Hashimoto's thyroiditis negatively influences IVF outcome in euthyroid women on T4 substitution therapy; a retrospective study

Tünde Herman (herman.tunde@med.unideb.hu)
University of Debrecen
Péter Török
University of Debrecen
Antonio Simone Laganà
ARNAS "Civico – Di Cristina – Benfratelli", University of Palermo
Vito Chiantera³
ARNAS "Civico – Di Cristina – Benfratelli", University of Palermo
Attila Jakab
University of Debrecen

Case Report

Keywords: infertility, Hashimoto thyroiditis, TPOAb, in vitro fertilization, fertilization rate

Posted Date: August 8th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3227758/v1

Abstract

Purpose: We retrospectively analyzed how the presence of thyroid autoimmunity (TAI positive) affected reproductive parameters in eighty-six (86) TAI positive women referred to IVF treatment.

Methods: All participants diagnosed with subclinical or overt hypothyroidism, while euthyroid on thyroxine replacement. Sixty-nine (69) female patients in the same IVF program, with no thyroid abnormalities served as controls (TAI negative group).

Results: Statistically significant baseline hormone profile differences were found in multiple parameters between the two groups. TAI positive women were older (mean age 34.7±5.42 vs. 32.32±5.04 years; p=0.002), had higher FSH (8.4±3.49 U/L vs. 7.04±2.32 U/L; p=0.024), higher E2 (53.94±47.61 vs. 42.93±18.92 pg/ml; p=0.025) levels, while their AMH (2.88±2.62 vs. 3.6±1.69 ng/ml; p=0.0002) was lower. There were no differences in TSH levels (1.64±0.96 vs. 1.66±0.65 uIU/ml; p= 0.652) between the two groups, FT3 (2.63±0.58 vs. 2.98±0.55 pg/ml; p=0.002) was lower and FT4 (1.304±0.29 vs. 1.13±0.21 ng/dl; p=0.0002) was higher in the TAI positive group.

Egg cell counts (6.0±3.82 vs. 7.5±3.95; p=0.015), clinical pregnancy rate (36.04 % vs. 69.56 %; p<0.001), live birth rate (23.25 % vs. 60.86 %) were lower, the miscarriage rate (35.48 % vs. 12.5 %; p=0.024) was higher in the TAI positive group. There was no difference in ICSI fertilization rate (69.12 % vs 62.91 %, p=0,12), although the fertilization rate was lower for patients under 35 in the TAI positive group, after adjusting for age.

Conclusion: TAI positive women carry a multitude of endocrine and metabolic parameters which may contribute to unfavorable effects on reproductive health.

Introduction

The prevalence of infertility has significantly increased worldwide over the past decades. To date, it is estimated that 8–12% of couples of reproductive age globally are infertile. Effects of lifestyle changes, dietary habits, environmental factors, postponement of childbearing and endocrine abnormalities may result in subsequent reproductive difficulties [1]. The incidence of autoimmune thyroid disease is significantly higher in infertile women [2]. According to a recent survey endocrinopathies occur with a high prevalence among women in IVF treatment programs. While the most common endocrinopathy was polycystic ovary syndrome (PCOS) until recently, thyroid disorders are now the most prevalent among females of reproductive age requiring in vitro fertilization. Nearly one third (32,5%) of the patients participating in IVF treatment suffer from thyroid disease. Hashimoto's thyroiditis is the most common with a rate of 68% in patients with thyroid abnormalities. Autoimmune thyroiditis is often associated with other endocrinological abnormalities: Diminished ovarian reserve (DOR) in 25,45%, PCOS in 20.0%, hyperprolactinaemia in 15,38% [3]. Of these, Grave's disease affects 1% of the population, while Hashimoto's thyroiditis prevalence is most common. These disorders adversely impact reproductive health, conception as well as pregnancy outcomes [4].

Thyroid hormone receptors are found in ovarian tissue as well. Together with follicular stimulating hormone (FSH), thyroid hormones play an important role in the proliferation of granulosa cells. Folliculogenesis is not only impaired at higher, but especially at lower thyroid hormone levels [5] as well. Triiodothyronine (T3) plays an important role in the biosynthesis of FSH and luteinizing hormone (LH). T3 receptors have been identified on the surface of human oocytes [6]. It is presumed that TPO is expressed in granulosa cells making them prone to the potential damage caused by anti-thyroid peroxidase antibodies (TPOAb), which may result in consequent decrease of anti-Müllerian hormone (AMH) production. TPOAb in serum also appears in the follicular fluid and is, assumed to be cytotoxic, TPOAb in high quantities bind to the oocytes surface, which is associated with poor oocyte quality, resulting in a decreased chance of sperm penetrating the oocyte. Thyroid hormones have also been shown to play a role in the production of progesterone by granulosa cells. The autoimmune process affecting the thyroid gland has an adverse effect on embryogenesis by causing subclinical or manifest hypothyroidism [7].

Several authors have investigated the negative effects of anti-thyroid antibodies on reproduction. It was assumed that certain fertility indicators, such as AMH levels, number of retrieved eggs (NOR), fertilization rates (FR), implantation rates, and pregnancy rates (PR) of infertile women with autoimmune thyroid disease are worse compared to healthy patients. Stagnaro-Green and colleagues were the first to demonstrate a strong positive association between the presence of anti-thyroid antibodies and the incidence of miscarriage [8]. Busnelli and colleagues reported in their meta-analysis that although thyroid autoimmunity does not appear to affect the outcome of IVF/ICSI, number of retrieved oocytes, fertilization, implantation, or clinical pregnancy rate, it may have a detrimental effect on the course of pregnancy [9]. A more recent meta-analysis by Venables et al of 14 studies on a comparable population, however, found no association between the pregnancy outcome in TAI positive euthyroid women compared to pregnancies of TAI negative women following in vitro fertilization (IVF) [10].

There is no significant evidence, however, on thyroid hormones affecting endometrial receptivity. Similarly, no clinical evidence has confirmed an interaction between endometrial cells and the TPOAb. Presumably, high TPOAb titers might alter or destroy endometrial cells, causing chronic endometritis, implantation abnormalities, and recurrent miscarriages [11].

In light of the above, we hypothesize that TAI positive women diagnosed with subclinical or overt hypothyroidism, but are euthyroid due to thyroid hormone substitution, may have poorer fertility indicators compared to TAI negative euthyroid women in an IVF program.

This study aims to evaluate the reproductive outcomes of TAI positive infertile women in an IVF program.

Materials and Methods

The research was approved by the Regional and Institutional Research Ethics Committee of the Clinical Center of the University of Debrecen (DE RKEB/IKEB 5684 – 2021). The design, analysis, interpretation of data, drafting, and revisions followed the Helsinki Declaration and the strengthening the reporting of observational studies in epidemiology (STROBE) statement, available through the enhancement of the quality and transparency of health research (EQUATOR) network (www.equator-network.org). Each patient enrolled in this study signed an informed consent form for all procedures and the collection and analysis of biological samples for research purposes. No remuneration was offered to enter or continue the study.

We retrospectively analyzed the data of eighty-six (86) TAI positive consecutive women presenting with either subclinical or overt hypothyroidism who received in vitro fertilization treatment, but euthyroid at the time of the investigation. Sixty-nine (69) TAI negative female patients in the same IVF program, with no thyroid abnormalities served as controls.

The following endocrine tests were performed between days 1–3 for included patients: follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), thyroid- stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), anti-thyroid peroxidase antibodies (TPOAb), antithyroglobulin antibodies (TGAb), total testosterone (TT), fasting glucose and insulin, and depending on the Homa-index an oral glucose tolerance test. AMH test was performed to determine the ovarian reserve capacity and to assess their responsiveness to stimulation. If the testosterone level was high or clinical hyperandrogenism was detected, dehydroepiandrosterone- sulfate (DHEAS), androstenedione, 17- hydroxyprogesterone (17-OHP) and sexual hormone binding globulin (SHBG) were also measured.

Following menstruation, a thorough ultrasound examination of the minor pelvis, antral follicular count (AFC) measurements and detailed sonographic assessment of the ovarian morphology were carried out to identify potential DOR (diminished ovarian reserve) or establish PCO (polycystic ovarian) morphology.

Patients were included based on TPOAb levels. The diagnosis of thyroid autoimmunity was based on elevated levels of TPOAb or TGAb according to the local laboratory reference values (TPOAb > 16 IU/ ml, TGAb > 60 IU/ml). Thyroid ultrasound and TSH receptor antibody measurement was used to exclude patients with Graves' or other thyroid diseases.

Subclinical hypothyroidism and overt hypothyroidism were treated according to current clinical guidelines. TSH levels were kept below 2.5 uIU/L and adjusted as required according to the European Society of Human Reproduction and Embryology (ESHRE) Guidelines [12] and European Thyroid Association ETA guidelines [24].

In the group of patients with thyroid dysfunction, we aimed for appropriate thyroxine hormone replacement and maintained TSH values within the normal range. Stimulation treatment was initiated if TSH was below 2.5 mU/L.

Prior to IVF treatments all endocrinological parameters of the patients were first assessed. IVF treatments were performed after the necessary endocrinological therapeutic correction was completed. IVF treatments were performed in all women using the standard treatment protocol at our center, irrespective of TPOAb levels. Stimulation was performed at a follicle size of at least 13 mm, using recombinant FSH according to the antagonist protocol with cetrorelix acetate supplementation. At a follicle size of 18 mm, 2,500 IU choriogonadotropin alfa was administered, which was followed by oocyte aspiration 36 hours later.

The oocytes were fertilized using intracytoplasmic sperm injection (ICSI).

One or two embryos (depending on patient's choice) were transferred into the uterine cavity on days 3 or 5. All women received vaginal, oral and subcutaneous progesterone replacement therapy. Low molecular weight heparin (LMWH) and aspirin were administered starting from the date of embryo transfer. Human choriogonadotropin hormone (HCG) levels were checked 12–14 days after embryo transfer. Pregnancy was defined as an hCG level exceeding 50 mIU/ml.

Patients' age, BMI, AMH, FSH, LH, E2, PRL, TSH, FT3, FT4, testosterone, DHEAS, 17-OHP and androstenedione levels.

The number of IVF cycles to achieve pregnancy, the number of retrieved oocytes, fertilization rate, clinical pregnancy rate, miscarriage rate, live birth rate were all analyzed and compared to those of TAI negative controls.

Statistical methods

Descriptive statistics were used. Variables were described using absolute and relative frequencies for categorical variables, and arithmetic means and standard deviations (SD) for continuous variables. Comparisons between categorical variables between the two cohorts were carried out using Fisher's exact tests. For continuous variables, comparisons were based on Student's two-sample t test (if distributional assumptions were satisfied) or Wilcoxon's rank-sum test (otherwise). The significance criterion was set at $\alpha \leq 0{,}05$. Data handling and analysis was performed using the Stata statistical package (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Age-adjusted comparisons of TAI positive and negative subjects in terms of pregnancy (binary categorical) and cycle count (three-level categorical: 1, 2, ≥ 3) were performed using logistic and ordered logistic multiple regression, respectively. Ordinary least-squares multiple linear regression was used to analyze the continuous outcomes of fertilization rate and oocyte count in the same relation. Additional explanatory terms included age squared or an interaction term between age and the TAI group identifier if such additions substantially improved model fit. Estimates were expressed as odds ratios (categorical outcomes) or additive differences (continuous outcomes) in TAI positives versus negatives, with 95% confidence intervals and p-values, either as a single estimate or a series of age-specific estimates.

Results

We compared the baseline characteristics and reproductive parameters of eighty-six (86) TAI positive subjects with those of sixty-nine (69) TAI negative IVF program participants. Baseline characteristics of both cohorts are shown in (Table 1.)

Table 1

Baseline characteristics of the patients * For statistical analysis, each case group (TAI positive) was compared with controls (TAI negative).

	TAI negative Controls	**TAI positive**	**p**
n	69	86	
Age	32.15 ± 4.87	35.31 ± 4.95	p = 0.0002
BMI	22.91 ± 3.33	23.88 ± 3.32	p = 0.0381
AMH (ng/ml)	3.6 ± 1.69	2.88 ± 2.62	p = 0.0002
baseline FSH(U/L)	7.04 ± 2.32	8.4 ± 3.49	p = 0.024
baseline LH(U/L)	6.34 ± 2.53	7.2 ± 3.34	p = 0.123
baseline E2(pg/ml)	42.93 ± 18.92	53.94 ± 47.61	p = 0.025
prolactin(ng/ml)	14.19 ± 4.96	15.11 ± 7.75	p = 0.634
sTSH (uIU/ml)	1.66 ± 0.65	1.64 ± 0.96	p = 0.652
FT3 (pg/ml)	2.98 ± 0.55	2.63 ± 0.58	p = 0.002
FT4 (ng/dl)	1.13 ± 0.21	1.304 ± 0.29	p = 0.0002
TPOAb (IU/ml)	11.25 ± 11.79	386.35 ± 488.97	p < 0.0001
TGAb (IU/ml)	8.28 ± 8.69	101.67 ± 169.56	p < 0.0001
TT (nmol/l)	1.05 ± 0.49	1.45 ± 2.8	p = 0.626
DHEAS (umol/L)	6.61 ± 3.88	5.78 ± 2.59	p = 0.632
17-OHP (nmol/L)	1.85 ± 1.08	1.13 ± 0.87	p = 0.008
androstenedione (ug/L)	1.84 ± 0.802	1.68 ± 0.96	p = 0.627
SHBG (nmol/L)	67.23 ± 45.59	59.88 ± 26.66	p = 0.883
baseline glucose (mmol/L)	4.78 ± 0.402	5.16 ± 0.58	p < 0.0001
baseline insulin (mIU/L)	7.23 ± 2.39	9.28 ± 4.71	p = 0.001

Values represent arithmetic means. P values pertain to comparisons between patients defined in column headers and patients with and without TAI

TAI autoimmune thyroiditis, BMI body mass index, AMH anti-Müllerian hormone, FSH follicle stimulating hormone, LH luteinizing hormone, E2 estradiol, TSH thyroid stimulating hormone, FT3 free triiodothyronine, FT4 free thyroxine, TPOAb anti-thyroid peroxidase antibodies, TGAb antithyroglobulin antibodies, TT total testosterone, DHEAS dehydroepiandrosterone sulfate, 17-OHP 17-hydroxyprogesterone, SHBG sex hormone binding globulin, min minute

	TAI negative Controls	TAI positive	p
60-min. glucose(mmol/L)	6.85 ± 1.18	6.7 ± 1.7	p = 0.61
60-min. insulin(mIU/L)	52.5 ± 5.37	58.24 ± 31.77	p = 0.86
120-min. glucose(mmol/L)	5.08 ± 1.54	5.7 ± 1.36	p = 0.028
120-min. insulin(mIU/L)	32.41 ± 13.76	47.96 ± 33.84	p = 0.118
HOMA index	1.55 ± 0.55	2.61 ± 3.11	p = 0.0004
Vitamin D (ng/ml)	76.53 ± 33.73	68.84 ± 34.43	p = 0.36
antisperm antibody(U/ml)	27.24 ± 24.13	28.68 ± 36.72	p = 0.58

Values represent arithmetic means. P values pertain to comparisons between patients defined in column headers and patients with and without TAI

TAI autoimmune thyroiditis, BMI body mass index, AMH anti-Müllerian hormone, FSH follicle stimulating hormone, LH luteinizing hormone, E2 estradiol, TSH thyroid stimulating hormone, FT3 free triiodothyronine, FT4 free thyroxine, TPOAb anti-thyroid peroxidase antibodies, TGAb antithyroglobulin antibodies, TT total testosterone, DHEAS dehydroepiandrosterone sulfate, 17-OHP 17-hydroxyprogesterone, SHBG sex hormone binding globulin, min minute

Initially, the entire TAI positive group was compared with the TAI negative control subjects.

TAI negative and TAI positive patients’ IVF outcomes are shown in (Table 2.)

Table 2
TAI negative and TAI positive patients' IVF outcomes

	TAI negative	TAI positive	p	age-adjusted
n	69	86		
number of cycles: 1, 2, ≥3	33 (48%) 29 (42%) 7 (10%)	42 (49%) 27 (31%) 17 (20%)	p = 0.182	p = 0.951
egg cell count	7.5 ± 3.95	6.0 ± 3.82	p = 0.015	p = 0.194
FR unadjusted fertilization rate	69.1%	62.9%	p = 0.123	
FR model-predicted fertilization rate at age 25 at age 27.5 at age 30 at age 32.5 at age 35 at age 37.5 at age 40 at age 42.5	72% 71% 70% 69% 68% 67% 66% 65%	50% 53% 56% 59% 63% 66% 69% 72%		p = 0.006 p = 0.005 p = 0.006 p = 0.023 p = 0.210 p = 0.820 p = 0.640 p = 0.380
CPR clinical pregnancy rate	48/69 69.6%	31/86 36.0%	p < 0.001	p < 0.001
MR miscarriage rate	6/48 12.5%	11/31 35.5%	p = 0.024	p = 0.089
LBR Live birth rate	42/69 60.9%	20/86 23.3%	p < 0.001	p < 0.001
Values represent counts, percentages, or arithmetic means ± standard deviations.				
Age-adjusted comparisons of TAI positive and negative subjects				

Comparison of TAI positive (n:86) and TAI negative (n:69) patients

Mean age (35.31 ± 4.95 vs. 32.15 ± 4.87 years; p = 0.0002), *BMI* (23.88 ± 3.32 vs. 22.91 ± 3.33 kg/m²; p = 0.038), start-of-cycle *FSH* level (8.4 ± 3.49 vs. 7.04 ± 2.32 U/l; p = 0.024), start-of-cycle *E2* level (53.94 ± 47.61 vs. 42.93 ± 18.92 pg/ml; p = 0.025) were all significantly higher in the TAI positive vs. the TAI negative groups, respectively.

TAI positive patients had significantly lower *AMH* levels (2.88 ± 2.62 vs. 3.6 ± 1.69 ng/ml; p = 0.0002) as well as *17-OHP* (1.13 ± 0.87 vs. 1.85 ± 1.08 nmol/l; p = 0.008) compared to the TAI negative group. *TPOAb* levels (386.35 ± 488.97 vs. 11.25 ± 11.79 IU/ml; p < 0.0001) and *TGAb* levels (101.67 ± 169.56 vs. 8.28 ± 8.69 IU/ml; p < 0.0001) were statistically significantly higher in the TAI positive study treatment arm.

No significant differences were observed in *start-of-cycle LH* (7.2 ± 3.34 vs. 6.34 ± 2.53 U/L; p:0.123*) and prolactin* (15.11 ± 7.75 vs. 14.19 ± 4.96; p: 0.634) levels between TAI negative and positive groups. Further, there was no significant difference in the *TSH* level (1.64 ± 0.96 vs. 1.66 ± 0.65 uUI/ml; p = 0.652) between the two groups, however, *FT4* (1.304 ± 0.29 vs. 1.13 ± 0.21 ng/dl; p = 0.0002) was significantly higher and *FT3* (2.63 ± 0.58 vs. 2.98 ± 0.55 pg/ml; p = 0.002) was significantly lower in TAI positive treatment cohort.

Carbohydrate metabolism parameters markedly differed between the two cohorts as well. *Baseline glucose* (5.16 ± 0.58 vs. 4.78 ± 0.402 mmol/l; p < 0.0001), *baseline insulin* (9.28 ± 4.71 vs. 7.23 ± 2.39 mIU/l; p = 0.001), *120-minute glucose* (5.7 ± 1.36 vs. 5.08 ± 1.54 mmol/l; p = 0.028), *HOMA- index* (2.61 ± 3.11 vs. 1.55 ± 0.55; p = 0.0004) were all statistically significantly higher in the TAI positive group.

No significant differences were observed in *60-min. glucose* (6.7 ± 1.7 vs. 6.85 ± 1.18 mmol/L; p = 0.61), *60-min. insulin* (58.24 ± 31.77 vs. 52.5 ± 5.37 mIU/L; p = 0.86), *120-min. insulin* (47.96 ± 33.84 vs. 32.41 ± 13.76 mIU/L; p = 0.118) between two groups. There were also no differences in *vitamin D* levels (68.84 ± 34.43 vs. 76.53 ± 33.73 ng/ml; p = 0.36) or *antisperm antibody* titers (28.68 ± 36.72 vs. 27.24 ± 24.13 U/ml; p = 0.58) between the treatment and control cohorts.

We observed no significant differences in the number of successful IVF cycles between the two cohorts.

The *number of oocytes* (6.0 ± 3.82 vs. 7.5 ± 3.95; p = 0.015) retrieved was significantly lower in the TAI positive group, however this difference did not persist after adjusting for age.

The *fertilization rate (FR)* was similar between the two groups *(62.91 vs. 69.12%; p = 0.12)*, but was significantly higher in the age-corrected analysis of TAI negative subjects younger than 35 at each cut-off point. Fertilization rates at age 25 (p = 0.0061), age 27.5 (p = 0.0052), age 30 (p = 0.0064) and age 32.5 (p = 0.023) were significantly lower in TAI positive compared to TAI negative group. No statistically significant differences, however, were found in subjects aged 35 years (p = 0.21), 37,5 years (p = 0.82), 40 years (p = 0.21), or 42.5 years (p = 0.21) between the TAI positive and negative treatment groups.

The *clinical pregnancy rate (CPR)* (36.04 vs. 69.56%; $p < 0.001$) was significantly higher in the TAI negative group, and this difference ($p < 0.001$) was consistently observed in age-corrected rates as well.

The *miscarriage rate (MR)* was significantly higher (35.48 vs. 12.5%; $p = 0.024$) and the *live birth rate (LBR)* was significantly lower (23.25% vs. 60.86%; $p < 0.001$) in the TAI positive treatment group. After adjusting for age, the *MR* ($p = 0.089$) did not differ significantly between the treatment groups, but the *LBR* ($p < 0.001$) remained significantly lower in the TAI positive cohort.

Discussion

The prevalence of autoimmune diseases is increasing. Following its onset, the disease itself progresses with higher antibody titers and a gradual increase in thyroxin demand can be observed with advancing age. Aging itself has a negative impact on reproductive health [13, 14]. Women over 40 making up 25% of the patients at IVF centers [2]. Changes in physical condition and genetic effects can be expected with advancing age, which may manifest as a decrease in ovarian reserve and abnormal oocyte meiotic division. The incidence of both endocrinological disorders (TAI) and anatomical changes (myomas) also increases-with advancing age [15].

In our study, the mean age of TAI positive patients (average: 35,31 years) was higher compared to the TAI negative (average: 32,15 years) controls.

We aimed to optimize BMI, thyroid function, carbohydrate metabolism, prolactin, androgen hormones and vitamin D level as much as possible prior to IVF treatment for all patients.

Significant differences could be observed in BMI between the TAI positive and the TAI negative groups. Based on our previous study 54% of polycystic ovary syndrome (PCOS) patients were overweight or obese [2]. PCOS is known to be common among women of reproductive age, and its incidence may be associated with autoimmune thyroid disease [16–19]. This may explain significantly higher BMI values in our TAI positive patients.

The association between TAI positivity and ovarian reserve capacity is not clear. Several authors hypothesize that there is a close relationship between the level of antithyroid antibodies and DOR; based on the same principle, auto-antibodies may destroy the granulosa cells of the ovaries in a similar fashion as they do the thyrocytes. However, no clear relationship was found for low, normal and high ovarian reserve in TAI positive patients [20–23]. Based on the current study, it may be concluded that AMH, a value referring to ovarian reserve capacity, is significantly lower in TAI positive women. The other parameter representing ovarian reserve capacity, FSH, was significantly higher in the TAI positive group compared to the TAI negative control group. Further evidence of decreased ovarian reserve as indicated by AMH and FSH was that significantly fewer oocytes were obtained during follicular aspiration. This result is different from previous investigation [14] in which the authors did not report an association between TPOAb levels and decreased ovarian reserve.

No significant differences in TSH were found among the individual patient groups, as thyroid hormone replacement was adjusted for all patients prior to IVF to maintain TSH below 2.5 uIU/L. This is probably because free T4 levels were significantly higher in the TAI positive group compared with negative controls. For the same reason, their T3 levels were significantly lower in the TAI positive group. According to the European Thyroid Association's most recent recommendation for thyroid hormone suppression in TAI positive patients is that TSH levels should remain below 2.5 uIU/ml, before IVF treatment is initiated. Further, thyroid hormone treatment of TAI negative euthyreoid infertile women is not recommended before IVF treatment [24].

Carbohydrate metabolism parameters, including both baseline blood glucose and insulin levels as well as the 120- minute glucose and the HOMA- index were all statistically significantly higher in the TAI positive group. Although we could not detect differences in TT, DHEAS and androstenedione levels in the patients of the TAI positive and the TAI negative groups, TAI positive patients had significantly lower 17-OHP levels, which suggests autoimmune involvement of the adrenal cortex.

Autoimmune processes exert their effects through to the pathologic activity of the immune system. There are several types of antibodies that can be detected in cases of recurrent implantation failure. We assessed the alloantibodies against sperm, but there was no significant difference between TAI negative and TAI positive group [25].

We did not rely on conventional fertilization at our center as all fertilization were performed using ICSI to circumvent potential challenges of sperm penetration of the zona pellucida. No significant differences in fertility rates were found between the TAI negative and the TAI positive patient groups using the ICSI technique. During age-corrected analyzes we found that the fertilization rate were significantly higher in TAI negative compared to TAI positive subjects ($p = 0.0236$) younger than 35 years, whereas no significant difference was noted in patients older than 35 years. Hashimoto's thyroiditis negatively impacts fertilization rates in younger women but this difference was no longer noticeable in women over 35 in our series as the negative overall effects of age prevail. Despite the lack of published clinical evidence, our present study supports the European Thyroid Association's latest recommendation for performing ICSI for IVF fertilization using ICSI in TAI positive patients. [24].

There were clear categorically significant differences in pregnancy rate between the two groups in this study. The pregnancy rate was 69.56% in TAI negative group, as compared to only 36.04% ($p < 0.001$). This difference persisted following, age-corrected analyzes as well. We believe that a reduced ovarian reserve capacity has an important background etiological causative component. Decreased oocyte quality, caused by the Monteleone effect, consecutive lower embryo quality and decreased endometrial receptivity are identified as contributing factors.

The miscarriage rate was significantly higher in the TAI positive group than the TAI negative group, finding which corroborates earlier studies with similar outcomes [26, 27]. The difference in the miscarriage rate did not maintain significance after adjusting for age.

We suppose that chronic autoimmune endometritis may be a possible culprit. Whether TPOAb itself damages the endometrium, or there are coincidental specific autoimmune processes towards endometrial cells remains to be elucidated. For this reason further studies are needed to ascertain whether chronic endometritis would be verified by endometrial biopsy [28].

Unlike the miscarriage rate did not maintain statistical significance following adjusting for the age of the patient, the live birth rate remained significantly lower in TAI positive patients (23.3% vs. 60.9%, $p < 0.001$).

Conclusion

We conclude based on our results the antithyroid antibody titer has a major adverse impact on reproductive health. TAI positive patients were diagnosed with overt hypothyroidism and undergoing treatment with thyroxine hormone replacement therapy and are euthyroid have a lower reproductive parameters. TAI positive patients have lower ovarian reserve resulting in a significantly lower number of retrieved oocytes during IVF. No differences in fertilization rates were observed between two groups, confirming that oocytes of TAI positive patients should be fertilized using ICSI technique. After adjusting for age, we conclude that Hashimoto's thyroiditis negatively impacts fertilization rates in women younger than 35. A lower clinical pregnancy rate, live birth rate and higher miscarriage rates can be expected among TAI positive patients.

Causative etiologies for decreased reproductive outcomes require further evaluation. Advancing age, anatomic and endocrinological disorders, decreased number and quality of oocytes, poor quality of embryos as well as impaired endometrial receptivity are all conditions that could effect reproductive characteristics, as well.

Normal thyroid function is essential for the success of assisted reproductive techniques (ART). Since thyroid autoimmunity fundamentally affects the outcome of ART, it is recommended that measuring autoantibody titers (TPOAb) should be added to laboratory tests to determine thyroid function and screen for autoimmunity in infertile women. These procedures are espirecially recommended in cases of infertile patients having multiple miscarriages or multiple unsuccessful IVF programs (RIF-recurrent implantation failure) in their history. Autoimmune thyroid disease was previously only considered as a partial indication for in vitro fertilization programs. Based on our results, IVF treatment is recommended for patients with TAI, taking into account older patients as well as those with diminished or aberrant reproductive indicators.

Declarations

Acknowledgements

Special thanks to our colleagues at the Assisted Reproduction Centre, Béla Bodnár and Zoltán Tándor for completing this clinical research. The authors wish to acknowledge László Kardos and Mónika Katkó for his continuous expert statistical support. We are grateful to Endre Nagy V. and Rayan Chakra, MD and Klara Boruzs for his assistance in editing the manuscript.

Statesment and declaration

Funding

No specific funding was received for this research.

Competing interest

There is no conflict of interest to disclose.

Author contribution

Tünde Herman: Project development, data collection, manuscript writing

Péter Török: project development, manuscript editing

Antonio Simone Laganà: manuscript editing, data management

Vito Chiantera: data management

Attila Jakab: Project development, data collection, manuscript writing

Availability of data and material

Data and material are available

Code availability

Not applicable

Ethics approval

UD CC REC/IEC No. 5684-2021

Consent to publish

All authors have consent to publish

References

1. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018;62:2–10. 10.1016/j.clinbiochem.2018.03.012.

2. Poppe K, Velkeniers B, Glinoer D. Thyroid disease and female reproduction. *Clin Endocrinol (Oxf.).* 2007; 66:309 – 21. doi: 10.1111/j.1365-2265.2007.02752.x.
3. Herman T, Csehely S, Orosz M, Bhattoa HP, Deli T, Torok P, Lagana AS, Chiantera V, Jakab A. Impact of Endocrine Disorders on IVF Outcomes: Results from a Large, Single-Centre, Prospective Study. Reprod Sci. 2023;30(6):1878–90. 10.1007/s43032-022-01137-0.
4. Ragusa F, Fallahi P, Elia G, Gonnella D, Paparo SR, Giusti C, Churilov LP, Ferrari SM, Antonelli A. Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. Best Pract Res Clin Endocrinol Metab. 2019;33(6):101367. 10.1016/j.beem.2019.101367.
5. Vissenberg R, Manders VD, Mastenbroek S, Fliers E, Afink GB, Ris-Stalpers C, Goddijn M, Bisschop PH. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum Reprod Update. 2015;21(3):378–87. 10.1093/humupd/dmv004.
6. Maruo T, Matsuo H, Mochizuki M. Thyroid hormone as a biological amplifier of differentiated trophoblast function in early pregnancy. Acta Endocrinol (Copenh). 1991;125:58–66. 10.1530/acta.0.1250058.
7. Monteleone P, Parrini D, Faviana P, Carletti E, Casarosa E, Uccelli A, Cela V, Genazzani AR, Artini PG. Female infertility related to thyroid autoimmunity: the ovarian follicle hypothesis. Am J Reprod Immunol. 2011;66(2):108–14. 10.1111/j.1600-0897.2010.00961.x.
8. Stagnaro-Green A, Glinoer D. Thyroid autoimmunity and the risk of miscarriage. Best Pract Res Clin Endocrinol Metab. 2004;18:167–81. 10.1016/j.beem.2004.03.007.
9. Busnelli A, Paffoni A, Fedele L, Somigliana E. The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis. Hum Reprod Update. 2016;22:775–90. 10.1093/humupd/dmw019.
10. Venables A, Wong W, Way M, Homer HA. Thyroid autoimmunity and IVF/ICSI outcomes in euthyroid women: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2020;25(1):120. 10.1186/s12958-020-00671-3.
11. Kushnir VA, Solouki S, Sarig-Meth T, Vega MG, Albertini DF, Darmon SK, Deligdisch L, Barad DH, Gleicher N. Systemic Inflammation and Autoimmunity in Women with Chronic Endometritis. Am J Reprod Immunol. 2016;75(6):672–7. 10.1111/aji.12508.
12. ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open. 2018;2018(2):hoy004. 10.1093/hropen/hoy004.
13. Poppe K, Velkeniers B, Glinoer D. The role of thyroid autoimmunity in fertility and pregnancy. Nat Clin Pract Endocrinol Metab. 2008;4:394–405. 10.1038/ncpendmet0846.
14. Negro R, Mangieri T, Coppola L, Presicce G, Casavola EC, Gismondi R, Locorotondo G, Caroli P, Pezzarossa A, Dazzi D, et al. Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study. Hum Reprod. 2005;20:1529–33. 10.1093/humrep/deh843.

15. Hart RJ. Physiological Aspects of Female Fertility: Role of the Environment, Modern Lifestyle, and Genetics. Physiol Rev. 2016;96:873–909. 10.1152/physrev.00023.2015.
16. Romitti M, Fabris VC, Ziegelmann PK, Maia AL, Spritzer PM. Association between PCOS and autoimmune thyroid disease: a systematic review and meta-analysis. Endocr Connect. 2018;26:7:1158–67. 10.1530/EC-18-0309.
17. Kowalczyk K, Franik G, Kowalczyk D, Pluta D, Blukacz Ł, Madej P. Thyroid disorders in polycystic ovary syndrome. Eur Rev Med Pharmacol Sci. 2017;21:346–60.
18. Serin AN, Birge Ö, Uysal A, Görar S, Tekeli F. Hashimoto's thyroiditis worsens ovaries in polycystic ovary syndrome patients compared to Anti-Müllerian hormone levels. BMC Endocr Disord. 2021;9(1):44. 10.1186/s12902-021-00706-9.
19. Ho CW, Chen HH, Hsieh MC, Chen CC, Hsu SP, Yip HT, Kao CH. Increased Risk of Polycystic Ovary Syndrome and It's Comorbidities in Women with Autoimmune Thyroid Disease. Int J Environ Res Public Health. 2020;17:2422. 10.3390/ijerph17072422.
20. Novosad JA, Kalantaridou SN, Tong ZB, Nelson LM. Ovarian antibodies as detected by indirect immunofluorescence are unreliable in the diagnosis of autoimmune premature ovarian failure: a controlled evaluation. BMC Womens Health. 2003;17:3:2. 10.1186/1472-6874-3-2.
21. Goswami R, Marwaha RK, Goswami D, Gupta N, Ray D, Tomar N, Singh S. Prevalence of thyroid autoimmunity in sporadic idiopathic hypoparathyroidism in comparison to type 1 diabetes and premature ovarian failure. J Clin Endocrinol Metab. 2006;91:4256–9. 10.1210/jc.2006-1005.
22. Warren BD, Kinsey WK, McGinnis LK, Christenson LK, et al. Ovarian autoimmune disease: clinical concepts and animal models. Cell Mol Immunol. 2014;11:510–21. 10.1038/cmi.2014.97.
23. Polyzos NP, Sakkas E, Vaiarelli A, Poppe K, Camus M, Tournaye H. Thyroid autoimmunity, hypothyroidism and ovarian reserve: a cross-sectional study of 5000 women based on age-specific AMH values. Hum Reprod. 2015;30:1690–6. 10.1093/humrep/dev089.
24. Poppe K, Bisschop P, Fugazzola L, Minziori G, Unuane D, Weghofer A. 2021 European Thyroid Association Guideline on Thyroid Disorders prior to and during Assisted Reproduction. *Eur Thyroid J*. 2021;9(6):281–295. doi: 10.1159/000512790.
25. Bobak L, Bobakova D, Vaczy Z, Rosocha J, Halagovec A. Incidence of antibodies in women after failure of assisted reproduction. Bratisl Lek Listy. 2014;115(3):145–9. 10.4149/bll_2014_031.
26. Negro R, Formoso G, Coppola L, Presicce G, Mangieri T, Pezzarossa A, Dazzi D. Euthyroid women with autoimmune disease undergoing assisted reproduction technologies: the role of autoimmunity and thyroid function. J Endocrinol Invest. 2007;30:3–8. 10.1007/BF03347388.
27. Poppe K, Glinoer D. Thyroid autoimmunity and hypothyroidism before and during pregnancy. Hum Reprod Update. 2003;9:149–61. 10.1093/humupd/dmg012.
28. Zeng S, Liu X, Liu D, Song W. Research update for the immune microenvironment of chronic endometritis. J Reprod Immunol. 2022;152:103637. 10.1016/j.jri.2022.103637.