Anticardiolipin antibody levels in women undergoing first in vitro fertilization/embryo transfer

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BACKGROUND: The clinical relevance of antiphospholipid antibodies (aPL) in women undergoing in vitro fertilization/embryo transfer (IVF/ET) and the role of IVF treatment in affecting antiphospholipid levels are controversial. The aim of this study was to evaluate anticardiolipin antibody (aCL) levels and the effect of IVF treatment on aCL in women undergoing their first IVF/ET cycle. METHODS: Immunoglobulin G (IgG)- and IgM-aCL were determined by enzyme-linked immunosorbent assay in 50 women undergoing IVF/ET, 18 due to endometriosis, 16 to tubal factor (TF) and 16 to male factor, before starting treatment (T0), on the day of oocyte retrieval (T1) and 14 days after ET (T2). A group of 31 age-matched fertile women served as controls. RESULTS: aCL levels detected at T0 in patients (TF) and 16 to male factor, before starting treatment (T0), on the day of oocyte retrieval (T1) and 14 days after ET (T2). A group of 31 age-matched fertile women served as controls. RESULTS: aCL levels detected at T0 in patients with endometriosis (T2), but not IgM-aCL significantly increased at T2 in comparison with T1 (P < 0.01) and T1 (P < 0.018). No relationship was found between aCL and pregnancy. CONCLUSIONS: Our results indicate that IVF treatment increases IgG-aCL levels in patients with endometriosis and TF, but their presence seems to have no clinical relevance.

Keywords: IVF/ET; hyperestrogenism; anticardiolipin antibodies; pregnancy

Introduction

Autoimmunity is a possible cause of reproductive failure and infertility, since an increased prevalence of both organ and non-organ specific autoantibodies has been documented in infertile women (Van Voorhis and Stovall, 1997; Shoefield and Blank, 2004). Antiphospholipid antibodies (aPL) are autoantibodies directed against negatively charged phospholipids and phospholipid binding proteins; they have gained much attention due to their association with pregnancy failure, particularly recurrent fetal loss (Branch, 2004). Since 1985, detection by enzyme-linked immunosorbent assay (ELISA) of anticardiolipin antibodies (aCL) is the most widely used test to assess the presence of aPL (Loizou et al., 1995).

Several aPL-mediated pathogenic mechanisms have been proposed, including inhibition of the protein C pathway, inhibition of β2-Glycoprotein-I (β2GPI) anticoagulant activity, enhancement of endothelial cell procoagulant activity and platelet aggregation, and reduced fibrinolysis (Espinosa et al., 2003). In any case, aPL are significantly associated with arterial and/or venous thrombosis and recurrent spontaneous abortion, the so-called Antiphospholipid Syndrome (APS) (Levine et al., 2002). Furthermore, it has been proposed that the same aPL-mediated dysfunctions responsible for recurrent abortions could also inhibit fertilization, embryonic development, implantation or post-implantation/placental development, but there is no agreement about this issue (Porter, 2001).

In previous investigations of the relationship between aPL and reproductive failure in women undergoing in vitro fertilization/embryo transfer (IVF/ET), conflicting results as to both their prevalence and pathogenic role have been obtained (Geva et al., 1994; Birdsall et al., 1996; Kaider et al., 1996; Denis et al., 1997; Chilcott et al., 2000). In this respect, a meta-analysis study indicated that the measurement of aPL is not warranted in patients undergoing IVF/ET (Hornstein et al., 2000). However, it should be pointed out that methodological variables, patients inclusion criteria and different characteristics of controls may affect comparison among the various studies. For instance, the methods used to determine the cut-off of normal aPL values are not consistent, since standard deviations (values > 2 or 3 or 5 SD), percentiles (95th, 97th or 99th percentiles) and multiple of medians have all been used. In some cases, information about demographic characteristics and the size of the control group from which the cut-off value was derived is missing.
Another interesting issue is the possible role of IVF treatment in inducing aPL release but, currently available results are again quite controversial.

Fisch et al. (1991) reported a relationship between IVF treatment and aPL demonstrating that women who had undergone at least one IVF/ET cycle in the past showed significantly higher aPL levels than controls. A role for estrogens was hypothesized but not supported by their results. Interestingly, the same group of investigators did not confirm this finding years later and proposed aPL as an inherent characteristic of the infertile state (Fisch et al., 1995). Birdsall et al. (1996) documented an aPL prevalence of 15% in patients undergoing IVF, but they failed to observe an increased aPL prevalence in women undergoing further attempts at IVF as compared with women undergoing their first cycle. By contrast, Delgado Alves et al. (2005) have recently reported a significant increase of aPL levels in infertile patients submitted to one or more IVF cycles in comparison both with infertile women as well as the analysis of the relationship between aCL and pregnancy were further objectives.

Materials and Methods

Patients
This prospective, single centre study was approved by the Institutional Review Board of Bari University Hospital and written informed consent was obtained from each participant.

A total of 50 patients undergoing ovarian stimulation and IVF with or without intracytoplasmic sperm injection (ICSI) at the Centre of Reproductive Medicine of Bari, University Hospital were consecutively included in the study from April 2005 until July 2006.

Inclusion criteria were: (i) the first IVF attempt; (ii) a normal menstrual cycle (range of 26–32 days); (iii) baseline FSH levels <12 IU/ml; (iv) body mass index (BMI) between 18–30 kg/m²; (v) no oral contraceptive pill taken in the last year. Exclusion criteria were: (i) diagnosed connective tissue disease or other autoimmune diseases; (ii) previous history of thromboembolism or recurrent abortions; (iii) treatments affecting the immune response, such as corticosteroids, immune-suppressive drugs or immune-modulators. According to the cause of infertility, patients were divided into three groups, i.e. endometriosis (18 patients), tubal factor (TF) (16 patients) and male factor (MF) (16 patients). Endometriosis and tubal damage were detected by laparoscopy.

Ovarian stimulation

Gonadotrophin-releasing hormone-agonist, 0.1 mg triptorelin (Decapeptyl 0.1 mg, IPSEN Biotech, Paris–France) was administered subcutaneously daily, starting in the late luteal phase (day 21) of the previous menstrual cycle. After down-regulation was achieved [serum oestradiol (E₂) ≤30 pg/ml], ovarian stimulation was started with recombinant FSH (rFSH) beginning on cycle day 2–3 at a dose of 225 IU/daily, for the first 5 days. In the following days, the dose was adapted according to the ovarian response to the treatment. Triptorelin was continued up to and including the day of human chorionic gonadotrophin (hCG) administration. Final oocyte maturation was achieved with 250 µg (6500 UI) of recombinant hCG (Ovitrelle, Serono International SA, Geneva, Switzerland) when two or more follicles reached a diameter ≥18 mm and E₂ concentrations dropped to 150–200 pg/ml for each leading follicle. Oocyte retrieval was performed 35–36 h after hCG administration. After oocyte retrieval, IVF with or without ICSI was performed. Embryo transfer was performed on day 2 or day 3 after oocyte retrieval.

The luteal phase was supplemented with progesterone in oil, 50 mg/die (Prontogest, Amsa srl, Roma, Italy) starting from the day after oocyte retrieval and continuing until 12 weeks’ gestation when pregnancy was achieved.

Monitoring of ovarian response during the treatment cycle consisted of blood sampling for hormonal analysis of E₂, LH and FSH on the first day of rFSH treatment, on the fifth treatment day and at regular intervals thereafter up to and including the day of hCG administration, using a commercially available enzyme immunoassay kit (AdaLitis, Bologna, Italy). On these days, vaginal ultrasound investigations were performed to monitor follicle development.

The pregnancy was tested 14 days after ET by quantitative definition of serum βhCG. Pregnancies progressing beyond the 12th week of gestation were considered to be ongoing.

Sera
Patient sera were obtained on three different days: (i) between day 19 and day 21 of the menstrual cycle, before starting triptorelin treatment (time 0; T0); (ii) on the day of oocyte retrieval (time 1, T1); (iii) 14 days after ET (time 2, T2), on the day of βhCG testing.

The control group consisted of 31 fertile women, matched for age, basal FSH and BMI. They were included in the study according to the following criteria: (i) last pregnancy >2 years before study entry; (ii) no oral contraceptive pill taken in the last year; (iii) absence of autoimmune diseases. Sera from controls were obtained in the luteal phase of the menstrual cycle.

Sera from 100 age-matched apparently healthy subjects (blood donors) were utilized to establish cut-off values for immunoglobulin G (IgG)- and IgM-aCL.

Samples from patients undergoing IVF were coded and the investigators performing aCL assays were unaware of either the cause of infertility or the IVF/ET outcome.

ELISA for IgG- and IgM-aCL determination

Half of the wells (alternate rows) of 96-well ELISA plates (NUNC PolySorp # 475094, Roskilde, Denmark) were coated with 1.5 µg/well of cardiolipin (CL) (Sigma–Aldrich Co, St Louis, MO, USA) in ethanol (30 µl/well) and the other half with 30 µl of ethanol alone (for sample blank determination); plates were air-dried overnight at 4°C. After two washings with phosphate-buffered saline (PBS), plates were blocked with 150 µl/well of PBS containing 10% (v/v) of fetal bovine serum (Sigma) (10% FCS/PBS) for 2 h at 25°C and then washed with PBS. Serum specimens diluted 1:100 in 10% FCS/PBS and standards were added (100 µl/well) in duplicate to both CL-coated and uncoated wells. Sera obtained from the same patient at T0, T1 and T2 were assessed in the same day and plate to minimize the effect of inter-assay variation. The assay blank value was obtained by identical treatment of wells using 100 µl of 10% FCS/PBS instead of serum. Serum from a patient affected by
Systemic Lupus Erythematosus with medium titers of IgG- and IgM-aCL (45 GPL and 52 MPL, respectively) was included as positive control in the IgG- or IgM-aCL assay. A negative control was also included in each plate. After a 30 min incubation at 25°C and three washings with PBS, horse-radish peroxidase-conjugated goat anti-human IgG or IgM (Fc specific) (Jackson Immunoresearch Laboratories, Avondale, PA, USA) was diluted 1:10 000 in 10% FCS/PBS and incubated (100 μl/well) for 30 min at 25°C. After washing, colour was developed with 100 μl/well of 0.4 mg/ml of orthophenylenediamine (Sigma) dissolved in citrate buffer (pH 5) and H₂O₂. Plates were incubated in the dark at 25°C and the colour reaction was stopped by addition of 50 μl/well of 2 M H₂SO₄. Optical densities (OD) were measured at 492 nm using an ELISA reader (Bio-Rad, Hercules, CA, USA).

The mean OD of the sample blank duplicates in the absence of antigen was subtracted from the mean OD achieved in the presence of antigen to obtain the net OD reading.

To express IgG- or IgM-aCL as GPL or MPL, respectively, a standard curve was obtained for each plate with the above-mentioned calibrators (non-linear regression, Best-fit curve). GPL and MPL curves (only R² > 0.98 was accepted as satisfactory) ranged from 0 to 80 and from 0 to 90, respectively.

The intra- and inter-assay coefficients of variation, estimated with positive controls were always <10%.

Cut-off values for IgG- and IgM-aCL were set at the 99th percentiles of values obtained in 100 apparently healthy blood donors, being 13.5 GPL and 7.5 MPL, respectively.

**Sample size**

The sample size was calculated planning a comparison for paired data between IgG-aCL levels detected at T0 and T2 (web site http://www.ddsresearch.com/). The expected effect was estimated on the basis of the first 15 patient samples analysed (2.5 ± 4.5 GPL, mean difference found between T2 and T0). On the basis of this expected result, planning an α error level of 1% and a statistical power (1−β) of 90%, a sample size of 42 subjects was calculated. In any case, we decided to study 50 patients.

**Statistical analysis**

Statistical analysis was performed with version 3.0 of GraphPad prism software (GraphPad, San Diego, CA, USA). Continuous variables are reported either as mean ± SD or median and 25th–75th percentiles. Numbers are presented as counts (percentage) for dichotomous data. According to the Gaussian or non-Gaussian distribution continuous data were compared using Student’s t-test, Wilcoxon signed rank test, ANOVA (Bonferroni post hoc test), Friedman test (Dunn’s post hoc test) or Kruskal–Wallis test (Dunn’s post hoc test) as appropriate. Spearman rank correlation coefficient analysis was used to examine the correlation between E₂ levels and aCL. Frequency data were compared using χ² or Fisher’s exact test. P-values <0.05 were taken as statistically significant.

**Results**

The baseline features of patients and control fertile women are shown in Table 1. No statistically significant differences were observed in terms of patients’ age, BMI, basal FSH and E₂ levels.

<table>
<thead>
<tr>
<th></th>
<th>Patients, n = 50</th>
<th>Fertile women, n = 31</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>34.4 (4.2)</td>
<td>33.3 (3.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>Basal FSH (UI/ml)</td>
<td>6.7 (2.3)</td>
<td>7.1 (1.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>BML (mean (SD))</td>
<td>21.8 (2.5)</td>
<td>21 (1.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>E₂ (pg/ml) (mean (SD))</td>
<td>1512 (858)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IgG-aCL levels; median (25th–75th percentile)</td>
<td>2 (0.8–4.7)</td>
<td>1.7 (0.9–3.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>IgM-aCL levels; median (25th–75th percentile)</td>
<td>2.5 (1.8–3.6)</td>
<td>1.8 (1.5–3.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>IgG-aCL positive, n (%)</td>
<td>2 (4)</td>
<td>1 (3.2)</td>
<td>1</td>
</tr>
<tr>
<td>IgM-aCL positive, n (%)</td>
<td>2 (4)</td>
<td>1 (3.2)</td>
<td>1</td>
</tr>
<tr>
<td>IgG- or IgM-aCL positive, n (%)</td>
<td>4 (8)</td>
<td>2 (6.4)</td>
<td>1</td>
</tr>
</tbody>
</table>

Unpaired Student’s t-test.

IgG- and IgM-aCL levels detected at T0 and expressed as GPL and MPL, respectively.

Mann–Whitney test.

aACL positive, according to cut-off values at T0.

Fisher’s exact test.

Scattergrams of IgG- and IgM-aCL levels at T0, T1 and T2 are reported in Fig. 1A and B, respectively. IgG-aCL detected at T2 (median, 25th–75th percentiles: 4.2, 1.4–9.5) were significantly higher in comparison with both those found at T0 (2.9, 1.1–6.6; P < 0.001) and T1 (2.9, 1.1–6.6; P < 0.05), whereas no significant difference was found between T0 and T1. In particular, 38/50 patients (14/18 endometriosis, 77.8%; 13/16 TF, 81.2% and 11/16 MF, 68.7%) showed a variable increase of IgG-aCL values at T2 compared with T0. The frequency of patients with IgG-aCL increase at T2 was not significantly different among groups (P = 0.69).

<table>
<thead>
<tr>
<th></th>
<th>Endometriosis, TF, n = 18</th>
<th>MF, n = 16</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>32.7 (3.9)</td>
<td>35.1 (4.1)</td>
<td>0.09</td>
</tr>
<tr>
<td>Basal FSH (UI/ml); mean (SD)</td>
<td>6 (2.6)</td>
<td>7.6 (2.4)</td>
<td>0.13</td>
</tr>
<tr>
<td>BML; mean (SD)</td>
<td>22.3 (3.1)</td>
<td>21.7 (2.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>E₂ (pg/ml); mean (SD)</td>
<td>1181 (786)</td>
<td>1637 (883)</td>
<td>0.08</td>
</tr>
<tr>
<td>IgG-aCL levels; median (25th–75th percentile)</td>
<td>2.7 (0.3–4.7)</td>
<td>1.4 (1–4.2)</td>
<td>0.66</td>
</tr>
<tr>
<td>IgM-aCL levels; median (25th–75th percentile)</td>
<td>3.3 (2–4.4)</td>
<td>2.7 (2.3–3.4)</td>
<td>0.09</td>
</tr>
<tr>
<td>βHCG positive, n (%)</td>
<td>5 (27.8)</td>
<td>4 (25)</td>
<td>0.98</td>
</tr>
<tr>
<td>Ongoing pregnancy, n (%)</td>
<td>5 (27.8)</td>
<td>3 (18.7)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Detected at T0.

ANOVA.

Kruskal–Wallis test.

χ².
Differences in IgM-aCL levels detected at the three different time points were not significant. Since the most significant differences were observed between T0 and T2, variations between these time points were analysed in each patient groups. IgG-aCL detected at T2 in patients with endometriosis (6.7, 1.7–12.7) and TF (4.2, 2.5–7.6) were significantly higher when compared with T0 in these groups (P = 0.003 and P = 0.018, respectively), whereas no significant differences were found with MF (Fig. 2A). In none of the three groups were significant differences found for IgM-aCL (Fig. 2B).

According to our cut-off values, patients were divided into aCL-positive or -negative groups. At T0, 2 out of 50 patients (4%), i.e. 1 patient with endometriosis and 1 with TF were IgG-aCL positive, whereas 2 patients (4%), i.e. 1 with endometriosis and 1 MF were IgM-aCL positive. Therefore, 4 patients (8%) were cumulatively IgG- or IgM-aCL positive. This prevalence was not significantly different in comparison with fertile women (1 IgG- and 1 IgM-aCL positive, 6.4%) (Table 1).

At T2, 7/50 of patients (14%), i.e. 4 endometriosis, 2 TF, and 1 MF, resulted IgG-aCL positive and 5/50 (10%), i.e. 3 endometriosis, 1 TF and 1 MF were IgM-aCL positive (Fig. 1A and B). Therefore, 12/50 patients (24%) resulted positive to IgG- (GPL ranging from 14.6 to 34.5) or IgM- (MPL ranging from 11.1 to 17.2). The comparison of cumulative aCL prevalence between T2 and T0 (24% versus 8%) showed no statistical significance (P = 0.054).

An increase of aCL values after IVF/ET was found in 3 out of 4 patients with baseline IgG- or IgM-aCL above the cut-offs, whereas in the remaining patient no modification was observed.

As illustrated in Fig. 3, there was no correlation between E2 levels and IgG—(r = −0.105, P = 0.47; Fig. 3A) nor IgM-aCL (r = 0.02, P = 0.9; Fig. 3B). E2 levels detected in the 12 aCL-positive patients at T2 (1345 ± 844 pg/ml) were not significantly different when compared with those in aCL-negative patients (1564 ± 867) (P = 0.45) (data not shown).

There were 13 out of 50 women (26%, i.e. 5 endometriosis, 4 TF and 4 MF) βhCG positive on day 14 after ET and ongoing pregnancy was documented in 12 of them (ongoing pregnancy rate 92%) (Table 2). Pregnancy was demonstrated in 1 of 4 aCL-positive patients at T0 (25%) and in 11 out of 46 aCL-negative (23.9%), with this difference not being significant (odds ratio 1.06, 95% confidence interval (CI) 0.1–11.3; P = 1). Analysis of the relationship between pregnancy and aCL detected at T2 showed that 5/12 aCL-positive (41.7%) and 7/38 aCL-negative (18.4%) patients became pregnant; also in this case, the difference was not significant (odds ratio 3.16, 95% CI 0.77–13; P = 0.13).

Discussion
Over the past two decades, studies on the relationship between aPL and IVF/ET have yielded controversial results concerning both the prevalence, ranging from <10% to >50%, and the
clinical relevance of these autoantibodies. Despite several efforts to standardize the aPL assay, the results have been disappointing and it is difficult to compare various studies, due to methodological variables (Geva et al., 1997). Besides laboratory methodologies, different patients inclusion criteria may explain the conflicting results, since patients who had previously undergone IVF/ET could exhibit higher values of aPL due to IVF-induced hyperestrogenism.

In our home-made aCL assay, the 99th percentiles of values obtained in 100 healthy subjects were utilized to establish cut-off values, as recommended by the European Forum on aPL (Tincani et al., 2004), and the sample blank was subtracted as indicated by the British Committee for Standards in Haematology (Greaves et al., 2000). In fact, non-specific binding could account for an overestimation of positive results (Tincani et al., 2001; Pellegrino and Caccavo, 2007) and commercially available kits for aCL detection do not include ‘no antigen’ wells for sample blank subtraction.

We have focused our investigation only on aCL, since (i) their determination still represents the most sensitive test for aPL screening (Nash et al., 2004) and the most common immunnoassay used in the routine detection of aPL; (ii) the pathogenic role of antibodies directed against other phospholipids, such as anti-phosphatidylserine, anti-phosphatidylethanolamine, etc. is controversial and requires further confirmation (Miyakis et al., 2006). Moreover, to rule out a possible interference of previous ovarian stimulation, only patients undergoing a first IVF/ET cycle were included in our study.

Our results show that neither IgG- nor IgM-aCL values detected before IVF/ET (T0) were significantly different in comparison with those found in fertile women, thus suggesting that the infertile state is not associated with increased aCL levels.

However, IgG-aCL detectable in sera obtained 14 days after ET (T2) were significantly higher than those found at T0, supporting a role of IVF/ET treatment in enhancing aCL levels. The differences documented for IgM-aCL were not significant, even though five women had IgM-aCL over the cut-off level at T2 compared with only two at T0. Since this trend is almost as large as that of IgG-aCL, the possibility cannot be excluded that in a larger group of patients a significant increase in IgM-aCL after IVF would also be found.

An interesting finding of our work is that differences in IgG-aCL between T0 and T2 reached statistical significance only in patients with endometriosis or TF, indicating that these patients are more prone to autoantibody production. This observation is not surprising, since an increased prevalence of several other autoantibodies, including anti-carboxic anhydrase or anti-laminin, has been reported in patients with endometriosis, even if there is no conclusive evidence on their pathogenic role (Gleicher et al., 1987; D’Cruz et al., 1996; Inagaki et al., 2003).

The significant increase of IgG-aCL levels suggests a secondary immune response. Thus, previous immunization with phospholipids, phospholipid-binding proteins or putative cross-reactive antigen derived from infectious agents could be hypothesized. An infectious aetiology of APS (either bacterial or viral) has been proposed, and molecular mimicry between the phospholipid binding protein β2-GPI and common pathogens was proposed as an important cause for aPL production (Blank et al., 2004). It is possible that previous tubal or endometrial infections (e.g. Chlamidia trachomatis or retrovirus, respectively) (Shibahara et al. 2003; Hu et al., 2006) could represent primary immunization against phospholipids or phospholipid-binding proteins, but this should be clarified in future research.

Another possible reason for aCL increase may be ovarian puncture, since it has been reported that ovarian trauma triggers the rise of anti-ovarian antibodies in patients undergoing IVF. In this respect, Barbarino-Monnier et al. (1991) detected high levels of anti-ovarian IgM in patients after the first puncture and increased anti-ovarian IgG and IgA when more than one IVF attempt had been performed.

Our data are consistent with those reported by Delgado Alves et al. (2005), who found no significant differences between IgG- and IgM-aCL detected in untreated infertile patients when compared with fertile controls, whereas patients submitted to one or three IVF/ET showed significantly higher aCL levels in comparison with both fertile women and untreated infertile women. It should be underlined that their results were obtained in distinct patient groups, whereas ours have been achieved analysing the same patients throughout the study.

Fisch et al. (1991) measured, at various time points, aCL in 30 patients undergoing IVF/ET but found no significant differences in aPL levels following IVF treatment. However, their patients had previously undergone other IVF/ET cycles (up to 8) and this inclusion criterion could explain their different results.

In this setting, it is worth mentioning that the cumulative aCL prevalence we found at T2 (24%) was 3-fold that observed at T0 (8%), even though this difference was not significant.

Several lines of evidence indicate that estrogens may enhance antibody production. Molecules involved in B cell activation, such as B cell surface CD22 receptor and intracellular tyrosine phosphatase SHP-1, as well as Bcl-2, which plays an important role in B cell survival, are significantly increased after E2 treatment (Grimaldi et al., 2002). In addition, an E2-induced protection of B lymphocytes against apoptosis has been demonstrated (Grimaldi et al., 2002). As to the relationship between estrogens and autoantibodies, it has been suggested that estrogens may enhance autoantibody production by allowing auto-reactive B lymphocytes to escape normal tolerance (Bynoe et al., 2000; Grimaldi et al., 2005).

With special reference to aCL, increased aCL levels have been found in healthy post-menopausal women during hormone replacement therapy (Todorova et al., 2004) and estrogen-induced aCL were documented in non-autoimmune C57BL/6J mice (Verthelyi and Ansar Ahmed, 1997). In our study, we failed to observe any correlation between E2 and aCL levels, and accordingly, our data do not support a role for estrogens in inducing aCL production. Nonetheless, it is possible that each patient could show a different degree of B cell response to hyperestrogenism.

A putative role for progesterone in triggering aCL rise cannot be excluded. Progesterone induces in vitro the
development of T helper 2 (Th2) cells producing Th2 type cytokines, which favour the antibody response (Piccinni et al. 1995). However, the effects of progesterone on B cells and antibody production are quite controversial, as in vitro and in vivo studies have documented either enhancing or inhibitory effects on B-cells (Vermeulen et al., 2001; Lu et al., 2002).

In animal models, aCL induce pregnancy failure by impairing embryonic implantation. In fact, BALB/c female mice immunized with aCL monoclonal antibodies showed low pregnancy rates and early embryos were severely impaired, demonstrating developmental delay, abnormal morphology, necrosis and a deficient implantation capacity (Sthoeget et al., 1993). Other possible aPL-mediated pathogenic mechanisms include vascular thrombosis and ischaemia at the site of implantation, impairment of endothelial cell receptivity and interference of aPL with syncytiotrophoblast formation (Rote et al., 1992; Silver et al., 1995).

Our results suggest that aCL do not affect ongoing pregnancy and this is, at least in part, consistent with data reported by Stern et al. (2003) who found in a placebo-controlled trial that treatment with heparin and aspirin did not improve pregnancy or implantation rate in aPL-positive patients with IVF implantation failure. Additionally, Nielsen and Christiansen (2005) have shown in a recent large prospective study that low and medium levels of IgG-aCL have no negative impact on pregnancy outcome in lupus anticoagulant negative patients with recurrent spontaneous abortion.

In previous studies, patients have been simply classified as aPL-positive or -negative without any indication of the degree of positivity, raising some pathogenic and clinical questions about this aspect.

In the revised classification criteria for the APS, it has been established that medium to high titers (i.e. >40 GPL or MPL) of IgG- and/or IgM-aCL represent a laboratory diagnostic criterion (Miyakis et al., 2006). Accordingly, in the V Meeting of the European Forum on aPL, a simple classification of aCL levels as low (<40 units) and high (>40 units) was suggested (Cervera et al., 2006).

In addition, it should be pointed out that medium to high aCL levels are more significantly associated with clinical manifestations of APS (Escalante et al., 1995; Silver et al., 1996; Levine et al., 1997).

Notably, in our work aCL were always below the threshold of 40 GPL or MPL, regardless of the detection time point and this finding should be taken into account when interpreting the lack of relationship between their presence and pregnancy. In this context, we are unable to compare our data with those of other investigators, due to the lack of information about the percentage of patients with low or medium to high aCL titers.

Another interesting issue is the persistence or not of aCL positivity after ovarian stimulation. Only a careful follow-up of aCL levels in patients showing an aCL increase could ascertain whether this autoantibody elevation is sustained or transient.

Our results suggest that in women undergoing the first IVF/ET cycle, aCL levels and prevalence are not different from those found in age-matched fertile controls but, at the same time, indicate that IVF/ET treatment increases IgG-aCL levels. However, in our cohort, aCL values fell in the range of ‘low positivity’ and this finding questions the biological and clinical relevance of aCL increase. Furthermore the aCL levels were not associated with the rate of pregnancy.

Therefore, our data do not support a need for routine testing of aCL in all patients submitted to IVF/ET.

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