Antiphospholipid antibodies and the outcome of pregnancy after the first in-vitro fertilization and embryo transfer cycle

Juan Balasch1,3, Montserrat Creus1, Francisco Fábregues1, Salvadora Cívico1, Juan C.Reverter2, Francisco Carmona1, Dolors Tassies2 and Juan A.Vanrell1

1Department of Obstetrics and Gynecology and 2Hemotherapy and Hemostasis Unit, Faculty of Medicine, University of Barcelona, Hospital Clinic i Provincial, Barcelona, Spain
3To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Hospital Clinic i Provincial, C/Casanova 143, 08036 Barcelona, Spain

Increased antiphospholipid antibody prevalence has been demonstrated by a number of recent studies in in-vitro fertilization (IVF) patients but the potential effects of antiphospholipid antibodies on the different components of the reproductive process and the consideration of whether to test IVF patients for antiphospholipid antibodies are controversial. The present study was undertaken to investigate the possible association between the presence of circulating antiphospholipid antibodies (namely the lupus anticoagulant and anticardiolipin antibodies), among a series of 21 consecutive IVF patients having a clinical spontaneous abortion after their first embryo transfer. As a control group (n = 42), the nearest IVF cycle resulting in an ongoing pregnancy before and after each miscarried IVF cycle (i.e. the closest cycles in temporal relationship to the index cycle) was used. One patient (4.8%) in the study group and two women (4.8%) among controls were seropositive for antiphospholipid antibodies. These low and similar seropositivity rates found in the two groups studied lead us to conclude that antiphospholipid antibodies testing in IVF patients should be considered only in those women having repeated failures of implantation/clini-cal abortion after embryo transfer but not in an infertile general population reaching an IVF programme.

Key words: abortion/anticardiolipin antibodies/antiphospholipid antibodies/in-vitro fertilization/lupus anticoagulant

Introduction

At present, there is convincing evidence that abnormal auto-immune function is an aetiological factor in ~10% of patients with recurrent pregnancy loss (Rai et al., 1995; Balasch et al., 1996; Hatasaka et al., 1997) and assessment of antiphospholipid antibodies, namely the lupus anticoagulant and anticardiolipin antibodies, has become routine in the evaluation of women having recurrent abortion (Balasch and Font, 1994; Balasch, 1995; Rai et al., 1996; Hatasaka et al., 1997). A particular subpopulation of anticardiolipin antibodies may be strongly represented in the male partners, the clinical significance of which remains to be established (Panton and Kilpatrick, 1997).

Though recurrent pregnancy loss involves the loss of clinically recognized (post-implantation) pregnancies, it has been postulated that the same immunological dysfunction that may lead to some cases of recurrent pregnancy loss could also affect earlier unrecognized pregnancies as well, leading to heretofore unexplained infertility (Tartakovsky et al., 1996; Hatasaka et al., 1997). Thus, recent research efforts have been focused on the prevalence of antiphospholipid antibody in an infertile population and to study the effect of their presence on in-vitro fertilization (IVF) cycle outcome.

Increased antibody prevalence has been uniformly demonstrated by a number of studies in an IVF general population (Gleicher et al., 1994; Sher et al., 1994; Fisch et al., 1995; Nip et al., 1995; Birdsall et al., 1996; Schenk et al., 1996; Denis et al., 1997; Kowalik et al., 1997; Kutteh et al., 1997). The magnitude of this increase, however, varies widely between reports ranging from 10 to 48%. On the other hand, although the majority of the studies conclude that antiphospholipid antibodies are a poor predictor of the IVF cycle outcome in an IVF general programme (Gleicher et al., 1994; Fisch et al., 1995; Nip et al., 1995; Birdsall et al., 1996; Schenk et al., 1996; Denis et al., 1997; Kowalik et al., 1997; Kutteh et al., 1997), some authors disagree (Sher et al., 1994) and four reports in the literature are in agreement that antiphospholipid antibody prevalence is higher among patients with repeated failure of embryo transfer (three or more) than in women who successfully conceive after IVF (Birkenfeld et al., 1994; Geva et al., 1995; Balasch et al., 1996; Kaider et al., 1996). An important reason for the above controversy may be the heterogeneity among different study populations. On the other hand, abnormal autoantibodies may be nothing but an epiphe-omenon with respect to IVF failure (Gleicher, 1997).

Interestingly, a very recent report (Kowalik et al., 1997) showed that antiphospholipid antibody was not predictive of IVF outcome but the rate of miscarriage was 2-fold higher in the IVF patients positive for antiphospholipid antibodies compared with antibody-negative women. The authors concluded that consideration whether to perform antiphospholipid antibody testing should be given to all patients whose IVF cycle results in a miscarriage after a clinical pregnancy has been documented. We therefore investigated the prevalence of antiphospholipid antibodies among IVF patients having a clinical spontaneous abortion after their first embryo transfer.

Materials and methods

We studied 21 frozen blood samples from consecutive infertile patients becoming pregnant after their first IVF–embryo transfer cycle.
but having a first-trimester spontaneous clinical abortion. As a control group (n = 42), the nearest IVF cycle resulting in an ongoing pregnancy before and after each miscarried IVF cycle (i.e. the closest cycles in temporal relationship to the index cycle) was used. In order to maintain the homogeneity of the two study groups only patients undergoing their first IVF–embryo transfer treatment were included.

No woman had had a previous pregnancy. Blood samples were obtained in keeping with approved protocols and all of them were obtained within 3 months before the IVF cycle. Patients having circulating antiphospholipid antibodies detected before their IVF cycle and a spontaneous clinical abortion after embryo transfer were screened again for antiphospholipid antibodies after miscarriage. Pregnancy was diagnosed by increasing serum concentrations of β-human chorionic gonadotrophin after embryo transfer, and the subsequent demonstration of an intrauterine gestational sac by ultrasound.

Antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies) were determined as described previously (Balasch et al., 1996). Briefly, immunoglobulin G (IgG) and immunoglobulin M (IgM) anticardiolipin antibodies were measured by enzyme-linked immunosorbent assay. Control serum samples from 100 normal blood donors were used to standardize the assay. The final value of the results was considered after correction by subtraction of non-specific binding to uncoated wells. A positive value was defined as a binding index >5 SD above the mean value obtained in normal control subjects. The following tests were carried out in all patients to detect lupus anticoagulant: prothrombin time, activated partial thromboplastin time, kaolin clotting time, diluted Russell’s viper venom time and tissue thromboplastin inhibition test. In order to rule out a deficit in a coagulation factor, each assay was also performed with a mixture and tissue thromboplastin inhibition test. In order to rule out a deficit in a coagulation factor, each assay was also performed with a mixture.

Results

Results are summarized in Table I. It can be seen that main demographic and baseline characteristics and incidence of antiphospholipid antibodies in the two groups studied. Values in parentheses are percentages.

<table>
<thead>
<tr>
<th>Variablea</th>
<th>IVF groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spontaneous clinical abortion (n = 21)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.0 ± 3.3</td>
</tr>
<tr>
<td>Cause of infertility</td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>10 (47.6)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>4 (19.0)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Male</td>
<td>4 (19.0)</td>
</tr>
<tr>
<td>Duration of infertility (years)b</td>
<td>6.2 ± 2.6</td>
</tr>
<tr>
<td>Previous infertility treatment</td>
<td></td>
</tr>
<tr>
<td>Ovulation induction + IUI</td>
<td>4 (19.0)</td>
</tr>
<tr>
<td>Ovulation induction</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Danazol or GnRH agonist</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Tubal surgery</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>No. of embryos/replacementb</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>LA positive women</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>aCL positive women</td>
<td>0</td>
</tr>
</tbody>
</table>

IVF = in-vitro fertilization; IUI = intrauterine insemination; LA = lupus anticoagulant; aCL = anticardiolipin antibodies.

No statistically significant differences were noted for any of the variables.

Values are mean ± SD.

Discussion

The association between autoimmune diseases, mainly systemic lupus erythematosus, and increased pregnancy wastage has long been recognized (Garsenstein et al., 1962). At present it is well known that women with lupus at increased risk for pregnancy loss are those having antiphospholipid antibodies, namely lupus anticoagulant and anticardiolipin antibodies (Kuteth et al., 1993). Apart from antiphospholipid antibodies, there is no convincing evidence to implicate disease-specific autoantibodies as causative in pregnancy failure (Hatasaka et al., 1997). Recently, it has been stressed that in addition to recurrent pregnancy loss, other reproductive processes such as endometriosis, unexplained infertility and IVF failure may be affected by phospholipid antibody abnormalities (the ‘reproductive autoimmune failure syndrome’) (Gleicher et al., 1993). However, in a recent study including a large series of 500 consecutive infertile patients and appropriate control women, we (Balasch et al., 1996) showed a low overall incidence (2.4%) of antiphospholipid antibody positivity which was not different from those found among our control groups of healthy women and was similar to the 2–3% reported in studies of normal pregnant or non-pregnant women (Petri, 1993; Lockwood and Rand, 1994). Furthermore, we were unable to find differences in antiphospholipid antibody positivity rates between different diagnostic categories of infertile patients including endometriosis and unexplained infertility (Balasch et al., 1988; Balasch, 1995).
Related infertility (Hatasaka, 1997). Therefore, the association of abnormal autoimmune function with infertility remains circumstantial and there is no specific autoantibody that may be a marker for autoimmune-related infertility (Hatasaka et al., 1997).

As IVF patients are those infertile women for whom standard infertility treatments have failed, it is surprising that a number of investigations have found an increased antiphospholipid antibody prevalence among women who reached an IVF programme (Gleicher et al., 1994; Sher et al., 1994; Fisch et al., 1995; Nip et al., 1995; Birdsall et al., 1996; Schenk et al., 1996; Denis et al., 1997; Kowalik et al., 1997; Kutteh et al., 1997). A very recent critical review of the literature on the subject leads to the conclusion that the autoantibodies do not appear to affect IVF outcome and several reasons have been proposed to explain the unexplained, mainly because no clear-cut factors (such as hormonal states or specific infertility diagnoses) have been identified as a source of autoantibody induction (Hatasaka et al., 1997). First, the populations studied are inconsistent. Second, among the different studies on the subject, no two used the same set of autoantibodies to establish a diagnosis. Third, different studies have either omitted a control group or have used control groups that are not acceptably comparable to the study population. Fourth, the majority of autoantibody assays used in the evaluation of infertile women are not standardized. Fifth, various definitions of ‘normal’ have been used with regard to the autoantibody assays. Sixth, when many tests are done that are independent or partially independent, then the chances of encountering a false positive increases exponentially (Hatasaka et al., 1997). Finally, it is important to stress that most studies on the subject discussed above measured antiphospholipid antibodies only on a single occasion (usually before the IVF cycle). This is notable because the concept of the ‘reproductive autoimmune failure syndrome’ was coined as an ‘expansion’ of the so-called ‘gynaeco-obstetrical primary antiphospholipid syndrome’ (Gleicher et al., 1993).

As recently stressed by Rai and Regan (1997), before a diagnosis of the primary antiphospholipid syndrome can be made, antiphospholipid antibody positive results should have been obtained on at least two occasions >8 weeks apart. This is particularly important as an association between adverse pregnancy outcome and antiphospholipid antibodies only holds for those with persistently positive titres. Even upper respiratory infections are known to induce ‘false positive’ autoantibody findings and we do not know if transient-positive results (which are a common occurrence) are normal or not among the various clinical categories or within an individual (Hatasaka et al., 1997; Rai and Regan, 1997). By applying this laboratory criterion, an increased incidence of antiphospholipid antibody abnormalities in a large series of 500 consecutive infertile patients was not found (Balasch et al., 1996).

Another essential criterion applying to antiphospholipid antibodies testing in patients with pregnancy loss, is that only patients with recurrent miscarriage should be tested and considered for treatment (Balasch and Font, 1994; Balasch, 1995; Cowchock et al., 1997). Since repeatedly unsuccessful embryo transfers after IVF are thought to be because of occult abortion (Clark, 1989; Gleicher et al., 1993; Hatasaka et al., 1997), a role for antiphospholipid antibodies in failure of nidation after repeated IVF–embryo transfer could be postulated. Favouring this concept is the fact that all the four studies in the literature investigating antiphospholipid antibodies in patients failing three or more IVF–embryo transfer attempts are in agreement that such patients have a higher incidence of antiphospholipid antibody seropositivity than women becoming pregnant with their first IVF attempt (Birkenfeld et al., 1994; Geva et al., 1995; Balasch et al., 1996; Kaider et al., 1996). Therefore, as with the ‘primary antiphospholipid syndrome’, antiphospholipid antibodies testing in IVF patients should be applied according to strict laboratory criteria in those women having repeated failures of implantation/clinical abortion after embryo transfer but not in an infertile general population reaching an IVF programme. This is well exemplified by the present study where only women becoming pregnant after their first IVF attempt were included. Patients having a clinical spontaneous abortion had a very low (and ‘transient’) seropositivity rate (4.7%) for antiphospholipid antibodies which was exactly the same as the incidence of antiphospholipid antibody positivity observed among women carrying to term and similar to the 2–3% reported in studies of normal pregnant or non-pregnant women (Petri, 1993; Lockwood and Rand, 1994). Unfortunately, in previous studies investigating the possible association between antiphospholipid antibodies and miscarriage after IVF and suggesting (Kowalik et al., 1997) or not (Birdsall et al., 1996; Denis et al., 1997; Kutteh et al., 1997) such an association, the number of IVF attempts/pregnancy for each patient was not provided. Thus, further studies similar to that reported here but including IVF patients having both just one or at least two abortions after embryo transfer are desirable.

Acknowledgements
The authors thank Mrs Paquita Antonell for her technical assistance. This work was supported by a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social (FIS 94/0564) to J.B.

References

1182
Antiphospholipid antibodies, IVF and abortion


Received on November 18, 1997; accepted on February 13, 1998