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# Study of the possible relations between seminal plasma antisperm antibodies and thyroid function tests in men infertility

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***Abstract*---**Infertility is defined as a man in a reproductive age who cannot conceive despite having regular unprotected intercourse for one year. It accounts for 40-50% of infertility in humans. In this study, the patients were gathered in 4 groups: 1. group - Prednisolone group, 2. group - Levothyroxine group, 3. group - control group, 4. group - Levothyron and Prednisolone group. In group 1, the drug taken in patients who only received prednisolone and undergone ovulation induction had no significant effect on Anti-T and Anti-TPO. Biochemically, conceiving rate was 16% higher in this group. In group 2, patients were diagnosed with thyroid disease and were taking levothyroxine for a long time, and they continued to take the drug during ovulation induction. The patients in group 3 (control group) did not take any medication except ovulation induction. Significant decrease was observed in Anti and Anti-TPO values in group 4 (P = 0.037 and P = 0.015, respectively). The combined use of both drugs significantly decreased the biochemical pregnancy rates in this group. In this study, we aimed to examine various parameters that define and process in this type of infertility and to determine the presence of possible relationships between antibodies and thyroid function tests.

***Keywords*---**Infertility male, Thyroid function tests, Levothyroxine.

## 1- Introduction

Male infertility is defined as the inability of a man in reproductive age to conceive despite having regular unprotected intercourse for one year (Vayena 2002). Some researchers prefer the term subfertility to describe men who are not sterile but have reduced fertility. About 80-90% of healthy young men conceive within a year, most within 6 months. Infertility affects approximately 15% of men of reproductive age (Speroff and Fritz 2005). Contrary to general perception, the overall incidence of infertility has remained relatively unchanged over the past 30 years. The evaluation and treatment of infertility has changed dramatically with major advances in this field. Evaluation of every infertile man deserves a careful history and physical examination. In this way, signs and symptoms suggestive of a specific cause can often be detected and help focus on possible responsible factors. In some patients, it is beneficial not to wait for the one-year period used for the diagnosis of infertility. This patient group includes those over 35 years of age, those who have reason to question their fertility, and men at a significant risk of infertility. In the history of the male partner, issues such as the duration of infertility, the frequency of coitus, previous infertility treatments, childhood diseases, surgical operations, systemic diseases, the presence of sexually transmitted diseases, toxin and heat exposure, drugs used, smoking and alcohol use, substance abuse should be questioned. (Yumru and Öndeş 2011). Physical examination of the male partner should include penile examination, testicular palpation and measurement of its size, presence and stiffness of the vasa and epididymis, presence of varicocele, evaluation of secondary sex characteristics, and rectal examination.

Human sperm are cells that can activate redox reactions that will lead to the formation of superoxide anion (O-2) and hydrogen peroxide (H2O2). This activity is of biological importance in the regulation of signal transduction pathways that control sperm capacitation. However, excessive production of reactive oxygen species (ROS) leads to a decrease in the fertilization ability of sperm and deterioration of its genetic integrity (Krassar et al. 2010). Oxidative DNA damage and consequently a decrease in pregnancy rates, an increase in the incidence of miscarriage, cancer and dominant genetic diseases are the serious harmful changes seen as a result of this situation.

Immunological factors play an important role in the reproductive process of fertilization, implantation and placental development. Anti-TPO (anti-microsomal) and Anti-T (anti-thyroglobulin) antibodies, called thyroid autoantibodies, are also measured in the diagnosis of reproductive failure and thyroid autoimmunity. High levels of these antibodies indicate that thyroid disease occurs due to a disorder of the immune system called autoimmune. Autoimmune disease is a condition in which the body perceives its own tissue (thyroid gland) as a foreign tissue and tries to destroy it. Therefore, our immune system produces thyroid autoantibodies Anti-TPO and Anti-T to destroy the thyroid gland. High levels of these autoantibodies indicate very mild subclinical hypothyroidism that cannot be diagnosed by measuring thyroid hormones and thyroid stimulating hormone (TSH) levels. Subclinical hypothyroidism occurs in patients with autoimmune thyroid disease or decreased thyroid reserve due to iodine deficiency (Krassar et al. 2010).

In this study, it was aimed to examine various parameters defining and emphasizing the oxidative process in infertile men in sperm and seminal plasma, to compare these parameters with the values of normozoospermic and fertile men, to determine the role of the oxidative process in this type of infertility, to determine the possible relationships between the relevant antibodies and thyroid function tests.

## 2. Materials and Method

### 2.1. Materiel

For this study, 65 normal and 125 infertile patients from Iraq Dhi Qar Governor's Hospital: Al Hussein Teaching Hospital were included in the application (The research was based on male infertility, but some men do not know the status of their wife, are they infertile or not? Therefore, women are in the research and follow-up of husband and wife status. According to the questionnaire applied to a series of male patients at the Al-Hussein Training Hospital, 190 men and women were selected to exclude those with chronic diseases, alcohol consumption and poor psychological status. were excluded from the study. In this survey, all the necessary details for sampling in this study are mentioned). Ages between group 1, group 2, group 3 and group 4 of the patients included in the study, -FSH, TSH, fT3, fT4, semen analysis, ASA, Anti-T before IVF, Anti-TPO before IVF, Anti-T during ET, The distribution of Anti-TPO and mean prednisolone intake time ± standard deviation (SD) values during ET was found.

All of the patients were evaluated by semen analysis, and the sperm count was divided into 4 groups as >15x106/ml and |<15x106/ml, and the patients were included in the study. Patient selection is based on screening and subsequent analysis of the following tests (FSH, TSH, T3, T4, semen analysis، ASA، Anti-T, Anti-TPO) values for each sample in venous blood routinely checked before treatment among patients receiving treatment for infertility. The image of the autoanalyzer of the company A (Roche Cobas E-411) where the analyzes were made is given in Figure 3.1. This hormone analyzer is the latest technology in the world to analyze hormones. Working principle: electrochemiluminescence (ECL) stands for electro electrical stimulation + chem indicates a chemical reaction + luminescence produces light = electrochemiluminescence (intensity of action signal from 460 to 610 nm) (Fig. 2.1)


Figure 2.1 Roche Cobas E-411 autoanalyzer

It works with the chemiluminescent microparticle immunoassay (CMIA) (Siemens Immulite 2000) method (Figure 3.2). For Anti-T, Anti-TPO, FSH tests:
intra-assay CVs < 4%, < 5%, < 6%, < 5%, < 3%, respectively.
The inter-assay CVs were <6%, <7%, <8%, <6%, <10%, respectively.
The Siemens Immulite 2000 system is a continuous random access immunoassay analyzer with a maximum throughput of 200 tests per hour. This design combines specialty and allergy testing on a single platform, increasing workflow and increasing productivity for medium to high volume immunoassay labs (density of action signal 540 nm) (Figure 2.2).
For Anti-T, Anti-TPO, FSH, tests,
intra-assay CVs were <5%, <8%, <3%, <2%, <2%, respectively.
The inter-assay CVs were <6%, <8%, <5%, <6%, <4%, respectively.

Figure 2.2 Siemens Immulite 2000 auto-analyzer

2.2. Semen Preparation

The semen sample given was kept in a 37°C incubator until liquefied. They were put into tubes (Falcon/2052) and their volumes were measured. Then its concentration, motility, vitality and pH (merck) were determined. Concentration and motility were evaluated by placing 10 µl of a maxillary counting camera from the semen sample and counting all frames under a 40X objective under a phase contrast microscope (Olympus, CX31).motility; They were divided into 4 groups as A motility (forward fast motile sperm group), B motility (forward slowly motile sperm group), C motility (sperm motile sperm group in situ) and D motility (immotile sperm group). At least 100 spermatozoa were examined and the ratios of A, B, C and D were determined as %. The total motility rate was determined as the sum of %A, %B and %C motility. For vitality, 10 µl of semen sample and 10 µl of dye (merck eosin y solution) were mixed on a slide and covered with a coverslip. The viability rate was determined by counting at least 100 sperm under a 40X objective under a phasecontrast microscope (Olympus, CX31).

## 3. Result

In the analyzed sperm parameters of male patients; Sperm concentration, sperm motility, sperm morphology, sperm concentration after preparation, sperm motility results after preparation were found to be statistically significantly higher in the patients in group 1 (Table 4.1). In line with these data, the pregnancy rate of the patients in group 1 was found to be high.

Table 3.1 Sperm parameters in patients

| | sperm in patients parameters | P* |
|---|---|---|
| Sperm concentration (mil/ml)<br>Sperm motility (%) | 53,16 ± 6,24 | 0,008 |
| Sperm morphology (%) | 30,85 ±15,52 | 0,008 |
| Prepared sperm concentration (mil/ml) 2 (mil/ml) | 20,47 ±20,78 10 | 0,008 |
| Prepared sperm motility (%)<br>conceiving | 69,24 ±22,41<br>%45 | 0,008 |

There was no statistically significant difference between the groups investigated in the study in terms of median anti-T values before IVF (p = 0.08). At the same time, a homogeneous distribution was observed in the median Anti-T values during ET between the groups, and no statistically significant difference was observed (p = 0.06). These statistics were examined to determine the homogeneous distribution of the cases, and in terms of the reliability of the data, no natural or selective bias was observed in any distribution. Table 3.2 shows Anti-T values according to the groups before IVF and during ET

Table 3.2 Anti-T in all cases before IVF and during ET according to groups

| | Before IV | during ET | p-value | Change |
|---|---|---|---|---|
| Group 1 | 144,2 (73,6-301,3) | 140,0 (56,1-296,9) | 0,06 | -11,0 (-45,8 - 11,5) |
| Group 2 | 187,1 (69,7-349,5) | 170,4 (56,4-281,5) | 0,02 | -11,2 (-69,6 - 4,6) |
| Group 3 | 108,6 (37,6-164,8) | 77,4 (28,7-162,6) | 0,02 | -15,1 (-51,7 - 6,6) |
| Group 4 | 159,3 (58,2-329,5) | 143,6 (42,8-279,3) | <0,001 | -13,8 (-35,7 - 1,2) |
| p-value | 0,08 | 0,06 | | 0,87 |

Data are shown in median (quartile width) format.

† Comparisons between groups before IVF and during ET Wilcoxon sign test.

‡ Comparisons between groups in terms of change before IVF, during ET, and during ET compared to before IVF Kruskal Wallis test

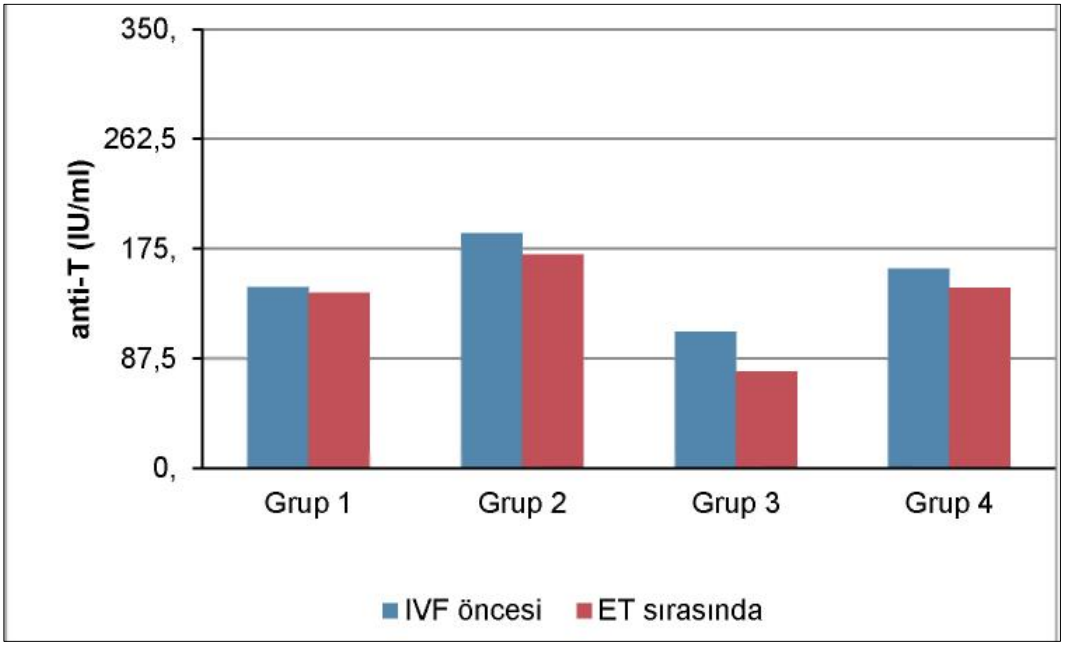


Figure 3.1 Anti-T values before IVF and during ET in all cases

There was no statistically significant difference between the anti-T measurements at the beginning of IVF and during the transfer between all groups, that is, the findings were similar (p = 0.87). However, among the IVF initiation values, especially the control group values are in a decrease in trend/tendency compared to the other group (p = 0.08). In the natural distribution, patients in the control group were recruited from patients whose Anti-T values tended to be low but had a higher than normal Anti-T or Anti-TPO value, but the distribution was normal. The same findings showed a decreasing trend/trend during ET in the control group compared to the other research groups (P=0.06). Although there was no statistical difference, the highest values were observed in the group with thyroid disease and taking thyroid medication. If so, the distribution is quite natural, that is, the antibody is higher in the patient cases and lower in the control. But there is no statistical difference.

If we examine the groups investigated in the study one by one as before IVF and during ET, there was no statistically significant difference between the values of median Anti-T IVF onset and during ET in group 1, but a trend/trend was observed within the statistical significance limit (p = 0.06). A statistically significant decrease was observed between the median Anti-T values during ET compared to the beginning of IVF in Group 2 (p = 0.02). There was a statistically significant decrease in median Anti-T values during transfer in Group 3 compared to pre-IVF (p = 0.02). In Group 4, a statistically significant decrease was observed in the median Anti-T values during ET compared to before IVF (p < 0.001).

In other words, a trend/trend with using prednisolone, a significant decrease in levathyroxine and levathyroxine prednisolone or no drug use groups indicates that a motif in ovulation induction or induction lowers Anti-T antibodies,

regardless of whether they are pregnant or not. There was no statistically significant difference between the groups in terms of median Anti-TPO values before IVF (p = 0.26). There was no statistically significant difference between the groups in terms of median Anti-TPO values in the order of transfer (p = 0.25) (Table 4.3).

Table 3.3 Anti-TPO values according to groups before IVF and during ET in all cases

| | Before IV | during ET | p-value | Change |
|---|---|---|---|---|
| Group 1 | 46,0 (13,0-201,1) | 48,7 (10,7-161,1) | 0,09 | -1,6 (-11,9 - 4,3) |
| Group 2 | 138,1 (17,9-290,0) | 99,5 (17,1 -224,8) | **0,02** | -8,6 (-38,3 - 5,2) |
| Group 3 | 67,8 (27,3-337,8) | 49,1 (12,7 -177,1) | 0,12 | -2,8 (-42,8 - 8,6) |
| Group 4 | 126,5 (42,7-262,9) | 103,4 (42,0 -270,7) | **0,008** | -11,8 (-37,9 - 0,002) |
| p-value | 0,26 | 0,25 | | 0,55 |

The data are displayed in median (interquartile range) format,
† Comparisons between groups before IVF and during ET, Wilcoxon Sign test.
‡ Comparisons between groups in terms of change before IVF, during ET, and during ET compared to before IVF Kruskal Wallis test.

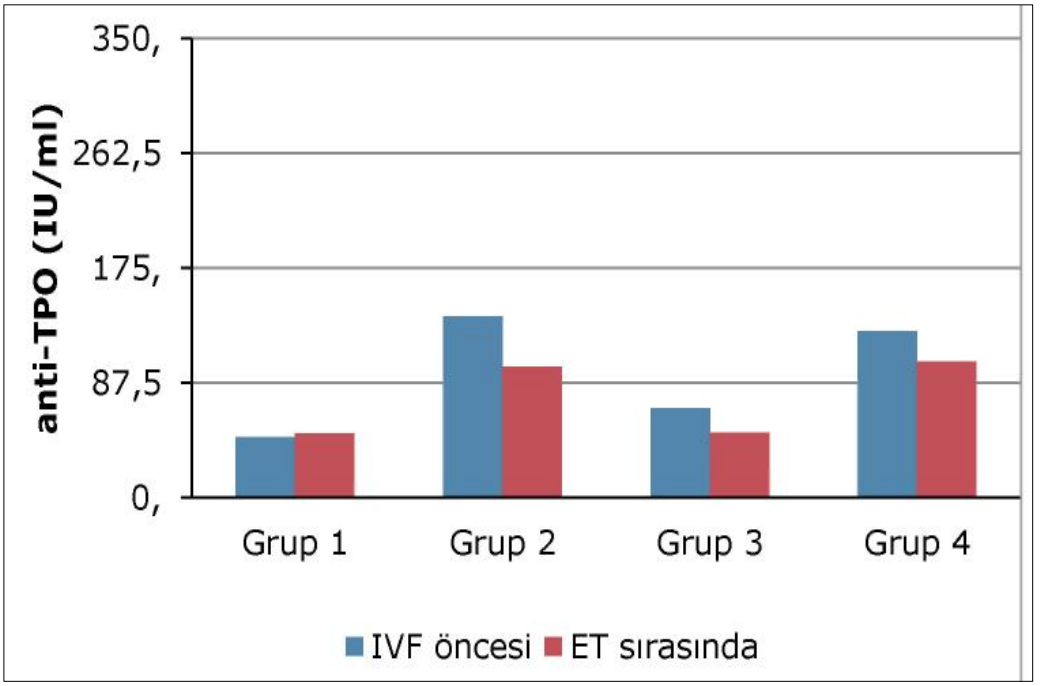


Figure 3.2 Anti-TPO values before IVF and during ET in all cases

There was no statistically significant difference between the anti-TPO measurements at the beginning of IVF and during ET among all groups, that is, the findings were similar (p = 0.26). The highest Anti-TPO values were also observed in the group with thyroid disease and taking thyroid medication (Group 2). If so, the distribution is quite natural, that is, the antibody is high in the

patient cases and low in the control. If we examine the groups investigated in the study individually as before IVF and during ET, no statistically significant difference was found between the median Anti-TPO IVF onset and during ET in group 1 (p=0.09). A statistically significant decrease was observed between the median Anti-T values during ET compared to the beginning of IVF in Group 2 (p=0.02). In group 3 (control group), the decrease in median Anti-TPO values during ET compared to pre-IVF was not statistically significant (p = 0.12). In Group 4, a statistically significant decrease was observed in the median Anti-T level during ET compared to before IVF (p = 0.008) (Figure 4.2).

Since there was no significant decrease in Anti-TPO values in the groups before IVF and during ET as in Anti-T, we can say here that ovulation induction alone does not decrease Anti-TPO values. In summary, it is possible to say that high Anti-TPO can show a significant decrease in the group receiving only levathyroxine with ovulation induction, adding prednisolone to this further increases the significance and lowers high Anti-TPO more effectively.

There was no statistically significant difference between all groups in terms of changes in Anti-TPO values during ET compared to before IVF (p = 0.55). Although the Wilcoxon Test was used because the cases did not have a normal distribution as a total group, the significance of the individual normally distributed groups and the significance of the difference between the two averages for comparison with the mean values (Student t test or Independent t test) analysis was carried out.

Table 3.4 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in all cases

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 239,3±393,9 | 222,1±360.4 | 0,247 |
| Anti-TPO | 255,6±548,2 | 197,0 ±457,1 | 0,085* |

*P >0,05

There was no statistical significance in the changes in Anti-T values before IVF and during ET in all cases, that is, the changes were similar in all cases (P=0.247) (Table 3.4)

A 22.7% decrease trend/trend was observed in the change in anti-TPO values before IVF and during ET. In other words, in normal distribution, ovulation induction alone is not a factor that significantly reduces Anti-T or Anti-TPO (Table 3.5)

When we performed a one-way analysis of aryance for the relationship between infertility and inability to conceive in all cases and the insignificant decrease in Anti-T and Anti-TPO before and after induction :

Table 3.5 One-way analysis of variance (One Way ANOVA) results for Anti-T and Anti-TPO values before IVF and during ET in all cases

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | | | 0,345 |
| Conceiving+ | 298,0 ± 470,8 | 212,1 ±245,2 | |
| Conceiving - | 233,1 ±416,5 | 227,2 ±407,0 | |

| | | | |
|---|---|---|---|
| Anti-TPO | | | 0,702 |
| Conceiving + | 230,7 ±463,3 | 153,9 ±253,9 | |
| Conceiving - | 263,1 ±563,8 | 219,9 ±530,2 | |
| P | 0,812 | 0,411 | |

The results of Table 3.5 report that the decrease in Anti-T and Anti-TPO is not significant between conception or inability to conceive (Table 3.5). In conclusion, in this study, Anti-T and Anti-TPO do not decrease or increase in a way that determines ovulation induction and conception or not.In the analysis in Table 3.3, after a significant difference was observed in the median values in groups 2, 3 and 4, before IVF and during ET, the significance of the difference between the two averages (Student t test or Independent t test) test was used to measure the significance of the mean difference between the groups.

Table 3.6 Double sample t-test results for Anti-T and Anti-TPO values before IVF and during ET in pregnant patients among all cases (N=49)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 261,0±311,3 | 212,1±245,2 | 0,018 * |
| Anti-TPO | 229,7±480,9 | 153,9±253,9 | 0,048* |

P>0,05

Table 3.7 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in patients who could not conceive among all cases

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 228,6±430,5 | 227,2±407,0 | 0,946 |
| Anti-TPO | 268,5±580,9 | 219,9±530,3 | 0,298 |

P>0,05

If we look at the changes in Anti-T and Anti-TPO values before IVF and during ET in patients who could not get pregnant among all cases, similarity was observed in the values (although there was a 19% decrease in Anti-TPO) and could not reach statistical significance (P=0.946 and P=0.298, respectively). (Table 3.6). In other words, those who cannot significantly lower their Anti-T and Anti-TPO values in patients who have undergone ovulation induction cannot get pregnant. The drop rate is predictive, and the decrease in induction in Anti-T and Anti-TPO values has a significant effect on fertility (Table 3.7 )

Table 3.8 Double sample t-test results for Anti-T and Anti-TPO values before IVF and during ET in Group 1 (prednisolone +)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 282,1±590,9 | 265,5±505,5 | 0,571 |
| Anti-TPO | 167,9 ±436,2 | 159,7±466,9 | 0,289 |

P>0,05

The changes in Anti-T and Anti-TPO values before IVF and during ET in Group 1 were similar and were not statistically significant (P=0.571 and P=0.289, respectively) (Table 3.8)

Table 3.9 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in patients who conceived in Group 1 (N=13)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 261,6 ±183,4 | 181,4±128,9 | 0,120 |
| Anti-TPO | 94,2 ± 97,6 | 80,3± 89,6 | 0,115 |

P>0,05

Changes in Anti-T and Anti-TPO values before IVF and during ET in cases who became pregnant in Group 1 were similar and were not statistically significant (P=0.120 and P=0.115, respectively) (Table 3.9).

Table 3.10 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in cases who could not conceive in Group 1

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 290,5±694,6 | 299,7±593,4 | 0,798 |
| Anti-TPO | 197,9±513,0 | 191,3±550,1 | 0,567 |

P>0,05

Changes in Anti-T and Anti-TPO values before IVF and during ET in cases who could not conceive in Group 1 were similar and were not statistically significant (P=0.789 and P=0.567, respectively) (Table 3.10).

Table 3.11 Double sample t results for Anti-T and Anti-TPO values before IVF and during ET in Group 2 (levothyroxine +)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 251,8±247,0 | 248,3±330,6 | 0,924 0,036* |
| Anti-TPO | 361,9±667,4 | 168,9±205,3 | |

*P<0,05

In group 2, the changes in Anti-T and Anti-TPO values before IVF and during ET in all patients with thyroid disease and using levothyroxine for treatment, only Anti-TPO at the end of ovulation induction decreased significantly as 53.4%, this statistic was observed to be significant. (P = 0.036). There was no such observation in Anti-T values in all cases in Group 2 (P = 0.924).

In all cases included in the study, Anti-TPO decreased by 22.7% at the end of ovulation induction as a trend/trend, while Anti-TPO values decreased significantly by 53.4% in those with thyroid disease and using levothyroxine at the end of ovulation induction. That is, the trend gains significance here (Table 3.12).

Table 3.12 Double sample t-test results for Anti-T and Anti-TPO values before IVF and during ET in cases who conceived in Group 2 (N = 16)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 213,9±269,9 | 192,5±222,0 | 0,590 |
| Anti-TPO | 227,5±398,7 | 123,6±127,1 | 0,169 |

In group 2, there was an insignificant decrease in Anti-T values before IVF and during ET in patients who had conceived (P=0.590). Although a decrease of 45.8% was observed in anti-TPO values, this could not reach statistical significance due to the low number of cases (N=16) (P=0.169). With a large case series, this rate may be significant.

Table 3.13 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in cases who could not conceive in Group 2 (N= 26)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 275,1±234,3 | 282,7±382,7 | 0,891 |
| Anti-TPO | 444,6±785,1 | 196,9±239,4 | 0,083 |

In Group 2, Anti-TPO decreases from 444.6±785.1 SD IU/ml to 196.9±239.4 SD IU/ml in patients who could not conceive. The decrease is insignificant with 55.8%, but it is in a trend/trend (Table 3.13). In summary; Although the anti-TPO values decreased to mean lower values during ET after ovulation induction (123.6 ± 127.1 SD IU/ml vs. 196.9 ± 239.4 SD IU/ml) compared to the patients who could not conceive, the number of cases (N=16) ) is not statistically significant. It does not yet predict conception. Interestingly, in the patients of this group, Anti-TPO at the beginning of induction was twice as high in the group who could not conceive. Despite receiving levothyroxine treatment in this group at the beginning of possible ovulation induction in patients with significantly higher anti-TPO values, when IVF treatment is started with high antibody values, induction cannot reduce it sufficiently and perhaps predicts inability to conceive. None of our patients with an initial anti-TPO value above 663 IU/ml could conceive in this group**.**

Table 3.14 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in Group 3 (control)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 166,6±275,1 | 151,9±232,4 | 0,416 |
| Anti-TPO | 283,6±650,2 | 294,7±700,2 | 0,889 |

Group 3 (control group) shows the effect of ovulation induction on pure Anti-T and Anti-TPO. In this group, the changes in Anti-T and Anti-TPO values before IVF and during ET were similar in all cases and were not statistically significant (P = 0.416 and P = 0.889, respectively) (Table 3.14). Already in the analysis of all our cases in Table 3.15, we have shown that ovulation induction reduces Anti-T and Anti-TPO insignificantly whether they use drugs or not. Group 3 analysis also confirmed this (Table 3.15).

Table 3.15 Double-sample t-test results for Anti-T and Anti-TPO values before IVF and during ET in cases who conceived in Group 3 (control) (N= 12)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 216,9±421,7 | 180,5±333,8 | 0,221 |
| Anti-TPO | 414,3±838,0 | 264,9±459,4 | 0,233 |

Although there was a 16.6% decrease in Anti-T values and 36.2% decrease in Anti-TPO values before IVF and during ET in patients in group 3 (control group), in cases that had conceived (N=12), statistical significance was not found.

Table 3.16 Double sample t-test results for Anti-T and Anti-TPO values before IVF and during ET in cases who could not conceive in Group 3 (control)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 143,4±178,7 | 138,7±174,2 | 0,839 |
| Anti-TPO | 223,3±551,9 | 308,5±795,1 | 0,403 |

In group 3 (control group), the decrease in Anti-T values before IVF and during ET was not statistically significant (P=0.839) (Table 4.16). Anti-TPO values of patients who could not conceive were increased by 27.5% during ET compared to before IVF. In other words, patients in the control group who had an increase in Anti-TPO values could not conceive. However, this increase is not significant (P= 0.403). However, an insignificant decrease was observed in the group able to conceive.

The question of whether the Anti-T or Anti-TPO value, who received ovulation induction would significantly decrease or could decrease at the end of the induction, would predict the fertility of the patients, unfortunately, did not give a result in this case size.

I wonder how is the situation in patients who do not have thyroid disease and have high antibodies and use levothyroxine and prednisolone drugs in combination (Group 4) (Table 3.17).

Table 3.17 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in Group 4 (levothyroxine+prednisolone)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 253,8±284,3 | 205,1±210,7 | 0,037* |
| Anti-TPO | 183,7±175,6 | 164,3±164,4 | 0,015* |

The use of levothyroxine and rednisolone together with ovulation induction in Group 4 caused a statistically significant decrease in both Anti-T and Anti-TPO values before IVF and during ET (P = 0.037 and P = 0.015, respectively).

Table 3.18 Double sample t-test results for Anti-T and Anti-TPO values before IVF and during ET in cases who conceived in Group 4 (N=8)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 420,3±366,1 | 348,8±172,8 | 0,091 |
| Anti-TPO | 177,9±278,5 | 168,0±169,3 | 0,337 |

In Group 4, when only pregnant women were examined, a trend/trend in decreasing Anti-T and values before IVF and during ET was not significant (P= 0.091). A lesser decrease was observed in anti-TPO values, but it was not statistically significant (P = 0.33) (Table 3.18).

Table 3.19 Double sample t test results for Anti-T and Anti-TPO values before IVF and during ET in cases who could not conceive in Group 4 (N=14)

| | Before IVF (X±SD) | During ET (X±SS) | p-value |
|---|---|---|---|
| Anti-T | 158,7±177,8 | 122,9±99,7 | 0,221 |
| Anti-TPO | 187,0±183,6 | 162,1±167,9 | 0,028 |

Considering the cases who could not conceive in Group 4, Anti-TPO values decreased by 13.3% before IVF and during ET (P = 0.028), and Anti-T values were not statistically significant (P = 0.221) (Table 3.19).
There was no statistically significant difference between the groups investigated in the study in terms of β hcg positivity (p = 0.41).
There was a statistically significant difference between the groups in terms of the frequency of biochemical conception (p=0.03), the reason for this difference was that the rate of biochemical conception was lower in group 4 compared to group 1 (p=0.05). ).
There was no statistically significant difference between the groups in terms of both clinical conception and abortion rates (p=0.49 and p=0.51) (Table 3.20).

Table 3.20 Findings found significant in our study

| | Group 1 | Group 2 | Group 3 | Group 4 | all cases |
|---|---|---|---|---|---|
| Anti-T | | | | P=0,037* (253→205) %18,9 fall | P=0,085± (255→197) %22,7 fall |
| Anti-TPO | | P=0,036* (361→168) %53,4 fall | | P=0,015* (183→164) % 10,3 fall | |
| Anti-T ability to conceive (+) | | P=0,018* (261→212) %18,7 fall | | P=0,091± (420→348) % 17,1 fall | |
| Anti-T ability to conceive (+) | | P=0,048* (229→153) %33,1 fall | | | |

| | | | | | |
|---|---|---|---|---|---|
| Ability to conceive (+) (Biochemical) | %16,0 | %6,0 | %4,6 | %0,0 | P=0,03* |
| Ability to conceive (+) (Abortion) | %12,0 | %8,0 | %13,8 | %20,0 | P=0,51 |

*P<0.05 (statistically significant)
± P>0.05 (trend/trend was observed at the statistical significance limit)

**4. Discussion and Conclusion**

Seminal ASA levels were found not only in infertile cases, but also in fertile men and women. These findings suggest that even fertile men have low concentrations of ASA. These findings were the results of detailed and systematic meta-analyses that showed that seminal ASAs do not directly inhibit pregnancy rates through IVF or ICSI. These assisted reproductive techniques are thought to be a viable option for infertile couples. However, more complex studies are warranted to address issues such as the determination of ASA threshold levels, among others. Circulating ASAs have been proven to play a partial role in the likelihood of miscarriage during pregnancy. Antibodies that react with human sperm can be produced in both men and women. Hypothyroidism can affect fertility in men and women, and it is also important for a pregnant woman to have problems with the thyroid gland in the early stages of pregnancy, as it can cause miscarriage.

— There was no significant effect of the drug on Anti-T and Anti-TPO in patients in group 1 who received only prednisolone and had ovulation induction. In this group, the rate of being able to conceive biochemically was 16% higher (compared to the control group), abortion rates were not different from the 6% control group.
— In Group 2, the patients were diagnosed with thyroid disease and were taking levothyroxine for a long time, and they continued the drug during ovulation induction. In group 2, long-term use of levothyroxine decreased significantly in Anti-T and Anti-TPO values in patients who were able to conceive (P=0.018 and P=0.036, respectively). In this group, patients who were able to conceive biochemically were 6% and abortion rates were 8%. Compared to the control group, no significant difference was observed in the rates of being able to conceive and abortion biochemically (P=0.02).
— In group 3 (control group), patients did not receive any medication other than ovulation induction. There was no significant decrease in Anti-T and Anti-TPO values in this group.
—A significant decrease was observed in Anti-T and Anti-TPO values in Group 4 (P = 0.037 and P = 0.015, respectively). The combined use of both drugs significantly decreased the rate of biochemical pregnancy in this group (0.0%). There was no significant difference between 20% in the abortion group and the control group.
— Giving Prednisolone 16 mg throughout induction, which we evaluated group 1 and group 3 critically compared to group 2, does not affect Anti-T and Anti-TPO as if it had never been given. Although prednisolone is used for fourteen days (average 24 days in total), it has no effect on the rate of conception. Abortion was insignificantly increased in group 1-3 with biochemical infertility (p = 0.06).

With this finding, when infertile patients who do not have a clinical diagnosis of hypothyroidism and only have high Anti-T or Anti-TPO use only levothyroxine or prednisolone in the IVF program, the drugs are ineffective on Anti-T and Anti-TPO blood levels during induction. However, in those diagnosed with clinical hypothyroidism and using levathyroxine for a long time, Anti-TPO decreases significantly with ovulation induction (from 361.9±667.4 SD to 168.9±205.3 SD) and Anti-T and Anti-TPO (from 261.0±311.3 SD to 212.1±245.2 SD) and (229.7±480.9 SD to 153.9±253.9 SD) decreased significantly, predicting possible previous pregnancy.
— When we evaluate group 1 and group 4 compared to group 2, although taking or not taking Prednisolone does not affect Anti-T and Anti-TPO on ET day, taking prednisolone and levothyroxine significantly reduces Anti-T and Anti-TPO on ET day. In other words, while Anti-TPO decreased 53.4% in the group that received levothyroxine alone (from 361.9±667.4 SS to 168.9±205.3 SD), adding prednisolone to the treatment also reduces Anti-T. (18.9% decrease in Anti-T, from 253.8±284.3 SD to 205.1±210.7 SD and 10.3% decrease in Anti-TPO, 183.7±175, 6 SD to 164.3±164.4 SD).

In a retrospective study on infertile patients with positive thyroid autoantibodies and euthyroid patients undergoing IVF cycles, a group of patients with positive thyroid autoantibodies (ATA+) did not receive treatment (Group A N=52 ATA+), and in another group patients who received only levothyroxine (Group B N). =56 ATA+) and other group patients used levothyroxine (LT), prednisolone (P) and aspirin (ASA) (Group C N=44 ATA+). In the study, fertilization, implantation and pregnancy outcomes of treated patients were compared with the results of untreated thyroid autoantibodies positive (ATA+) and control group thyroid autoantibodies negative (ATA-) patients (group D N=200). Pregnancy and implantation rates were significantly higher in ATA+ patients who received LT+ASA+P compared to untreated ATA+ patients (GO/ET 25.6% and IO 17.7% P<0.01 versus GO/ET 7.5% and IO 4.7% (P<0.01). As another result of this study, when the IVF results of all patients were examined, it was found that the pregnancy and implantation results were higher in patients with ATA - (control group) (GO/ET 32.8% and IO 19%) (P<0.01). In the study, abortion rates were found to be higher in ATA+ patients compared to ATA- patients (25-28.5% vs. 12%) (P<0.01)( Revelli et al. 2009).

The results of conception in our study were compared with the results of untreated ATA+ patients (although in the above study, the two control groups ATA+ and ATA- patient groups were compared with the conceiving results). In our study, the abortion rate of patients who received adjuvant treatment in the form of prednisolone, levothyroxine or a combination of levothyroxine and prednisolone compared to the control group was observed to be the highest in group 4 (prednisolone and levothyroxine) with 20%, although there was no statistical significance in the comparison of all groups (P=0, 51).

Yi-ping Zhong et al. In a study comparing IVF and conception results of 90 infertile patients with positive thyroid autoantibodies (ATA+) with IVF and conceiving results of 676 ATA- (control group) infertile patients, no difference was found in the number of cells collected from both patient groups (10.9±6.1). versus 11.8±6.9) (P=0.166). Fertilization rates (64.3% vs. 74.6% P<0.001), implantation

rates (17.8% vs. 27.1% P<0.001) and infertility rates (33.3% vs. 46.7% P=0.002) and abortion rates (26.9% vs. 11.8% P=0.002) found favorable results for ATA-patients in the control group. The small number of patients (compared to the control group) in the study was considered a limitation in terms of the strength of the study (Zhong et al. 2012).

According to the design of our study, ATA+ patients who did not take any medication were included in our control group. Therefore, although we did not have the opportunity to compare the rates of conceiving and abortion in our study with the results of the ATA-patient group, when we compared the patient groups in our study with the control group, there was no significant difference in the rate of being able to get pregnant (P=0.49) clinically. Compared to the control group in our study, we noticed that the abortion rates were higher in group 4 (13.8% vs. 20%).
Roberto Negro et al. prospective study investigating the effect of levothyroxine (LT4) treatment on the pregnancy outcomes of anti-TPO positive euthyroid infertile patients 412 Anti-TPO negative control group (group C) versus 72 Anti-TPO (positive) patients (group A- 36 patients given LT4 and group B- 36 patients given placebo). Group A patients in the study used LT4 for one month before and after IVF.

In another study, Busnelli et al. discussed the effect of hypothyroidism treatment on IVF results. The study was designed as a comparison of 137 hypothyroid patients versus 274 euthyroid patients (control group) and IVF results. According to the results of the study, no significant difference was observed in clinical conceiving rates (28% vs. 22%, respectively) (p = 0.11). However, fertilization rates were found to be statistically significant, being 75% in the patient group (lower) and 86% in the control group (P=0.017) (Busnelli et al.2013).

Similar to both studies, in our study, patients with clinical hypothyroidism in group 2 and patients with euthyroidism in group 4 used prednisolone and levothyroxine as adjuvant therapy. When we compared the conceiving results of both groups with the control group, the abortion rates in euthyroid patients in group 4 compared to the control group Negro et al.

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