DOI: 10.7860/JCDR/2019/40760.12872

Original Article

Obstetrics and Gynaecology Section

Antithyroid Antibodies and Fertility Outcome in Euthyroid Women Undergoing In Vitro Fertilisation

AHEIBAM BIDYA DEVI[1], SUDHA PRASAD[2], BIDHAN CHANDRA KONER[3]

ABSTRACT

Introduction: Antithyroid Antibodies (ATA) are suspected to cause poor outcome of In Vitro Fertilisation (IVF) even in the absence of thyroid dysfunction.

Aim: To study the effect of ATA on fertilisation rate, implantation rate and clinical pregnancy rate in euthyroid women undergoing IVF.

Materials and Methods: The study was a prospective cohort study conducted at an IVF centre of a Medical College. A total of 81 euthyroid patients planned for IVF were recruited and divided into two groups depending upon the presence of ATA. All of them underwent the standard stimulation protocol. The results were compared using unpaired t-test for continuous variables and Chi-square/Fishers'-exact test for categorical variables.

Results: The prevalence of ATA was 20.99% (17/81) in our study population. The fertilisation rate was significantly lower ($p<0.05$) in women with ATA (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 ATA was associated with a lower fertilisation rate even in euthyroid state in women undergoing IVF. However, the implantation and clinical pregnancy rate were not correlated with the presence of such antibodies.

Keywords: Anti-thyroglobulin antibody, Anti-thyroperoxidase antibody, Pregnancy rate

INTRODUCTION

The introduction of Assisted Reproductive Technology (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 of many infertile couples. Although the results of In Vitro Fertilisation-Frozen Embryo Transfer (IVF-FET) 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 32.4-33.0% per IVF transfer [1]. The chances that one will get pregnant, decreases with each successive round and the cost increases. The chances of live birth IVF success rate for women under 35 years after three full cycles of IVF is 59-67% [2]. The probability for success with IVF relates to several prognostic factors like maternal age, ovarian reserve, causes of infertility and past reproductive performance [3].

Autoimmune thyroid diseases are rather frequent in women of reproductive age affecting 5-20% of them [4]. They are characterised by the presence of Antithyroid Antibodies (ATA) like anti-Thyroglobulin (anti-Tg) and anti-Thyroperoxidase Antibodies (anti-TPO). These antibodies are often detected in subjects complaining of hypo- or hyperthyroidism, but are also found in patients without any sign and laboratory evidence of thyroid dysfunction [4]. ATA are suspected to cause poorer outcome of IVF even in the absence of thyroid dysfunction. It is evident that ATA can have a negative influence on the female reproductive potential. Women with no sign of thyroid dysfunction but are positive for ATA have three to five fold more risk of spontaneous miscarriage [5] and increased incidence of preterm birth and adverse neurodevelopmental sequelae in children than those who are negative for ATA [6,7].

Some authors claimed that high ATA prevalence in euthyroid women who had three or more unsuccessful IVF cycle [8] and a lower pregnancy rate of IVF with ATA positivity [9]. While other studies failed to detect any difference in IVF success rate between ATA positive and ATA negative patients [10]. Till date, there is lack of robust data to provide evidence regarding the impact of ATA on the outcome of IVF-ET. These issues need to be investigated and a common universal protocol on the optimal management of euthyroid ATA positive women in assisted reproductive medicine needs to be implemented.

Hence, the study was conducted to determine the relationship between ATA and fertility outcome in euthyroid women undergoing IVF.

MATERIALS AND METHODS

A cross-sectional study was conducted at the IVF and Reproductive Biology Centre of a Medical College in New Delhi from November 2014 to April 2016 and a prospective cohort design was used to establish the relationship between ATA and fertility outcomes following IVF. Sample size was calculated as 81, it was the sample size of convenience. Ethical clearance was taken from the ethical committee of the concerned Medical College vide letter no F.No. /11/IEC/MAMC/2011/147 dated 05/12/14.

Thyroid function test was routinely done for all infertile women in our centre. Those patients with normal free triiodothyronine (fT3) (3.1-6.8 pmol/L) and free thyroxine (fT4) (12-22 pmol/L) levels with serum Thyroid Stimulating Hormone (TSH) level between 0.5 to 2.5 mIU/L, the reference range being taken as per institutional protocol, were considered as euthyroid. A total of 81 euthyroid women fulfilling the inclusion and exclusion criteria were recruited. Euthyroid infertile women between 20-35 years of age with tubal or peritoneal or unexplained factors were included in the present study. Women with clinically significant systemic disease, uterine anomalies, polycystic ovarian syndrome, thrombophilias or other autoimmune disease like anticardiolipin antibody or lupus anticoagulant positivity and those with male factor infertility were excluded from the study. A written informed consent was taken from each patient recruited for the study. A fasting venous blood sample of 2 mL was withdrawn by antecubital venepuncture for the estimation of ATA 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 giving injection Leuprolide acetate 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 Progesterone4 (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. In patients with markedly suppressed LH (less than 1 IU/L), hMG 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 (ethinyl estradiol 0.030 mg and levonorgestrel 0.150 mg) 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 or HMG from day 2 or 3 of menstrual cycle and continued till oocyte trigger. Injection Cetrorelix, GnRH antagonist 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 measurement of serum estradiol levels and transvaginal sonographic imaging of ovarian follicles. Endometrial development was also monitored during stimulation by measuring the endometrial thickness and the endometrial blood flow using Doppler ultrasound. When at least two follicles measuring 17-18 mm in mean diameter and a serum estradiol concentration of approximately 200 pg/mL per follicle measuring 14 mm or greater was achieved, trigger was given with injection hCG (5000-10000 IU) administered intramuscularly on the same day to induce follicular maturation. Oocyte retrieval was performed after 34-36 hours of hCG trigger under ultrasound guidance. Fertilisation was achieved by conventional micro-insemination. After three days of oocyte retrieval, at most three embryos were transferred into the uterine cavity. Micronized progesterone was started from the day of oocyte collection for luteal phase support. Two weeks after embryo transfer, Urine Pregnancy Test (UPT) was done and serum level of βhCG was measured. In those women with positive UPT and/or serum βhCG more than 50 mIU/mL, transvaginal ultrasonography was done for the detection of gestational sac/cardiac activity four weeks after embryo transfer.

Outcome of the study was measured as below:

1. Fertilisation rate-defined as the percentage of oocytes fertilised out of the total number of oocytes retrieved.
2. Implantation rate-defined as the number of gestational sacs observed by transvaginal ultrasonography per embryo transfer, expressed in percentage.
3. Clinical pregnancy rate-defined as the percentage of patients with cardiac activity in foetus observed by transvaginal ultrasonography done four weeks after embryo transfer.

In the present study, serum TSH, fT3 and fT4 levels were measured using 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 [11]. As the potential benefits to mother and foetus outweighed the negligible risks associated with treatment, we considered the upper level of TSH as 2.5 mIU/L.

STATISTICAL ANALYSIS

Statistical analysis of differences between the two groups was done using Statistical Package for Social Science version 17.0. (SPSS Inc, Chicago). The results were compared using unpaired t-test for continuous variables and Chi-square/Fisher's-exact test for categorical variables. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Out of 81 euthyroid women, 17 were positive for ATA (16 were positive for anti-TPO antibody, two for anti-Tg antibody and one woman for both antibodies) and hence, were included in group 1 while 64 women who were negative for both antibodies were included under group 2. Hence, the prevalence of ATA in the study population was 20.99% (17/81). One woman in group 1 and two women in group 2 failed to respond to the stimulation protocol i.e., no oocyte could be retrieved from all the three of them. Further study could not be carried out on these three patients and hence, were dropped from further study. A woman in group 2 had total fertilisation failure i.e., none of the oocytes retrieved were fertilised leading to failure of development of embryo with cancellation of subsequent embryo transfer. The remaining 77 women underwent embryo transfer successfully and were followed up till the completion of the study.

There was no difference between the two groups in terms of demographic features, duration of infertility, serum level of follicle stimulating hormone and luteinizing hormone [Table/Fig-1] as well as the type of infertility and stimulation protocol used [Table/Fig-2].

Parameter	Group 1 (ATA+) N=17	Group 2 (ATA-) N=64	p-value
Age (year)	29.8±3.5	30.7±3.3	0.173
BMI (kg/m^2)	23.3±2.6	24.7±3.4	0.062
Duration of infertility (years)	8.7±4.6	7.0±3.6	0.062
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

[Table/Fig-1]: Comparison of various variables between the two groups.
Test used: T-test

Parameter	Group 1 (ATA+) N=17	Group 2 (ATA-) N=64	p-value
Type of infertility (%) (Primary/Secondary)	82.4/17.6	67.2/32.8	0.112
Stimulation protocol (%) (Agonist/Antagonist)	41.2/58.8	32.8/67.2	0.260

[Table/Fig-2]: Comparison of type of infertility and stimulation protocol between the two groups.
Test used: Chi-square test/Fisher's-exact test

Unexplained infertility accounted for 47.1% of women in group 1 and 51.5% in group 2 followed by tubal factors with 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 mild endometriosis with evidence of tubal or peritoneal adhesions were included under peritoneal factors. The differences were not statistically significant. The mean serum TSH level was 2.0±0.4 mIU/L in group 1 and 2.1±0.5 mIU/L in group 2 which was statistically not significant (p-0.378).

The mean number of oocytes retrieved and that fertilised were comparable in both the groups. As mentioned earlier, no oocyte was retrieved in a woman in group 1 and two women in group 2. The fertilisation rate in group 1 was found to be 66.3% which was significantly lower as compared to 78.9% in group 2 [Table/Fig-3]. This showed that the presence of thyroid autoantibodies had some detrimental effect on the fertilisation of ovum.

Parameter	Group 1 (ATA+) *N=16	Group 2 (ATA-) *N=62	p-value
Oocytes retrieved	10.4±5.7	9.0±6.9	0.235
Oocytes fertilised	6.2±3.2	6.6±4.6	0.359
Fertilisation rate (%)	66.3±23.9	78.9±17.9	0.011

[Table/Fig-3]: Comparison of fertilisation rate between the two groups.
Test used: T-test
*N=16 in group 1 and 62 in group 2 because no oocyte was retrieved in one woman in group 1 and two women in group 2

Embryo transfer was done in 16 women in group 1 and 61 women in group 2 as another woman in group 2 failed to fertilise any of the oocytes retrieved. In both the groups, comparable number of embryos was transferred (2.0±0.6 vs. 2.1±0.7). Urine pregnancy test done after two weeks of embryo transfer showed positivity in 37.5% (6/16) of women in group 1 and 42.6% (26/61) of women in group 2. On transvaginal sonography done in those women with positive pregnancy test, gestational sac was seen in 5 women in group 1 (4 with singleton pregnancy and one with twin pregnancy). While in group 2, gestational sac was observed in 24 women (19 with singleton pregnancy, 4 with twin pregnancy and 1 triplet pregnancy). There was no statistically significant difference in the implantation rate per embryo transfer (18.2% vs 26.8%) between the two groups. Moreover, clinical pregnancy rate was 31.3% in group 1 and 39.3% in group 2. However, the difference was not statistically significant [Table/Fig-4].

Parameter	Group 1 (ATA+) N=16	Group 2 (ATA-) *N=61	p-value
Number of gestational sacs observed	6	30	
Total number of embryos transferred	33	112	
Implantation rate (%) per embryo transfer	18.2 (6/33)	26.8 (30/112)	0.226
Cardiac activity present	5	24	
Clinical pregnancy rate (%)	31.3 (5/16)	39.3 (24/61)	0.276

[Table/Fig-4]: Comparison of implantation and clinical pregnancy rate between the two groups.
Test used: Chi-square test/Fisher's-exact test
*N=61 in group 2 as one woman had total fertilisation failure and hence, embryo transfer was not done

DISCUSSION

In the study population, it was observed that the prevalence of ATA in euthyroid infertile women was 20.99%. Our finding was consistent with the study conducted by Geva E et al., [12] with the prevalence of 20.5% and Kutteh WH et al., [10] where it was 19.2%. However, Revelli A et al., showed the prevalence of ATA in euthyroid infertile women to be 10.5% [13]. While Poppe K et al., and Negro R et al., found the prevalence of anti-TPO antibodies to be 14% and 15%, respectively [14,15]. In both the studies, levels of anti-Tg antibodies were not measured and hence, the prevalence of ATA was lower compared with the present study. The higher prevalence of ATA in the present study might be due to the fact that being a referral centre, most of the women referred here belonged to that category of women who failed to conceive despite repeated attempts, due to some undiagnosed factors. Moreover, it was also observed that the prevalence of ATA in euthyroid infertile women (20.99%) was much higher than the one reported in euthyroid fertile women (13.8%) [16].

In the present study, the mean number of oocytes retrieved was comparable in both the groups. However, the fertilisation rate was significantly lower in ATA positive group (66.3%) as compared to ATA negative group (78.9%) [Table/Fig-3]. Similarly, the fertilisation rate was significantly lower in ATA positive group compared with ATA negative group in a study by Revelli A et al., (74.2% vs. 83.0%) [13] and another study by Zhong YP et al., (64.3% vs. 74.6%) [17]. However, no statistical difference was seen between the two groups in a study by Geva E et al., (64.8% vs 66.1%) [12]. The association of thyroid autoantibodies with a lower fertilisation rate might be due to the reason that ATA might bind to the surface of the oocyte interfering with its fertilisation and subsequent embryo development.

Up to a maximum of three embryos were transferred in both the groups in the present study. Both the implantation rate (18.2% vs 26.8%) and clinical pregnancy rate (31.3% vs 39.3%) were lower in ATA positive group compared with ATA negative group but the results were not statistically significant [Table/Fig-4]. The presence of ATA in the endometrium might exert detrimental effect on embryo implantation and induce early pregnancy loss [17]. These autoantibodies might be an indicator of an underlying enhanced global autoimmune state which could adversely affect foetal and placental development. In the study conducted by Revelli A et al., and Zhong YP et al., the implantation rate and clinical pregnancy rate were significantly lower in ATA positive group compared with ATA negative group [13,17]. Moreover, Bussen S et al., Kim CH et al., and Geva E et al., also observed that clinical pregnancy rate was significantly lower in ATA positive group [8,9,12]. However, Kutteh WH et al., and Negro R et al., could not detect any adverse outcome of IVF due to presence of ATA in the patients [10,15]. A systemic literature review and meta-analysis conducted by Busnelli A et al., on published research articles from January 1990 to November 2015 found that thyroid autoimmunity did not impact on IVF/ICSI outcome in terms of number of oocytes retrieved and likelihood of fertilisation, implantation and clinical pregnancy in euthyroid infertile women [18]. Another systemic review done by Simopoulou M et al., following evaluation of the evidences published from 2006 to 2018 also showed that ATA were not associated with poorer outcomes following IVF/Intracytoplasmic Sperm Injection (ICSI) cycles in euthyroid women [19].

LIMITATION

The main limitation was the small sample size of the study population due to which the strength of the provided evidence was restricted.

CONCLUSION

The prevalence of ATA in euthyroid infertile women undergoing IVF was 20.99% and the presence of these antibodies was associated with a lower fertilisation rate compared with those without these antibodies (p-value-0.011) but the clinical pregnancy rate was not correlated with the presence or absence of these antibodies. These autoantibodies are an important marker of reproductive failure, so their identification is important for good outcome of an IVF-ET programme. Therefore, the determination of thyroid antibodies seems to be essential in the evaluation of euthyroid women undergoing IVF. However, to conclusively prove the hypothesis of our study, randomised controlled trials with larger sample size are required.

ACKNOWLEDGEMENTS

We are thankful to the Planning Branch of the concerned institute for providing us the kit required for the study.

REFERENCES

[1] De Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, et al. Assisted reproductive technology in Europe, 2006: Results generated from European registers by ESHRE. Hum Reprod. 2010;25(8):1851-62.
[2] Berek JS. Berek & Novak's Gynecology, 15th ed, 2nd India reprint. New Delhi: Lippincott Williams & Wilkins; 2013; Chapter 32: Infertility and Assisted Reproductive Technology; Pp. 1174.
[3] Speroff L, Fritz MA. Clinical Gynecologic Endocrinology and Infertility, 8th ed, 5th Indian reprint. New Delhi: Lippincott Williams & Wilkins; 2015; Chapter 32: Assisted Reproductive Technologies; Pp. 1337.
[4] Poppe K, Velkeniers B, Glinoert D. Thyroid disease and female reproduction. Clin Endocrinol. 2007;66(3):309-21.
[5] Poppe K, Velkeniers B, Glinoer D. The role of thyroid autoimmunity in fertility and pregnancy. Natl Clin Pract Endocrinol Metab. 2008;4(7):394-405.

[6] Glinoer D, Soto MF, Bourdoux P, Lejeune B, Delange F, Lemone M, et al. Pregnancy in patients with mild thyroid abnormalities: maternal and neonatal repercussions. J Clin Endocrinol Metab. 1991;73:4217.
[7] Glinoer D, Delange F. The potential repercussions of maternal, fetal, and neonatal hypothyroxinemia on the progeny. Thyroid. 2000;10(10):871-87.
[8] Bussen S, Steck T, Dietl J. Increased prevalence of thyroid antibodies in euthyroid women with a history of recurrent in vitro fertilization failure. Hum Reprod. 2000;15(3):545-48.
[9] Kim CH, Chae HD, Kang BM, Chang YS. Influence of antithyroid antibodies in euthyroid women on in vitro fertilization embryo transfer outcome. Am J Reprod Immunol. 1998;40(1):2-8.
[10] 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(5):843-48.
[11] Surks MI, Goswami G, Daniel GH. The thyrotropin reference range should remain unchanged. J Clin Endocrinol Metab. 2005;90(9):5489-96.
[12] Geva E, Vardinon N, Lessing JB. Organ-specific autoantibodies are possible markers for reproductive failure: A prospective study in an in-vitro fertilization-embryo transfer programme. Hum Reprod. 1996;11(8):1627-31.
[13] Revelli A, Casano S, Piane LD. A retrospective study on IVF outcome in euthyroid patients with antithyroid antibodies: Effects of levothyroxine, acetylsalicylicacid and prednisolone adjuvant treatments. Reprod Biol Endocrinol. 2009;7:137.
[14] Poppe K, Glinoer D, Van Steirteghem A, Tournaye H, Devroey P, Schiettecatte J, et al. Thyroid dysfunction and autoimmunity in infertile women. Thyroid. 2002;12(11):997-1001.
[15] 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(6):1529-33.
[16] Prummel MF, Wiersinga WM. Thyroid peroxidase autoantibodies in euthyroid subjects. Best Pract Res Clin Endocrinol Metab. 2005;19(1):1-15.
[17] Zhong YP, Ying Y, Wu HT, Zhou CQ, Xu YW, Wang Q, et al. Relationship between antithyroid antibody and pregnancy outcome following in vitro fertilization and embryo transfer. Int J Med Sci. 2012;9(2):121-25.
[18] Busnelli A, Paffoni A, Fedele L, Somigliana E. The impact of thyroid autoimmunity on IVF/ICSI outcome: A systemic review and meta-analysis. Hum Reprod Update. 2016;22(6):775-90.
[19] Simopoulou M, Sfakianoudis K, Maziotis E, Grigoriadis S, Giannelou P, Rapani A, et al. The impact of autoantibodies on IVF treatment and outcome: A systemic review. Int J Mol Sci. 2019;20(4)piiE:892.

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Obstetrics and Gynaecology, Regional Institute of Medical Sciences, Imphal, Manipur, India.
2. Director Professor, Department of Obstetrics and Gynaecology, Maulana Azad Medical College, New Delhi, India.
3. Professor, Department of Biochemistry, Maulana Azad Medical College, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Aheibam Bidya Devi,
Heirangoithong Aheibam Leirak, P.O. Singjamei, Imphal-795008, Manipur, India.
E-mail: aheibambidya@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 01, 2019**
Date of Peer Review: **Feb 05, 2019**
Date of Acceptance: **Mar 30, 2019**
Date of Publishing: **May 01, 2019**