

## REVIEW

# Risk of spontaneous miscarriage in euthyroid women with thyroid autoimmunity undergoing IVF: a meta-analysis

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## Abstract

**Objective:** To investigate whether thyroid autoimmunity (TAI) is associated with increased risk for spontaneous miscarriage in subfertile, euthyroid women undergoing IVF.

**Design:** Meta-analysis of observational studies.

**Patient(s):** Four prospective studies that reported data on 1098 subfertile women undergoing IVF (141 with TAI and 957 controls) were included in the meta-analysis.

**Main outcome measure:** Miscarriage risk ratio (RR).

**Secondary outcome measures:** Clinical pregnancy rate and delivery rate.

**Result(s):** Euthyroid, subfertile women with TAI undergoing IVF demonstrated significantly higher risk for miscarriage compared with controls (four studies—fixed effects RR: 1.99, 95% confidence interval: 1.42–2.79,  $P < 0.001$ ). No significant difference in clinical pregnancy and delivery rates was detected between groups.

**Conclusion:** Based on the currently available evidence, it appears that the presence of TAI is associated with an increased risk for spontaneous miscarriage in subfertile women achieving a pregnancy through an IVF procedure.

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## Introduction

The association between spontaneous miscarriage and thyroid autoimmunity (TAI) defined as the presence of autoantibodies against thyroid peroxidase (TPOab) and/or thyroglobulin (TGab) was initially reported by Stagnaro-Green *et al.* (1), subsequently investigated in numerous studies and finally confirmed by two meta-analyses (2, 3). Causality remains unclear; co-presence of TAI with other autoimmune syndromes, direct action of anti-TPO and TGab on placenta, hampered adaptability of the thyroid gland to the increased demands of pregnancy in the presence of TAI and higher age of women with TAI have all been implicated. The obscure pathophysiology is probably one of the reasons why adequate treatment strategies have not yet been developed. A well-designed randomized controlled trial, in which sodium levothyroxine ( $l$ -T<sub>4</sub>) supplementation was associated with a risk of miscarriage similar to that observed in healthy controls (4), needs to be confirmed before  $l$ -T<sub>4</sub> supplementation could be routinely recommended in the course of a TAI-positive pregnancy (5).

Although devastating on a personal level, pregnancy loss could be regarded as a natural outcome of an inefficient process, considering that only ~23% of conceptions result in live births (6). However, pregnancy outcome becomes a matter of copious and costly efforts in subfertile women undergoing artificial reproduction techniques (ART), where any factor that could potentially affect the outcome should be adequately analyzed and carefully considered. Currently, it is not clear whether there is an association between TAI and poor IVF outcome (5, 7). Several studies which examined the association between TAI status and miscarriage provided rather controversial results (8–18), although it appears that 'TAI *per se* does not alter the implantation of embryo' (19). Moreover, observations regarding TAI and miscarriage risk from the general population could not be applied to an IVF setting and vice versa, since women undergoing IVF are considered as a distinct subgroup, representing a 'special population' (20). An answer to whether the presence of thyroid autoantibodies is associated with an increased risk for miscarriage in IVF might assist the IVF counseling in women with TAI. For that purpose,

a meta-analysis of observational studies evaluating this research question was performed.

In short, the ideal study should be prospective in design, include only first-time ART users and analyze only first cycle ART outcomes, measure both TPOab and TGA in all the subjects before ART procedure, control for the possible confounding effect of other autoantibodies and/or causes of spontaneous miscarriages and involve euthyroid, without TAI, age-matched women undergoing IVF as controls.

## Methods

### Search strategy

A preliminary search was conducted in the electronic database MEDLINE on various combinations of the terms 'thyroid gland' (MeSH), 'thyroid microsomal antibodies' (substance name), 'anti-thyroglobulin' (substance name), 'autoantibodies' (MeSH), 'thyroiditis, autoimmune' (MeSH), 'thyroid diseases', 'IVF' (MeSH) and 'reproductive techniques, assisted' (MeSH) in order to evaluate the size of the relevant literature and orientate to the keywords which would be used in the main search. To identify eligible studies, the main search was conducted in the electronic databases MEDLINE and EMBASE from inception through to June 2009 and was restricted to English literature. The terms used for the MEDLINE search were ('autoantibodies' (MeSH) and 'thyroid gland' (MeSH)) or 'thyroid diseases' (MeSH) and 'reproductive techniques, assisted' (MeSH). The procedure was concluded by the perusal of the reference sections of all relevant studies or reviews and a manual search of key journals and abstracts from the major annual meetings in the field of reproduction. The main search, as well as screening of titles, abstracts, and full-text articles, was completed independently by two reviewers (K T and D G). Any discrepancy was solved unanimously by discussion.

### Eligibility of relevant studies

Studies eligible for the systematic review were those that reported data on IVF outcome in subfertile women whose TAI status had been determined, independently of design (prospective or retrospective, IVF protocol). Exclusion criteria were i) recurrent miscarriages during IVF, ii) subfertile women with known autoimmune disease other than TAI, iii) no control group or control group other than women without TAI who underwent IVF, iv) ovulation induction without IVF and v) reviews or letters to the editors.

To ensure interpretable data synthesis, a study was included into the meta-analysis, only if i) it was prospective in design, ii) euthyroid women had been enrolled, iii) TAI and control groups were comparable in age and iv) follow-up period lasted at least until the end of the first trimester of pregnancy. Studies in which it

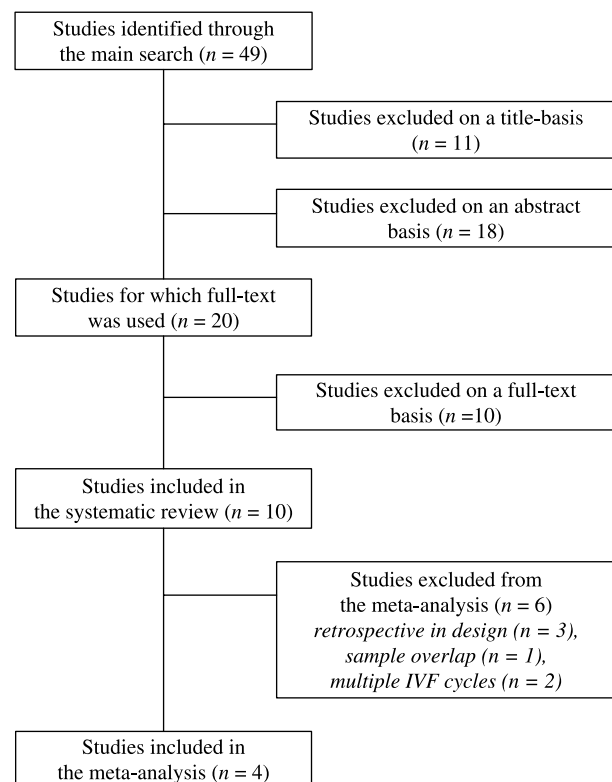
was clearly stated that analysis included multiple-cycle IVF outcomes were not considered eligible for the meta-analysis.

### Data extraction

Information from each study was extracted independently by two reviewers (K T and D G), using a standardized data extraction form. General characteristics of the study (author, year of publication, country, study design, study period, sample size), characteristics of the study groups, their comparability on baseline characteristics (age, body mass index, thyroid function tests), methodology (IVF protocol, number of oocytes retrieved and embryos transferred, biochemical and clinical pregnancy definition, thyroid autoantibodies measurement method, threshold and time of measurement, study quality), and outcomes (biochemical and clinical pregnancies, deliveries, miscarriages) were recorded, where available, and double-checked. Where appropriate, an effort was made to complete the data set through communication with the authors.

### Outcomes

The main outcome of the meta-analysis was the miscarriage risk, which was defined as the loss of



**Figure 1** Flow chart for the systematic review and the meta-analysis.

**Table 1** Characteristics of the studies considered for the meta-analysis.

Author (year) country	Design, sample size, study period	Inclusion criteria, subject characteristics	Study groups (control and TAI <sup>a</sup> )	ART protocol, pregnancy definition	Comparability between groups	Type, method, threshold and time of Ab assay (IU/ml)
Kilic <i>et al.</i> (2008) Turkey	Prospective, cohort, single-center including 69 euthyroid, subfertile women undergoing ART, from Jan 2006 to Feb 2007	Women with unexplained infertility, first ART procedure, with no history of miscarriage, thyroid nodules or systemic disease	n = 31 control n = 23 TAI, euthyroid without treatment, n = 15 TAI, euthyroid on treatment	Standard long protocol <sup>b</sup> , leuprolide, gonadotropins, hCG, ICSI, ET Biochemical pregnancy: $\beta$ -hCG > 50 IU/ml (12th day of ET) Clinical pregnancy: U/S fetal cardiac activity (4–6th week after ET)	No difference in age, BMI, FT <sub>4</sub> , TSH, PRL, FSH, LH levels, number of oocytes retrieved, fertilized oocytes and embryos transferred	TPOab and TGab, ECL immunoassay, threshold > 34 (TPOab) and > 115 (TGab), time not clearly stated
Negro <i>et al.</i> (2007) Italy	Retrospective, cohort, single-center including 416 euthyroid, subfertile women that underwent ART, from Jan 2000 to Jan 2006	Subfertile women < 35 years (various etiology), without overt thyroid dysfunction, first ART procedure, male factor etiology 50%	n = 374 control n = 42 TAI (positive TPOab)	Controlled ovarian superovulation, conventional IVF, r-FSH, GnRH antagonist, hCG, ICSI Biochemical pregnancy: $\beta$ -hCG > 20 IU/ml (10th day of ET) Clinical pregnancy: U/S fetal cardiac activity (5 weeks after ET)	No difference between groups in age, number of oocytes retrieved and embryos transferred TSH levels higher in TPOab(+) group	TPOab only, RIA, threshold > 100, before ART and during pregnancy
Negro <i>et al.</i> (2005) Italy	RCT within prospective, cohort, single-center including 662 euthyroid, subfertile women undergoing ART, from Jan 1999 to Jan 2003	Subfertile women < 35 years (various etiology), without overt thyroid dysfunction, first ART procedure	n = 576 control n = 86 TAI (positive TPOab), 43 on L-T <sub>4</sub> and 43 on placebo	Controlled ovarian superovulation, conventional IVF, ICSI, GnRH antagonist, u/r-FSH, hCG, ICSI Biochemical pregnancy: $\beta$ -hCG > 20 IU/ml (10th day of ET) Clinical pregnancy: U/S fetal cardiac activity (5 weeks after ET)	No difference between groups in age, number of oocytes retrieved and embryos transferred After 1 month, TSH lower and FT <sub>4</sub> higher in TPOab(+) group on L-T <sub>4</sub>	TPOab only, RIA, threshold > 100, before ART and during pregnancy
Poppe <i>et al.</i> (2004) Belgium	Prospective, cohort, single-center including 69 euthyroid, subfertile women undergoing ART (recruitment period not clearly stated)	Only euthyroid women with an ongoing pregnancy after OH were included (infertility of various etiology), not clearly stated whether first ART procedure	n = 27 control n = 9 TAI (positive TPOab)	Controlled ovarian superovulation, conventional IVF, ICSI, GnRH antagonist, u/r-FSH, hCG, ICSI Biochemical pregnancy: $\beta$ -hCG > 20 IU/ml (at least 10th day of ET) Clinical pregnancy: U/S fetal cardiac activity (5 weeks after ET)	No difference between groups in age, TSH, FT <sub>4</sub> , and serum hCG levels at different times	TPOab only, RIA, threshold > 100, before ART and during pregnancy

Table 1 Continued

Author (year) country	Design, sample size, study period	Inclusion criteria, subject characteristics	Study groups (control and TAI <sup>a</sup> )	ART protocol, pregnancy definition	Comparability between groups	Type, method, threshold and time of Ab assay (IU/ml)
Poppe <i>et al.</i> (2003) Belgium	Prospective, cohort, single-center including 234 subfertile women undergoing ART from Oct 1999 to Nov 2000	Subfertile women (various etiology) without overt thyroid dysfunction, first ART procedure, male factor etiology 50%, restricted to first cycle (clearly stated)	n = 202 control n = 32 TAI (positive TPOab)	Controlled ovarian superovulation, conventional IVF, ICSI Biochemical pregnancy: $\beta$ -hCG > 20 IU/ml (10th day of ET) Clinical pregnancy: U/S fetal cardiac activity (5 weeks after ET)	No difference between groups in age, TSH, FT <sub>4</sub> levels, number of embryos transferred	TPOab only, RIA, threshold > 100, before ART
Kutteh <i>et al.</i> (1999) USA	Retrospective, multi-center, cohort including 873 subfertile women who had undergone ART from Apr 1996 to Apr 1997	Subfertile women (various etiology) without thyroid dysfunction, restricted to first cycle (clearly stated), women with > 1 prior miscarriage or known AI disease were excluded	n = 730 control n = 143 TAI (also included 200 normal women)	Standard protocol, GnRH agonist, gonadotrophins, hCG, ET, ICSI Definition of pregnancy not clearly stated	Abnormal TSH values in 43 out of 730 TAI (-) (5.9%) and 27 out of 143 TAI (+) (18.9%)	TPOab and TGab, ELISA, threshold > 65 (TPOab) and > 120 (TGab), frozen sera before ART
Muller <i>et al.</i> (1999) The Netherlands	Prospective, nested case-control study including 173 subfertile women undergoing IVF from Mar 1994 to Mar 1996	Subfertile women (various etiology), women with a history of miscarriage were excluded, not clearly stated whether first ART procedure	n = 148 control n = 25 TAI (positive TPOab)	ART protocol described in a previous publication Biochemical pregnancy: hCG > 25 U/ml (18th day after ET) Clinical pregnancy: vaginal U/S on positive hCG, 2 weeks later	No differences in age, TSH, cause of infertility, number of previous pregnancies and deliveries, miscarriage rate and smoking habits	TPOab only, RIA, threshold > 80, frozen sera at initial visit and during pregnancy
Kim <i>et al.</i> (1998) Korea	(Unclear design), cohort, single-center including 79 euthyroid women from Oct 1995 to Nov 1996	Subfertile, euthyroid women (unexplained or tubal factor), women positive for ANA, ACA, LpA, Rf were excluded, not clearly stated whether first ART procedure	n = 51 control n = 28 TAI (9 positive TPOab alone, 7 positive TGab alone, 12 positive both TPOab and TGab)	Multiple cycles, standard long protocol <sup>b</sup> Biochemical pregnancy: $\beta$ -hCG > 10 IU/ml, rising titres, no gestational sac on U/S Clinical pregnancy: U/S fetal cardiac activity	No difference in age, TSH, FT <sub>4</sub> and infertility duration, number of oocytes retrieved and embryos transferred	TPOab and TGab, radioligand assay, threshold > 100 (for both), measured on the day that superovulation began
Geva <i>et al.</i> (1996) Israel	Prospective, cohort, single-center including 78 subfertile women from Jan 1993 to Dec 1994	Subfertile, euthyroid women (unexplained or mechanical), with no history of miscarriage, first ART procedure	n = 55 control n = 16 TAI (also another 7 women with anti-ovarian Ab alone)	Multiple cycles, controlled OS, GnRH agonists, hMG, definition of pregnancy not clearly stated	No difference in age, etiology and duration of infertility, number of oocytes retrieved, number of hMG ampoules per cycle, number of OI per cycle	TPOab and TGab, indirect passive agglutination assay, threshold > 1:1600 (TPOab) and > 1:400 (TGab), samples 1 day before OS

Table 1 Continued

Author (year) country	Design, sample size, study period	Inclusion criteria, subject characteristics	Study groups (control and TAI <sup>a</sup> )	ART protocol, pregnancy definition	Comparability between groups	Type, method, threshold and time of Ab assay (IU/ml)
Singh <i>et al.</i> (1995) USA	Retrospective, cohort, single-center including 487 subfertile women from Jan 1991 to Dec 1992 who had undergone ART	Subfertile women (various etiology) who had a $\beta$ -hCG > 5 IU/ml, 19 women had $\geq 3$ miscarriages, no data on previous ART, 18 subjects on L-T <sub>4</sub> replacement, 9 with positive TPOab and/or TGab	n = 381 control n = 106 TAI	ART protocol not reported Biochemical pregnancy: $\beta$ -hCG increasing in two subsequent measurements, no further details Clinical pregnancy: presence of gestational sac, no further details	No difference in age, gravidity, etiology of infertility or number of prior miscarriages	TPOab and TGab, EIA semi-quantitative, threshold > 3.8, stored frozen sera 14 days after ET

ACA, anticardiolipin autoantibody; AI, autoimmune; ANA, antinuclear autoantibody; ART, assisted reproduction techniques; ECL, electrochemiluminescence; EIA, enzyme immunoassay; ET, embryo transfer; FT<sub>4</sub>, free thyroxine; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin (75 IU FSH + 75 IU LH); ICSI, intra-cytoplasmic sperm injection; IU, international units; LpA, lupus anticoagulant; L-T<sub>4</sub>, levothyroxine; OI, ovulation induction; OS, ovarian stimulation; PRL, prolactin; RCT, randomized controlled trial; RF, rheumatoid factor; TAI, thyroid autoimmunity; TGab, thyroglobulin autoantibodies; TPOab, thyroid peroxidase autoantibodies; TSH, thyroid stimulating hormone; U/S, ultrasonography.  
<sup>a</sup>TAI refers to positive TPOab and/or TGab.  
<sup>b</sup>Standard long protocol: superovulation with the use of oral contraceptives and GnRH-agonists or antagonists for down-regulation, gonadotropins for ovarian stimulation, hCG for ovulation triggering, ICSI, ET, luteal progesterone support.

pregnancy after a positive confirmation, either biochemical or clinical. In order to gain a more thorough perception of IVF outcome in women with TAI, clinical pregnancies (ultrasound confirmed fetal cardiac activity, 5 weeks after embryo transfer (ET)) and deliveries served as secondary outcome measures.

**Statistical analysis**

As a dichotomous outcome, miscarriage risk in each study was expressed as risk ratio (RR) with 95% confidence interval (CI) and combined using a fixed effects model and Mantel–Haensel method as the weighting scheme. When significant heterogeneity was detected, random effects model was preferred. Secondary outcomes were analyzed similarly.

Heterogeneity between the results of different studies was examined by *I*<sup>2</sup> test (*I*<sup>2</sup> > 50%: significant heterogeneity, *I*<sup>2</sup> = 50–25%: moderate heterogeneity, *I*<sup>2</sup> < 25%: insignificant heterogeneity), which can be interpreted as the percentage of total variation across several studies due to heterogeneity (21). To assess the extent of publication bias, Egger’s test for publication bias was used (22). Sensitivity analyses were undertaken with the exclusion of a study with borderline eligibility. Meta-analysis was conducted using Stata/SE 10.0 for Windows (StataCorp. LP, 4905 College Station, TX, USA). The report of the study was complemented in adherence with the meta-analysis of observational studies in epidemiology group standards for reporting meta-analysis of observational studies (23).

**Results**

**Search results**

The search strategy identified 49 potentially relevant studies. Seven of them were identified through reference sections of relevant publications or manual search. A flow chart summarizing search results is provided in Fig. 1. Eleven publications were excluded, because it was clear from the title that they did not fulfill the selection criteria. From the remaining 38 publications, 18 were excluded on the basis of the abstract. Twenty articles were read in full, independently by two reviewers, to assess their accordance with the predefined inclusion criteria. Four studies were excluded because TAI status had not been determined (24–26) or women with TAI had been excluded (27). Three studies were excluded because women with recurrent spontaneous miscarriages had been recruited (28–30) and another three studies because the IVF outcome had not been recorded or presented (14, 31, 32).

From the remaining ten studies included in the systematic review, three were excluded from the meta-analysis on the basis of their retrospective design (15–17), two because women involved underwent multiple IVF procedures (8, 9), and one (18) because

Table 2 Major reported outcomes of the studies considered for the meta-analysis.

Author (year)	Biochemical pregnancies <sup>a</sup>			Clinical pregnancies			Miscarriages			Deliveries			Notes/original authors' conclusions
	TAI	Control	TAI	TAI	Control	TAI	Control	TAI	Control	TAI	Control		
Kilic <i>et al.</i> (2008)	10/23 (43.5%)	16/31 (51.6%)	7/23 (30.4%)	7/23 (30.4%)	13/31 (41.9%)	3 <sup>b</sup>	3	NR	NR	NR	NR	Positive TPOab above 69.78 IU/ml affected the clinical but not the biochemical pregnancy rate	
Negro <i>et al.</i> (2007)	NR	NR	21/42 (50.0%)	21/42 (50.0%)	234/374 (62.4%)	5/21 (23.8%)	27/234 (11.5%)	16/21 (76.2%)	207/234 (88.5%)	16/21 (76.2%)	207/234 (88.5%)	Women with positive TPOab, whose ART was unsuccessful or complicated by miscarriage showed an initial thyroid function worse than women who delivered	
Negro <i>et al.</i> (2005)	NR	NR	21/43 <sup>c</sup> (48.8%)	21/43 <sup>c</sup> (48.8%)	318/576 (55.2%)	11/21 <sup>c</sup> (52.4%)	82/318 (25.8%)	10/21 <sup>c</sup> (47.6%)	236/318 (74.2%)	10/21 <sup>c</sup> (47.6%)	236/318 (74.2%)	Miscarriages included early pregnancy loss (biochemical pregnancies). Miscarriage risk was similar between control and TAI subjected to L-T <sub>4</sub>	
Poppe <i>et al.</i> (2004)	Only pregnant included	Only pregnant included	Only pregnant included	Only pregnant included	Only pregnant included	5/9 <sup>d</sup>	11/27 <sup>d</sup>	NR <sup>d</sup>	NR <sup>d</sup>	NR <sup>d</sup>	NR <sup>d</sup>	The changes in serum TSH and FT <sub>4</sub> during the first trimester were comparable to the thyroid function observed in spontaneous pregnancies	
Poppe <i>et al.</i> (2003)	NR	NR	17/32 (53.0%)	17/32 (53.0%)	87/202 (43.0%)	9/17 (52.9%)	20/87 (23.0%)	8/17 (41.1%)	67/87 (67.0%)	8/17 (41.1%)	67/87 (67.0%)	Miscarriages included early pregnancy loss (biochemical pregnancies). The age of the woman was an independent risk factor for miscarriage	
Kutteh <i>et al.</i> (1999)	5/143 (3.5%)	34/730 (4.7%)	92/143 (64.3%)	92/143 (64.3%)	478/730 (65.5%)	14/92 (15.2%)	82/478 (17.2%)	78/92 (84.8%)	396/478 (82.8%)	78/92 (84.8%)	396/478 (82.8%)	No difference in biochemical or clinical pregnancy rate. Levels of autoantibodies did not affect ART outcome	
Muller <i>et al.</i> (1999)	NR <sup>e</sup>	NR <sup>e</sup>	12/25 (48.0%)	12/25 (48.0%)	42/148 (28.4%)	4/12 (33.0%)	8/42 (19.0%)	8/12 (67.0%)	34/42 (81.0%)	8/12 (67.0%)	34/42 (81.0%)	Levels of TSH were not different: i) in women who became pregnant compared with those who did not, ii) in women with ongoing pregnancies compared with those who miscarried. Similar levels of anticardiolipin antibodies in cases (miscarriages) and controls	
Kim <i>et al.</i> (1998)	7/28 (18.4% per cycle)	5/51 (5.6% per cycle)	10/28 (26.3% per cycle)	10/28 (26.3% per cycle)	35/51 (39.3% per cycle)	4/10 (40.0%)	4/35 (11.4%)	6/10 (60.0%) <sup>f</sup>	31/35 (88.5%) <sup>f</sup>	6/10 (60.0%) <sup>f</sup>	31/35 (88.5%) <sup>f</sup>	18 TAI women were euthyroid under treatment, higher autoantibodies titers in TAI women who miscarried compared with those who did not	
Geva <i>et al.</i> (1996)	1/16 <sup>g</sup>	3/24 <sup>g</sup>	6/16 (13.8% per cycle)	6/16 (13.8% per cycle)	24/55 (25.0% per cycle)	2/6 <sup>g</sup>	4/24 <sup>g</sup>	1/16 <sup>g</sup>	6/24 <sup>g</sup>	1/16 <sup>g</sup>	6/24 <sup>g</sup>	Pregnancy rate per cycle was higher in the control group. TSH, FT <sub>4</sub> levels were not reported	
Singh <i>et al.</i> (1995)	15/106 (14.2%)	67/381 (17.6%)	87/106 (85.8%) <sup>h</sup>	87/106 (85.8%) <sup>h</sup>	301/381 (82.4%)	28/87 (32.2%)	49/301 (16.3%)	59/87 (67.8%)	252/301 (83.7%)	59/87 (67.8%)	252/301 (83.7%)	No difference in the incidence of biochemical or ectopic pregnancy between groups. In the subgroup of recurrent miscarriages, pregnancy loss was not associated with the presence of thyroid autoantibodies. TSH, FT <sub>4</sub> levels were not reported	

ART, assisted reproduction techniques; FT<sub>4</sub>, free thyroxine; IU, international units; L-T<sub>4</sub>, levothyroxine; NR, not reported; TAI, thyroid autoimmunity (presence of TPO and/or TG autoantibodies); TG, thyroglobulin; TPO, thyroid peroxidase; TSH, thyroid stimulating hormone.

<sup>a</sup>Authors refer to biochemical pregnancies either as the biochemical confirmation of a pregnancy (by β-hCG rise) (Kilic 2008) or as the loss of a pregnancy that had been confirmed only biochemically (Kutteh 1999, Muller 1999, Singh 1995)

<sup>b</sup>Refers to TAI group, euthyroid without treatment (in the TAI euthyroid under treatment group, three miscarriages occurred (unpublished data from the original authors)).

<sup>c</sup>Refers to TAI on placebo.

<sup>d</sup>Study was ended after the first trimester.

<sup>e</sup>Authors did not provide exact numbers of biochemical pregnancy losses in groups; however, they stated that there were four in total and their exclusion did not alter the findings.

<sup>f</sup>Deliveries and ongoing pregnancies were summed up.

<sup>g</sup>Full data not provided, information is missing and ongoing pregnancies were excluded.

<sup>h</sup>Clinical pregnancies, all the subjects recruited had a confirmed conception, ectopic pregnancies excluded.

of a sample overlap with a previous study (12). Finally, four studies were included in the meta-analysis (10–13, 18). Disagreement between reviewers was recorded on whether multiple IVF procedures were involved in some of the included studies (10, 11, 13, 18) and was resolved by consensus.

**Systematic review**

The ten studies included in the systematic review were published between 1995 and 2008 and reported data on 3107 subfertile women undergoing IVF procedures. Main data are summarized in Tables 1 and 2. Five of them were held in Europe, three in Asia, and two in the United States. Substantial diversity was observed in the methodology researchers had chosen to investigate the hypothesis. Six studies were prospective in design, three were retrospective, while in one study, the design was not clearly stated (8). In five studies, it was clearly stated that first ART procedure was an inclusion criterion, whereas in three studies, no specific statement was provided (8, 13, 15, 18). In two studies, it was clearly reported that only first cycle results were analyzed (12, 15), in another two, multiple IVF cycles were reported (8, 9), whereas in the rest no relevant information could be retrieved. Groups were comparable in age in all of the studies but differed in mean thyroid stimulating hormone (TSH) values in two studies (15, 17). TSH and free T<sub>4</sub> (FT<sub>4</sub>) levels were not reported in two studies (9, 16).

Diversity in the methodology applied to measure thyroid autoantibodies was observed too. Both TPOab and TGab were determined in five studies, whereas in the remaining five, only TPOab was measured (11–13, 17, 18). Most studies (generally the most recent) applied RIA for the measurements (five studies), whereas electrochemiluminescence assays (ECL), enzyme immunoassays, ELISA, indirect passive agglutination, and radioligand assays were also used with various thresholds (further details in Table 1). In one

study, sera were drawn 14 days after ET (16), and in another, no relevant information was provided (10).

Overall, the reporting of studies was fair: total numbers, inclusion criteria, baseline characteristics, and IVF protocols were generally clearly stated; however, reporting on secondary outcomes (gestational age when miscarriage occurred, biochemical pregnancies, levels of antibodies) and on limitations was incomplete.

**Meta-analysis**

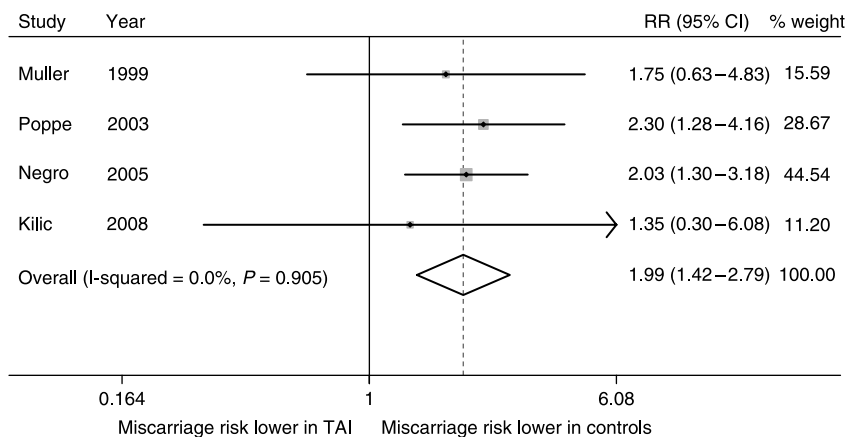
The four studies that were included in the meta-analysis reported data on 1098 subfertile women undergoing IVF procedures (141 with TAI and 957 controls). Study size varied from 69 to 622 women (median = 203.5). The percentage of miscarriage (to clinical pregnancies) reported in individual studies ranged from 33.3 to 52.9%. Main results of the meta-analysis are summarized below.

**Miscarriage risk**

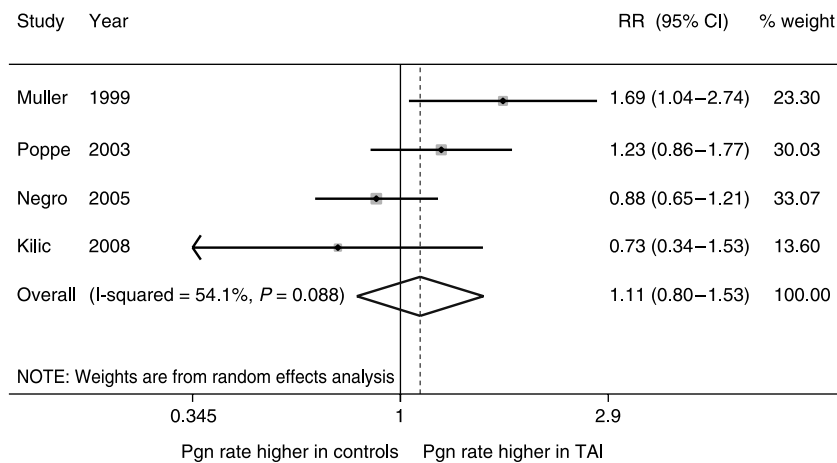
Subfertile women with TAI undergoing IVF demonstrated significantly higher risk for miscarriage compared with subfertile women without TAI undergoing IVF (four studies—fixed effects RR: 1.99, 95% CI: 1.416–2.793, *P* < 0.001; Fig. 2). No sign of heterogeneity among studies was detected (*I*<sup>2</sup> = 0%). No sign of publication bias was detected (Egger’s test, *P* = 0.298). Sensitivity analysis, with the exclusion of the study by Kilic *et al.* revealed similar findings (three studies—fixed effects RR: 2.07, 95% CI: 1.47–2.71).

**Clinical pregnancy rate**

Subfertile women with TAI undergoing IVF demonstrated similar clinical pregnancy rate compared with subfertile women without TAI undergoing IVF (four studies—random effects RR: 1.107, 95% CI: 0.80–1.530, *P* = 0.504; Fig. 3); however, substantial heterogeneity



**Figure 2** Miscarriage risk in euthyroid women with TAI undergoing IVF.



**Figure 3** Clinical pregnancy rate in euthyroid women with TAI undergoing IVF.

among studies was detected ( $I^2 = 54.1\%$ ). Sensitivity analysis, with the exclusion of the study by Kilic *et al.* revealed similar findings (three studies—random effects RR: 1.18, 95% CI: 0.88–1.69).

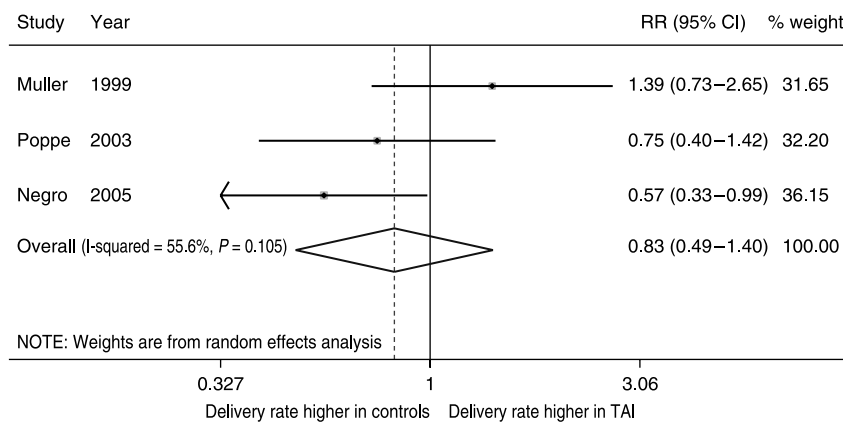
**Delivery rate**

Subfertile women with TAI undergoing IVF demonstrated similar delivery rate compared with subfertile women without TAI undergoing IVF (three studies—random effects RR: 0.826, 95% CI: 0.488–1.398,  $P=0.477$ ; Fig. 4). Substantial heterogeneity among studies was detected ( $I^2 = 55.6\%$ ).

**Discussion**

The present meta-analysis attempted to provide an estimation of the risk for miscarriage for euthyroid women undergoing IVF in the presence of thyroid autoantibodies. Despite the relative paucity of primary data (four studies) that undermine the reliability of the results, it was estimated that a subfertile euthyroid

woman with TAI is at a twofold higher risk of miscarriage in an IVF pregnancy compared with a counterpart without TAI. The result is in agreement with those of similar studies on spontaneous (non-IVF) pregnancies (3), both in the direction and in the magnitude of the effect. Furthermore, it appears to be consistent across studies, since no sign of heterogeneity was detected. On the other hand, the present meta-analysis failed to detect a significant effect of TAI status on the clinical pregnancy rate and delivery rate in women undergoing IVF. However, the latter finding should be regarded with caution, since only three studies reported delivery rates; thus, the analysis was probably underpowered. In fact, a *post hoc* power analysis revealed that a sample size of ~300 women with TAI would be needed to detect a difference in delivery rates (study power 0.8, TAI:control allocation 1:9). Alternatively, it could be suggested that, despite a twofold relative increase in the risk of miscarriage, the actual difference in the number of miscarriages between euthyroid women with TAI undergoing IVF and controls (the attributable risk) is small, resulting in a rather ‘silent’ effect on delivery or clinical pregnancy rate.



**Figure 4** Delivery rate in euthyroid women with TAI undergoing IVF.



The findings of the present meta-analysis could potentially provide useful inferences about the etiology of the association between TAI and miscarriage in IVF. First, the comparison between the TAI-related miscarriage risk in IVF pregnancies and similar risk in spontaneous pregnancies (3) is consistent with the fact that the exogenous use of gonadotropins in IVF procedures does not convey additional risk for miscarriage in the presence of TAI. Despite evidence for a pattern of dissociation for both serum TSH (higher) and FT<sub>4</sub> (lower) in women with TAI compared with those without TAI after ART-induced pregnancies (18), the clinical importance of the changes provoked by gonadotropins in terms of miscarriage risk appears to be minimal.

On the contrary, it appears that the role of TSH levels and L-T<sub>4</sub> supplementation are important for IVF outcome. Poppe *et al.* investigated the thyroid function after an IVF procedure and its association with the reproductive outcome (18). Although it failed to reach statistical significance in thyroid function parameters between miscarriage and ongoing pregnancy groups, the study did confirm a significantly different pattern of change in thyroid function during 'the very first period of pregnancy' between TAI groups. Negro *et al.* provided evidence that L-T<sub>4</sub> supplementation in women with TAI who became pregnant through IVF did lower the miscarriage risk to the level of controls (11). Subsequently, the same group reported in a retrospective study that women with TAI, whose ART was unsuccessful or complicated by miscarriage, showed worse thyroid function profile than women who delivered (17). Unfortunately, although subtle alterations in thyroid function could potentially explain the observed difference in miscarriage risk in the present meta-analysis, their impact was not investigated, since thyroid function tests were not applied in the course of IVF pregnancies in most studies. The lack of an assessment of the dynamics of thyroidal function or the actual thyroid status at miscarriage, which could have favored an etiopathogenic approach, inserts bias in the analysis that can actually affect the interpretation of clinical data.

In regard to alternative explanations for the observed difference in miscarriage, the influence of age is rather minimal, since all the TAI groups were comparable in the mean value in all the studies included in the present meta-analysis. A 'dose-response' pattern between autoantibody titers and miscarriage risk, which could potentially imply a direct action, could not be supported by the findings of the systematic review; the unique report of an association between them (8) was not confirmed subsequently (15). On the other hand, influence of other causes of spontaneous abortions was not routinely controlled for; the presence of other autoantibodies was an exclusion criterion in only one study (8), whereas another one retrospectively estimated the prevalence of anticardiolipin antibodies (13).

Etiology of infertility was reported to be similar in TAI groups in four studies (9, 11, 16, 33), while three evaluated personal history of miscarriages as an exclusion criterion (9, 10, 13). Thus, the potential confounding effect of other causes of spontaneous miscarriages could not be neglected.

Recently, another hypothesis has been suggested to explain the association between TAI and miscarriage (34), compatible with the notion of thyroid autoantibodies as a marker that should be regarded as a reflection of an undefined autoimmunity. According to this hypothesis, the presently undefined autoimmunity is the blocking TSH receptor autoantibodies (TSHRab-blocking), not infrequently detected in Hashimoto's thyroiditis (35), which could putatively inhibit, through a cross-reactivity process (34), the action of the human chorionic gonadotropin (hCG) on the corpus luteum. This inhibition could lead to a decrease in progesterone and estrogen production that is essential for the support and maintenance of pregnancy during the first trimester (36). Unfortunately, neither TSHRab nor progesterone levels were reported in the studies included in the systematic review. In one study where hCG levels were measured (18), their mean value appeared to grow slower in the TAI group than in the control group, yet in a non-significant fashion.

In summary, it could be argued that euthyroid, subfertile women with TAI face an approximately twofold higher risk for miscarriage when undergoing an IVF compared with subfertile women without TAI. However, due to the relative paucity of evidence, absence of an established intervention and cost, the routine screening of thyroid autoantibodies before an IVF procedure should be reserved in a research setting.

## 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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