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Antithyroid Antibodies and Fertility Outcome in Euthyroid Women Undergoing In-vitro Fertilization: A Clinical Article

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Abstract

Background: The presence of antithyroid antibodies is associated with poor IVF outcomes in euthyroid infertile women. Hence, the objective of our study is to find out the effect of antithyroid antibodies on fertilization rate, implantation rate and clinical pregnancy rate in euthyroid women undergoing IVF.

Methods and findings: The study was a prospective cohort study conducted at Maulana Azad Medical College, New Delhi. Eighty one euthyroid patients planned for IVF were recruited and divided into 2 groups depending upon the presence of antithyroid antibodies. All of them went through the standard stimulation protocol. The results were compared using unpaired t-test for quantitative variables and Chi-square/Fisher's exact test for qualitative variables.

The prevalence of antithyroid antibodies was 21% in our study population. The fertilization rate was significantly lower ($P<0.05$) in women with antithyroid antibodies (66.3% versus 78.9%). However, no statistically significant difference was observed in implantation rate (18.2% versus 26.8%) and clinical pregnancy rate (31.3% versus 39.3%).

Conclusion: The presence of antithyroid antibodies is associated with poor IVF outcomes in euthyroid infertile women.

Abbreviations: AR: Assisted Reproductive Technologies; ATA: Anti-Thyroid Antibodies; BMI: Body Mass Index; ET: Embryo Transfer; E2: Estrogen 2; fT3: Free Triodothyronine; fT4: Free Thyroxine; GnRH: Gonadotropin Releasing Hormone; hCG: Human Chorionic Gonadotropin hMG: Human Menopausal Gonadotropins; IVF: In Vitro Fertilization; LH: Leutinising Hormone; P4: Progesterone 4; FSH: Follicle Stimulating Hormone; SPSS: Statistical Package of Social Sciences; Tg: ThyroGlobulin; TPO: ThyroPerOxidase; TSH: Thyroid Stimulating Hormone; TVS: Transvaginal Sonography; UPT: Urine Pregnancy Test

Keywords: Infertility; *In-vitro* fertilization; Antithyroid antibodies; Euthyroid

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Introduction

The introduction of Assisted Reproductive Technologies (ART) and its recent advances have provided the means to study reproductive processes in new and more revealing ways and have markedly improved the prognosis for many infertile couples. Although the results of In Vitro Fertilization-Frozen Embryo Transfer have been steadily improving over recent years, still six out of ten women are unable to conceive successfully with each treatment cycle and the clinical pregnancy rate is only 32.4-33% per IVF transfer [1]. Approximately two out of ten women fail to conceive even after two or three IVF attempts [2]. Several factors such as woman's age, duration of infertility, body mass index, type of stimulation protocol, quality of oocytes retrieved, nature of embryos transferred etc influence the outcome of IVF programme. Autoimmune thyroid diseases are frequently seen in women of child bearing age, affecting about 5-20% of them [3]. They are characterized by the presence of

antithyroid antibodies (ATA) like anti-thyroglobulin (anti-Tg) and anti-thyroperoxidase antibodies (anti-TPO). ATA are often detected in subjects complaining of hypo or hyperthyroidism, but are also frequently found in patients without any signs and laboratory evidences of thyroid dysfunction [3]. Anti-thyroid antibodies, even if not associated with thyroid dysfunction, are suspected to cause poorer outcome of In-Vitro Fertilization. Some evidences suggest that ATA can exert a negative influence on the female reproductive potential. Women with no sign of thyroid dysfunction but are positive for ATA have three to five folds more risk of spontaneous miscarriage [4] and increased incidence of preterm birth and adverse neurodevelopmental sequelae in children than those who are negative for ATA [5,6].

To date, no consensus has been achieved regarding the impact of ATA on the outcome of IVF-ET. Whether to give adjuvant therapy to regulate the thyroid autoimmunity before and during IVF is still controversial. These issues are required to be investigated and clarified. Hence, the objective of this study is to determine the relationship between ATA and fertility outcomes in euthyroid women undergoing In-Vitro Fertilization.

Methods

A cross-sectional study was conducted for estimating the prevalence of antithyroid antibodies and a prospective cohort design was used to establish the relation between ATA and fertility outcomes following IVF. The study was conducted at the IVF and Reproductive Biology Centre, Department of Obstetrics and Gynecology, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi from Nov'14 to April'16. Of all women attending the IVF centre during this period, a total of 81 euthyroid women fulfilling the inclusion and exclusion criteria were recruited for the study. The inclusion criteria included the euthyroid infertile women between 20-35 years with tubal or peritoneal or unexplained factors. Women with clinically significant systemic disease, uterine anomalies, polycystic ovarian syndrome, thrombophilias or other autoimmune disease like anticardiolipin antibody or lupus anticoagulant positivity were excluded from the study. After obtaining a written informed consent, a comprehensive history was taken from each patient followed by general physical and gynecological examination. All routine and specific investigations for infertility were done. A fasting venous blood sample of 2 ml was withdrawn by antecubital venepuncture for the estimation of antithyroid antibodies by commercially available ELISA anti-TPO and anti-Tg kit (Calbiotech Inc, United States) prior to IVF cycle. The cut off value for anti-TPO antibody was 50 IU/ml and that of anti-Tg antibody was 100 IU/ml (the cut off being fixed according to manufacture's instruction). Those women who were positive for either anti-TPO or anti-Tg antibodies or both were included in group 1 (ATA positive group) while those women who were negative for both were included in group 2 (ATA negative group). All the 81 women went through the stimulation protocol with either agonist or antagonist protocol.

In the agonist protocol, downregulation of the cycle was achieved by injection leuprolide acetate (Lupride, manufactured by Sun Pharmaceuticals, India) 0.5 mg/day, subcutaneously, starting from day 21 of previous menstrual cycle. On the second day of next menstrual cycle, serum FSH, LH, Estrogen 2 (E2), and Progesterone 4 (P4) were estimated by electrochemiluminescence based immunoassay method. In case of successful downregulation, controlled ovarian hyperstimulation was started using recombinant gonadotropins or hMG. The starting dose was 225 IU/day of recombinant FSH (Foligraf, manufactured by Bharat Serum, India). In patients with markedly suppressed LH (less than 1 IU/L), hMG (Humog HP, manufactured by Bharat Serum, India) was given. Simultaneously, on day 2 of cycle, the dose of injection leuprolide was decreased to 0.3 mg/day.

In the antagonist protocol, women were started on oral contraceptive pills, Ovral-L (ethinyl estradiol 0.030 mg and levonorgestrel 0.150 mg, manufactured by Wyeth, a subdivision of Pfizer Pharmaceuticals Ltd, India), 1 tab daily from day 2 of their previous menstrual cycle for 21 days after which they had their menses. On the second day of next cycle, serum FSH, LH. E2 and P4 were estimated. The women were then started on recombinant FSH (Foligraf) or hMG (Humog HP) from day 2 or 3 of menstrual cycle and continued till oocyte trigger. Injection Cetrorelix, GnRH antagonist (Cetrotide, Sereno laboratory, Mumbai, India) 0.25 mg was initiated from day 6/7 of stimulation when follicle reached an average dimension of 14 mm on ultrasonography. The response to stimulation was monitored with serial measurements of serum estradiol and transvaginal sonographic imaging of ovarian follicles. Most women required a total of 9-11 days of stimulation. It was aimed to have at least 4-5 follicles measuring 17-18 mm in mean diameter in each ovary, and a serum estradiol concentration that was consistent with the overall size and maturity of the cohort (approximately 200 pg/mL per follicle measuring 14 mm or greater). Endometrial development was also monitored during stimulation by measuring the endometrial thickness and the endometrial blood flow using Doppler ultrasound. Once the targeted thresholds of response were met with atleast two leading follicles of more than 18 mm, injection hCG (5000-10000 IU) was administered subcutaneously to induce follicular maturation. Oocyte retrieval was performed after 34-36 hours of hCG trigger, TVS guided aspiration under general anesthesia being the technique. Fertilization was achieved by conventional microinsemination. Embryo transfer was performed three or five days after oocyte retrieval. Luteal phase support was started in all women in the form of micronized progesterone. Urine pregnancy test (UPT) was done and serum level of βhCG was measured 14 days after embryo transfer. Positive UPT and/or serum βhCG more than 50 mIU/mL indicated successful implantation. Patients with successful implantation were followed up for detection of gestational sac/cardiac activity four weeks after embryo transfer.

In our study, serum TSH, fT3 and fT4 levels were measured with electrochemiluminescence based immunoassays. The normal range of serum TSH was taken to be between 0.5 to 2.5 mIU/L and for fT3 between 3.1-6.8 pmol/L and fT4 between 12-22 pmol/L. Previous studies showed that lowering the threshold of

serum TSH from 4.5 to 2.5 mIU/L would identify an additional 9.7% of patients who would also be diagnosed as subclinical hypothyroid if the upper TSH limit were decreased [7]. As the potential benefits to mother and foetus outweighed the neglible risks associated with treatment, we considered the upper level of TSH as 2.5 mIU/L.

Statistical analysis of differences between the two groups was done using Statistical Package for Social Science version 17.0. (SPSS Inc, Chicago). Quantitative variables were expressed as mean ±standard deviation and compared by unpaired t test. Qualitative variables were expressed as frequencies/percentages and compared using Chi-square test or Fisher's exact test. A p-value of <0.05 was considered statistically significant. Ethical clearance was taken from the ethical committee of Maulana Azad Medical College.

Results

Out of 81 women, one woman in group 1 and two women in group 2 failed to respond to the stimulation protocol and hence, all the three of them were dropped from further study. A women in group 2 had total fertilization failure. The remaining 77 women were followed up till the completion of the study. In our study, it was found that 16 were positive for anti-TPO antibody, 2 for anti-Tg antibody and one women was positive for both the antibodies. Hence, the prevalence of anti-thyroid antibodies in the study population was 21% (17/81) which was much higher than the one reported in euthyroid fertile women between 18-45 years [8]. The mean age of women in group 1 was 29.8 ± 3.5 years and that in group 2 was 30.7 ± 3.3 years while the mean BMI (kg/m^2) in group 1 was 23.3 ± 2.6 and that in group 2 was 24.7 ± 3.4. The difference between the two groups was not statistically significant. As shown in **Table 1**, there was also no statistical difference between the two groups in term of type of infertility, duration of infertility, basal serum level of follicle stimulating hormone and luteinizing hormone, the type of stimulation protocol, the number of oocytes retrieved and the number of embryos transferred. In both the groups, most of the women were having unexplained infertility,47.1% in group 1 and 51.5% in group 2 followed by tubal factors, 29.4% in group 1 and 34.4% in group 2 and peritoneal factors, 23.5% in group 1 and 14.1% in group 2. Women with endometriosis with evidence of tubal or peritoneal adhesions were included under peritoneal factors. The differences were not statistically significant.

Table 1 Two groups in terms of type and duration of infertility.

Variables	ATA±group	ATA- group	p-value
Age (year)	29.8+3.5	30.7+3.3	0.173
BMI(kg/m2)	23.3+2.6	24.7+3.4	0.062
Duration of infertility (years)	8.7+4.6	7.0+3.6	0.062
Primary infertility (%)	82.4	67.2	0.112

Table 2 Comparison of various variables between ATA positive and ATA negative groups.

Agonist protocol (%)	41.2	32.8	0.26
S.FSH (IU/L)	9.2+6.6	7.3+4.3	0.078
S.LH (IU/L)	5.3+4.9	3.9+2.4	0.052
Number of oocytes retrieved	10.4+5.7	9+6.9	0.235
Fertilization rate (%)	66.3	78.9	0.011
Number of embryos transferred	2.0+0.6	2.1+0.7	0.122
Implantation rate (%)	18.2	26.8	0.226
Clinical pregnancy rate (%)	31.3	39.3	0.276

The fertilization rate in group 1 was found to be 66.3% which is significantly lower as compared to 78.9% in group 2. In both the groups, comparable number of embryos were transferred. However, there were no statistically significant difference in implantation rate (18.2% vs 26.8%) and clinical pregnancy rate (31.3% vs 39.3%).

Discussion

In our study population of euthyroid infertile women, we observed an increased prevalence of antithyroid antibodies i.e., 21%. However, previous research showed the prevalence of ATA in euthyroid infertile women to be 10.5% [8] which was similar to the one reported in euthyroid fertile women between 18 and 45 years (13.8%) [9]. The incidence was reported to be higher in women with pelvic endometriosis (21.8%) or reduced ovarian reserve (22.5%) when compared to women with tubal disease (5.6%) or with idiopathic infertility (13.9%) or male-related infertility (6.4%) [8]. However, we have included only those women with unexplained or mechanical causes of infertility in our study group and hence, such comparison could not be made.

The fertilization rate was significantly lower in ATA positive group as compared to ATA negative group while the implantation rate and clinical pregnancy rate was lower in ATA positive women than ATA negative women although the results were not significant statistically. The exact mechanisms of these associations were unknown, though two had been proposed. Firstly, the presence of thyroid autoantibodies in women with normal thyroid function could be associated with a subtle deficiency in the availability of thyroid hormones (a fall in circulating free thyroid hormones within the reference range) or a lower capacity of the thyroid gland to adequately rise to the demand for augmented synthesis of thyroid hormones required in pregnancy [10]. Secondly, thyroid autoantibodies might be an indicator of an underlying enhanced global autoimmune state. This itself could have a direct adverse effect on placental or foetal development [10].

It is also believed that ATA may bind to either the surface of the egg or embryo and interfere with fertilization and subsequent embryo development. Alternatively, the presence of ATA in the endometrium may exert detrimental effect on embryo implantation and induce early pregnancy loss [11]. Various conflicting studies were found regarding the effect of anti-thyroid antibodies on pregnancy rate. Kutteh et al. [12] and Negro et al. [13] could not detect any adverse outcome of IVF due to presence of ATA in the patients. On the contrary, the present study detected an association of lower fertilization rate following IVF with the positive serum ATA status. The disagreement might be attributed to several factors. Unlike ours which was a prospective cohort study, the above two were retrospective studies. We excluded

all thyroid dysfunction cases by selecting a very stringent criteria but the above two studies were not devoid of such bias.

The studies conducted by Geva et al. [14], Kim et al. [15], Bussen et al. [16], Revelli et al. [8] and Zhong et al. [11] were in agreement with the present study that the presence of ATA antibodies in blood adversely affected the outcome of IVF. Contrary to observations made in the above studies, we found that only one outcome parameter i.e., fertilization rate following IVF was lower in ATA positive euthyroid cases in comparison to that in ATA negative euthyroid subjects indicating that ATA positivity might be an independent risk factor for adverse outcome among the patients undergoing IVF-ET as major confounder like thyroid dysfunction was controlled in this study. Although other outcome parameters like implantation rate and clinical pregnancy rate were lower following IVF in ATA positive euthyroid subjects, the differences were not statistically significant when compared to those of ATA negative subjects.

In conclusion, the prevalence of thyroid antibodies appears to be much higher in euthyroid infertile women and the presence of these antibodies is associated with a lower fertilization rate, 66.3% compared to 78.9% in absence of antibodies (p value 0.011) and a poorer IVF outcomes. Since these autoantibodies seem to be distinct and independent markers for reproductive failure, their identification provides the opportunity to identify women at risk for an adverse outcome in an IVF-ET programme. Therefore, we suggest to include the determination of thyroid antibodies in the evaluation of euthyroid women undergoing IVF.

However, to conclusively prove the hypothesis of our study, randomized controlled trials with larger sample size are required.

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