.0%)	0.340
	PCOS	9 (22.5%)	5 (12.5%)	
	Endometriosis	5 (12.5%)	6 (15.0%)	
	Tubal	7 (17.5%)	3 (7.5%)	
	Unexplained	8 (20.0%)	8 (20.0%)	
Number of previous trials		1.1 ± 0.21	1.5 ± 0.3	0.100
Basal FSH		6.0 ± 1.4	6.7 ± 1.34	0.46
AMH		3.3 ± 0.74	3.8 ± 0.8	0.36
TSH		2.9 ± 0.6	2.8 ± 0.7	0.43

*P value ≤0.05: Significant

No statistical significant differences according to presence of anti-thyroid antibodies regarding age Induction duration, EC, fertilized oocytes, M2 oocytes and transferred embryos. Induction ampoules were significantly higher in cases that had Anti-thyroid antibodies.

Table (2): Comparison of IVF/ICSI cycle characteristics of the study population.

IVF/ICSI cycle characteristics	Anti-thyroid antibodies Positive	Anti-thyroid antibodies Negative	P value*
Induction duration (days)	11.4 ± 1.6	12 ± 1.7	0.164
Induction ampoules (days)	36.9 ± 7.3	33.4 ± 6.1	0.025*
Endometrial thickness (mm)	11 ± 2.3	10.8 ± 2.1	0.228
EC	15.6 ± 3.8	15.1 ± 3.4	0.829
Fertilized oocytes	8.7 ± 2.1	8.9 ± 2.2	0.897
M2 oocytes	12.4 ± 2.8	12.6 ± 2.9	0.900
Transferred embryos	2.8 ± 0.5	2.7 ± 0.6	0.318

*P value ≤0.05: Significant

Biochemical and chemical pregnancy was non-significantly less frequent in cases that had anti-thyroid antibodies. Abortion among pregnant was significantly more frequent in cases that had Anti-thyroid antibodies.

Table (3): Comparison of pregnancy outcomes of the study population.

Pregnancy outcomes	Anti-thyroid antibodies positive	Anti-thyroid antibodies Negative	P value*
Biochemical pregnancy	16 (40.0%)	23 (57.5%)	#0.117
Clinical pregnancy	16 (40.0%)	23 (57.5%)	#0.117
Abortion among pregnant (Total=16,23)	5 (31.3%)	1 (4.3%)	§0.033*

*P value ≤0.05: Significant.

DISCUSSION

There is ongoing debate on how thyroid autoimmunity affects intracytoplasmic sperm injection (ICSI) cycles. Despite the fact that some women had euthyroid thyroid function, certain research revealed thyroid autoimmune disease and dysfunction increased the likelihood of infertility. In order to assess how anti-thyroid antibodies affected ICSI cycle parameters, embryo quality, and pregnancy outcomes in women, we looked at anti-thyroid antibody levels.

We recruited 40 cases with negative Anti-thyroid antibodies and another 40 cases with positive Anti-thyroid antibodies, amongst them 21 (52.5%) had positive anti-TG, 28 (70.0%) had positive anti-TPO, cases that had both anti-TG and TPO were 9 (22.5%), while those who had either anti-TG or TPO were 31 (77.5%). Hence, we compared cases that had Anti-thyroid antibodies with those who had no, then we compared between cases that had doubles, single and none anti-thyroid antibodies.

Both groups were homogenous with no significant difference as regard demographic data (age, BMI, cause and duration of infertility or number of previous trials), or base line hormonal profile.

As regard induction of ovulation, significantly higher number of gonadotrophins was used in thyroid Abs positive group (the mean number of ampoules 36.9±7.3 versus 33.4±6.1, P=0.025*). However, there was no discernible change in the amount of time required to induce ovulation. There was no discernible change in endometrial thickness between the two groups on the HCG trigger day. There was no difference in the quantity or quality of oocytes retrieved on the day of retrieval, and the fertilization rate was comparable between the two groups. There was no discernible difference in the number of embryos transferred between the two groups.As regard pregnancy outcome, the result was better in negative antithyroid group. This group showed higher biochemical pregnancy rate (57.5% versus 40.0%) and clinical pregnancy rate (57.5% versus 40%) but this difference was statistically insignificant. On the other hand thyroid Ab positive group had significantly higher abortion rate (31.3% versus 4.3% in the other group, P=0.033*).

The anti-thyroid peroxidase antibody (anti-TPO Ab) levels of 52 women who underwent IVF or ICSI at Milann - The Fertility Centre, Bangalore, between January 2014 and December 2014 (Cases) and 21 women who had anti-TPO Ab levels over 35 IU/mL (Controls) were retrospectively analyzed. There was no difference between the cases and controls in terms of embryo quality, implantation rate, cleavage rate, or maturation rate. However, cases significantly outnumbered controls in terms of both abortion and biochemical pregnancy rates (P = 0.027 for each measure). However, the rate of clinical pregnancy was considerably lower in the instances (P = 0. 045) [(9)].

Anti-thyroid antibody-positive individuals had a considerably lower incidence of fertilization than anti-thyroid antibody-negative patients, according to **Tokgoz and his colleagues** [(10)] (97.1 (SD 10.5) vs. 91.5 (SD 19.8), respectively, P=0.003). The incidence of clinical pregnancy, miscarriage, and continuing pregnancy were comparable among research groups. Patients with anti-thyroid antibody positivity had fewer high-quality embryos present, although this difference did not achieve statistical significance (56.5% vs. 67.8%, P=0.09). Except for the miscarriage rate based on the TSH threshold, there were no significant changes in the subgroup analysis [(10)].

Finally, in a retrospective cohort study conducted by **Hamad and his colleagues** [(11)], 584 Syrian women who received IVF/ICSI treatment at the Orient Hospital in Damascus between November 2012 and April 2017 were included. Prior to IVF/ICSI, patients had TAI tests.

Between the TAI positive and TAI negative groups, CPR did not significantly differ (P >0.05). Only patients with primary infertility were included in the subgroup analysis, and it revealed a statistically significant difference in CPR between the TAI positive and TAI negative groups [(11)].

In conclusion, infertile women with TAI, compared to women without TAI, treated with ICSI had a significantly increased risk for a first-trimester miscarriage but no increased risk for poor IVF/ICSI outcomes.

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REFERENCES

1. **Unuane D, Velkeniers B (2020):** Impact of thyroid disease on fertility and assisted conception. Best Pract Res Clin Endocrinol Metab., 34(4):101378. doi: 10.1016/j.beem.2020.101378.
2. **Huang N, Chen L, Lian Y *et al.* (2021):** Impact of Thyroid Autoimmunity on In Vitro Fertilization/Intracytoplasmic Sperm Injection Outcomes and Fetal Weight. Frontiers in Endocrinology, 12:822. doi: 10.3389/fendo.2021.698579.
3. **Chai J, Yeung W, Lee C *et al.* (2014):** Live birth rates following in vitro fertilization in women with thyroid autoimmunity and/or subclinical hypothyroidism. Clinical Endocrinology, 80(1):122-7.
4. **Sakar M, Unal A, Atay A *et al.* (2016):** Is there an effect of thyroid autoimmunity on the outcomes of assisted reproduction? Journal of Obstetrics and Gynaecology, 36(2):213-7.
5. **Poppe K, Velkeniers B, Glinoer D (2007):** Thyroid disease and female reproduction. Clinical Endocrinology, 66(3):309-21.
6. **Aljarad M, Alhalabi N, Hamad A *et al.* (2019):** Prevalence of thyroid autoimmune antibodies in women seeking fertility care in Damascus, Syria. Cureus, 11(8):5315. doi: 10.7759/cureus.5315.
7. **Krassas G, Poppe K, Glinoer D (2010):** Thyroid function and human reproductive health. Endocrine Reviews, 31(5):702-55.
8. **Busnelli A, Paffoni A, Fedele L *et al.* (2016):** The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis. Human Reproduction Update, 22(6):775-90.
9. **Gadwal B (2021):** Effects of Anti-TPO Antibodies on the Outcome of In Vitro Fertilization-Embryo Transfer Cycles. Journal of Evolution of Medical and Dental Sciences, 39:3487-91
10. **Tokgoz V, Isim B, Tekin A (2020):** The impact of thyroid autoantibodies on the cycle outcome and embryo quality in women undergoing intracytoplasmic sperm injection. Middle East Fertility Society Journal, 25(1):1-8.
11. **Hamad A, Alhalabi N, Nmr N *et al.* (2021):** Impact of Thyroid Autoimmunity in euthyroid women on the outcomes of In Vitro Fertilization. Annals of Medicine and Surgery, 67:102473. doi: 10.1016/j.amsu.2021.102473.