Increased anticardiolipin antibodies are positively related to the uterine artery pulsatility index in unexplained infertility

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The objective of the study was to evaluate, in patients with unexplained infertility, the possible relationship between anticardiolipin antibodies and indices of uterine artery Doppler measurements. A total of 46 infertile women participated in the study and underwent ovarian stimulation. Transvaginal ultrasonography and colour Doppler were performed on the day of embryo transfer and patients were divided on the basis of pulsatility index (PI): group I, PI <2.5; group II, PI 2.5–3.0; and group III, PI >3.0. On the same day that Doppler analysis took place, peripheral blood was obtained and circulating anticardiolipin antibodies were assayed. The response to ovarian stimulation was similar in the three studied groups. No significant differences in oestradiol and ultrasonographic parameters were observed between the groups. A significant increase in anticardiolipin antibodies was observed in those patients with higher resistance to flow at the level of the uterine artery. A significant relationship was found between the uterine artery PI and anticardiolipin immunoglobulin G class (F = 14.35; P = 0.001), and immunoglobulin M class (F = 5.88; P = 0.020). It is concluded that, in unexplained infertility, anticardiolipin antibodies may be involved in uterine vascular modifications and that Doppler flow analysis of uterine arteries may be an important tool in the assessment and management of ovarian stimulation.

Key words: anticardiolipin antibodies/autoimmune/Doppler/infertility/ultrasound

Introduction
It is estimated that 10–20% of couples attending fertility clinics have unexplained infertility (American Fertility Society, 1991). As the definition implies, the mechanisms involved are unknown. Factors that contribute to unexplained infertility have been postulated to include: occult problems in either the oocyte (Hertig, 1975) or the spermatozoon (Haxton et al., 1987), leading to fertilization failure or dysfunctional embryos, infections (Fedele et al., 1989), minimal tubal damage (Edwards and Steptoe, 1983), luteinization of unruptured follicles (Edwards and Steptoe, 1983), peritoneal factors (Vasquez et al., 1984), endometrial receptivity (Lessey et al., 1995) and the immune system (Taylor et al., 1989).

The association between recurrent pregnancy loss and autoimmune diseases has been recognized since Nilsson et al. (1975) first reported the association between the presence of antiphospholipid antibodies (APA) and adverse pregnancy outcomes. Recently, it has been underlined that in addition to recurrent pregnancy loss, the autoimmune system may be involved in other reproductive processes such as endometriosis, unexplained infertility and in-vitro fertilization (IVF) failure (Gleicher et al., 1993). However, despite the current interest in immunological factors leading to unexplained infertility and reproductive failure, the relationship between APA, which can be immunoglobulin G (IgG), IgM or IgA classes, and reproductive performance is still controversial (Balash et al., 1996). The rationale for the role of APA in infertility may be merely speculative. Proposed theories include: inhibition of prostacyclin production with modified thromboxane/prostacyclin ratio (Carreras and Vermlyen, 1982), an increase in platelet activation (Harris et al., 1985), and a decrease in the activation of the antithrombotic action of protein C (Cariou et al., 1988). Furthermore, it has been shown that the attachment of APA to surface phospholipids on the trophoblast may result in direct cellular injury, with inhibition of the cytotoxic trophoblastic conversion to syncytiotrophoblast (Sessions and Horowitz, 1982; Rote et al., 1992; Kowalik et al., 1997). The above hypotheses stress the role of APA in modulating uterine vascularization and implantation processes.

Transvaginal colour Doppler ultrasound is an important tool for studying the female reproductive system. It has been suggested that uterine blood flow has an important role in endometrial receptivity (Battaglia et al., 1997); and it has been demonstrated that, during IVF, embryos fail to implant in women with impaired uterine perfusion (Battaglia et al., 1990; Steer et al., 1992). In addition, Steer et al. (1994) suggested that decreased uterine perfusion might be a cause of unexplained infertility.

The aim of the present observational study was to evaluate prospectively, in patients with unexplained infertility, the possible relationship between phospholipid antibodies and uterine vascularity.

Materials and methods

Patients and protocols
The study protocol was approved by the local ethics review committee. In all, 46 patients attending the Infertility Clinic participated in the study after giving informed consent. The women, all with unexplained infertility, were selected and subdivided into three groups on the basis...
of the uterine artery pulsatility index (PI) on the day of embryo transfer. Group I included patients with a PI < 2.5 (n = 19); group II included women with a PI of 2.5–2.99 (n = 18) and group III included women with a PI ≥ 3.0 (n = 9). For this study, only women aged < 42 years with a follicle stimulating hormone (FSH) concentration of < 15 IU/L, and an oestradiol concentration of < 200 pmol/l, on day 3 of the menstrual cycle, and a normal uterine cavity were included. Excluded from the study were patients with two or more clinical pregnancy losses (not including ectopic pregnancies), one or more prior stillbirths, clinical signs of autoimmune diseases or allergies. All patients had a complete evaluation, including history and physical examination, transvaginal ultrasonography, post-coital test, hysterosalpingography and/or laparoscopy, and a hormonal evaluation including mild luteal serum progesterone and prolactin concentrations. A semen analysis was performed in all the partners.

Infertility was defined as the inability to achieve pregnancy after a minimum of 1 year of unprotected intercourse. Unexplained infertility was defined according to the following criteria: duration of infertility > 1 year, regular menstrual cycles (25–34 days), patent tubes, absent endometriosis, no hormonal pathologies, normal post-coital test, and a normal semen analysis according the World Health Organization criteria for normality.

The groups were similar in age, duration of infertility, and number of previous IVF attempts. The mean age was 34.7 ± 4.3 years, and the mean duration of infertility was 5.2 ± 3.6 years. The mean number of previous IVF attempts was 2.1 ± 1.8.

Ovarian stimulation was achieved by an injection on day 20 of the cycle of i.m. gonadotrophin-releasing hormone agonist (GnRHa) triptorelin (Decapeptyl 3.75; Ipsen, Milan, Italy) and pure follicle stimulating hormone (FSH) (pFSH; Metrodin 75 HP; Serono, Rome, Italy) administered i.m. after pituitary desensitization (plasma oestradiol (E2) concentration < 100 pmol/l; ovaries with no follicles > 5 mm in diameter and endometrial thickness < 5 mm) in an individual assessed dosage. When at least three follicles < 17 mm in diameter were present and a serum oestradiol concentration of > 700 pmol/l follicle was achieved, pFSH was withdrawn and 10 000 IU human chorionic gonadotrophin (HCG; Profasi; Serono) was administered i.m. Transvaginal ultrasonographic oocyte recovery was carried out transvaginally 35 h after HCG injection. The retrieved oocytes were classified as mature, intermediate, immature, and atretic on the basis of the morphology and the appearance of the oocyte cumulus–corona complex according to the criteria of Acosta et al. (1984). To study the impact of embryo quality on implantation, the embryos were graded morphologically before replacement. The embryos were scored as follows: grade A, equal sized blastomeres, no fragmentation; grade B, equal or unequal sized blastomeres, < 20% fragmentation; grade C, equal or unequal sized blastomeres, 20–50% fragmentation; grade D, equal or unequal sized blastomeres, > 50% fragmentation. Transfer was performed 48–72 h after oocyte retrieval. Between one and three of the best embryos were replaced at the 6–12-cell stage. In none of the patients studied was intracytoplasmic sperm injection and/or assisted hatching found to be necessary. Transcervical transfer was carried out using a Frydman catheter (SCS International, Genoa, Italy). The remaining cleaved embryos with < 20% fragmentation were allocated to a cryopreservation protocol. Profasi (2000 IU) was prescribed i.m. as luteal phase support on alternate days until the serum β-HCG assay. Patients with a clinical pregnancy (ultrasonographic evidence of embryonic heart activity) were followed-up until after delivery.

During the ovarian stimulation regimen the patients were submitted to hormonal and ultrasonographic evaluation. On the day of embryo transfer, all the patients underwent colour Doppler analysis of uterine arteries and serum evaluation of IgG and IgM classes of anticardiolipin antibodies (ACA).

Ultrasound and Doppler examinations

Transvaginal ultrasonographic assessment of endometrial thickness and echographic pattern were performed to confirm pituitary desensitization and on the day of HCG administration using a 6.5 MHz vaginal transducer (AU4 Idec; Esaote, Milan, Italy). Measurements of follicular number and size (after confirmation of pituitary desensitization) were performed daily beginning on day 7 of the cycle until the day of oocyte retrieval.

Doppler flow measurements of uterine arteries were performed transvaginally with a 6.5 MHz (AU4 Idec) colour Doppler system. The Doppler evaluation was performed on the day of embryo transfer. All the patients were studied between 08.00 and 11.00 to exclude the effects of circadian rhythmicity on blood flow (Zaidi et al., 1995). They rested for at least 15 min before being scanned, and completely emptied the bladder to minimize any external effects on blood flow (Battaglia et al., 1994). A 50 Hz filter was used to eliminate low frequency signals originating from vessel wall movements. Colour flow images of the ascending branches of the uterine arteries were sampled lateral to the cervix in a longitudinal plane. The angle of insonation was altered to obtain the maximum colour intensity. When good colour signals were obtained, blood flow velocity waveforms were recorded by placing the sample volume across the vessel and entering the pulsed Doppler mode. The PI, defined as the difference between peak systolic (S) and end diastolic (D) flow velocity divided by the mean flow velocity, was calculated electronically by the machine. The PI has been shown to reflect blood flow impedance and may be used when the end diastolic frequency shift is absent or reversed. For each examination the mean value of three consecutive waveforms was obtained. No significant differences between the PI of the left and right uterine arteries were observed. Therefore, the average value of both arteries was used. In the results, the PI has not been corrected for heart rate (Battaglia et al., 1997). The ultrasonographic and Doppler analyses were performed by one examiner (C.B.).

Hormonal assay and ACA determination

Peripheral blood was obtained from all patients between 08.00 and 11.00, after an overnight fast, on the day that the Doppler examination took place. Oestradiol concentrations were determined by a radio-immunoassay (Radim, Pomezia, Italy).

ACA were detected using a non-competitive sandwich enzyme immunoassay system (Autozyme ACL; Cambridge Life Sciences, Cambridge, UK). Serum samples were frozen at −20°C prior to ACA assay. Control serum samples from 100 normal blood donors were used to standardize the assay. The intra-assay variations were 8 and 6% respectively for IgG and IgM classes. The upper limits of normal values were 7.2 and 6.5 IU/ml respectively for IgG and IgM ACA classes.

Statistical analysis

A statistical analysis was performed using the unpaired Student’s t-test, χ² test, and a one-way analysis of variance where indicated. The relationship between the parameters analysed was assessed using the linear regression method. P < 0.05 was considered to be statistically significant. Data are presented as mean ± SD, unless otherwise indicated.

Results

All 46 patients completed the study and were compliant. The number of pFSH ampoules used in the 46 completed cycles, the duration of pFSH treatment and plasma oestradiol con-
Table I. Response to ovulation induction on the day of human chorionic gonadotrophin administration

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>Group III (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pFSH ampoules</td>
<td>25.3 ± 5.7</td>
<td>27.0 ± 4.2</td>
<td>27.6 ± 2.1</td>
</tr>
<tr>
<td>No. of days of pFSH treatment</td>
<td>11.0 ± 2.9</td>
<td>11.7 ± 2.1</td>
<td>11.0 ± 1.7</td>
</tr>
<tr>
<td>Oestradiol concentration (pmol/l)</td>
<td>6057 ± 1945</td>
<td>5818 ± 1343</td>
<td>4963 ± 1343</td>
</tr>
<tr>
<td>No. of follicles of size &gt;1.7</td>
<td>4.4 ± 1.6</td>
<td>4.6 ± 1.4</td>
<td>4.0 ± 2.1</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>10.9 ± 1.5</td>
<td>11.0 ± 1.8</td>
<td>10.4 ± 1.2</td>
</tr>
<tr>
<td>Multilayered endometrium (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</table>

FSH = follicle stimulating hormone.

Table II. Response to ovulation induction

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>Group III (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of follicle aspirated</td>
<td>8.7 ± 3.1</td>
<td>7.2 ± 1.2</td>
<td>6.9 ± 4.1</td>
</tr>
<tr>
<td>No. of oocytes collected</td>
<td>7.1 ± 3.7</td>
<td>6.2 ± 4.1</td>
<td>6.2 ± 3.2</td>
</tr>
<tr>
<td>Oocyte morphology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>54</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>Intermediate</td>
<td>24</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>Immature</td>
<td>18</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Atretic</td>
<td>4</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>68</td>
<td>71</td>
<td>64</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.7 ± 0.6</td>
<td>2.8 ± 0.3</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>Embryo morphology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade A</td>
<td>42</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>Grade B</td>
<td>48</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td>Grade C</td>
<td>7</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Grade D</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pregnancy rate per cycle (%)</td>
<td>22.2</td>
<td>5.5</td>
<td>0*</td>
</tr>
</tbody>
</table>

*p < 0.05.

Discussion

Connective tissue disorders, mainly systemic lupus erythematosus (SLE), are associated with thromboembolic pathologies and adverse pregnancy outcomes (Garsenstein et al., 1962). Furthermore, it is well known that SLE patients at most risk for pregnancy loss are those with elevated APA (Kutteh et al., 1993; Balash et al., 1996), a group of autoantibodies that bind to negatively charged phospholipids. Recently, it has been underlined that in addition to pregnancy wastage (i.e. recurrent miscarriage, placental abortion, intrauterine growth retardation, and pre-eclampsia), other reproductive phenomena such as endometriosis, unexplained infertility and repeated IVF failures, may be related to increased circulating values of APA (Gleicher et al., 1993). Many different autoantibodies may be implicated in reproductive failure, however, ACA have been more often described in association with such anomalies (Kutteh et al., 1993). Fisch et al. (1991) affirmed that the increased circulating APA levels in infertile patients may be related to IVF treatment; however, recently, it has been suggested that increased circulating APA concentrations are not dependent on the treatment but must be considered in relation to the infertile state (Fish et al., 1995; Royburt et al., 1996).

Reproductive autoimmune failure syndrome (Aoki et al., 1995), is a relatively new autoimmune disorder with a continuous evolution of clinical and laboratory criteria. Rigorous definition is difficult because the clinical features of the syndrome are relatively non-specific, and low values of APA have been found in 1–4% of normal individuals (Harris and Spinnato, 1991). The concept of subclinical autoimmune disease (presence of autoantibodies in apparently healthy women) has been recently established and, probably, anomalies of the autoimmune system affecting infertile women do not promote the same high production of APA as patients with clinical and laboratory findings of connective tissue diseases (Gleicher et al., 1993; Balash and Font, 1994). Despite the accumulation of a large body of literature, the relationship between APA and reproductive failure is still one of the most controversial areas of reproductive medicine (Christiansen, 1997; Gleicher, 1997; Gleicher and Coulam, 1997).

The current use of different ovulatory agents and laboratory techniques allows high rates of oocyte retrieval and embryo transfer, although pregnancy rates remain disappointingly low (Yaron et al., 1994). This may be related to embryo quality (Erenus et al., 1991; Plachot, 1992; Tarlatzis, 1992) and/or endometrial receptivity (Noyes et al., 1995).

There are no accepted standard criteria for evaluating endometrial receptivity, although attempts have been made to relate it to ultrasound parameters (Gonen and Casper, 1990; Dickey et al., 1992; Khalifa et al., 1992; Coulam et al., 1994; Yaron et al., 1994; Noyes et al., 1995; Bustillo et al., 1995).

Here we obtained similar results in terms of endometrial texture and thickness. These data are in accordance with those of Sterzik et al. (1991), who affirmed that ultrasound determination of these parameters was not helpful in evaluating endometrial receptivity. Recently, the measurement of impedance to uterine blood flow in IVF cycles has provided...
an indirect measure of endometrial receptivity (Battaglia et al., 1990, 1997; Steer et al., 1992; Bassil et al., 1995; Zaidi et al., 1995, 1996). Steer et al. (1992) reported that 35% of women who failed to conceive in an IVF programme had a mean uterine PI value >3.0. Therefore, they suggested embryo cryopreservation in those patients with a PI >2.99 for transfer in subsequent cycles. The best uterine receptivity was assumed to be expressed by a PI of 2.0–2.99. In our study we observed the highest pregnancy rate (22.2%) in the group with lower resistance at the level of the uterine arteries, whereas no pregnancies were observed in those patients with a PI >3.0. This confirms that the decrease in peripheral impedance in the uterine vasculature bed, reflected by a low PI, is a consequence of increased blood flow and tissue perfusion, which may improve uterine receptivity. In our study, a significant direct relationship between uterine artery PI and ACA was found with significantly higher circulating ACA in those patients with a PI >2.5 on the day of embryo transfer. Uterine artery PI and ACA were not related to the age of the patients, duration of infertility, number of previous IVF attempts, number of retrieved oocytes and number of transferred embryos. This excludes possible interactions between ACA, ovarian response, and oocyte and embryo quality supporting the notion that decreased uterine artery perfusion may be a cause of unexplained infertility and that even moderate increases of ACA may have a possible role in the failure of embryo nidation after embryo transfer. The theories by which APA cause poor endometrial receptivity include: inhibition of prostacyclin production with resultant thromboxane preponderance and increased thromboxane/thromboxane ratio (Bassolino et al., 1980; Carreras et al., 1982), increased platelet aggregation (Harris et al., 1985) and cytokine dysfunction (Wada et al., 1994). We recently demonstrated that significantly lower endometrial cell thromboxane concentrations are present in pregnant compared with non-pregnant patients, and this was found to be related directly to Doppler flow indices of spiral arteries (Battaglia et al., 1997). Hence, an improved prostacyclin/thromboxane ratio may improve endometrial perfusion, prevent expulsion of the nidating blastocyst and prepare the endometrium for nidation (Harper, 1989; Harper et al., 1989; van der Weiden; 1991). The above considerations support the hypothesis that, in ACA positive patients with impaired uterine perfusion, modulation of endometrial vascularity by heparin and/or aspirin may improve pregnancy rates by drug-induced immunosuppression, and antithromboxane and anti-

aggregant effects (Birkenfeld et al., 1994; Geva et al., 1994; Sher et al., 1994; Wada et al., 1994). However, whether the supposed impaired uterine perfusion may be mediated through an antibody-independent mechanism remains to be proven by other study designs.

In conclusion, our data suggest that, in unexplained infertility, ACA may be involved in uterine vascular modifications and that Doppler flow analysis of uterine arteries may be an important tool in the assessment and management of ovarian stimulation. In accordance with Steer et al. (1992), Zaidi et al. (1996), and our previous data (Battaglia et al., 1990), we suggest that, in the presence of elevated uterine artery PI and increased ACA, any embryos produced should be cryopreserved for transfer in a subsequent cycle. Further wider, prospective clinical studies are necessary to elucidate the possible interaction between ACA, decreased uterine perfusion and unexplained infertility.

Table III. Doppler and anticardiolipin antibody findings

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 19)</th>
<th>Group II (n = 18)</th>
<th>Group III (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine artery pulsatility index (PI)</td>
<td>1.99 ± 0.30*</td>
<td>2.76 ± 0.14**</td>
<td>3.45 ± 0.48***</td>
</tr>
<tr>
<td>Anticardiolipin antibodies (IU/ml)</td>
<td>1.76 ± 1.0†</td>
<td>4.4 ± 4.3††</td>
<td>10.9 ± 4.7†††</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>0.96 ± 0.65‡‡</td>
<td>1.72 ± 1.0‡‡‡</td>
<td>2.21 ± 0.8‡‡‡‡</td>
</tr>
<tr>
<td>Immunoglobulin M</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significantly different:

* versus ** (P = 0.019); * versus *** (P = 0.027); ** versus *** (P = 0.028);
† versus †† (P = 0.038); † versus ††† (P = 0.002); †† versus ††† (P = 0.048);
‡ versus ‡‡ (P = 0.014); ‡ versus ‡‡‡ (P = 0.004); ‡‡ versus ‡‡‡ (P = 0.036).

References


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