Adjuvant L-arginine treatment in controlled ovarian hyperstimulation: a double-blind, randomized study

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BACKGROUND: Enhanced vascularization appears to be important for follicular selection and maturation in both spontaneous and stimulated IVF cycles. Nitric oxide, formed in vivo from L-arginine, may play a key role in follicular maturation and ovulation. METHODS: To evaluate the role of L-arginine supplementation in controlled ovarian hyperstimulation, 37 IVF patients were divided into two groups according to ovarian stimulation protocols: group I, GnRH agonist plus pure (p)FSH plus oral L-arginine (n = 18); and group II, GnRH agonist plus pFSH plus placebo (n = 19). Hormonal, ultrasonographic and Doppler evaluations were performed, and plasma and follicular fluid nitrite/nitrate concentrations were monitored. RESULTS: Thirty-two patients completed the study. In group I (n = 16), plasma L-arginine concentrations increased from (basal) 87 ± 12 µmol to 279 ± 31 µmol (P = 0.002) on the day of β-HCG administration. In this group, pFSH treatment was shorter (P = 0.039) than in group II (n = 16). The number of the follicles ≥17 mm was lower (P = 0.038) in group I than group II. The ‘good quality’ embryos were fewer in number (P = 0.034) and pregnancy rate, both per patient (P = 0.024) and per embryo transfer (P = 0.019), was lower in group I. In the L-arginine group, an increased follicular fluid concentration of nitrite/nitrate was observed. On day 8 of the cycle, elevated plasma estradiol levels were associated with decreased blood flow resistances of perifollicular arteries. Follicular fluid concentrations of nitrite/nitrate were inversely correlated with embryo quality (r = –0.613; P = 0.005) and perifollicular artery pulsatility index (r = –0.609; P = 0.021). CONCLUSIONS: L-Arginine supplementation may be detrimental to embryo quality and pregnancy rate during controlled ovarian hyperstimulation cycles.

Key words: controlled ovarian hyperstimulation/Doppler/IVF/L-arginine/nitric oxide

Introduction

Helping infertile couples to have healthy children is one of the primary tasks of assisted reproductive technologies. In order to fulfil this task, reproductive medicine constantly needs to obtain information on physiology and pathophysiology of infertility, and to develop efficient strategies for controlled ovarian hyperstimulation.

The regulation and significance of ovarian and uterine haemodynamics in human reproductive pathophysiology is becoming an important research area, and transvaginal colour flow Doppler ultrasound facilitating the detection of small vessels and the measurement of impedance to flow in the utero-ovarian circulation may represent an important tool for studying the female reproductive system and pelvic haemodynamics.

An increased vascularization of ovarian follicles during the course of their development occurs in experimental animals (Koning et al., 1989). In women, an enhanced vascularization seems to be responsible for the selection and maturation of follicles both in spontaneous and stimulated IVF cycles (Weiner et al., 1993; Balakier and Stronell, 1994; Bassil et al., 1997). Gonadotrophins, steroids, prostaglandins and other vasoactive molecules are involved in the regulation of ovarian blood flow (Taymor, 1996). The importance of nitric oxide (NO) as an intra- and intercellular modulator has been recognized in many biological processes, including ovarian physiology (Anteby et al., 1996). NO is a labile and diffusible molecule which forms stable oxidized metabolites (nitrite/nitrate; NO2-/NO3–) detectable in many biological fluids. In vivo, NO is formed from L-arginine either by a constitutive calcium-dependent, or a pro-inflammatory cytokine-inducible, NO synthase (Moncada et al., 1991). Although the precise role of NO has not been elucidated, it has been suggested that it is involved in follicular maturation and ovulation (Anteby et al., 1996; Tao et al., 1997). It has also been suggested that NO may participate in
periovulatory vasodilatory modulation of rat ovarian blood flow (Ben-Shlomo et al., 1994).

The role of NO in IVF has been recently evaluated (Manau et al., 2000). These authors showed a lack of relationship between intrafollicular nitrate/nitrite concentrations and ovarian response and IVF outcome (i.e. fertilization and pregnancy rate). However, in a previous paper (Battaglia et al., 1999), it was shown that oral L-arginine supplementation during controlled ovarian hyperstimulation in poor responder patients decreases blood flow resistance in both perifollicular and uterine arteries. Hence it was speculated that L-arginine, by modulating the permeability of follicular epithelium to plasma proteins and increasing uterine perfusion, might improve ovarian response, endometrial receptivity and pregnancy rate.

The aim of the present study was to evaluate, prospectively, blindly and randomly, the possible role of orally administered L-arginine in modifying vascular parameters and improving ovarian response to gonadotrophins in IVF cycles in normally responding women.

Materials and methods

Patients and protocols

The study protocol was approved by the Institutional Ethics Review Committee. The patient sample size calculation was computed with regard to the number of follicles >17 mm maximum diameter. This parameter was considered the primary outcome. It was calculated that to obtain an arbitrarily chosen potential difference of 1.7 follicles of >17 mm diameter among treated and untreated women, a sample size of 15 patients would provide 90% power at a significance level of 0.05. All 37 women attending the Modena University Infertility Clinic who participated the study provided their informed consent.

The mean (± SD) age of the patients was 33.8 ± 3.1 years (range 28–37), and the mean duration of infertility was 3.7 ± 2.4 years (range 2–6). All patients were selected from women who suffered from tubal infertility. All had regular menstrual cycles (28 ± 4 days), and their partners were fertile according to World Health Organization standards. Patients with concurrent illness were excluded from the study. Other exclusion criteria included body mass index [BMI = weight (kg)/height (m)²] ≥ 30, endometriosis, ovarian functional cyst, polycystic ovarian syndrome, unilateral ovarian resection or ovariectomy. Likewise, patients who took regular exercise, were heavy smokers (>10 cigarettes/day), and were hypertensive (systolic blood pressure >140 mmHg and/or diastolic pressure >90 mmHg) were excluded from the study. None of the women had received hormonal treatments for at least 4 months before the IVF attempt.

In order to assess ovarian reserve, peripheral blood was obtained from all patients between 08:00 and 11:00 on day 3 of the cycle preceding the IVF attempt, after an overnight fast. Basal plasma estradiol (E₂), FSH and LH concentrations were determined using a radioimmunoassay (RIA; Radim, Pomezia, Italy).

Patients were assigned randomly to two different stimulation protocols: a long GnRH agonist protocol and pure (p)FSH plus oral L-arginine (group I; n = 18); or a long GnRH agonist protocol and pFSH plus placebo (group II; n = 19). The placebo resembled (in terms of ampoule appearance, smell and flavour) the corresponding L-arginine preparation, and both the participating women and investigators were unaware which treatment was received. Randomization was performed by opening sequentially numbered sealed envelopes containing treatment allocation determined by a random number table.

Controlled ovarian hyperstimulation was achieved by an i.m. injection on day 20 of the cycle of GnRH agonist triptorelin (Decapeptyl 3.75; Ipsen, Milan, Italy) and, after pituitary desensitization (plasma E₂ concentration <100 pmol/l; ovaries with no follicles >5 mm in diameter and endometrial thickness <5 mm), i.m. administration of pFSH (Metrodin 75 HP; Serono, Rome, Italy; 225 IU in the first 3 days of the cycle, then in an individually assessed dosage).

Patients were supplemented with 2×4 ampoules/day of either oral L-arginine (Bioarginina; Damor, Napoli, Italy; one ampoule = 2 g L-arginine) or placebo.

The IVF cycles were cancelled when E₂ plasma levels were <1.1 pmol/l and/or fewer than three follicles were recruited by cycle day 8. Similarly, the IVF cycles were cancelled in those patients at risk of ovarian hyperstimulation syndrome (>15 follicles per ovary and/or plasma E₂ levels >9000 pmol/l).

When at least two follicles >17 mm in diameter were present, triptorelin, pFSH and L-arginine or placebo were withdrawn and 10 000 IU HCG (Profasi; Serono) were administered i.m. Ultrasonographic oocyte recovery was performed transvaginally 35–36 h after HCG injection. The retrieved oocytes were classified as mature, immature or atretic on the basis of the morphology and appearance of the oocyte cumulus–corona complex according to published criteria (Acosta et al., 1984). In order 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; and grade D, equal- or unequal-sized blastomeres, >50% fragmentation. Embryo transfer was performed 72 h after oocyte retrieval. Between one and three embryos were replaced at the 6- to 12-cell stage. 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. Vaginal progesterone (Esolut; Angelini, Rome, Italy) was prescribed as luteal phase support until the serum β-HCG assay was performed. A clinical pregnancy was diagnosed by ultrasonographic evidence of embryonic heart activity.

During the ovarian stimulation regimen the patients were submitted to hormonal (E₂), biochemical (L-arginine) and ultrasonographic (follicular number and diameter, endometrial thickness) and Doppler (uterine and perifollicular arteries) evaluations. Plasma and follicular fluid concentrations of NO₂/NO₃⁻ were assayed.

Ultrasound and Doppler examinations

Transvaginal ultrasonographic assessments of endometrial thickness were performed on days 1 and 8 of ovarian stimulation, and on the day of HCG administration in both groups, using a 6.5 MHz vaginal transducer (A4 Idea; Esaote, Milan, Italy). Measurements of follicular size were performed daily beginning on day 8 of the cycle until the day of oocyte retrieval. A modified ovarian synchrony index (OSI = number of follicles >17 mm/number of follicles 10–14 mm × 100) (Franco et al., 1994) was calculated.

Doppler flow measurements of uterine and perifollicular arteries were performed transvaginally with a 6.5 MHz (A4 Idea) colour Doppler system. The Doppler examination was performed at the beginning of pFSH administration, on day 8 of controlled ovarian hyperstimulation and on the day of oocyte retrieval. All patients were studied between 08:00 and 11:00 in order to exclude the effects of circadian rhythm on blood flow (Zaidi et al., 1995b). Patients were allowed to rest for at least 15 min before being scanned, and completely emptied their bladder in order 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. The maximum ultrasonographic energy was <80 mW/cm². The intensity was within the safety limits suggested by the American Institute for Ultrasound in Medicine (Lizzi and Mortimer, 1989). 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 pulsatility index (PI), defined as the difference between peak systolic (S) and end-diastolic (D) flow velocity divided by the mean flow velocity (S – D/mean) was calculated electronically. 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, and hence the average value of both arteries was used. The perifollicular arteries, starting from day 8 of controlled ovarian hyperstimulation, were identified around the follicles (>1.0 cm maximum diameter), in the ovarian stroma at the maximum distance from the surface of the ovary. Recorded spectra were analysed and the resistance index (RI) was obtained (RI = S – D/S). Arteries demonstrating the lowest downstream impedance were selected for measurements, assuming that these were the branches supplying the developing follicles directly. When calculating results, the PIs of both uterine and perifollicular arteries were not corrected for heart rate. An indication of within-patient precision of the Doppler procedures was obtained by analysing the flow velocity waveforms recorded on three occasions either from uterine and perifollicular arteries at 1 min intervals. An analysis of variance of the results from 15 patients gave a mean coefficient of variation of 5.3% for uterine and 6.7% for perifollicular arteries, and showed no significant differences between the replicate analyses. Ultrasonographic and Doppler analyses were performed by one examiner (C.B.).

**Hormonal and biochemical assays**

Peripheral blood was obtained between 08:00 and 11:00 (after an overnight fast) on day 1, day 8, and on the day of HCG administration. The blood was immediately centrifuged, and the serum removed and stored at -70°C until taken for assay. Estradiol was measured by RIA as described previously (Facchinetti et al., 1998). NO production was assessed by monitoring (on day 1, day 8 and day of oocyte retrieval) plasma levels of stable oxidation products of NO metabolism (NO2⁻/NO3⁻). Since very little or no NO2⁻ is normally found in the serum, no attempt was made to differentiate between NO2⁻ and NO3⁻ amounts; hence, results were reported as NO2⁻/NO3⁻. NO2⁻/NO3⁻ were assayed using the Greiss reaction with previously described procedures (Clancy and Abramson, 1992; Facchinetti et al., 1997).

NO2⁻/NO3⁻ levels were also assayed in follicular fluid in those patients who reached oocyte retrieval. Following transvaginal needle aspiration of the accessible follicles, in order to homogenize the fluids and to reduce possible interfollicular differences, the follicular fluids of follicles ≥17 mm were pooled and immediately centrifuged (2000 g for 20 min). The supernatant was removed and stored at -70°C until bioassayed. Similarly, aliquots of follicular fluid obtained by aspiration of all accessible follicles (<17 mm) were pooled, centrifuged, stored at -70°C, and subsequently assayed. The analyses were performed using the same methods as for serum assays. All samples from each subject were analysed in duplicate in the same assay. On the basis of two quality control samples, the average intra- and inter-assay coefficients of variation were 5.1 and 7.7% for LH, 4.8 and 7.1% for FSH, 4.9 and 7.5% for E2, and 6.8 and 11.3% for L-arginine respectively. In addition the NO2⁻/NO3⁻ intra- and inter-assay coefficients of variation were 6.6 and 8.9% respectively. No differences were observed between follicular fluid and serum assays.

**Statistical analysis**

A statistical analysis was performed using the Mann–Whitney, χ², Fisher–Irwin exact and Wilcoxon tests and a one-way analysis of variance, where indicated. The relationship between the parameters analysed was assessed using the linear regression method. A P-value ≤ 0.05 was considered to be statistically significant. Data were presented as mean ± SD, unless otherwise indicated.

**Results**

Hormonal evaluation on day 3 of the cycle preceding the IVF attempt confirmed normal ovarian reserve in both patient groups (Table I).

Thirty-two patients completed the study. The cancellation rate, due to a poor response, was 2/18 (11.1%) and 3/19 (15.7%) in groups I and II respectively. No significant side-effects were reported by patients in either the L-arginine- or placebo-treated groups. The number of pFSH ampoules and pFSH units/day did not differ significantly between groups (Table II). However, the duration of pFSH treatment was significantly longer in the placebo-treated group (12.3 ± 3.5 days) than the L-arginine group (10.6 ± 2.4 days; P = 0.039). The number of recruited follicles on the day of HCG

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**Table I. Ovarian reserve on day 3 of the cycle preceding the IVF attempt in L-arginine- and placebo-treated groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-Argininea</th>
<th>Placebob</th>
<th>Normal rangec</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of recruited follicles</td>
<td>15.3 ± 3.3</td>
<td>10.4 ± 3.1</td>
<td>10.4 ± 3.1</td>
</tr>
<tr>
<td>Ovarian synchrony index (%)</td>
<td>32.3 ± 11.6</td>
<td>48.5 ± 12.8</td>
<td>48.5 ± 12.8</td>
</tr>
<tr>
<td>Endometrial thickness (cm)</td>
<td>1.07 ± 0.11</td>
<td>1.09 ± 0.17</td>
<td>1.09 ± 0.17</td>
</tr>
</tbody>
</table>

**Table II. Response to controlled ovarian hyperstimulation in the L-arginine- and placebo-treated groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-Argininea</th>
<th>Placebob</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pFSH ampoules</td>
<td>31.5 ± 10.2</td>
<td>37.6 ± 6.1</td>
<td>NS</td>
</tr>
<tr>
<td>No. of days of pFSH treatment</td>
<td>10.6 ± 2.4</td>
<td>12.3 ± 3.5</td>
<td>0.039</td>
</tr>
<tr>
<td>FSH units/day</td>
<td>3.2 ± 0.8</td>
<td>2.5 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. of recruited follicles</td>
<td>15.3 ± 3.3</td>
<td>10.4 ± 3.1</td>
<td>0.021</td>
</tr>
<tr>
<td>Follicles ≥17 mm</td>
<td>4.3 ± 2.1</td>
<td>6.1 ± 2.1</td>
<td>0.038</td>
</tr>
<tr>
<td>Follicles 14–16 mm</td>
<td>3.7 ± 1.8</td>
<td>2.2 ± 1.1</td>
<td>0.057</td>
</tr>
<tr>
<td>Follicles 10–14 mm</td>
<td>7.4 ± 1.7</td>
<td>3.3 ± 2.0</td>
<td>0.034</td>
</tr>
</tbody>
</table>

**a**No significant differences were observed between the groups.
**b**Values are mean ± SD.
**c**Normal range derived from normally ovulating patients attending the infertility clinic.
administration was higher in group I (15.3 ± 3.3) than in group II (10.4 ± 3.1; P = 0.021). The number of large (≥17 mm maximum diameter) and small (10–14 mm maximum diameter) follicles, as well as the OSI (an index of follicular growth homogeneity) and endometrial thickness on the day of HCG administration are reported in Table II. Although, between groups, the number of large (≥17 mm) and small (<14 mm) follicles reached only a weak significant difference, the OSI was significantly higher in placebo-treated patients (48.5 ± 12.8%) than in those receiving L-arginine (32.3 ± 11.6%; P = 0.004).

The number and quality of oocytes collected, and the fertilization rate (number of oocytes fertilized/number of oocytes collected ×100) did not differ significantly between the two groups (Table III). However, embryo morphology (an indirect expression of embryo quality) was significantly better in group II (grade A + grade B = 72.1 ± 15.6%) than in group I (grade A + grade B = 50.0 ± 26.3%; P = 0.034). The number of embryos transferred was similar in both groups (2.6 ± 0.5 versus 2.8 ± 0.3). The pregnancy rate per cycle (16.6 versus 31.6%; P = 0.024) and pregnancy rate per embryo transfer (18.7 versus 37.5%; P = 0.019) was significantly higher in the placebo- than the L-arginine-treated group. Among the nine pregnancies obtained, seven resulted in live births and two are currently near-term ongoing pregnancies.

During ovarian stimulation, serum E2 levels were increased (Figure 1). The plasma concentration of L-arginine was increased in the treated group, from 87 ± 12 (basal) to 279 ± 31 μmol/l (day of HCG administration) (P = 0.002). No significant differences were observed between basal and HCG day plasma L-arginine levels in the placebo group (68 ± 11 versus 56 ± 19 μmol/l). Plasma NO2−/NO3− levels were also increased during ovarian stimulation (Figure 2). In the follicular fluid, NO2−/NO3− concentrations were higher in the L-arginine (9.87 ± 1.35 μmol/l) than placebo (8.68 ± 1.82 μmol/l; P = 0.048) -treated group, the difference becoming more evident (11.56 ± 3.42 versus 8.75 ± 1.51 μmol/l; P = 0.033) when considering only the NO2−/NO3− concentration in follicles with maximum diameter >17 mm (Figure 2). Uterine and perifollicular blood flow resistances were also changed during ovarian stimulation (Figure 3).

Among the study population, follicular fluid NO2−/NO3− concentrations were inversely correlated with embryo quality (r = −0.613; P = 0.005) and perifollicular artery PI (r = −0.609; P = 0.021). Furthermore, plasma NO2−/NO3− concentrations were inversely correlated with uterine artery PI (r = −0.476; P = 0.046).

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### Table III. Response to controlled ovarian hyperstimulation in the L-arginine- and placebo-treated groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-Arginine&lt;sup&gt;a&lt;/sup&gt; (n = 16)</th>
<th>Placebo&lt;sup&gt;a&lt;/sup&gt; (n = 16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of oocytes collected</td>
<td>9.9 ± 3.4</td>
<td>9.2 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Oocyte morphology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>64.2 ± 15.7</td>
<td>78.5 ± 24.6</td>
<td>NS</td>
</tr>
<tr>
<td>Immature</td>
<td>32.5 ± 13.4</td>
<td>21.4 ± 24.6</td>
<td>NS</td>
</tr>
<tr>
<td>Atretic</td>
<td>6.5 ± 6.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>68.4 ± 23.7</td>
<td>71.3 ± 22.9</td>
<td>NS</td>
</tr>
<tr>
<td>Embryo morphology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade A + Grade B</td>
<td>50.0 ± 26.3</td>
<td>72.1 ± 15.6</td>
<td>0.034</td>
</tr>
<tr>
<td>Grade C + Grade D</td>
<td>49.9 ± 36.1</td>
<td>27.3 ± 14.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean ± SD. NS = not significant.
it might be speculated that NO derivatives act as vasodilators that cause too early an increase in the permeability of the follicular epithelium to plasma proteins, and that this results in the follicles being susceptible to circulating FSH and growth hormone (GH) action. The simultaneous early action of FSH and GH may promote a wide and incongruent follicular recruitment with consequent early and not synchronous increased production of insulin-like growth factor-I, which most likely favours a non-homogeneous follicular maturation and differentiation of granulosa cells by modifying various physiological mechanisms (Erikson et al., 1989; Adashi et al., 1991; Artini et al., 1994).

In the present study, although the fertilization rate and the number of transferred embryos were similar in both groups, the quality (type A+B) of embryos and pregnancy rate were higher in placebo-treated patients. Furthermore, in l-arginine-treated women, the intrafollicular NO$_2$/NO$_3^-$ concentrations were higher than in the placebo-supplemented group, whilst in the entire study population the follicular fluid NO$_2$/NO$_3^-$ concentrations were inversely correlated with embryo quality.

Each embryo has its own developmental potential, and few cleaved embryos are competent to implant after IVF and develop through gestation (Van Blerkom et al., 1997). It is known that mature oocytes often contain chromosomal and cytoplasmic structural defects that prevent the fertilized oocytes from adequate developmental growth. How and when such anomalies intervene are not well understood. Although differences in follicle cell function and follicular fluid biochemistry have been suggested to influence the developmental potential of the human oocyte, no single factor—whether secreted into the circulation or present in the follicular fluid—has been shown to provide definitive prediction of the developmental competence of the oocyte-embryo complex. It has been suggested (Gaulden, 1992) that intrafollicular hypoxia might negatively influence spindle organization and chromosomal segregation in the human oocyte. However, the intra-ovarian regulatory system is composed of various substances including growth factors, cytokines, neuropeptides and vasoregulatory molecules and, among these, NO and its derivatives may serve an important role.

Several studies have demonstrated that elevated NO concentrations can reduce cellular ATP levels by inhibiting the cells’ ATP-generating ability (Moncada et al., 1991). This cytostatic and cytotoxic mechanism induces a direct inhibition of mitochondrial respiration and DNA synthesis. Furthermore, elevated NO concentrations react actively with oxygen, yielding strongly oxidizing molecules (nitrogen dioxide and peroxy nitrates) that are potentially more toxic than NO itself (Anggard, 1994).

The above considerations allow us to speculate that l-arginine supplementation with the consequent elevated intrafollicular NO$_2$/NO$_3^-$ concentrations may have detrimental effects on embryo quality and pregnancy rate. This may be appear to be in contrast to a previous study, where it was affirmed that in ‘poor responder’ patients the adjuvant l-arginine supplementation during controlled ovarian hyperstimulation improved follicular growth, oocyte quality, fertilization rate, and arguably also pregnancy rate (Battaglia

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**Discussion**

In natural cycles, only one follicle is dominant and reaches a mean size of >17 mm maximum diameter, while the remaining follicles attain a maximum diameter <10 mm (Hackeloer et al., 1979). Thus, whilst in the natural cycle the ideal OSI is 100%, in stimulated cycles many follicles are dominant and it is not possible to obtain an ideal OSI. However, a progressive increase of OSI may reflect an increasing homogeneity of follicular cohort. The use of l-arginine resulted in quick, intense and incongruent follicular growth, as indicated by the OSI. It has previously been shown (Eldar-Geva et al., 1998) that incongruent follicular development during controlled ovarian hyperstimulation may have a negative influence on the outcome of gamete intra-Fallopian transfer cycles. Moreover, because it occurred in older patients who were submitted to higher doses of FSH and presented lower serum E$_2$ concentrations and fewer retrieved oocytes, the authors speculated that incongruent follicular distribution might be considered an expression of poor follicular development during controlled ovarian hyperstimulation. The results of the present study do not appear to confirm the findings of Eldar-Geva et al. as the doses of pFSH, the plasma E$_2$ levels on the day of HCG, and the number and quality of oocytes collected did not differ between the two groups. In contrast, because in the l-arginine-treated group (on day 8 of the stimulated cycle) elevated plasma E$_2$ levels were associated with high plasma NO$_2$/NO$_3^-$ levels and also with decreased blood flow resistances of perifollicular arteries,
et al., 1999). However, when comparing the intrafollicular content in normal and ‘poor’ responders, it was noted that L-arginine-supplemented ‘poor responders’ showed follicular fluid NO$_2$ /NO$_3$$^{-}$ concentrations (6.7 ± 1.11 μmol/l) (Battaglia et al., 1999) that were similar to those in placebo-treated patients (8.68 ± 1.82 μmol/l; P = 0.064) but significantly lower than in L-arginine-supplemented, normally responding women (9.87 ± 1.35 μmol/l; P = 0.022). It was speculated that follicular fluid NO derivatives are most likely necessary for oocyte activation at fertilization and have beneficial effects when produced within physiological limits, but at higher doses they can cause cytostatic and cytotoxic effects and have detrimental consequences on embryo quality, implantation and pregnancy rate.

The pregnancy rate was significantly higher in the placebo-treated than the L-arginine-treated group, and this might be due to better embryo quality and/or improved endometrial receptivity. There are no accepted standard criteria for evaluating endometrial receptivity, although attempts have been made to correlate it with ultrasound parameters (Gonen and Casper, 1990; Khalifa et al., 1992; Coulam et al., 1994; Yaron et al., 1994; Noyes et al., 1995). In those patients who reached oocyte retrieval, similar results were obtained in terms of endometrial texture and thickness, with or without L-arginine. These data confirm that endometrial ultrasonography is not helpful in evaluating endometrial receptivity.

The measurement of impedance to uterine blood flow in IVF cycles has provided an indirect measure of endometrial receptivity (Battaglia et al., 1990; Steer et al., 1992; Bassil et al., 1995; Zaidi et al., 1995a, 1996). In the present study, a significantly lower downstream impedance in uterine arteries resulted, on the day of oocyte retrieval, in placebo-supplemented patients. These data confirm that the decrease in peripheral impedance in the uterine vascular bed, reflected by a low PI, is a consequence of increased blood flow and tissue perfusion, which may improve uterine receptivity (Goswamy et al., 1988; Battaglia et al., 1990, 1997; Steer et al., 1992).

In both groups, an inverse correlation between plasma NO$_2$ /NO$_3$$^{-}$ concentrations and uterine artery Doppler PI was seen. Furthermore, on the day of oocyte retrieval, a significantly lower uterine artery PI was observed in the placebo-treated women. Hence, it might be suggested that, in accordance with data reported by others (Ramsay et al., 1994, 1995) who found that human uterine blood flow can be increased by the administration of a NO donor drug, the relaxation of vascular smooth muscle of endometrial vessels may be partially mediated by NO and its derivatives. However, a sudden reduction in plasma NO$_2$ /NO$_3$$^{-}$ concentration, seen after the circulation of large quantities of NO$_2$ /NO$_3$$^{-}$ for a relatively long period, might induce an intense rebound effect on vascular tone, increase the impedance to flow in the uterine vascular bed, and reduce endometrial receptivity.

The above considerations support the hypothesis that an adequate modulation of endometrial vascularity might improve the implantation and pregnancy rate.

Although further larger randomized studies are necessary to elucidate the factors that influence intra-ovarian regulation of ovarian function, it may be concluded that oral L-arginine supplementation in normally responding patients increases follicular recruitment and reduces the duration of pFSH treatment, but might also have detrimental effects on embryo quality and pregnancy rate.

Acknowledgements
The authors thank Dr Michela Salvatori and the staff of the Modena University Infertility Clinic for their invaluable help and cooperation. They also thank Daniele Radi for expert assistance in laboratory assays, and E.Rossi for help with the statistical analysis.

References


