IVF/ICSI outcomes of euthyroid infertile women with TAI: does treating with aspirin plus prednisone matter?

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Abstract

Background

Thyroid autoimmunity (TAI) has been associated with adverse pregnancy outcomes. To settle with fertility problem, prescribing aspirin combined with prednisone (P+A) to women positive for antithyroid antibodies (ATA) is frequent in clinical practice, but the real effect remains controversial.

Methods

A multicenter, retrospective study was conducted in three reproductive centers from 2017 to 2020. We recruited 494 euthyroid infertile women positive for anti-thyroperoxidase and/or thyroglobulin antibodies (TPOAb and TgAb, respectively) with thyroid stimulating hormone (TSH) levels ranging 0.35-4.0mIU/L who were undergoing their first in vitro fertilization and embryo transfer (IVF-ET) cycle. Ultimately, 346 women were included of which 150 women were treated with prednisone (10mg/d) and aspirin (100mg/d), while the remaining 196 women were untreated (control group). Treatment started on the day of embryo transfer and continued until clinical pregnancy was determined.

Results

Clinical pregnancy rate (CPR) was 57.5% vs. 63.5% in the control and treated groups (P=0.414) for first fresh embryo transfer cycles and 57.8% vs. 61.8% for frozen-thawed embryo transfer cycles (P=0.606). Additionally, the live birth rate (LBR) at the fresh embryo transfer was 49.6% vs. 47.3% in the control and treated groups (P=0.762). Logistic regression revealed that P+A did not improve CPR or miscarriage rates (MR). Furthermore, we observed that low free triiodothyronine (FT3) was associated with high MR.

Conclusions

Utilizing an adjuvant treatment of P+A after the embryo transfer may be unnecessary in euthyroid women with TAI undergoing their first IVF-ET, regardless of embryo type (fresh or frozen).

1. Background

Despite numerous advances in assisted reproductive technology (ART) such as controlled ovarian stimulation, assisted hatching, and pre-implantation genetic testing, implantation remains a long-standing rate-limiting step in IVF treatments [1]. A successful implantation relies on the intricate collaboration between good-quality embryos and a receptive human endometrium, both of which are indispensable requisites [2–4]. Therefore, when good-quality embryos or even euploid embryos are prepared for the transfer, the endometrium may be responsible for implantation failures [4, 5]. The major factors determining uterine receptivity for implantation and further embryo development are progesterone, estrogens, and the immune system [6]. In addition, the process of reprogramming the maternal immune system from rejection to temporary tolerance towards the fetal (paternally derived) semi-allograft depends on the endocrine-immune interaction [7–9]. A healthy female immune system usually induces tolerance towards the embryo, whereas this process fails in a hyperactive immune system, thus reducing fertility and increasing the risk of miscarriage [7]. Among the various studies investigating immunological mechanisms, thyroid autoimmunity, as a predictor of generalized autoimmune disturbance, has been closely linked to recurrent embryo implantation failure, early pregnancy loss, and adverse pregnancy outcomes [10–12]. Furthermore, several reports demonstrated that ATA did not affect embryo quality but decreased CPR, partly because of the impaired maternal immune modulation [13]. When addressing the reproductive challenges faced by infertile woman who are positive for ATA pursuing pregnancy, prednisone (P) for immunosuppression and aspirin (A) as an antithrombotic agent are frequently and customarily prescribed in clinical practice.

As a therapeutic alternative, corticosteroid hormones in combination with aspirin may potentially benefit blood perfusion to the ovaries and endometrium and decrease the local inflammatory reaction to the transfer procedure, thus inducing a more favorable micro-environment for the transferred embryo [14, 15]. However, insufficient evidence exists to date to determine whether P+A therapy improves the likelihood of pregnancy following ART in thyroid Ab-positive euthyroid women.

Hence, the aim of the present study was to evaluate the effects of P+A treatment on improving pregnancy outcomes of the first IVF/ICSI cycles in euthyroid infertile women who only present positive thyroid autoimmune antibodies.

2. Methods

2.1 Patients

We conducted a multicenter retrospective study involving 494 infertile women who tested positive for TPOAb and/or TgAb and were being treated for infertility at the Second Affiliated Hospital of Zhejiang University School of Medicine, Ningbo Women and Children's Hospital, and People's Hospital of Jinhua from October 2017 to July 2020. Both TPOAb and TgAb were measured in all subjects before ART procedure, and their TSH level ranged from 0.35-4.0mIU/L. They were treated with P+A or untreated during the ART procedure. Additionally, we screened and analyzed only the first embryo transfer (fresh or frozen-thawed embryo transfer) and first cycle. Inclusion criteria were as follows: age under 40 years old, regular spontaneous menstrual cycle (21–35 days), normal uterine cavity, presence of both ovaries, normal ovarian reserve as defined by basic follicle stimulating hormone (bFSH)<10 IU/L, and antral follicle count (AFC)>5.

We excluded from the study women with known autoimmune diseases or clinical presentations of autoimmune disorders including systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS) (n=2), those whose thyroid function was not normal (n=38), those who were diagnosed with diseases affecting the uterine cavity (n=12), and those whose infertility was caused by severe oligoasthenospermia and azoospermatism (n=16). Similarly, we ruled out eight women because they or their partners presented aberrant chromosome karyotypes with particularly significant parental balanced translocations or Turner mosaicism. One woman was excluded due to medical history of insulin-dependent diabetes mellitus (DM), seven women due to a lack of mature oocytes to retrieve and 64 women on account of not undergoing any embryo transfers for various reasons.

Of the selected 346 ATA (+) women, 150 (43.4%) patients received P+A treatment during ART (Fig. 1). The study was reviewed and approved by the Ethics Committees of the Second Affiliated Hospital of Zhejiang University School of Medicine, Ningbo Women and Children's Hospital, and People's Hospital of Jinhua.

2.2 Laboratory assays

Women's serum samples were analyzed by the standard third-generation electrochemiluminescence (ECL) immunoassay (CobasElesys 601, Roche) in the three reproductive centers. Thyroid autoimmunity (TAI) was defined as the presence of serum antibodies directed against TPO and/or TG. The reference range was 0–5.61 IU/mL for TPOAb and <4.11 IU/mL for TgAb. Women were diagnosed with euthyroidism when serum levels of TSH were within the reference range of 0.35–4.0mIU/L and none of the free thyroxine parameters (FT4) or FT3 was outside their reference values, which were 0.7-1.48ng/dL for FT4 and 1.71-3.71pg/mL for FT3. These reference values were provided by the manufacturer of these assay kits.

2.3 ART procedure and collection of clinical information

In all groups, ovarian stimulation was carried out using standard protocols in each of the participant sites. Patients underwent ovulation induction with recombinant follicle-stimulating hormone (rFSH) (Gonal F; Serono, Switzerland) or human menopausal gonadotropin (HMG) (Livzon, China) to obtain a cohort of mature oocytes at the time of oocyte retrieval. Pituitary inhibition was achieved using a gonadotropin-releasing hormone (GnRH) analog (Decapeptyl; Ferring, Switzerland) or GnRH antagonist (Centrotide; Serono, Germany). The doses of these drugs were adjusted according to the women's age, AFC, and day three bFSH values. Cycles were monitored using transvaginal sonography together with laboratory assays. A dose of 5000–10,000 IU of human chorionic gonadotropin (hCG) (Livzon, China) was administered when a minimum of three leading follicles

reached 17–18 mm and paired with appropriate serum E2 levels. Cumulus oocyte complexes (COCs) were aspirated 36–38 hours after hCG injection. Subsequently, whether conventional IVF or ICSI was utilized depended on the semen condition and clinical indication. All patients were transferred good-quality embryos. Luteal phase support was added in the form of micronized progesterone capsules and oral dydrogesterone. Fourteen days after embryo transfer (ET), serum hCG was assessed and clinical pregnancy was determined 5 weeks after ET by ultrasonography. Clinical data including women's age, body mass index (BMI), duration of infertility, previous history of miscarriage, bFSH, anti-Mullerian hormone (AMH), and AFC were recorded and analyzed. The following laboratory parameters and pregnancy outcomes were also documented: total gonadotropin doses (Gn), days of gonadotropin treatment, E2 levels on hCG day, endometrial thicknesses on hCG day and embryo transfer day, oocytes retrieved, fertilization rate, number of embryos for transferring, implantation rate of cleavage and blastocyst stage embryos, pregnancy rate (PR), CPR, MR, LBR.

2.4 Adjuvant medical treatments

A total of 346 euthyroid infertile women with TAI were divided into two groups—the control group (n=196) and the treated group (n=150), which was treated with orally administered prednisone (Xianju pharmaceutical factory, China) and aspirin (Bayer, Germany) in a daily dose of 10 mg prednisone and 100 mg aspirin from the day of ET until the determination of successfully clinical pregnancy. Treatment was discontinued if a persistent hCG decline occurred.

2.5 Outcome measures

Primary outcome was CPR after the first embryo transfer. Secondary outcomes were: implantation rate of cleavage stage embryos, MR, and LBR after the first embryo transfer.

We defined PR as the percentage of transfers with positive serum levels of hCG (≥5 mIU/mL), whereas clinical pregnancy was defined as the existence of a viable embryo within an intrauterine gestational sac. The spontaneous abortion rate was defined as the ratio between the number of pregnancy losses after sonographic visualization of an intrauterine gestational sac and the number of clinical pregnancies. Recurrent miscarriage (RM) was determined by the loss of two or more clinical pregnancies. Implantation rate was calculated as the number of sacs with fetal heart beat divided by the total number of embryos transferred, whereas LBR was the percentage of transfers resulting in a live birth.

2.6 Statistics

Data analysis was performed using the Statistics Package for Social Sciences (SPSS 24.0). First, a Kolmogorov-Smirnov test was applied to both groups and variables to evaluate whether the distribution was symmetrical or not. Continuous data were expressed as median (25th–75th) when not normally distributed, and as mean±SD for normally distributed data. Categorical data were calculated as the number (percentage) of cases. Comparisons of quantitative data were analyzed using the Mann–Whitney U test or independent T test and Chi-square or two-sided Fisher's exact test in the case of categorical data.

For the logistic regression analysis, the independent variables were age and FT3 levels in the whole range. CPR and MR were considered as dependent outcomes.

The significance level of alpha was defined at 0.05, where a value of $p<0.05$ was considered statistically significant.

3 Results

3.1 Clinical characteristics

The characteristics of women with ATA (+) are shown in Table 1. The clinical descriptive characteristics were broadly comparable between the P+A and non-treated groups and consisted of age, BMI, number of previous miscarriages, duration of infertility, the proportion of primary infertility, bFSH, AMH, AFC, TSH, FT4, the ratio of only TPOAb positivity, only TgAb positivity, or TPOAb and TgAb positivity. Furthermore, the cause of infertility was comparable between those groups (Supplemental Table SI). The value of FT3 in the P+A treated group was 2.90±0.39pg/mL, which was significantly lower than that of ATA-positive untreated subjects (3.05±0.44, P=0.017).

Table 1
Characteristics of women with positive antithyroid antibodies

Cycles	Fresh embryo transfer cycles			Frozen-thawed embryo transfer cycles		
	Control group n=113	P+A group n=74	P value	Control group n=83	P+A group n=76	P value
Age (yrs.)	31.0 (29.0-35.0)	31.5 (29.0-35.0)	0.832	30.0 (28.0-34.0)	30.0 (28.0-34.8)	0.737
<31	44 (38.9%)	26 (35.1%)	0.829	44 (53.0%)	39 (51.3%)	0.727
31-37	63 (55.8%)	43 (58.1%)		35 (42.2%)	31 (40.8%)	
>37	6 (5.3%)	5 (6.8%)		4 (4.8%)	6 (7.9%)	
BMI (kg/m^2)	22.0 (20.0-24.0)	22.3 (20.2-25.1)	0.336	22.38±2.97	22.09±3.26	0.557
BMI≥25 (kg/m^2)	21 (18.6%)	21 (28.4%)	0.117	16 (19.3%)	14 (18.4%)	0.890
Previous miscarriages	0.0 (0.0-1.0)	1.0 (0.0-1.0)	0.636	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.752
≥2	21 (18.6%)	15 (20.3%)	0.775	7 (8.4%)	10 (13.2%)	0.336
Duration of infertility (yrs.)	3.0 (2.0-5.5)	3.0 (1.0-5.0)	0.961	3.0 (1.3-4.0)	2.0 (2.0-4.0)	0.973
<3	53 (46.9%)	32 (43.2%)	0.623	39 (47.0%)	40 (52.6%)	0.477
≥3	60 (53.1%)	42 (56.8%)		44 (53.0%)	36 (47.4%)	
Primary infertility	50 (44.2%)	30 (40.5%)	0.616	42 (50.6%)	35 (46.1%)	0.566
Basal FSH (IU/L)	5.74±1.57	5.67±1.36	0.732	6.2 (4.7-7.1)	5.2 (4.4-6.3)	0.094
AMH (ng/mL)	2.7 (1.7-3.7)	2.9 (1.9-4.2)	0.429	4.3 (2.4-6.5)	3.9 (1.9-6.0)	0.327
AFC	11.0 (7.0-18.0)	14 (10.0-17.0)	0.103	12.0 (9.0-18.0)	14.0 (8.0-17.0)	0.882
TSH (mIU/L)	2.2 (1.5-2.8)	2.0 (1.4-2.7)	0.422	2.22±0.90	1.99±0.86	0.109
FT4 (ng/dL)	1.00±0.14	1.01±0.09	0.475	1.0 (0.9-1.1)	1.0 (1.0-1.1)	0.867
FT3 (pg/mL)	3.05±0.44	2.90±0.39	0.017*	2.99±0.48	2.89±0.39	0.145
Only TPOAb positivity	9 (8.0%)	7 (9.5%)	0.542	9 (10.8%)	12 (15.8%)	0.592
Only TgAb positivity	52 (46.0%)	28 (37.8%)		33 (39.8%)	31 (40.8%)	
Both positivity	52 (46.0%)	39 (52.7%)		41 (49.4%)	33 (43.4%)	
P<0.05 was considered statistically significant. * represents a statistically significant difference between the two groups.						
Continuous data are expressed as mean±SD when normally distributed, or as median (25th -75th) otherwise. Categorical variables are expressed as number and percentage.						
BMI, body mass index; FSH, follicle-stimulating hormone; AMH, anti-Mullerian hormone; AFC, antral follicle count; TSH, thyroid stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine; TPOAb, anti-thyroperoxidase antibody; TgAb, thyroglobulin antibody.						

3.2 Cycle characteristics and embryological data

No significant differences were observed in the ratio of GnRHant/GnRHa, days of ovarian stimulation, total Gn doses, E2 levels on hCG day, endometrial thicknesses on hCG day, number of oocytes retrieved, or the type of ART used between the two groups for both fresh and frozen embryo transfer cycles (Table 2).

Table 2
Cycle characteristics and embryological data of studied groups

Cycles	Fresh embryo transfer cycles				Frozen-thawed embryo transfer cycles			
	Control group n=113	P+A group n=74	F value	P value	Control group n=83	P+A group n=76	F value	P value
GnRHant/GnRHa	28/85	23/51	0.895	0.344	38/45	27/49	1.727	0.189
Total Gn Dose (IU)	2250.0 (1575.0-2700.0)	2250.0 (1762.5-2925.0)		0.373	1950.0 (1425.0-2550.0)	1875.0 (1425.0-2225.0)		0.437
Stimulation length (d)	10.0 (9.0-11.0)	10.0 (9.0-11.0)		0.442	9.0 (8.0-11.0)	9.0 (8.0-11.0)		0.897
E2 level on HCG day (pg/mL)	2246.77±980.18	2287.10±865.47		0.547	3449.1 (2076.4-4496.1)	3811.1 (2293.9-5959.9)		0.486
Endometrial thickness on hCG day (mm)	12 (10-12)	12 (10-12)		0.444	/	/		/
Number of oocytes retrieved	9.0 (5.0-12.0)	10.0 (6.0-15.0)		0.053	12.0 (9.0-17.0)	15.5 (9.0-21.8)		0.057
IVF/ICSI	102/11	70/4	1.136	0.287	70/13	65/11	0.044	0.834
Fertilization rate (%)	82.6 (846/1024)	80.4 (634/789)	1.521	0.217	81.0 (856/1057)	78.0 (973/1247)	3.056	0.080
Cleavage rate (%)	92.7 (784/846)	93.5 (593/634)	0.416	0.519	95.7 (819/856)	94.3 (918/973)	1.687	0.194
Available embryo rate (%)	61.2 (480/784)	56.2 (333/593)	3.588	0.058	56.8 (465/819)	55.4 (509/918)	0.311	0.577
P<0.05 was considered statistically significant.								
Continuous data are expressed as mean±SD when normally distributed, or as median (25th-75th) otherwise. Categorical variables are expressed as number and percentage.								
GnRHant, gonadotropin-releasing hormone antagonist; GnRHa, gonadotropin-releasing hormone agonist; Gn dose, gonadotropin dose; E2, estradiol; hCG, human chorionic gonadotropin; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection;								

3.3 Reproductive outcomes

As for fresh embryo transfers, we meticulously observed and documented the outcomes (Table 3). Implantation rate of cleavage stage embryos was slightly higher (non-significant difference) in treated women than in untreated women (44.7% vs. 40.2%, respectively; P=0.407). As for PR, it was similar between the treated and control groups (64.9% vs. 63.7%; p = 0.873), although we observed a higher but non-significant prevalence of clinical pregnancy in treated patients (63.5% vs. 57.5%; p=0.414). More miscarriages were reported in the treated than in the control group at the first attempt (25.5% versus 13.8%), but this difference was not significant (P=0.118). The prevalence of live births among treated women was 47.3% compared with a 49.6% among untreated women (P=0.762). The results regarding frozen embryo transfers were comparable to those obtained with fresh embryo transfers. Notably, an increased but nonsignificant prevalence of cleavage stage embryo implantation and clinical pregnancy was observed in treated women compared with untreated women (45.5% vs 39.7%, P=0.341; 61.8% vs 57.8%, P=0.606). Additionally, the incidence of treated women that suffered a miscarriage was 27.7% vs. 18.8% in the control group (P=0.303). At the first frozen embryo transfer, the treated group bore similar likely to delivering a live birth compared with what was observed in the control group (44.7% vs 47.0%, P=0.776).

Table 3
Reproductive outcomes at the first embryo transfer and cumulative outcomes after 1-year follow-up

Cycles	Fresh embryo transfer cycles				Frozen-thawed embryo transfer cycles			
	Control group n=113	P+A group n=74	F value	P value	Control group n=83	P+A group n=76	F value	P value
Embryo stage, n (%)								
Cleavage stage	108 (95.6%)	72 (97.3%)	0.368	0.544	73 (88.0%)	71 (93.4%)	1.389	0.239
Blastocyst stage	5 (4.4%)	2 (2.7%)			10 (12.0%)	5 (6.6%)		
Number of embryos transferred	1.88±0.33	1.93±0.25		0.214	1.81±0.40	1.84±0.37		0.565
Endometrial thickness on embryo transfer day (mm)	/	/		/	9.1 (8.5,11.0)	9.1 (8.5,11.0)		0.984
Implantation rate (%)	40.1 (85/212)	44.8 (64/143)	0.762	0.355	42.0 (63/150)	46.4 (65/140)	0.576	0.448
Implantation rate of cleavage stage embryos (%)	40.2 (82/204)	44.7 (63/141)	0.688	0.407	39.7 (54/136)	45.5 (60/132)	0.906	0.341
Implantation rate of blastocyst stage embryos (%)	37.5 (3/8)	50.0 (1/2)	/	/	60.0 (9/14)	62.5 (5/8)	/	/
Pregnancy rate (%)	63.7 (72/113)	64.9 (48/74)	0.026	0.873	65.1 (54/83)	65.8 (50/76)	0.009	0.923
Clinical pregnancy rate (%)	57.5 (65/113)	63.5 (47/74)	0.668	0.414	57.8 (48/83)	61.8 (47/76)	0.265	0.606
Miscarriage rate (%)	13.8 (9/65)	25.5 (12/47)	2.445	0.118	18.8 (9/48)	27.7 (13/47)	1.059	0.303
Neonatal mortality rate (%)	0 (0/113)	0 (0/74)			0 (0/83)	0 (0/76)		
Live birth rate (%)	49.6 (56/113)	47.3 (35/74)	0.091	0.762	47.0 (39/83)	44.7 (34/76)	0.081	0.776
P<0.05 was considered to be statistically significant.								
Continuous data are expressed as mean±SD when normally distributed, or as median (25th-75th) otherwise. Categorical variables are expressed as number and percentage.								

3.4 Logistic regression analysis

Since there was a significant difference in FT3 at the fresh embryo transfer cycle between women with or without P+A treatment, we performed a multiple logistic regression analysis. Besides, age, the clinically relevant variable, was also included in the regression analysis (Table 4). In women transferred with fresh embryos, after adjusting for age and additional treatments, FT3 within the normal reference appeared to have a negative relationship with MR (odds ratio [OR] 0.248 [95% confidence interval, CI 0.063-0.984], P=0.047). Furthermore, P+A treatment exerted no influence on MR or CPR.

Table 4
Multivariable logistic regression analysis.

Dependent outcomes	Fresh embryo transfer cycles				Frozen-thawed embryo transfer cycles			
	Miscarriage rate		Clinical pregnancy rate		Miscarriage rate		Clinical pregnancy rate	
Independent variables	aOR [CI]	P	aOR [CI]	P	aOR [CI]	P	aOR [CI]	P
Age (yrs.)	0.983[0.850-1.135]	0.812	1.040[0.957-1.130]	0.352	1.002[0.886-1.133]	0.975	0.971[0.895-1.054]	0.486
FT3 (pg/mL)	0.248[0.063-0.984]	0.047*	1.323[0.651-2.690]	0.439				
P+A treatment	0.548[0.204-1.468]	0.232	0.748[0.404-1.382]	0.353	0.603[0.229-1.587]	0.306	0.839[0.443-1.586]	0.588
P<0.05 was considered statistically significant. * represents a statistically significant difference between the two groups.								
FT3, free triiodothyronine; P, prednisone; A, aspirin								

3.5 Recurrent pregnancy loss and IVF outcome

Baseline demographics and clinical characteristics were comparable between groups (Supplemental Table SII). Based on our analysis, there was no association between P+A treatment and subsequent pregnancy outcomes in women suffering from recurrent pregnancy loss (RPL) who had autoimmune thyroid disease (Supplemental Table SIII). However, the small sample size in this study did not provide the adequate power required to evaluate this outcome. Thus, future investigations, preferably focusing on randomized controlled trials (RCT), are urgently needed to assess the value of additional treatment in RPL women with TAI.

3.6 Comparison of IVF outcomes of continuous embryo transfers

The comparison of IVF outcomes of continuous embryo transfers in women receiving nothing at the first embryo transfer but obtaining therapy at the subsequent frozen embryo transfers at the same IVF cycle was depicted in Supplemental Table SIV. In other words, this part was before-after study in the same patients. As shown in the Supplemental Table SIV, the presence of P+A was not beneficial to final reproductive outcomes. Nevertheless, there were considerable shortcomings in the limited eligible evidence, discrepant ratio of cleavage to blastocyst stage embryo, and various types of embryos transferred, fresh or frozen-thawed, inevitably reaching a questionable conclusion.

4 Discussion

The correlation between antithyroid antibodies, fecundity, and pregnancy outcomes is quite debatable and conflicting. Previously, meta-analysis of four prospective studies that included 1098 subfertile women undergoing IVF revealed a significant two-fold increase in the risk of miscarriage of subfertile euthyroid women with TAI compared with a counterpart without TAI [16]. Among those four studies, three of them only measured TPO-Ab and the remaining one measured both TPOAb and TgAb. Of them, one study recruited participants only with unexplained infertility and without previous history of miscarriages, whereas the other investigations included subfertile women irrespective of causes for infertility or previous history of miscarriages. Under the circumstances of different ART/IVF protocols, dissimilar underlying etiologies contributing to infertility, and changeable cut-off values for euthyroidism and subclinical hypothyroidism, the 2017 American Thyroid Association pregnancy guidelines was unable to reach a definite conclusion on the link between TAI and ART outcomes. Moreover, levothyroxine treatment was recommended for subclinical hypothyroidism, defined as a TSH >2.5 mIU/L, and considered for euthyroid infertile women with TAI when they attempted to conceive by virtue of ART after weighing the pros and cons of levothyroxine supplement [17].

However, in the past few years following the publication of the 2017 guidelines, two large RCTs assessing the value of levothyroxine on pregnancy outcomes in euthyroid TPO-Ab positive women reported that the use of this drug did not improve MR and LBR significantly [18, 19]. Despite several limitations (mainly involving fixed levothyroxine doses, undetermined TSH values during early pregnancy following medicine supplement, uncertain population compliance, and the exclusion of women with RM or positive for other autoimmune antibodies), the large-sample RCT results were essential to evaluate levothyroxine effectiveness specifically in euthyroid women with TAI [20]. Furthermore, a recent meta-analysis of six RCTs demonstrated that levothyroxine could not improve clinical pregnancy outcomes among women positive for TPOAb. Indeed, of the meta-analyses based on high- to moderate-quality evidence, two trials involved ART, two studies used fixed levothyroxine doses and one investigation enrolled euthyroid or subclinical women [21]. Thus, further large-scale high-quality research on this particular population is still urgently needed.

Based on the decreased effectiveness of levothyroxine and generalized autoimmune imbalance resulting from thyroid autoimmunity, we retrospectively explored the impact of P+A treatment on euthyroid women with TAI undergoing their first IVF/ICSI procedure. A dynamic and responsive immune system is critical for a successful pregnancy—the first trimester begins in a pro-inflammatory stage that allows implantation and placentation, then it shifts to an anti-inflammatory environment, pivotal for fetal growth, and finally returns to a pro-inflammatory stage suitable for labor and delivery[22]. The pro-inflammatory process initiated during embryo implantation and trophoblast invasion better promotes cell clearance, angiogenesis, cell growth, and tolerance, as it is characterized by the presence of angiogenic, growth, and survival factors, as well as cytokines and chemokines [22]. Following implantation, the female immune system usually induces tolerance towards the embryo, whereas tolerance induction is incomplete in a hyperactive immune system. Subfertile women with autoimmune thyroid disease usually express increased levels of IFNγ from pro-inflammatory Th1 immune cells, along with lower IL-4 and IL-10 from Th2 immune cells compared with control patients without antithyroid antibodies. This suggests that excessively activated pro-inflammatory Th1 cells hamper the onset of a successful pregnancy [23]. Moreover, pinopodes, the spherical protrusions of the epithelial plasma membrane into the lumen, are characterized as classic morphological biomarkers of receptive endometrium favoring implantation. Recently, a euthyroid Hashimoto's thyroiditis (HT) mice model was established to explore the correlation of HT and endometrial receptivity defects. The resulting evidence indicated that HT alone inhibited luminal epithelium development, retarded the formation and development of pinopodes, and decreased expression of receptivity markers, thereby inducing a nonreceptive endometrial milieu and leading to implantation failure [24]. Prednisone, a type of glucocorticoid, is readily absorbed from the gastrointestinal tract and used primarily for its anti-inflammatory effects in many disorders [25]. Numerous trials revealed that low doses of corticosteroids (10 mg/day) improved IVF pregnancy outcomes in women experiencing immunological infertility and RMs, even in cases with a prior history of 19 consecutive miscarriages [26–29]. Furthermore, by virtue of exposing cleavage stage mouse embryos to 3 and 30 μM concentrations of prednisolone in vitro to assess the embryonic response to direct prednisolone exposure, a recent animal study revealed that exposure to 30 μM prednisolone delayed the embryonic progression, decreased hatching potential, and increased apoptosis in blastocysts. However, 3 μM prednisolone increased inner cell mass proliferation, which was incorporated to predict the implantation potential [30]. It is worth mentioning that 3 μM is close to the therapeutic dose and 30 μM to reflect the ten-times higher than the initial level. Experimental evidence in animal models demonstrated that glucocorticoids at higher concentrations could negatively affect oocyte maturation and early embryogenesis. The therapeutic dose of prednisolone reduced post-implantation demise, possibly due to its effects on choriocarcinoma cell lines. Similarly, the latest trial that investigated the role of prednisolone on decidualization and decidual-trophoblast interactions reported that this treatment enhanced trophoblast outgrowth, elevated trophoblast mRNA expression of cell motility gene PLCG1, and altered decidual-trophoblast interactions, yet the clinical consequences of these changes were unknown [31]. Thus, a great need for further research on this topic still remains.

Simultaneously, low dose of aspirin plays an essential role in improving uterine and ovarian blood flow, enhancing embryo implantation and sustaining early pregnancy. This stems from its capacity to decrease blood viscosity and increase blood flow, which is secondary to the inhibition of cyclooxygenase-1 and decreased production of thromboxane-2. In addition, daily low-dose aspirin use is considerably safe as it does not affect menstrual cycle, follicular phase, luteal phase length, or hormone levels across the menstrual cycle [32]. The adjuvant treatment of P+A is recommended to patients with autoantibodies

undergoing IVF as its benefits are demonstrated in several investigations [26, 27, 33]. However, these trials were published long ago and do not demonstrate the efficacy of this approach for infertile women positive only for antithyroid antibodies.

In our study, we classified patients according to their age in three categories: <31 years, 31–37 years, and >37 years, based on our understanding of natural fertility, since its decline begins at 31 years and 37 years old has been recorded as the pivotal age for success rates in treatment programs [34, 35]. Notably, the distribution among age groups was comparable and thus reduced the potential confounding risk of age, as advanced age increases the chance of de novo chromosomal aberrations in oocytes and, in turn, in the embryo [11, 36, 37]. As for ovarian reserve, age, AMH, AFC, and bFSH were all comparable between treated and untreated patients for both fresh and frozen embryo transfer cycles. Additionally, couples with significant parental chromosome abnormality, severe oligoasthenospermia, and azoospermatism were excluded from our study, as the rate of chromosomal anomaly was 0.24% in normal semen group, 4.7% in moderate-to-severe oligoasthenospermia group, and 9.59% in azoospermia group[38]. The consistency between both groups allowed us to confidently reach the final conclusion as 30-50% of implantation failures can be attributed to poor embryo quality and that embryo quality is determined by a number of parameters, primarily the women's age, ovarian reserve, underlying causes of infertility, and sperm quality. Furthermore, decreased endometrial receptivity is thought to account for two thirds of these failures [5]. Typically, endometrium is the direct or indirect target of antithyroid antibodies, prednisone, and aspirin.

Because of uncertain harm caused from single antibody and combined antibodies, we recorded and analyzed the proportion of positive isolated TPOAb, positive isolated TgAb, and double positive TPOAb and TgAb in our study, and no significant differences were observed. Moreover, in our study, euthyroidism was defined by a TSH reference value range of 0.35–4.0 mIU/L and the value was comparable between two groups. The threshold between euthyroid and subclinical hypothyroidism changes over time. Nowadays, the association between elevated maternal TSH concentrations and pregnancy-specific complications appears to be more pronounced when adopting the the cut-off point of 4.0 mIU/L, or a population-based reference value than the level of 2.5 mIU/L[39]. Newer guidelines suggested that an upper limit of 4.0 mIU/L should be considered diagnostic compared with the previous guideline of 2.5 mIU/L [17]. Based on the TSH threshold of 4.0mIU/L and the 2017 American Thyroid Association recommendations, levothyroxine supplement was not included in our study.

Interestingly, we observed no association between P+A treatment and reproductive outcomes including CPR, MR, and LBR at the first embryo transfer regardless of embryo type (fresh or frozen). Assessed infertile women exhibited normal thyroid tests and no autoimmune antibodies except anti-thyroid ones. This finding has not been replicated in other studies and should be interpreted with caution. In 2009, Alberto Revelli et al. performed a retrospective study of 329 euthyroid women who were positive for TPOAb, TgAb, or both. The medication prescribed was prednisolone (10 mg/d) and aspirin (100 mg/d), from the day of stimulation to 10 weeks of gestational age and, during that period, P was increased to 30 mg/d for 5 days starting from the day of ET. This approach was deemed beneficial to pregnancy and implantation rates in contrast with untreated ATA+ patients [33]. In our study, treatment started on the day of embryo transfer and lasted for 2–6weeks, mainly focusing on improving the implantation micro-immune environment. In a prospective case-control study including 233 consecutive patients, dexamethasone (0.5 mg/d) and acetylsalicylic acid (100 mg/d) starting from the day of embryo transfer and until the end of the 12th week of gestation increased the PR and implantation rate when compared with the control group [40]. Nevertheless, the inclusion criteria of this prospective study included inherited and acquired thrombophilia, compound heterozygous polymorphisms, positive anti-nuclear and anti-thyroglobulin antibodies, which were the strong indication for steroid hormone and anticoagulant drug [40]. Coincidentally, its inclusion criteria coincided with the exclusion criteria in our study, which largely explained the conflicting results. Thus, the effective value of treatment may be not evident in women with unaffected thyroid function and only thyroid antibodies when compared with those with multiple types of autoantibodies or the history of RPL.

In terms of the mixed correlation between TAI and infertility, a recent review published in 2020 contributed to a better understanding of its relevance. By summarizing and analyzing the latest studies since the 2017 guidelines, this review documented that anti-TPO Abs were associated with infertility in subsets of women, mainly in those with unexplained infertility or polycystic ovarian syndrome (PCOS), but not in all women [20]. Such a conclusion was primarily dependent on a secondary analysis of data from two multicenter RCTs involving 1650 euthyroid infertile women either with unexplained infertility or PCOS

[41]. The weak correlation between TAI and IVF reproductive outcomes of general infertile population partially explained the negative results of our study. Furthermore, a 2020 meta-analysis of 17 studies pinpointed a statistically significant association between RPL and TAI (odds ratio 1.94; 95% CI, 1.43–2.64). The statistical significance and magnitude of the results remained unchanged following sensitivity analyses [42], similarly to the findings in our previous work [43]. Up to now, for euthyroid infertile women with unexplained infertility, PCOS, or RPL, little evidence exists concerning the effect of replacement therapy of P+A. In our study, due to the scarcity of a great number of subjects, stratified research does not achieve valid results at the subgroup level. However, based on the fact that P +A treatment improved adverse IVF reproductive outcomes in women with positive antinuclear antibodies [44], with unexplained RPL[45] and with other immune-related antibodies[15], combined treatment is likely to benefit euthyroid infertile women with TAI and unexplained infertility or RPL, although it still requires prospective large-sample trials to justify its potency.

Additionally, in our study, we observed that regardless of the embryo type being transferred, incidence of abortion was higher but not significant in the treated group than in control patients. As illustrated above, the value of FT3 in the P+A treated group was 2.90±0.39 pg/mL, significantly lower than that of ATA-positive untreated subjects (P=0.017). Multivariable logistic regression demonstrated the negative role of FT3 in fetal loss incidence at the first fresh embryo transfer. This is consistent with a preliminary observational study that reported that low serum FT3 levels compromised the beneficial effect of levothyroxine substitution in women with HT [46]. It is widely acknowledged that thyroid hormone (TH) transporters, receptors, and their associated proteins are expressed in the ovary, early embryo, endometrium, uterus, and placenta[7]. Simultaneously, the expression of these proteins in the endometrium is dynamic throughout the various phases of the menstrual cycle [47]. It has been documented that receptive endometrium is accompanied with strengthened expression of TH receptors in normal women [48], whereas decreased expression of thyroid-related proteins in the uterus was observed at the day of implantation in hypothyroid pregnant rats [49]. By binding to TH receptors on the placenta and endometrium, as well as regulating the invasive potential of extravillous trophoblasts, thyroid hormone can affect implantation and early fetal development [7]. Furthermore, an optimal T3 value is crucial for ovulation and folliculogenesis, as T3 in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis via the PI3K/Akt pathway [7, 50]. To conclude, the aforementioned evidence seemingly suggests that additional levothyroxine should be supplemented in euthyroid infertile women with low but yet normal values of FT3. It seems dissimilar to other investigations. A novel pathogenesis model of the link between thyroid autoimmunity and fertility may offer us a new perspective [20]. During the early stages of autoimmunity, the main detrimental effects comprise the hostile immune environment impacting the ovary, with TPO as the direct antigen. At this stage, the thyroid hormone response is still intact and levothyroxine treatment is inefficient. As thyroid autoimmunity progresses, thyroid response to hCG stimulation is impaired and thus unable to meet the high thyroid hormone demand during pregnancy. In that situation, treatment with thyroid hormone would prove beneficial [20]. Based on this potential model, distinguishing the different stages is key to achieving an efficient treatment.

Although progress has been made in some areas of the autoimmune disorders, little is known about the underlying mechanisms of autoimmune antibodies on reproductive outcome, which represents a challenge for effective treatment research. Organs-on-a-chip, advanced in vitro models of multicellular tissue complexes or functional organ units, may help illuminate this intricate connection. Exploiting organ-on-a-chip approaches to model decidualization, implantation and placentation, would enable an in-depth study of the invasive and remodeling behavior of extravillous trophoblast cells, and of uteroplacental circulation that provides vascular supply to the growing fetus[51]. Furthermore, interaction between antibodies and endometrium and variable expression of immunological factors, as well as glucocorticoid targets, are all stand a chance of being explored.

Overall, there were several advantages in our study. Firstly, by establishing strict inclusion and exclusion criteria, we controlled for the possible confounding factors of other autoantibodies and severe detrimental elements of spontaneous miscarriages, which allowed us to minimize any patient-related variation and concentrate solely on P+A effects on isolated euthyroid infertile women with TAI. Secondly, we only included first-time ART users and analyzed only first-cycle ART outcomes to investigate a homogeneous, good-prognosis population and provide relevant suggestions for targeted subjects. However, our study also had some limitations. Firstly, it was inevitably limited by its retrospective nature. Secondly, given the variation in TPOAb and TH

concentrations in the context of pregnancy, the measurement of longitudinal thyroid parameters during pregnancy was reasonable and necessary, but we did not record the changes[52]. While P+A supplement did not improve LBR or PR in euthyroid TAI women at the first embryo transfer, the potential benefits of P+A supplementation during pregnancy cannot be ruled out. Additional RCTs are required to determine whether P+A would yield different results on women who test positive for antithyroid antibodies with recurrent implantation failure or with unexplained infertility. Similarly, it is possible that a higher risk population with increased recurrent pregnancy loss might be affected in a different manner.

5 Conclusion

To conclude, according to the reality of routine thyroid screening, a large number of euthyroid women who test positive for antithyroid antibodies are discovered. Among them, patients underwent the first IVF cycle without the history of recurrent miscarriages or unexplained infertility are not recommended for the combined treatment of prednisone and aspirin.

Abbreviations

thyroid autoimmunity	TAI
antithyroid antibody	ATA
thyroperoxidase antibody	TPOAb
thyroglobulin antibody	TgAb
in vitro fertilization and embryo transfer	IVF-ET
clinical pregnancy rate	CPR
miscarriage rate	MR
free triiodothyronine	FT3
prednisone and aspirin	P+A
assisted reproductive technology	ART
thyroid stimulating hormone	TSH
basic follicle stimulating hormone	bFSH
antral follicle count	AFC
systemic lupus erythematosus	SLE
antiphospholipid syndrome	APS
diabetes mellitus	DM
electrochemiluminescence	ECL
free thyroxine	FT4
recombinant follicle-stimulating hormone	rFSH
human menopausal gonadotropin	HMG
gonadotropin-releasing hormone	GnRH
human chorionic gonadotropin	hCG
cumulus oocyte complexes	COCs
body mass index	BMI
anti-Mullerian hormone	AMH
antral follicle count	AFC
gonadotropin	Gn
pregnancy rate	PR
live birth rate	LBR
recurrent miscarriage	RM
Statistics Package for Social Sciences	SPSS
fertilization rate	FR
cleavage rate	CR
recurrent pregnancy loss	RPL
randomized controlled trial	RCT

Hashimoto's thyroiditis	HT
prednisolone	PRDL
inner cell mass	ICM
polycystic ovarian syndrome	PCOS
thyroid hormone	TH

Declarations

Ethics approval and consent to participate

All trials were performed in strict accordance with the Ethics Committee at the Second Affiliated Hospital of Zhejiang University School of Medicine. The Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine, Ningbo Women and Children's Hospital, and People's Hospital of Jinhua approved the protocols. Our study was exempted from informed consent requirements by the Ethics Committee at the Second Affiliated Hospital of Zhejiang University School of Medicine because of the retrospective use of preexisting data.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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Authors' contributions

Patient selection: P. Z and Y.Y; sample collection and processing: J. X, Q.Z and L.Y; data analysis and interpretation: P. Z and Q.Y; study design: C.F, L.Z, and M.J; manuscript drafting: P. Z and M.J. All authors read and approved the final manuscript.

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Figures

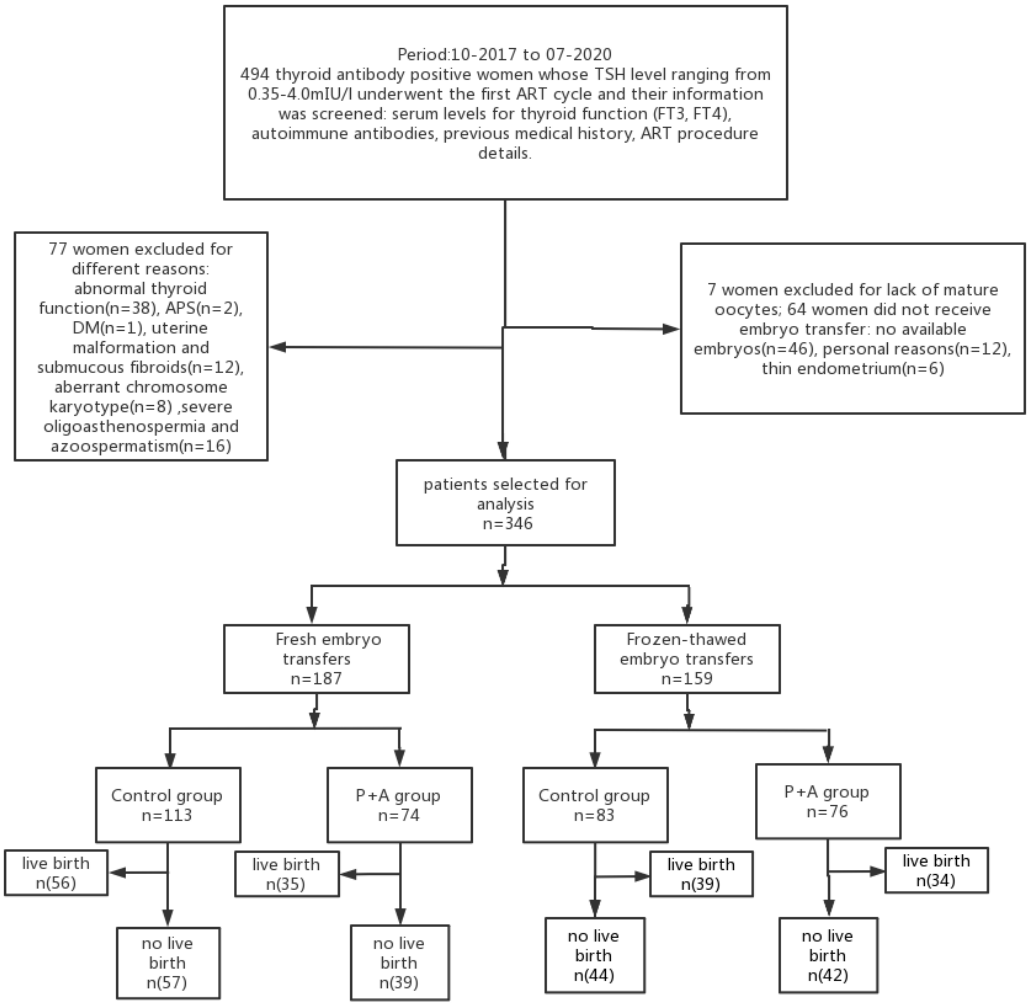

Figure 1

Flowchart illustrating the selection of the infertile women, their grouping and IVF outcomes

Supplementary Files

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