

# Thyroid autoantibodies *per se* do not impair intracytoplasmic sperm injection outcome in euthyroid healthy women

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## Abstract

**Objective:** Autoimmune thyroid disease (AITD) has been associated with adverse pregnancy outcomes in subfertile women with spontaneous and assisted reproductive technology-induced pregnancies. The underlying pathophysiology is still elusive and an association with thyroid dysfunction or other infertility causes is discussed. However, whether thyroid autoimmunity (TAI) *per se* has a negative impact on female fertility has not yet been clarified. In this study, we investigated whether TAI in healthy women undergoing intracytoplasmic sperm injection (ICSI) for male infertility may affect pregnancy outcome.

**Design:** A retrospective, single-centre study.

**Methods:** The ICSI outcome data obtained from 835 euthyroid women (age:  $31.4 \pm 4.3$  years, BMI:  $23.7 \pm 4.2$  kg/m<sup>2</sup>) were correlated with pre-ICSI TAI status. The known causes of female subfertility were excluded. Outcome parameters included rates of pregnancy, birth, miscarriage and preterm delivery. Blood analysis was carried out retrospectively using blood samples drawn before ICSI. TAI was defined by elevation of anti-thyroperoxidase- or anti-thyroglobulin-antibodies > 100 U/l.

**Results:** TAI-positive and -negative groups did not differ in age, BMI or TSH levels. TAI status did not influence any ICSI outcome parameters. In contrast, increasing maternal age was significantly correlated with lower pregnancy rate (odds ratio (OR): 0.94 (95% CI: 0.91–0.97);  $P=0.0003$ ) and birth rate (OR: 0.93 (95% CI: 0.09–0.97);  $P<0.0001$ ).

**Conclusions:** Our study suggests that TAI *per se* does not influence ICSI outcome. A strict definition of AITD and TAI and consideration of TAI-associated and -independent confounders are important to further elucidate the interplay between TAI and reproduction.

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## Introduction

Thyroid dysfunction and thyroid autoimmunity (TAI) are common findings in women of reproductive age. The prevalence of thyroid dysfunction is estimated to be 2–3%, with autoimmune thyroid disease (AITD) as the most frequent underlying cause (1). The term AITD is commonly used to describe an immunological effect on the thyroid gland characterised by lymphocytic infiltration and follicle destruction with typical ultrasound findings, presence of thyroid autoantibodies and variable degrees of

thyroid dysfunction. The term TAI, as used here, is a serological finding and refers to the presence of thyroid autoantibodies, i.e. anti-thyroperoxidase (TPO) or anti-thyroglobulin (Tg) antibodies. TAI is present in up to 15% of women of reproductive age (2) and is thus more frequent than thyroid dysfunction. Both AITD and TAI have been associated with adverse pregnancy outcomes (3), but the relevance of TAI in particular is not yet understood. Thus, it is far from clear whether there is a

causal link, such as a reduced thyroid functional reserve in TAI-positive women or whether other factors must be considered. For example, a direct interference of thyroid autoantibodies with normal placental function has been proposed (4). Furthermore, TAI may coexist with other (autoimmune) syndromes and a higher prevalence of TAI has been found in women with endometriosis or polycystic ovarian syndrome (PCOS) conditions, which themselves can hamper fertility and pregnancy outcome (5, 6, 7). Moreover, women seeking treatment for sub- or infertility tend to be older, and this *per se* influences fertility (8, 9, 10). Importantly, Krassas *et al.* (11) have recently highlighted in their excellent review that despite the vast study data, an interpretation of findings on TAI and reproduction is still hampered by weaknesses in study design and sample bias, such as focussing on women seeking help for sub- or infertility in fertility clinics rather than assessing the 'natural' effect of TAI on reproduction in the normal population. For these reasons, we designed the Dortmund intracytoplasmic sperm injection study (DICS), which includes a cohort of euthyroid healthy women receiving assisted reproductive technology exclusively for male infertility reasons.

## Subjects and methods

### Study design

**Inclusion** ► Totally, 1066 healthy women, aged 18–40 years, undergoing intracytoplasmic sperm injection (ICSI) for male infertility were included in the study. The female infertility factors such as PCOS, congenital adrenal hyperplasia, hyperprolactinaemia, primary and secondary ovarian insufficiency, endometriosis or tubal occlusion had previously been excluded in all women by personal interview, gynaecological examination, transvaginal ultrasonography, endocrine laboratory investigation, screening for infectious diseases and, whenever indicated, hysterosalpingography and/or laparoscopy. Weight was measured before starting ICSI treatment. Details about height, smoking status, information on thyroid disorders or intake of thyroid medication were obtained by a questionnaire at baseline. Age and BMI were calculated based on obtained data. Patients with reported thyroid disease and intake of thyroid medication, besides iodine supplementation before or during fertility treatment, and with incomplete questionnaire data, such as missing information on BMI or smoking status, were excluded from the analysis.

ICSI was carried out at a single centre (Dortmund Fertility Center) between 1997 and 2006 using the published institutional protocol for ICSI (12). All patients underwent their first ICSI procedure and only the outcome of the first ICSI cycle was analysed. Before ICSI, all patients were asked to provide a blood sample for the analysis of thyroid autoantibodies and thyroid-stimulating hormone (TSH) levels. The blood samples were analysed *post-hoc* in 2006 ensuring that the treating physician and the patient were blinded to the existence of TAI at the time of ICSI. Patients with newly diagnosed thyroid dysfunction in the *post-hoc* analysis were also excluded from further analysis.

The study protocol was approved by the Ethics Committee of the University of Witten-Herdecke.

**Blood sampling and biochemical analysis** ► The blood samples were obtained at a visit before ICSI and samples were stored at  $-80^{\circ}\text{C}$  until analysis. Automated chemiluminescence immunoassay systems were used for the determination of TSH and free thyroxine levels ( $\text{FT}_4$ ; ADVIA Centaur, Siemens, Munich, Germany), and TPO- and Tg-antibodies were determined immunometrically (IMMULITE 2000, Siemens). The reference ranges of TSH and  $\text{FT}_4$  were 0.3–3 mU/l and 11.5–22.7 pmol/l respectively. Assay-specific cut-offs for thyroid autoantibodies were  $\geq 35$  U/l (anti-thyroperoxidase antibodies; TPO-ab) and  $\geq 40$  U/l (anti-thyroglobulin antibodies; Tg-ab). An intra-assay variation was  $< 5\%$  and inter-assay variation was  $< 8\%$  for all measured variables. Biochemical analyses were carried out at the Division of Laboratory Research at the University Hospital Essen in 2006.

**Definition of thyroid dysfunction and TAI** ► Thyroid dysfunction was defined as a TSH above or below the reference range. TAI was defined as TPO- or Tg-ab levels  $> 100$  U/l.

**ICSI outcome parameters** ► The endpoints of ICSI outcome were clinical pregnancy rate (PR) (%), birth rate (BR) (%) vs miscarriage rate (MR) (%) as well as gestational age and the prevalence of preterm delivery (PD) and very preterm delivery (VPD) (%). PD and VPD were defined as birth before the 37th and 32nd week of pregnancy respectively.

### Statistical analyses

Statistical analyses were carried out using SAS (SAS Institute, Inc., Cary, NC, USA, version 9.2) and GraphPad Prism

**Table 1** Characteristics of TAI-positive vs TAI-negative women in DICI cohort. Data are given as mean  $\pm$  s.d. or percentage affected.

Parameter	TAI-positive women	TAI-negative women	P
Age (years)	31.5 $\pm$ 4.0	31.3 $\pm$ 4.4	NS
BMI (kg/m <sup>2</sup> )	23.4 $\pm$ 3.7	23.7 $\pm$ 4.3	NS
Smoking (%)	32.7	31.9	NS
TSH (mU/l)	1.7 $\pm$ 0.7	1.5 $\pm$ 0.6	0.0052
TPO-ab (U/l)	253.8 $\pm$ 295.5	10.2 $\pm$ 14.1	<0.0001
Tg-ab (U/l)	232.3 $\pm$ 392.9	17.8 $\pm$ 21.7	<0.0001

TPO-ab, thyroid peroxidase-antibodies; Tg-ab, thyroglobulin-antibodies; TSH, thyroid-stimulating hormone; NS, not significant.

(version 5.01; GraphPad Software, Inc., La Jolla, CA, USA) software. The correlation of TAI status with ICSI outcome parameters was analysed retrospectively by using a multiple regression analysis or independent-sample *t*-tests, Fisher's exact and  $\chi^2$  tests wherever appropriate. TSH, age, BMI, status of smoking and multiple pregnancies were used as covariates as these variables are known as potential influencing factors on ICSI outcome. Nominal two-sided *P* values, effect sizes estimates and 95% CI for the estimates were derived. GraphPad Prism was used for general statistics. Correlations between variables were assessed using Spearman's test and frequency distributions of dichotomous variables, by means of Fisher's exact test. All statistical tests were considered statistically significant when *P* levels were  $\leq 0.05$ .

## Results

### Epidemiology of the study cohort

Data were obtained from 1066 patients. In total, 231 patients were excluded retrospectively because of missing information in the questionnaire or newly diagnosed thyroid dysfunction in the *post-hoc* analysis of blood samples. Thus, a total of 835 patients (mean age 31.4  $\pm$  4.3 years) were included in the further analysis and formed the DICI study cohort (Table 1). The mean BMI in the DICI cohort was 23.7  $\pm$  4.2 kg/m<sup>2</sup>. In sum, 3.2% of patients were underweight and 7.3% were overweight. About 32.0% of patients were smokers and reported an average consumption of 13.0  $\pm$  7.0 cigarettes per day (range 1–30). Among the 835 included patients, none indicated autoimmune diseases in their personal medical history.

**TAI and thyroid function** ► TAI as defined by TPO- or Tg-ab levels >100 U/l was present in 110 patients (13%)

of the cohort. Among them, 23.6% showed elevation of both TPO- and Tg-ab. An elevation of either TPO- or Tg-ab was detected in 42.7 and 33.6% of TAI-positive patients respectively.

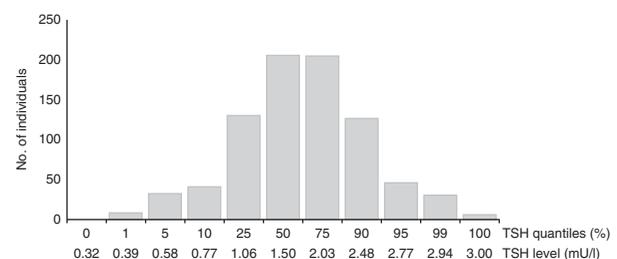
By definition, all patients in the DICI cohort were euthyroid (TSH levels 0.3–3.0 mU/l). Mean TSH levels were 1.6  $\pm$  0.6 mU/l (median: 1.5 mU/l, Table 1 and Fig. 1). Mean FT<sub>4</sub> levels were 16.4  $\pm$  2.4 pmol/l (median: 16.0 pmol/l). TSH level is higher in the TAI group but FT<sub>4</sub> levels were comparable between the TAI-positive and the TAI-negative group. Furthermore, age, BMI and smoking status did not differ between the TAI-positive and the TAI-negative group (Table 1).

**ICSI outcome** ► The overall PR was 41.4% (*n*=346/835). Ultrasound showed one gestational sac in 67.5%, two in 27.5% and three in 4.9% of treated women. Miscarriages occurred in 11.3% (*n*=39/346) of pregnancies with loss of one embryo in 33 cases and of two embryos in six cases. Pregnancy resulted in 315 births with 214 singletons (67.9%), 88 twins (27.9%) and 13 triplets (4.1%). The mean gestational age was 37.2  $\pm$  3.1 weeks. PD and VPD occurred in 31.1 and 4.7% of the offspring respectively.

### Correlation of TAI and covariates with ICSI outcome

**Thyroid autoimmunity** ► In women with TAI (*n*=110), PR was 40.9% (*n*=45). Of these 45 women, 11.1% had a miscarriage of at least one foetus and 95.6% delivered. In the TAI-negative group (*n*=275), PR was 41.5% (*n*=301). Of these 301 women, 5.5% had a miscarriage of at least one foetus and 90.4% delivered.

TAI-positive women delivered at 37.5  $\pm$  3.0 gestational weeks and PD and VPD occurred in 35.6 and 3.4% cases



**Figure 1**

TSH distribution in DICI cohort. The DICI study comprised 835 women aged 31.4  $\pm$  4.3 years undergoing ICSI for male infertility reasons. Data are given as TSH quantiles with numbers of individuals. The according TSH level (given in mU/l) is presented in the line below the TSH quantile.

**Table 2** Correlation of positive vs negative TAI status with ICSI outcome parameters. Data are given as OR and 95% CI or parameter estimate  $\pm$  s.d.

Parameter	OR (95% CI)	P
PR	1.00 (0.66–1.52)	0.9880
BR	1.10 (0.72–1.66)	0.6633
MR	0.46 (0.11–1.96)	0.2941
Gestational age	0.56 $\pm$ 0.40	0.1631
PD	1.23 (0.52–2.92)	0.6360
VPD	1.23 (0.52–2.92)	0.8312

PR, pregnancy rate; BR, birth rate; MR, miscarriage rate; PD, preterm delivery; VPD, very preterm delivery. Definition of TAI: presence of TPO or TG antibodies > 100 U/l.

respectively. TAI-negative mothers delivered at  $37.1 \pm 3.2$  gestational weeks and PD and VPD occurred in 30.4 and in 4.9% cases respectively. PR, BR and MR as well as prevalence of PD and VPD were statistically not different in both groups. Both analyses of TAI status as categorical variable (Table 2) and of TPO respectively, Tg-ab levels as continuous variables (data not shown) had no statistically significant influence on any examined ICSI outcome parameters.

**Age, BMI, smoking and ICSI-induced multiple pregnancies** ▶ Maternal age had a statistically negative influence on PR (odds ratio (OR): 0.942 (95% CI: 0.91–0.97);  $P=0.0003$ ) and BR (OR: 0.933 (95% CI: 0.90–0.97);  $P<0.0001$ ). Using age as an independent variable, the generalized additive model procedure provides a parameter estimate of  $-0.057$  ( $P=0.0007$ ) and  $-0.063$  ( $P=0.0003$ ) for PR and BR respectively. However, MR was not significantly affected by age. BMI or smoking status of the mother neither influenced PR or BR nor MR or neonatal outcome parameters such as PD and VPD. Of note is that the presence of multiple pregnancies vs singleton pregnancy negatively influenced the chance of PD (twins: OR: 6.2, 95% CI: 3.4–11.4,  $P<0.0001$ ; triplets: OR: 110.4, 95% CI: 13.6–898.6,  $P<0.0001$ ) and VPD (triplets: OR 18.4, 95% CI: 3.4–100.1,  $P=0.0007$ ) but did not influence PR, BR or MR.

## Discussion

The signs of TAI are common in women of childbearing age; the assessment of its impact on reproduction is of clinical relevance. Since the initial description of an association between TAI and miscarriage some 20 years ago (13), the relevance of thyroid antibodies on fertility has been widely discussed. Several meta-analyses showed a

high risk for negative pregnancy outcome in the presence of TAI in spontaneous as well as in assisted reproductive technology (ART)-induced pregnancies (14, 15, 16). A meta-analysis including studies of spontaneously pregnant women with unexplained subfertility or recurrent abortion showed an OR of 3.7 for miscarriage and an OR of 2.3 for recurrent miscarriage as well as a nearly doubled risk for PD (14). Another meta-analysis including eight case-control studies of women with recurrent abortion and 14 cohort studies including five IVF studies showed a higher prevalence of TAI in patients with recurrent abortions and a 2.3-fold higher MR in spontaneous and IVF-induced pregnancies (15). Furthermore, Toulis *et al.* (16) demonstrated a relative risk of 1.99 for miscarriage in IVF-induced pregnancies in subfertile women in a meta-analysis of four studies comprising 141 women with TAI and 957 controls. The shortcoming of these studies is, however, that the study population comprises a negative selection of female patients who seek treatment for impaired fertility. Hence, bystanders and confounders will be difficult to distinguish from true causal factors.

In the DICI cohort, we tried to avoid this likely bias by using the selection criterion of male infertility and by consequently studying the female partners of infertile men. In these women, known causes of female infertility had been excluded by a work-up programme suggesting that the DICI cohort may be close to the normal population. In this cohort, we did not find an association between presence of TAI and negative outcome of assisted reproductive techniques, but we confirm a significant inverse association between maternal age and ICSI success.

Our study has some limitations. First, even though all women had been investigated for female infertility before ICSI planning, we retrospectively identified a group of women in our *post-hoc* lab analysis (14.0%) that showed thyroid dysfunction at the start of ICSI. These women were excluded from further analysis and are thus not part of the DICI cohort. Secondly, this is a long-term study over 9 years. In this period, methodological improvements were implanted in ART procedures. This could be a reason for the good outcome in our study theoretically but its contribution remains speculative. Thirdly, one may argue that this is yet another retrospective study. However, our study design comprising correlation of retrospective data analysis with *post-hoc* endocrine laboratory analysis ensured that the ICSI procedure and the resulting pregnancy course could not have been affected by the knowledge of TAI status. Furthermore, the finding that age was identified as a strong confounder is reassuring. Interestingly, a difference in the mean age and mean TSH

in TAI-positive and TAI-negative women has been highlighted in two meta-analyses (15, 17), whereby TAI-positive women were 0.7–1.3-years older than the controls. Thus, age rather than TAI may be a relevant cause of miscarriage in TAI-positive women. As TAI-positive and TAI-negative women in our DICI cohort did not differ in age, it is not surprising that no 'TAI' effect was observed. This observation underscores the importance of confounder inclusion to avoid bias and erroneous data interpretation.

In summary, our study suggests that the presence of thyroid autoantibodies *per se* is not associated with negative outcome in ART such as ICSI. This may be a relief to TAI-positive patients worrying about a reduced fertility perspective because of positive thyroid autoantibodies. Furthermore, it is a warning to all eager physicians and may protect euthyroid healthy women, who wish to become pregnant, from unnecessary (over) treatment with L-T<sub>4</sub> because of positive thyroid autoantibodies. At the same time, our observations from the DICI study are by no means an argument to neglect AITD and TAI in a woman of childbearing age. On pathophysiological grounds, the thyroid functional reserve will be reduced in AITD and most likely also in some women with TAI. Along this line, the recent Rotterdam R study underscores that TPO-positivity is the crucial risk factor for development of hypothyroidism in pregnancy but only a small portion of the TPO-positive women will develop thyroid dysfunction in pregnancy (18). This warrants special attention, and for the time being, the Endocrine Society's and the ATA's recent recommendations of an upper TSH limit of 2.5 mU/l in women wishing to start a family and in the first trimester may provide some helpful guidance for initiation and adjustment of L-T<sub>4</sub> treatment (19, 20). What we thus need is a better understanding and subsequent definition of TAI. This will be a pivotal prerequisite to unravel the role of TAI in fertility and reproduction.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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