

Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study

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BACKGROUND: Infertile women positive for thyroid antibodies suffer from a poor pregnancy/delivery outcome, although conflicting data have been published. Our objective was to investigate if levothyroxine (LT4) exerts any effect on pregnancy and/or delivery rates in thyroid peroxidase antibody (TPOAb)-positive (+) women undergoing assisted reproductive technologies. **METHODS:** Patients undergoing treatment were screened for TPOAb, thyroid-stimulating hormone (TSH) and free thyroxine (FT4). A total of 72 (15%) out of the 484 euthyroid women selected were TPOAb (+). These 72 patients were randomly divided into two groups: group A ($n = 36$) underwent LT4 treatment, group B ($n = 36$) placebo. Group C consisted of 412 women (85%) who were TPOAb negative (–). All patients received controlled ovarian stimulation. The endpoints of treatment were pregnancy rate, miscarriage rate and delivery rate. **RESULTS:** No differences in pregnancy rate were observed between the three groups. Miscarriage rate was higher in TPOAb (+) in comparison to TPOAb (–) [relative risk: 2.01 (95% CI = 1.13–3.56), $P = 0.028$]. **CONCLUSIONS:** The pregnancy rate is not affected either by presence of TPOAb or treatment with LT4. However, TPOAb (+) women show a poorer delivery rate compared to TPOAb (–). LT4 treatment in TPOAb (+) does not affect the delivery rate.

Key words: assisted reproductive technologies/autoimmunity/levothyroxine/thyroid

Introduction

The prevalence of infertile couples in European countries is estimated to be between 10 and 25%. Of these, 45% are due to female disorders, which include endometriosis, tubal disease, and ovulatory dysfunction (Poppe *et al.*, 2002).

In infertile women, the prevalence of those positive for thyroid peroxidase antibodies [TPOAb (+)] is higher than their fertile control (Poppe *et al.*, 2002). The pregnancy rate of those undergoing assisted reproduction technologies appears to be significantly lower in subjects who are positive for organ-specific autoantibodies such as antithyroid and antiovarian antibodies (Geva *et al.*, 1996). Furthermore, women who are positive for thyroid antibodies (either anti-peroxidase or anti-thyroglobulin) show an increased miscarriage rate (Kim *et al.*, 1998) and a poor pregnancy/delivery outcome (although conflicting data have been published on this subject) (Kutteh *et al.*, 1999). The issues raised are whether positive thyroid autoantibodies in patients undergoing assisted reproduction

technologies are: (i) only a marker of autoimmunity; (ii) directly responsible for reduced pregnancy and/or delivery rate; or (iii) an indirect sign of a mild thyroid dysfunction.

Aim of the study was to investigate if the poor pregnancy and delivery outcome of TPOAb (+) women may be due to a mild thyroid dysfunction and also whether levothyroxine (LT4) treatment may improve this situation.

Materials and methods

A prospective analysis was undertaken in infertile women (mean \pm SD age 30.2 ± 4 years; range 20–45), who had to undergo a first assisted reproduction technology procedure between January 1999 and January 2003. The study was carried out at the Division of Physiopathology of Human Reproduction. Causes of infertility were of female origin in 57% of cases; they comprised ovarian dysfunction (36%), tubal factors (28%), endometriosis (17%) and idiopathic (19%). All patients were screened for the presence of TPOAb, serum TSH and free thyroxine (FT4) before undergoing assisted

reproduction technologies. Women with overt thyroid dysfunction were excluded. Seventy-two (15%) out of the 484 women selected were TPOAb (+). These 72 patients were divided into two groups, an intervention group treated with LT4 and another group with placebo. Medical doctors attended different phases of the protocol so that each was blinded to which group the patients belonged. A computer program was used to randomly assign the patients to one or the other group (T.M.). A sealed opaque envelope was assigned to each patient and only the doctor (R.N.) applying the treatment knew which group each patient had been assigned to, and did not participate in any subsequent phase of the study (L.C. and G.P. for assisted reproduction technologies; E.C.C., R.G., G.L. and P.C. for visits during pregnancy; H.H. for laboratory tests; A.P., D.D. for data analyses). Subsequently, 1 month before assisted reproduction technologies, group A (*n* = 36) underwent LT4 treatment (1 mg/kg/day), group B (*n* = 36) placebo. Group C was composed of the 412 women (85%) who were TPOAb (–). In group A, LT4 treatment was maintained throughout pregnancy. In this group, thyroid function tests were checked 1 month after treatment, before assisted reproduction technologies was begun.

The Institutional Review Board approved the study protocol and all the participants gave written informed consent. All study patients completed the protocol.

All patients underwent ovulation induction. Recombinant FSH (rFSH) (Puregon; NV Organon, The Netherlands) and GnRH antagonist (Orgalutran; NV Organon) were used for ovarian stimulation. rFSH was started on day 2 of the menstrual cycle at 200 IU per day; the dosage remained the same in all patients during stimulation. Ovulation triggering was performed using 10 000 IU of hCG (Pregnyl; NV Organon) as soon as at least three 17 mm follicles were present on ultrasound scan. Using conventional IVF, each oocyte was inseminated within 3–4 h after retrieval by adding 5000–20 000 motile sperm per oocyte. If the semen sample on the day of oocyte recovery contained few motile sperm, ICSI was performed. For each patient, six or seven oocytes were retrieved by the vaginal route and after fertilization one to three embryos were transferred depending on their morphological quality. Pregnancy was diagnosed on two occasions ≥10 days after transfer by rising serum hCG levels of

≥20 IU/ml. Clinical pregnancies were diagnosed by ultrasonography performed 5 weeks after embryo transfer. The endpoints of assisted reproduction technologies were pregnancy rates, miscarriage rates and delivery rates. Miscarriage rates also included early pregnancy loss (biochemical pregnancies).

Serum TSH and FT4 were measured using a third-generation electrochemiluminescence immunoassay (Roche, Germany). The reference values were 0.27–4.2 mIU/l for TSH and 9.3–18.0 ng/l (12–33.5 pmol/l) for FT4. TPOAb were determined using a radioimmunoassay kit (B.R.A.H.M.S. Diagnostica, Germany). The reference range was 0–100 kIU/l. TPOAb titres >100 kIU/l were considered positive.

Statistical analysis was performed using an SPSS (SPSS, Inc., USA) program, by means of Fisher’s exact test. Correlations between variables were assessed using Spearman’s test, and differences between mean values were determined by the Mann–Whitney *U*-test. A multivariate approach was used, starting with a univariate model for each individual variable. All statistical tests were considered statistically significant whenever *P* < 0.05.

Results

Clinical characteristics of patients are shown in Table I. There were no differences between groups concerning the number of oocytes retrieved and embryos transferred. Distribution of causes of female infertility were not different between groups.

After 1 month of treatment with LT4, TSH was lower in group A (1.1 ± 0.3 mIU/l) compared to group B (1.7 ± 0.7 mIU/l) (*P* = 0.011) and group C (1.6 ± 0.6 mIU/l) (*P* = 0.033); and FT4 was higher in group A (14.1 ± 2.5 ng/l) compared to group B (11.7 ± 2.1 ng/l) (*P* = 0.018) and group C (12.2 ± 1.9 ng/l) (*P* = 0.031).

Outcomes for patients (pregnancy rate, delivery rate and miscarriage rate) are shown in Figure 1.

Table I. Characteristics of patients. Age, thyroid parameters and outcome of the women submitted to ART; Group A: TPOAb (+) before and after 1 month treatment with levothyroxine; Group B: TPOAb (+) not treated; Group C: TPOAb (–)

	Total	Group A		Group B	Group C
	(<i>n</i> = 484)	TPOAb (+) before LT4 (<i>n</i> = 36)	TPOAb (+) on LT4 (<i>n</i> = 36)	TPOAb (+) (<i>n</i> = 36)	TPOAb (–) (<i>n</i> = 412)
Age (years) ^a	30.2 ± 4	29.2 ± 4	29.2 ± 4	30.1 ± 5	30.4 ± 5
Thyroid-stimulating hormone (mIU/l) ^a	1.6 ± 0.8	1.9 ± 0.7	1.1 ± 0.3	1.7 ± 0.7	1.6 ± 0.6
Free thyroxine (ng/ml) ^a	12.0 ± 2.0	11.2 ± 1.8	14.1 ± 2.5	11.7 ± 2.1	12.2 ± 1.9
Deliveries ^a	0.05 ± 0.16		0.06 ± 0.16	0.04 ± 0.15	0.05 ± 0.16
Miscarriages ^a	1.75 ± 0.73		1.86 ± 0.76	1.75 ± 0.73	1.94 ± 0.74
Ovarian dysfunction, <i>n</i> (%)	174 (36)		11 (31)	13 (36)	150 (37)
Tubal factors, <i>n</i> (%)	136 (28)		10 (28)	9 (25)	117 (28)
Endometriosis, <i>n</i> (%)	82 (17)		7 (19)	9 (25)	66 (16)
Idiopathic, <i>n</i> (%)	92 (19)		8 (22)	5 (14)	79 (19)
Oocytes retrieved ^a	6.1 ± 0.76		6.19 ± 0.74	6.08 ± 0.79	6.05 ± 0.79
Oocytes transferred ^a	2.51 ± 0.68		2.61 ± 0.64	2.52 ± 0.69	2.5 ± 0.67

^aValues are mean ± SD.
TPOAb = thyroid peroxidase antibody; (+) = positive; (–) = negative; LT4 = levothyroxine.
¹years.
²mU/liter (0.05–3.6).
³ng/liter (9.1–18.0).
⁴Number of deliveries before ART.
⁵Number of miscarriages before ART.
⁶Cause of female infertility.

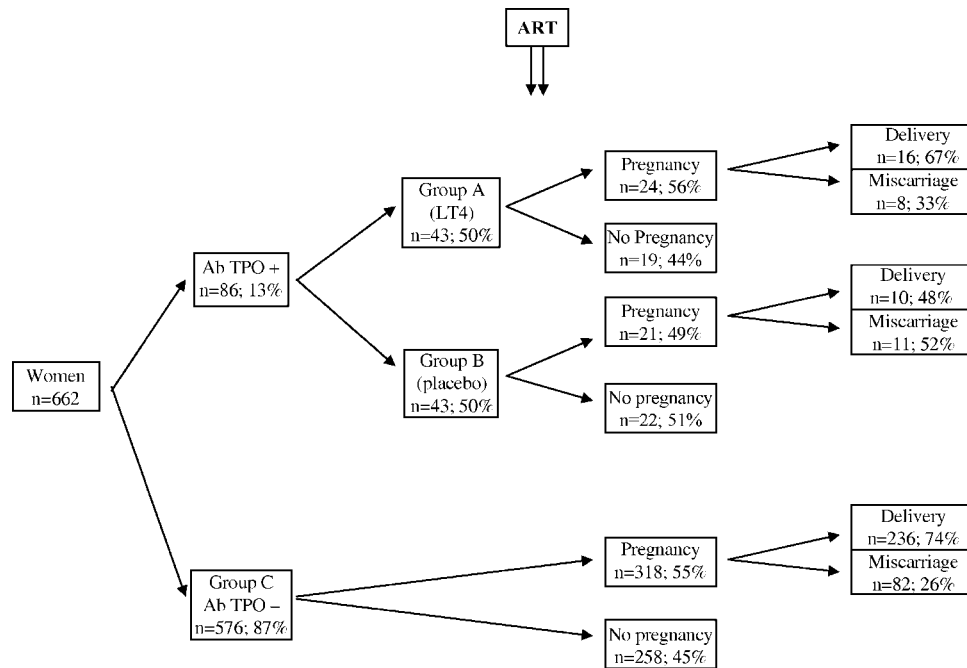


Figure 1. Outcome of ART in women with and without thyroid autoimmunity.

Pregnancy rate

Our study showed that pregnancy rate was not significantly influenced by autoimmunity. The pregnancy rate was comparable between TPOAb (+) (groups A + B) and TPOAb (–) women. There was no difference in pregnancy rate between groups A and B. LT4 treatment did not influence the pregnancy rate in TPOAb (+) women.

Miscarriage rate

The miscarriage rate was significantly higher in TPOAb (+) women in comparison to TPOAb (–) women [relative risk: 2.01 (95% CI 1.13–3.56), $P = 0.028$]. There was a significantly increased miscarriage rate in group B compared to group C. The risk of pregnancy loss was doubled in the former group (the group not subjected to LT4 treatment) compared to the latter [relative risk: 1.89 (95% CI 1.2–3.2), $P = 0.034$].

Discussion

To summarize the main findings, in the present prospective study we found no difference in the pregnancy rates of women with or without thyroid autoantibodies. An increased miscarriage rate was present in TPOAb (+) compared to TPOAb (–) women, but TPOAb (+) women treated with LT4 showed no difference in miscarriage rate when compared to those TPOAb (–).

Our data suggest that the presence of thyroid autoimmune disease should be taken into consideration when approaching investigation of female infertility. However, since the prevalence of TPOAb observed in our study population was not different from that of the general population (Knudsen *et al.*, 1999; Pedersen *et al.*, 2003) we suggest that thyroid autoim-

munity may not be a cause of infertility *per se* because its prevalence would have otherwise been higher. Poppe *et al.* (2002) reported a non-significantly higher number of TPOAb (+) women among infertile women in whom elevated TSH values represent a strong factor against fertility.

In our study, the pregnancy rates were not different between TPOAb (+) and TPOAb (–) women, while thyroid autoimmunity appeared to significantly influence the miscarriage rate. These findings confirm those of Poppe *et al.* (2003), who showed that women with positive TPOAb before the first assisted reproduction treatment cycle have a significantly increased risk for miscarriage but a similar pregnancy rate when compared to those without TPOAb. The underlying mechanisms for this are unclear, but three hypotheses have been proposed: (i) the miscarriage seen in women with thyroid antibodies may be due to subtle deficiency of thyroid hormone; (ii) there may be a direct effect(s) of thyroid autoantibodies on the placenta; (iii) thyroid autoantibodies may only be markers of an abnormal immune state, responsible for unstable implant (Abramson and Stagnaro-Green, 2001). Conflicting data exist on the association between thyroid autoimmunity and pregnancy (Van Voorhis and Stovall, 1997). Some authors have found no correlation between TPOAb and the incidence of recurrent abortions (Muller *et al.*, 1999; Rushworth *et al.*, 2000). A study by Kutteh *et al.* (1999) has shown that the number of pregnancies, miscarriages and deliveries were not different in thyroid Ab (+) and (–) women. Bussen and Steck (1997) have found that the incidence of thyroid autoantibodies in women with recurrent miscarriages was significantly increased compared to controls. Some studies have confirmed the association between recurrent miscarriages and autoimmunity, in particular by showing an increased number of CD5⁺/20⁺ positive

cells and an abnormal T helper (Th-1) preponderance (Roberts *et al.*, 1996; Gleicher, 2002). Thus, the presence of TPOAb may only be a marker of autoimmunity, whereas the presence of a peripheral marker for abnormal T cell function may be responsible for a poor pregnancy outcome (Stagnaro-Green *et al.*, 1992).

In the same context, subclinical hypothyroidism can also exert a negative influence on early pregnancy (Abalovich *et al.*, 2002; Vaquero *et al.*, 2002). Some studies have shown that thyroid hormones may play a determinant role in the physiology of early pregnancy and that LT4 treatment of infertile women may improve the conception rate (Maruo *et al.*, 1992; Raber *et al.*, 2003). A meta-analysis performed by Prummel and Wiersinga (2004), who looked at the association between thyroid autoimmunity and miscarriage rates, suggested that the increased miscarriage rate in TPOAb (+) women may be due to mild thyroid failure, as serum TSH concentrations in TPOAb (+) women are higher than in TPOAb (–) women (Marx and Bucher, 2003; Prummel and Wiersinga, 2004). Calvo *et al.* (2002) have shown differences between the maternal thyroid status and the fetal concentrations of thyroid hormones. The fetal compartment shows 100-fold lower serum concentrations of T3 and T4 compared to the mother and serum FT4 concentrations reach values that are about one-third of those biologically active in the respective euthyroid mothers (Calvo *et al.*, 2002). As a consequence, even slight reduction in maternal serum FT4 concentrations may cause a relevant and significant decrease in fetal serum FT4 concentrations. Furthermore, it has been shown that, in euthyroid women undergoing assisted reproduction treatment, controlled ovarian stimulation during early pregnancy leads to a decrease in serum FT4 and a concomitant rise in serum TSH concentrations (Muller *et al.*, 2000).

Lastly, the presence of thyroid autoimmunity may be associated with an increased incidence of miscarriage rate due to the phenomenon of microchimerism (Ando and Davies, 2004). Fetal microchimerism is defined as the presence of fetal cells in maternal tissues occurring during pregnancy. It involves transplacental passage of fetal cells into the maternal thyroid and may be one mechanism explaining thyroid disorders and an enhanced immune response against the fetoplacental unit (Ando and Davies, 2003). The study by Imaizumi *et al.* (2001) has shown that in murine experimental autoimmune thyroiditis, microchimerism is responsible for increased miscarriage rate without differences in thyroid function between the control mice and the Tg-immunized pregnant mice. In addition, thyroid autoantibodies were associated with higher miscarriage rates. This study was carried out in mice but suggests that microchimerism in humans may be involved in the setting of autoimmunity and hypothyroidism.

In summary, our study shows that in women undergoing assisted reproduction technologies: (i) the pregnancy rate is not influenced by either the presence of TPOAb or treatment with LT4; (ii) the miscarriage rate is greater in TPOAb (+) compared to TPOAb (–) women; (iii) LT4 treatment in TPOAb (+) women does not improve the delivery

rate. Thyroid autoimmunity represents a risk factor for miscarriage, which appears to be linked to an abnormal immune response rather than to subsequent mild thyroid failure.

Acknowledgements

We are grateful to Dr Mario Salvi for his skilful revision of the manuscript.

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Submitted on December 17, 2004; resubmitted on February 8, 2005; accepted on February 15, 2005