The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion

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BACKGROUND: The role of thrombophilia and thyroid autoimmunity in unexplained infertility (UI), implantation failure (IF) and recurrent spontaneous abortion (RSA) is controversial and poorly understood. METHODS: From March, 2004 to January, 2007, 119 women were prospectively included: 32 oocyte donors, 31 patients with UI, 26 with IF and 30 with RSA. The IF and RSA groups presented normal preimplantation genetic screening. Protein C, protein S, antithrombin III, lupus anticoagulant, activated protein C resistance (APCR), immunoglobulin M and G anticardiolipin antibodies, homocystine, Factor V Leiden, prothrombin G20210A mutation, methylentetrahydrofolate reductase C677T mutation, thyroid-stimulating hormone (TSH), free thyroxine, anti-thyroid peroxidase (TPO) and anti-thyroglobulin (TG) antibodies were assessed. RESULTS: The prevalence of thrombophilia was high and similar among groups. In the IF group, the prevalence of APCR (15.4%), lupus anticoagulant (11.5%) and combined thrombophilia (19.2%) was higher, but not significantly different, than the other three groups. The prevalence of thyroid autoimmunity in women with IF (anti-TPO antibodies, \( P = 0.009 \); anti-TPO plus anti-TG antibodies, \( P = 0.04 \)) and UI (anti-TPO, \( P = 0.002 \); anti-TG, \( P = 0.019 \); anti-TPO plus anti-TG antibodies, \( P = 0.005 \)) was significantly increased in comparison to those with RSA. There was also a trend towards a higher prevalence of thyroid autoimmunity in the UI and IF groups than in the control group. TSH and free thyroxine levels all remained within a normal range. CONCLUSIONS: When embryo aneuploidy is ruled out, thrombophilia could constitute an etiologic factor in IF. Furthermore, thyroid autoimmunity is strongly related to UI and IF.

Keywords: thrombophilia; thyroid autoimmunity; recurrent spontaneous abortion; implantation failure; unexplained infertility

Introduction

Infertility is a common problem among the general population, with a prevalence of 9% in our society (Boivin et al., 2007). Although it has many known causes, some couples are still classified as having unexplained infertility (UI) because the underlying mechanism(s) is never found. Some of the infertile couples undergo unsuccessful IVF attempts after the replacement of several embryos and are thus defined as having implantation failure (IF). Moreover, they may experience early pregnancy losses in both natural conception and assisted reproduction cycles, which go unnoticed by the woman (Simón et al., 1999). In addition, there is another subgroup of infertile couples that conceive but lose the pregnancy at some point, and these are classified as having recurrent spontaneous abortion (RSA). These three conditions are frustrating, and may have common mechanisms by which the embryo does not implant in the uterus, or fails to continue normal development to term. Some of these common mechanisms have been clearly identified, whereas others remain mere speculation (Christiansen, 2006). This is the case for chromosome aberrations, which can be inherited from a balanced structural chromosomal rearrangement in one of the parents that is unbalanced in the offspring, or can appear de novo in the embryo as a consequence of a meiotic error in the oocyte or spermatozoid segregation (Rai and Regan, 2006). While chromosomal abnormalities are considered to be the main cause of RSA, their role in IF or UI is not fully understood (Rubio, 2005).

Antiphospholipid syndrome (APS) is another known cause of RSA (Empson et al., 2005) and has been related to IF (Stern et al., 1998). It has recently been proposed that patients with this syndrome have a defective trophoblastic invasion, which would explain other pregnancy complications, such as pre-eclampsia or placental insufficiency (Rai and Regan, 2006). However, although a similar mechanism has been
described in some inherited thrombophilias, such as Factor V Leiden (Rey et al., 2003) and in some types of hyperhomocysteinemia (Nelen et al., 2000), the relationship between inherited thrombophilia and RSA or IF is a somewhat grey area (Christiansen, 2006).

Recently, thyroid autoimmunity has been highlighted by several studies as forming part of the aetiology of RSA (Roberts et al., 1996; Matalon et al., 2001), IF (Bussen et al., 2000) and infertility (Poppe et al., 2002). The mechanism by which antithyroid antibodies (ATA) may operate consists of an alteration of the endometrium through a general immune dysfunction that affects the foetal allograft, and/or thyroid failure during assisted reproduction treatments (ART) and subsequent pregnancy (Roberts et al., 1996; Kutteh et al., 1999a; Poppe et al., 2003; Prummel and Wiersinga, 2004). An evolutionary explanation suggests that reproductive problems in women with high titres of ATA exist in order to prevent the transmission of autoimmune genes to the next generation (Matalon et al., 2001).

Thus, this study was designed to evaluate the potential role of both inherited and acquired thrombophilia and thyroid autoimmunity in UI, IF and RSA. To focus on these particular disorders and to rule out potentially confounding variables, the study was prospectively designed to include four groups of young women (<38 years old) in whom no organic uterine abnormality, or other autoimmune disease or endocrine disorder were detected prior the study. The women in two groups (RSA and IF) had undergone a previous preimplantation genetic screening (PGS) cycle in which a substantial percentage of chromosomally normal embryos had been detected.

Materials and Methods

Study design
This is a prospective study, in which women were enrolled between first March, 2004 and first January, 2007. Four groups were formed based on the following inclusion criteria:

- The control group included 32 oocyte donors of 18–35 years of age, all Caucasian, with normal karyotype, no history of spontaneous abortions, autoimmune disorders or endocrine diseases, and previous term pregnancies without complications.
- The UI group consisted of 31 Caucasian women with >1-year infertility, <38 years of age, absence of autoimmune or endocrine disorders, with normal ovarian function, normal hysterosalpingography and transvaginal ultrasound scan of the uterus and ovaries, normal karyotype, and whose partner had normal karyotype and normozoospermia. These couples underwent 4.0 ± 1.8 (mean ± SD) failed intrauterine insemination cycles before performing an IVF/ICSI procedure which led to pregnancy following the replacement of good quality embryos. Two out of the 31 patients (6.4%) had previously given birth.
- The IF group was composed of 26 couples that had experienced 3.9 ± 1.1 IVF failures with embryo transfer of at least two good quality embryos. To be specific, said couples received a mean number of 6.9 ± 2.5 embryos considering all the performed cycles. The female partners were <38 years old, Caucasian, presented normal ovarian function and a normal uterus by vaginal ultrasound and/or hysterosalpingography/hysteroscopy. Moreover, endocrine disorders and autoimmune diseases were ruled out, and a normal karyotype was confirmed in both parents. The male partner’s sperm concentration was >5 × 10⁶/ml. In the last IVF cycle performed, PGS was added: a total of 4.0 ± 2.2 embryos were analysed (range: 1–10), of which 79.3 ± 16.8% (range: 60–100%) were found to be chromosomally normal.
- RSA was considered with at least two clinical spontaneous abortions. The group of women with RSA (n = 30) had had 3.2 ± 1.3 (range: 2–9) previous spontaneous abortions. They were <38 years old, Caucasian, presented normal ovarian function and had a normal uterus, as confirmed by vaginal ultrasound and/or hysterosalpingography/hysteroscopy. No endocrine anomalies or autoimmune diseases were detected, and both partners had a normal karyotype. Sperm concentration of the male partner was >5 × 10⁶/ml. Only 6 women had 2 spontaneous abortions (20%) and the remaining 24 patients had suffered ≥3, all occurring between week 5 and 13 of gestation, except for one case, which occurred at week 20. Five women (16.7%) had previously given birth. Subjects had a PGS cycle with ≥60% of normal embryos. The mean number of analysed embryos was 5.0 ± 2.5 (range: 2–12), of which 79.2 ± 16.4% (range: 60–100%) were chromosomally normal.

In the study period, in the Instituto Valenciano de Infertilidad, Valencia, of the 472 PGS cycles performed due to RSA, only 44 cycles (9.3%) could be accepted in the study due to the inclusion criteria of women <38 years of age, with <40% of chromosomally abnormal embryos, and whose partners had >5 × 10⁶/ml spermatozoa in their ejaculates, as was the case with the 528 PGS cycles performed due to IF, in which only 46 cycles (8.7%) could be accepted. From those 44 and 46 cycles of RSA and IF, respectively, only 30 and 26 were finally included in the study after ruling out patients with uterine, endocrine or autoimmune alterations. Thus, a similar number of women were included in the other two study groups (32 and 31 in the oocyte donor controls and UI groups, respectively).

The study was approved by the Institutional Research and Ethics committee. All subjects were contacted by telephone. The study purposes and procedures were carefully explained, and an informed consent was obtained from those willing to participate. Since estrogen levels may alter protein S, activated protein C resistance (APCR) and homocysteine levels, and given that the latter levels can be modified after meals, all the blood tests were performed in fasting conditions while the women were not pregnant, at least 3 months after the last pregnancy or spontaneous abortion, and in the absence of any recent or concurrent hormonal therapy.

We defined isolated thrombophilia as the presence of a specific thrombophilic marker. Combined thrombophilia was considered as the presence in the same patient of at least two thrombophilic markers.

Blood measurements
Functional protein C levels were measured using a chromogenic assay, with Berichron protein C reagent (normal range: 70–140%). Functional protein S levels were measured using a clotting assay with protein S antibodies (normal range: 50–120%). Antithrombin III levels were measured using a clotting assay with Berichron protein C reagent (normal range: 75–125%). Screening for lupus anticoagulant (LA) was performed using a clotting assay with LA1 screening reagent/LA2 confirmation reagent (result was expressed as positive or negative). APCR was measured using a clotting assay with ProC® Global reagent (normal range in standard ratio: 0.69–1.56). Dade Behring reagents were employed for all these parameters and were measured in a Dade Behring coagulometer BCS® 2.2.1. (Dade Behring Marburg GmbH, Marburg, Germany).

Immunoglobulin (Ig) M and IgG anticardiolipin antibodies (ACAs) were assayed using an enzyme-linked immunosorbent assay for the semi-quantitative detection of IgM or IgG ACAs in human serum with QUANTA LITE™ ACA IgG III and IgM, respectively.
Homocysteine levels were determined using competitive immuno-assay (normal range: 5–15 μmol/l). Serum thyroid-stimulating hormone (TSH) was measured by IMMULITE 2000 Third Generation TSH in a solid-phase, two-site chemiluminiscent immunometric assay (normal range: 0.25–5 μU/ml). Free thyroxine was determined using a solid-phase, chemiluminiscent competitive analogue immunoassay (normal range: 0.73–2.2 ng/dl). All these parameters were measured by IMMULITE 2000 (DPC®, Los Angeles, USA).

Anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies were studied with a two-stage immunoluminometric assay (sandwich principle). For the coating of the solid-phase (anti-TG) antibodies were studied with a two-site immunoluminometric assay (sandwich principle). For the coating of the solid-phase (anti-TG) antibodies were studied with a two-site immunoluminometric assay (sandwich principle).


data were expressed as number and percentage, and numerical data

were compared by analysis of variance, with Bonferroni and Scheffe’s post hoc analysis were employed. *Comparison of controls with the other three groups. †Comparison of controls with the other three groups. ‡Comparison of UI and IF groups with controls and RSA group. BMI, body mass index; RSA, recurrent spontaneous abortion; IF, implantation failure; UI, unexplained infertility.

Table I. Clinical characteristics of the patients of each group.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 32)</th>
<th>UI (n = 31)</th>
<th>IF (n = 26)</th>
<th>RSA (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.5 (3.8)</td>
<td>32.4 (2.4)</td>
<td>34.2 (2.4)</td>
<td>34.0 (3.6)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8 (3.5)</td>
<td>22.8 (3.2)</td>
<td>22.7 (4.1)</td>
<td>22.7 (2.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous live births (n)</td>
<td>1.4 (0.8)</td>
<td>0.1 (0.2)</td>
<td>0.2 (0.4)</td>
<td>0.2 (0.4)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>0</td>
<td>3.4 (2.3)</td>
<td>3.8 (2.0)</td>
<td>0.3 (0.9)</td>
<td>&lt;0.0001‡</td>
</tr>
</tbody>
</table>

Values expressed as mean (SD). Analysis of variance, with Bonferroni and Schefè’s post hoc analysis were employed. *Comparison of controls with the other three groups. †Comparison of controls with the other three groups. ‡Comparison of UI and IF groups with controls and RSA group. BMI, body mass index; RSA, recurrent spontaneous abortion; IF, implantation failure; UI, unexplained infertility.

Table II. Prevalence of isolated thrombophilia in the four groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 32)</th>
<th>UI (n = 31)</th>
<th>IF (n = 26)</th>
<th>RSA (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR</td>
<td>0 a</td>
<td>2 (6.5)</td>
<td>4 (15.4)</td>
<td>0 c</td>
<td>0.03</td>
</tr>
<tr>
<td>Anthrombin III deficiency</td>
<td>1 (3.1)</td>
<td>1 (3.2)</td>
<td>1 (3.8)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>1 (3.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>3 (9.4)</td>
<td>1 (3.2)</td>
<td>2 (7.7)</td>
<td>2 (6.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Factor V Leiden (homozygous)</td>
<td>0</td>
<td>2 (6.5)</td>
<td>1 (3.8)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Factor II mutation (homozygous)</td>
<td>2 (6.3)</td>
<td>1 (3.2)</td>
<td>1 (3.8)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>MTHFR C677T mutation (homozygous)</td>
<td>5 (15.6)</td>
<td>7 (22.6)</td>
<td>4 (15.4)</td>
<td>6 (21.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperhomocystinemia</td>
<td>1 (3.1)</td>
<td>0</td>
<td>1 (4.2)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>0</td>
<td>3 (11.5)</td>
<td>1 (3.3)</td>
<td>0</td>
<td>(P = 0.056)</td>
</tr>
<tr>
<td>ACA IgG</td>
<td>0</td>
<td>3 (9.7)</td>
<td>1 (3.8)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>ACA IgM</td>
<td>6 (18.8)</td>
<td>3 (9.7)</td>
<td>1 (3.8)</td>
<td>6 (20.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values expressed as number (%). Chi-square tests were applied. APCR, activated protein C resistance; MTHFR, methylenetetrahydrofolate reductase; ACA, anticardiolipin antibody; Ig, immunoglobulin.

and P = 0.235, respectively), showing only a trend. Similarly, a trend was observed towards a higher incidence of LA in the IF group, but one that did not reach statistical significance (P = 0.056).

Table III shows the prevalence of one or more thrombophilia in each of the groups, which was frequent in all (between 40 and 52%). When ACAs IgG/IgM and LA were excluded in order to compare the incidence of thrombophilia without APS, no difference was detected. In addition, when ACAs and LA were only considered in the RSA group, given that the definition of APS would be only applied to this group, a similar incidence of thrombophilia was observed again (Table III).

Moreover, when the four groups were compared excluding the most prevalent condition, the homozygous MTHFR C677T mutation, the prevalence of thrombophilia did not change (Table III). No difference was found when only inherited thrombophilia (without APCR, LA and ACAs) was considered (Table III).
Combined thrombophilia was present in 3 (9.4%) controls, in 3 (9.7%) patients with UI, in 5 (19.2%) women with IF and in 2 (6.7%) patients with RSA. Despite a trend towards a higher incidence of combined thrombophilia (>1) in the IF group, no difference was noted among the groups (Table III).

The incidence of the heterozygous MTHFR C677T mutation (data not shown in the tables), which does not have a clinical impact, was high in all the study groups: 65.6% (n = 21) within oocyte donors, 35.5% (n = 11) in those with UI, 38.5% (n = 10) in those with IF and 46.7% (n = 14) in those with RSA (NS).

Table IV shows the prevalence of thyroid autoimmunity, which was very low among women with RSA, but was significantly higher in the IF than RSA group when anti-TPO antibodies were considered alone (P = 0.009) or together with anti-TG antibodies (P = 0.04). Similarly, prevalence of thyroid autoimmunity was also high in the UI group in comparison to the RSA group when the two ATAs (anti-TPO and anti-TBG) were considered separately (anti-TPO: P = 0.002; anti-TG: P = 0.019) or together (P = 0.005) (Table IV). Oocyte donor controls tended to have a lower prevalence of thyroid autoimmunity than women with UI and IF; however, no significant differences were observed when the three infertile groups were compared with controls. In the four groups, TSH and free thyroxine levels were within a normal range.

### Discussion

This study was designed to explore the implication of thrombophilia and thyroid autoimmunity in three common and contentious problems encountered in reproductive medicine, namely UI, IF and RSA. With that aim, four groups of patients were selected in order to rule out potentially confounding variables for each of the clinical problems under scrutiny. To the best of our knowledge, our study is the first to contemplate thrombophilia and thyroid autoimmunity after excluding embryonic chromosomal abnormalities by PGS.

RSA is classically defined as ≥3 pregnancy losses before the foetus has reached viability (Rai et al., 2006). However, the risk of a new spontaneous abortion after 2 or 3 spontaneous abortions is similar and an underlying cause could be identified (Hill, 1994). In addition, cytogenetic analysis in abortuses and PGS studies have revealed the same incidence of aneuploidies (60–70%) in women with two, or more spontaneous abortions (Ogasawara et al., 2000; Ferro et al., 2003; Rubio, 2005). Thus, we included women with at least 2 spontaneous abortions, although we tried to recruit especially those with ≥3 spontaneous abortions (80% of cases).

The RSA group comprised women in whom described causes of RSA had been ruled out. In order to exclude chromosomal aberrations from the causes of RSA in our patients, both a normal karyotyping of the parents (ACOG, 2002; Rai and Regan, 2006) and a PGS cycle in which at least 60% of the embryos were found to be euploid (Rubio, 2005) were established as criteria for inclusion in the group. This proportion of normal embryos was chosen because PGS studies in RSA and IF have shown a 60–70% incidence of de novo numerical chromosomal abnormalities in embryos analysed by fluorescence in situ hybridization after IVF, in comparison to 33–36% in fertile patients that were assessed for sex-linked diseases (Pehlivan et al., 2003; Rubio, 2005). Other possible mechanisms, such as acquired and congenital uterine anomalies, endocrine disturbances and autoimmune or alloimmune diseases, have not been clearly confirmed as causes of RSA due to the lack of well-designed studies and ineffectiveness of the proposed therapies employed to improve the reproductive performance of affected couples (ACOG, 2002; Christiansen, 2006; Rai and Regan, 2006). Nevertheless, uterine anomalies, and endocrine or autoimmune diseases were ruled out in our patients in order to focus exclusively on thrombophilia and ATA.

Advanced maternal age (>38 years) (Gianaroli et al., 1999; Rubio, 2005; Findikli, 2006) and severe oligozoospermia
(≤5 × 10⁹/ml) (Rubio et al., 2001) have been related to an increased risk of de novo chromosomal aberrations in both oocytes and sperm, and, in turn, in the embryo. Moreover, the presence of thyroid antibodies (anti-TPO and anti-TG) increases with maternal age (Prummel and Wiersinga, 2004). Therefore, we only considered couples of <38-year-old women and non-severe oligozoospermic men.

In our study, the prevalence of thrombophilia in RSA patients did not differ from that in fertile controls. APS is related to RSA as the administration of heparin plus low dose aspirin to affected women seems to dramatically reduce the probability of a further spontaneous abortion (Empson et al., 2005). However, there is a lack of randomized studies in inherited thrombophilia, and very few meta-analysis and systematic reviews have indicated that Factor V Leiden, G20210A prothrombin mutation and perhaps protein S deficiency, APC prothrombin and hyperhomocystinemia, could be related to RSA (Rey et al., 2003; Kujovich, 2004; Kovalevsky et al., 2004; Krabbendam et al., 2005).

A possible reason why we were unable to detect differences in the incidence of thrombophilia in RSA patients was the higher than expected prevalence among controls. Specifically, some genetic mutations, such as heterozygous MTHFR mutation, were more frequent than the normal genotype. In our study, despite the differences not reaching significance, the heterozygous MTHFR mutation was more frequent in the RSA group than in the IU and IF groups, but less frequent than in the control group. This finding (data not shown in the tables) is not clinically relevant. In fact, when the MTHFR mutation is heterozygous it is not considered a thrombophilic defect owing to the lack of impact of on human health, but shows the high incidence of mutations which occurs in the fertile population. Other authors have described an incidence of inherited thrombophilic defects of 15–17% in the healthy population (Kupferminc et al., 1999), while we found an incidence of 28.1%. This leads us to believe that the negative impact of this condition on human fertility is less significant than expected, and indicates that universal screening of thrombophilia in Caucasian women should not be recommended in order to avoid over-diagnosis and over-treatment of healthy subjects.

Thyroid autoimmunity has recently been related to RSA (Roberts et al., 1996; Matalon et al., 2001). Kutteh et al. (1999a) observed a higher prevalence of ATA in women with RSA (22.5%) than in fertile controls (14.5%), although this finding has been challenged by other authors (Esplin et al., 1998). Poppe et al. (2003) and Prummel and Wiersinga (2004) have described an odds ratio of 2.3–3.77 for the risk of spontaneous abortion in women with positive ATA. However, Kutteh et al. (1999b) did not find differences in terms of biochemical pregnancies or clinical pregnancy losses between women undergoing ART with and without positive ATA. Similarly, Negro et al. (2007) has recently described that pregnancy and delivery rates in euthyroid women undergoing ART were not affected by the presence of anti-TPO antibodies. However, the same authors (Negro et al., 2006) showed the benefit of levothyroxine for decreasing spontaneous abortion risk in infertile and pregnant women with positive ATA and normal thyroid function. A recent prospective study comparing 134 women with RSA, who were ATA positive and had normal thyroid function, with 710 women with RSA who were ATA negative and received no treatment, did not detect any difference in pregnancy outcome (Rushworth et al., 2000). Thus, it is doubtful that the presence of positive ATA with normal thyroid function is a cause of RSA. We endorse this conclusion, since, in our RSA group, the incidence of positive ATA in normothyroid young women was not significantly different from our young controls.

It has been postulated that, in some circumstances, RSA and IF could represent different manifestations of the same pathogenetic spectrum (Christiansen, 2006). In fact, several studies have found common aetiologies for both clinical entities. This is why we excluded from our IF group any possible influence of chromosomal aberrations by karyotyping the couple and performing a PGS cycle (Pehlivan et al., 2003; Rubio, 2005). Age was also considered.

The role of antiphospholipid antibodies in IF is more controversial. In some recent reports (Stern et al., 1998; Qublan et al., 2006), LA and ACAs were significantly more prevalent in women with IF. Similarly, inherited thrombophilia have recently been associated with IF (Qublan et al., 2006; Coulam, 2006), in particular Factor V Leiden and prothrombin gene mutation (Grandone et al., 2001; Qublan et al., 2006). Bare et al. (2000) described that Factor V Leiden mutation carriers presented a 1.5-fold risk of one spontaneous abortion, and a 2.5-fold risk of two or more spontaneous abortions or infertility problems. Glueck et al. (2000) also implicated protein S deficiency and hypofibrinolisis in RSA and IF. Azem et al. (2004) studied a group of 45 women with a history of ≥4 failed IVF cycles, in whom at least 3 good quality embryos were transferred, and found an incidence of inherited thrombophilia of 44.4% in comparison to that of 18.2% among fertile controls (P < 0.05). A subgroup of 21 cases in the IF group with UI presented an even higher incidence of thrombophilia both with (61.9%) and without (42.8%) the inclusion of the most prevalent condition (homozygous MTHFR mutation). In this way, this study demonstrated a relationship between thrombophilia and IF, especially in cases of unknown origin. However, subjects were aged between 24 and 45 years old and the distribution of maternal ages among the study groups was not reported. Qublan et al. (2006) also detected at least one inherited or acquired thrombophilic factor in 69% of young women with ≥3 IVF IF in comparison to 25% of controls.

In our IF group, we detected a similar prevalence of thrombophilia (46.2%) to that found in Azem’s study (2004), and a similar prevalence to that of the individual thrombophilic defects considered. The prevalence of APCR was significantly different among groups (control versus IF and IF versus RSA) and highest in the IF group, but the differences did not reach significance when multiple comparisons were performed between groups (Bonferroni’s correction), probably due to the sample size considered. Similarly, although not significant, in the IF group there was also an increased prevalence of both positive LA and combined thrombophilia. These trends could indicate that some thrombophilias, and especially combined
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Thrombophilias, would undermine the possibility of embryo implantation after ART, as previously suggested. In fact, Qublan et al. (2006) described a prevalence of combined thrombophilia of 35.6% in women with at least three previous IVF failures, and of only 3–4% in fertile controls and women with a successful pregnancy after their first IVF attempt. Coulam (2006) observed more than 3 gene mutations among 10 thrombophilic genes studied in 74% of women with IF and in only 20% of fertile controls.

The presence of thyroid autoimmunity has also been related to IF (Bussen et al., 2000). In our healthy population, its prevalence (15.6%) was similar to that described by other authors (Vianna et al., 1991; Kutteh et al., 1999a; Poppe et al., 2002). Interestingly, we found a significant increase of positive ATA titres in IF patients, which were mainly anti-TPO antibodies. In a study by Bussen et al. (2000), an increased prevalence of ATA (54.2%), in particular TG antibodies, was observed in euthyroid women with three or more IF failures.

In the present study, we also addressed the influence of thrombophilia and thyroid autoimmunity in UI. Thrombophilia has never been addressed with respect to infertility, whereas thyroid autoimmunity has been widely related to this problem (Poppe et al., 2002). We found that women with UI did not show a higher incidence of thrombophilia than the other three groups. However, a high incidence of ATA was observed in UI subjects (compared with those with RSA), highlighting a possible autoimmune dysfunction in the origin of their infertility, as some authors have previously described (Kutteh et al., 1999a; Poppe et al., 2002). The lack of significant differences between the UI and IF groups and the control group, despite the lower incidence of positive ATA in the latter, could be related to the sample size of the study. Further studies with more patients need to be carried out to determine if any significant differences are present.

Our study may be limited by the sample size, but it is the first in which all the ‘known’ and ‘suspected’ causes of UI, IF and RSA have been discarded in order to focus the real relevance of thrombophilia and thyroid autoimmunity. It is worth mentioning that the selection criteria for our study and the blood analysis performed made our task tremendously difficult and expensive. During the study period, only 26 and 30 couples with IF or RSA, respectively, were eligible for inclusion.

In summary, thrombophilia seems to be more frequent than expected among the healthy population, and could impair implantation in some subgroups of infertile women. Similarly, thyroid autoimmunity appears to play a negative role in both UI and IF. Further research in this field and effective therapies for improving the reproductive performance of the affected couples should be developed.

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