

Adjuvant L-arginine treatment for in-vitro fertilization in poor responder patients

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The objective of the present study was prospectively and randomly to evaluate the role of L-arginine in improving uterine and follicular Doppler flow and in improving ovarian response to gonadotrophin in poor responder women. A total of 34 patients undergoing assisted reproduction was divided in two groups according to different ovarian stimulation protocols: (i) flare-up gonadotrophin-releasing hormone analogue (GnRH_a) plus elevated pure follicle stimulating hormone (pFSH) ($n = 17$); and (ii) flare-up GnRH_a plus elevated pFSH plus oral L-arginine ($n = 17$). During the ovarian stimulation regimen, the patients were submitted to hormonal (oestradiol and growth hormone), ultrasonographic (follicular number and diameter, endometrial thickness) and Doppler (uterine and perfollicular arteries) evaluations. Furthermore, the plasma and follicular fluid concentrations of arginine, citrulline, nitrite/nitrate ($\text{NO}_2^-/\text{NO}_3^-$), and insulin-like growth factor-1 (IGF-1) were assayed. All 34 patients completed the study. In the L-arginine treated group a lower cancellation rate, an increased number of oocytes collected, and embryos transferred were observed. In the same group, increased plasma and follicular fluid concentrations of arginine, citrulline, $\text{NO}_2^-/\text{NO}_3^-$, and IGF-1 was observed. Significant Doppler flow improvement was obtained in the L-arginine supplemented group. Three pregnancies were registered in these patients. No pregnancies were observed in the other group. It was concluded that oral L-arginine supplementation in poor responder patients may improve ovarian response, endometrial receptivity and pregnancy rate.

Key words: Doppler/IVF/L-arginine/poor responders/ultrasonography

Introduction

Despite advances in assisted conception, poor ovarian response to gonadotropin stimulation remains one of the major challenges for in-vitro fertilization (IVF). The incidence is comprised of between 5% and 18% of the IVF cycles (Ben-Rafael

et al., 1991; Jenkins *et al.*, 1991). Although there is a lack of standard definitions, poor responders are patients failing to achieve an adequate number of mature follicles and/or an adequate serum oestradiol levels after gonadotrophin stimulation. This condition leads to cycle cancellation, or, in cases in which oocyte retrieval is possible, a very low pregnancy rate (Keay *et al.*, 1997). Furthermore, a poor response identifies patients who on subsequent IVF attempts present a high cancellation rate (24–68%) (Tanbo *et al.*, 1990). The above condition may be considered the result of diminished ovarian reserve and can be due to advanced age, prior ovarian surgery, environmental and genetic factors (Toner *et al.*, 1991; Keay *et al.*, 1997). Furthermore, severe endometriosis (Wardle *et al.*, 1985) and pelvic infections (Keay *et al.*, 1998) may impair ovarian function either through direct damage to the ovaries or through indirect mechanisms. In most patients, the inadequate responses remain unexplained (Ben-Rafael *et al.*, 1986; Rodgers *et al.*, 1995).

For appropriate counselling and management it would be helpful to identify these patients before initiating hormonal stimulation for IVF. Advanced female age (≥ 40 years) (Piette *et al.*, 1990), elevated serum oestradiol (Smotrich *et al.*, 1995), inhibin (Halvorson and De Cherny, 1996), follicle stimulating hormone (FSH) (Muasher *et al.*, 1988) and increased FSH/luteinizing hormone (LH) ratio (Droesch *et al.*, 1989) on cycle day 3 have been associated with suboptimal response to stimulation. On the contrary, a rise in endogenous growth hormone (GH) secretion is associated with significantly higher plasma oestradiol concentrations and more oocytes per stimulated cycle (Stone and Marrs, 1992).

Many strategies for the treatment of such patients have been proposed (Sandow *et al.*, 1978; Check *et al.*, 1990; Ibrahim *et al.*, 1991; Manzi *et al.*, 1994; Schoolcraft *et al.*, 1997). However, despite multiple different stimulation protocols for IVF, the ideal stimulation for poor responders still remains unknown.

The regulation and significance of the ovarian and uterine haemodynamics in human reproductive pathophysiology is becoming an important tool. Increased vascularization of ovarian follicles in the course of their development occurs in experimental animals (Koning *et al.*, 1989). In women, 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). *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). The precise role of NO has not been elucidated; however, it has been thought to be involved in follicular maturation and ovulation (Anteby *et al.*, 1996; Tao *et al.*, 1997). It was suggested (Ben-Shlomo, 1994) that NO may participate in periovulatory vasodilatory modulation of rat ovarian blood flow.

Transvaginal colour flow Doppler ultrasound is an important tool to study the female reproductive system and pelvic haemodynamics. It facilitates the detection of small vessels in the utero-ovarian circulation and the measurement of impedance to flow in this vascular tree.

The aim of the present study was prospectively to evaluate, in a group of poor responder patients, a possible role of orally administered L-arginine in modifying vascular parameters and improving ovarian response to gonadotrophins in IVF cycles.

Materials and methods

Patients and protocols

The study protocol was approved by the local ethics review committee. In all, 34 women attending the infertility clinic participated in the study after giving informed consent. The women had previously undergone a failed IVF attempt. The IVF cycles were cancelled when oestradiol plasma concentrations were <1.100 pmol/l and/or fewer than three follicles were recruited by cycle day 8. Briefly, in cancelled cycles, ovarian stimulation was achieved as follows: mid-luteal gonadotrophin-releasing hormone analogue (GnRHa) leuprorelin acetate (Enantone 3.75; Takeda, Roma, Italy) and pure FSH (pFSH; Metrodin 75 HP; Serono, Roma, Italy); leuprorelin was administered from day 20 of the menstrual cycle (3.75 mg i.m.) and pFSH (in an individually assessed i.m. dosage) commenced after pituitary desensitization (plasma oestradiol concentration <35 mg/ml; ovaries with no follicles >5 mm in diameter and endometrial thickness <5 mm).

The mean age (mean \pm SD) of the above poor responder patients was 40.2 ± 2.1 years (range 37–44 years), the mean duration of infertility was 6.8 ± 3.8 (range 4–12 years). All the patients were selected among women who suffered from tubal infertility. They had regular menstrual cycles (28 ± 4 days) and their partners were fertile according to World Health Organization standards. Patients with intercurrent illness were excluded from the study. Other exclusion criteria were body mass index [BMI = weight (kg)/height² (m²)] >30 , endometriosis, ovarian functional cyst, polycystic ovarian syndrome, unilateral ovarian resection or ovariectomy. Furthermore, patients who took regular exercise, heavy smokers (>10 cigarettes/day), and with hypertension (systolic blood pressure >140 mmHg and/or diastolic pressure >90 mmHg) were excluded from the study. Women had not received hormonal treatments for at least 4 months before the first IVF attempt.

To assess ovarian reserve, on day 3 of the subsequent cycle, peripheral blood was obtained from all patients between 8.00 and 11.00 a.m., after an overnight fast, and different hormonal parameters were analysed. Basal plasma oestradiol, FSH and LH concentrations were determined by a radioimmunoassay (Radim, Pomezia, Italy). The FSH/LH ratio was then calculated. Serum GH levels were similarly measured by radioimmunoassay (Sorin Biomedica, Saluggio, Italy).

After a 3 month hormonal 'washing-out' period, all the poor

responder patients further underwent ovarian stimulation. To suppress the endogenous elevated FSH levels a combined monophasic oral contraceptive, Mercilon (20 μ g ethinyl oestradiol + 150 μ g desogestrel/day for 21 days; Organon Italia, Roma, Italy), was administered prior to gonadotrophin stimulation. Patients were randomly assigned to two different stimulation protocols: (i) flare-up GnRHa protocol and elevated pFSH (group I; $n = 17$); (ii) flare-up GnRHa protocol, elevated pFSH and oral L-arginine (group II; $n = 17$). Randomization was performed by opening sequentially numbered sealed envelopes containing treatment allocation determined by a random number table.

Ovarian stimulation was achieved as follows: (i) group I: triptorelin (Decapeptyl 0.1; Ipsen, Milano, Italy) was daily administered from day 1 of the menstrual cycle (0.1 mg s.c.) in association with i.m. pFSH (450 IU in the first 3 days of the menstrual cycle, then in an individually assessed dosage); (ii) group II: triptorelin and pFSH were administered as in group I. In addition, patients were daily supplemented (16 g) with oral L-arginine (Bioarginina; Damor, Napoli, Italy). The IVF cycles were cancelled when oestradiol plasma levels were <1.100 pmol/l and/or fewer than three follicles were recruited by cycle day 8. When at least one follicle >17 mm in diameter was present, triptorelin, pFSH and L-arginine were withdrawn and 10 000 IU human chorionic gonadotrophin (HCG; Profasi; Serono) were administered i.m.. Ultrasonographic oocyte recovery was performed transvaginally 35 h after HCG injection. Embryo transfer was performed 48 h after oocyte retrieval and one to three embryos were replaced. Human chorionic gonadotrophin (2000 IU) was prescribed i.m. as luteal phase support on alternate days until the serum β -HCG assay. A clinical pregnancy was diagnosed by ultrasonographic evidence of embryonic heart activity.

During the ovarian stimulation regimen the patients were submitted (day 0, day 8, day of HCG administration, day of oocyte retrieval) to hormonal (oestradiol, GH), ultrasonographic (follicular number and diameter, endometrial thickness) and Doppler (uterine and perfollicular arteries) evaluations. Plasma (day 0, day 8, and day of oocyte retrieval), and follicular fluid concentrations of arginine (Arg), citrulline (Cit) and nitrites/nitrate (NO₂/NO₃) were assayed. Furthermore, the concentration of follicular fluid insulin-like growth factor-1 (IGF-1) was also assayed.

Ultrasound and Doppler examinations

Transvaginal ultrasonographic assessments of endometrial thickness were performed on day 1 and 8 of ovarian stimulation, and, where possible, on the day of HCG administration in both groups, using a 6.5 MHz vaginal transducer (A4 Idea, Esaote; Milano, Italy). Measurements of follicular size were performed daily beginning on day 8 of the cycle until the day of oocyte retrieval.

Doppler flow measurements of uterine and perfollicular arteries were performed transvaginally with 6.5 MHz (A4 Idea) colour Doppler system. The Doppler examination was performed at the beginning of pFSH administration, on day 8 of ovarian stimulation and on the day of oocyte retrieval. All the patients were studied between 08.00 and 11.00 h to exclude the effects of circadian rhythmicity on blood flow (Zaidi *et al.*, 1995b). 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. The maximum ultrasonographic energy was <80 mW/cm². The intensity is within the safety limits suggested by the American Institute for Ultrasound in Medicine (Lizzi and Mortimer, 1988). 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

Table I. Ovarian reserve on day 3 of the cycle subsequent to the first failed IVF attempt

	Poor responders (n = 34)	Normal range*
FSH (IU/l)	16.6 ± 8.7	1.5–10
LH (IU/l)	6.3 ± 4.2	1.5–10
FSH/LH ratio	2.6 ± 0.9	1–5
GH (µg/l)	0.8 ± 0.9	1–1.4
Oestradiol (pmol/l)	215 ± 77	<110

FSH = follicle stimulating hormone, LH = luteinizing hormone, GH = growth hormone.

*The normal range is derived from normally responsive patients attending the infertility clinic.

Table II. Response to ovulation induction

	Group I (n = 17)	Group II (n = 17)	Significance (P)
No. of pFSH ampoules	35.0 ± 6.4	45.9 ± 114.6	0.543
No. of days of pFSH treatment	9.8 ± 2.2	11.4 ± 2.7	0.135
No. of ampoules/day	4.4 ± 1.2	3.7 ± 0.7	0.612
No. of follicles <17 mm	1.5 ± 0.5	5.2 ± 2.3	0.048
Endometrial thickness (mm)	0.75 ± 0.17	0.95 ± 0.21	0.144
No. of oocytes collected	1.6 ± 0.5	4.1 ± 1.9	0.049
No. of transferred embryos	1.0 ± 0.5	2.4 ± 0.5	0.50

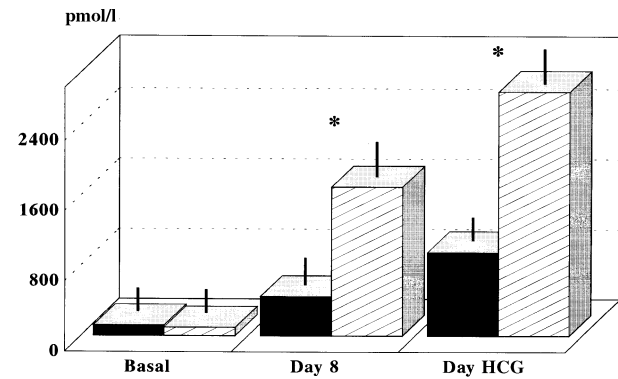
pFSH = pure follicle stimulating hormone.

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 = S–D/mean), 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. The perifollicular arteries, starting from day 8 of ovarian stimulation, were identified around the follicles (>1.0 cm in maximum diameter), in the ovarian stroma at the maximum distance from the surface of the ovary. Recorded spectra were analysed and PI was obtained. Arteries demonstrating the lowest downstream impedance were selected for measurements, assuming that these are the branches directly supplying the developing follicles. In the results the PIs of both uterine and perifollicular arteries have not been corrected for heart rate. Ultrasonographic and Doppler analyses were performed by one examiner (C.B.) who was unaware of the patients' treatment group.

Hormonal and biochemical assay

Peripheral blood was obtained between 08.00 and 11.00 h, after an overnight fast, on the same day that Doppler examination took place. Blood was immediately centrifuged and serum stored at –20°C, until assays. Oestradiol and GH were measured as reported above. L-Arginine (Arg) and L-citrulline (Cit) concentrations were assessed as previously described (Facchinetti *et al.*, 1998). Briefly, 1 ml of serum was added to 30 mg sulphosalicylic acid powder, vortexed and centrifuged at 2000 g for 20 min. A fraction of clear supernatant was mixed with an equal volume of *o*-phthalaldehyde for 1 min. The fluorescent derivative was then injected into a high pressure liquid chromatography apparatus equipped with a RP C-18 column. Flow

Oestradiol



GH

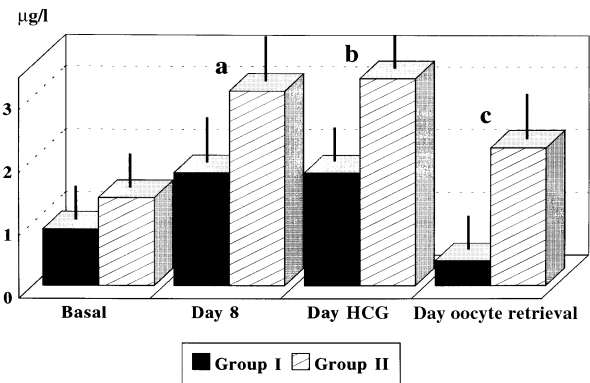


Figure 1. Serum oestradiol and growth hormone (GH) concentrations during ovarian stimulation. Group I, flare-up of gonadotrophin-releasing hormone analogue (GnRH_a) protocol and elevated pure follicle stimulating hormone (pFSH) (n = 17); group II, as group I plus oral L-arginine (n = 17). Significance: *P < 0.001; ^aP = 0.028; ^bP = 0.021; ^cP ≤ 0.001.

rate was adjusted to 1 ml/min. The mobile phase was constituted by a mixture of 0.012 mol/l phosphate buffer with acetonitrile and methanol (91:4:5), pH 5.9. Cit and Arg had retention times of 6.8 and 10.5 min respectively. Nitric oxide production was assessed by monitoring plasma levels of stable oxidation products of NO metabolism (NO₂⁻/NO₃⁻). Since very little or no NO₂⁻ is normally found in the serum, we did not attempt to differentiate between the respective amounts of NO₂⁻ and NO₃⁻; therefore, results are reported as NO₂⁻/NO₃⁻. The concentrations of NO₂⁻/NO₃⁻ were assayed with the Greiss reaction with procedures previously described (Clancy and Abramson, 1992; Facchinetti *et al.*, 1997).

L-Arginine, L-citrulline, and NO₂⁻/NO₃⁻ concentrations were also assayed in follicular fluid in those patients who reached the stage of oocyte retrieval: after transvaginal needle aspiration of the accessible follicles, the follicular fluids of follicles >17 mm were pooled and immediately centrifuged (2000 g for 20 min) and the supernatant stored at –20°C until bioassay. The analyses were performed with the same methods used for serum assays. Follicular fluid levels of IGF-1 were determined using a double-antibody radioimmunoassay (Immuno Nuclear Corp, Stillwater, USA).

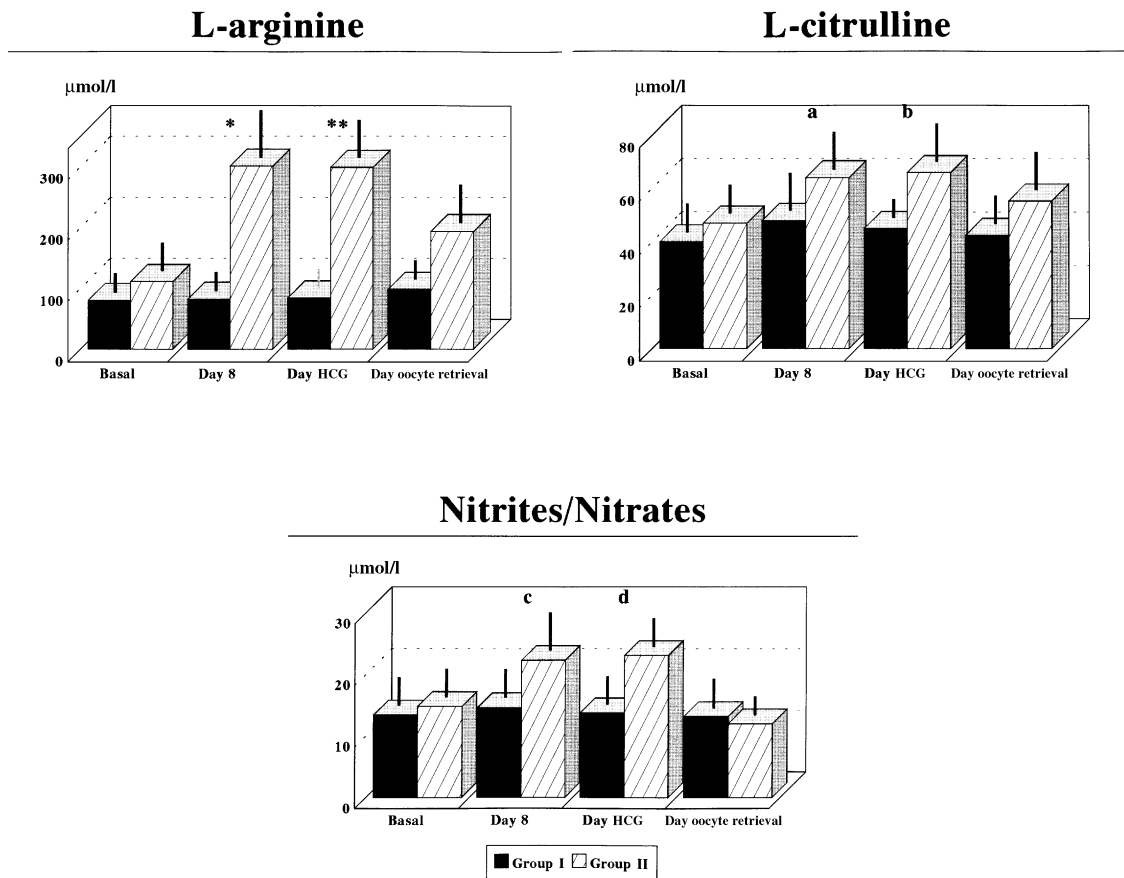


Figure 2. L-Arginine, L-citrulline, and $\text{NO}_2^-/\text{NO}_3^-$ plasma concentrations during ovarian stimulation. HCG = human chorionic gonadotrophin. See Figure 1 for definition of groups. Significance: * $P < 0.001$; ** $P = 0.009$; ^a $P = 0.041$; ^b $P = 0.021$; ^c $P = 0.036$; ^d $P = 0.029$.

Statistical analysis

A statistical analysis was performed using the Mann–Whitney test, χ^2 test, Fisher–Irwin exact test and a one-way analysis of variance where indicated. The relationship between the parameters analysed was assessed using the linear regression method. $P \leq 0.05$ was considered to be the limit of statistical significance. Data are presented as mean \pm SD, unless otherwise indicated.

Results

All 34 patients completed the study. Hormonal evaluation, on day 3 of the cycle subsequent to the first failed IVF attempt, confirmed a poor ovarian reserve in the whole population (Table I).

In the L-arginine group the cancellation rate (2/17; 11%) was lower than in group I (13/17; 76%, $P < 0.001$). No significant side-effects were reported by patients of L-arginine treated group. The number of pFSH ampoules, the duration of pFSH treatment, and the number of pFSH ampoules/day used in the two groups are shown in Table II, as well as the number of large follicles (>17 mm in maximum diameter), and the endometrial thickness on the day of HCG administration. The number of oocytes collected and the number of embryos transferred were significantly higher in group II than in group I (Table II). Three pregnancies (17%) were obtained in the

L-arginine supplemented group, even though all of them resulted in early pregnancy loss. No pregnancies were obtained in group I patients. During ovarian stimulation, serum oestradiol and GH levels changed as indicated in Figure 1. Plasma (Figure 2) and follicular fluid (Figure 3) L-arginine, L-citrulline and $\text{NO}_2^-/\text{NO}_3^-$ concentrations were significantly higher in group II. Follicular fluid IGF-1 concentrations were higher (Figure 3), and uterine and perfollicular blood flow resistances lower (Figure 4) in the L-arginine treated women.

In the study population, plasma oestradiol concentration was highly correlated with the number of follicles ($r = 0.819$; $P < 0.001$), and inversely related with uterine ($r = -0.503$; $P = 0.033$) and perfollicular ($r = -0.695$; $P = 0.002$) artery PI. Serum GH was directly correlated with follicular fluid concentrations of L-arginine ($r = 0.521$; $P = 0.025$), and inversely correlated with uterine artery PI ($r = -0.266$; $P = 0.044$). Plasma L-arginine concentrations were correlated with the number of developed follicles ($r = 0.811$; $P = 0.027$). Plasma $\text{NO}_2^-/\text{NO}_3^-$ concentration resulted inversely correlated with both uterine ($r = -0.437$; $P = 0.046$) and perfollicular ($r = -0.487$; $P = 0.039$) arteries PI. Follicular fluid L-arginine concentrations were inversely correlated with those in the perfollicular artery PI ($r = -0.671$; $P = 0.024$). Follicular fluid concentrations of IGF-1 were directly correlated

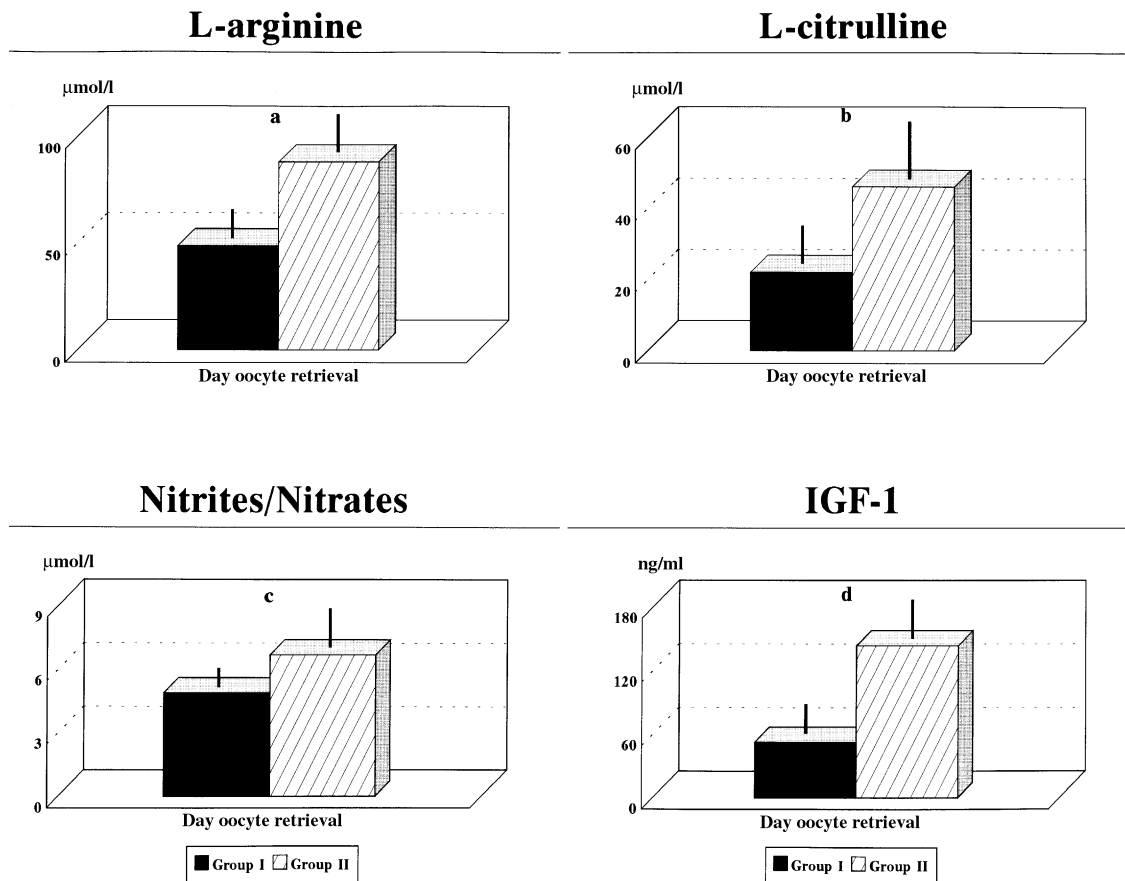


Figure 3. Follicular fluid content in patients who reached the stage of oocyte retrieval. See Figure 1 for definition of groups. Significance: $^aP = 0.044$; $^bP = 0.039$; $^cP = 0.011$; $^dP = 0.024$.

with retrieved oocytes ($r = 0.942$; $P = 0.017$), and fertilized embryos ($r = 0.849$; $P = 0.048$).

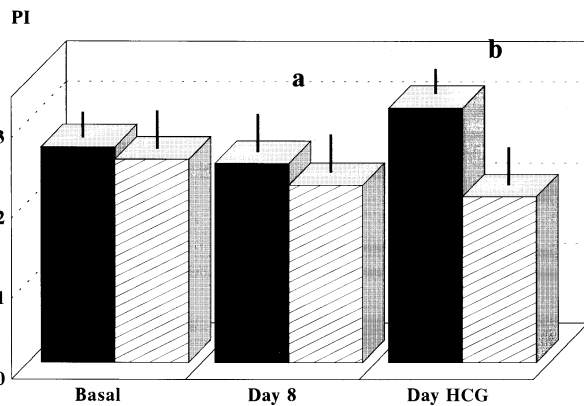
Discussion

In the present study the patients, on day 3 of a spontaneous cycle, presented plasma oestradiol and FSH concentrations and FSH/LH ratio above the normal range, while plasma GH concentrations were lower than normal range. These parameters were prognostic for subsequent impaired ovarian response to ovarian stimulation, resulting, in group I, in a cancellation rate of 76%. To suppress the elevated endogenous FSH levels, both groups were treated with a cycle of oral contraceptive pill prior to gonadotrophin stimulation. In both groups, the GnRHa has been used with a flare-up protocol for taking advantage of the initial release of endogenous gonadotrophins, and, during the first 3 days of the therapy, the daily stimulation dose was increased to 450 IU pFSH. The two ovulation induction protocols, with and without L-arginine, resulted in no significant differences in the number of pFSH ampoules, the days of pFSH treatment, the number of ampoules/day and endometrial thickness. However, the numbers of large follicles (>17 mm), the number of collected oocytes and transferred embryos were significantly higher in the L-arginine group. On day 8 and on day of HCG administration, oestradiol and GH plasma concentrations were significantly increased in the treated group. Plasma L-arginine, L-citrulline and $\text{NO}_2^-/\text{NO}_3^-$

concentrations were also significantly higher in the arginine supplemented group. In these patients oestradiol and L-arginine plasma concentrations correlated with the number of developed follicles. The above data agree with previous data (Stone and Marrs, 1992) confirming that women with increased GH concentrations produce significantly more oocytes and high oestradiol concentrations at each stage of ovarian stimulation and suggest that L-arginine supplementation may minimize poor response.

Nitric oxide is an established regulator of ovarian function, and in the present study the L-arginine oral supplementation during ovarian stimulation induced an increase in plasma L-arginine, L-citrulline and nitrite/nitrate levels and was associated with increased follicular fluid concentrations of L-arginine and its derivatives. The above data were correlated with decreased blood flow resistance in the perifollicular arteries. We speculated that NO acts as vasodilator increasing the permeability of follicular epithelium to plasma proteins. The concept of the blood–follicle barrier was first introduced 40 years ago (Zachariae, 1958), when it was found that the influx of Evans blue dye was greater into stimulated pre-ovulatory follicles than into unstimulated follicles. As the follicles mature, they become more permeable to plasma proteins and result more susceptibility to circulating FSH and GH action. The simultaneous action of FSH and GH may promote an increased production of IGF-I. Growth factors such as IGF-I play a role in follicular maturation and differenti-

Uterine arteries



Perifollicular arteries

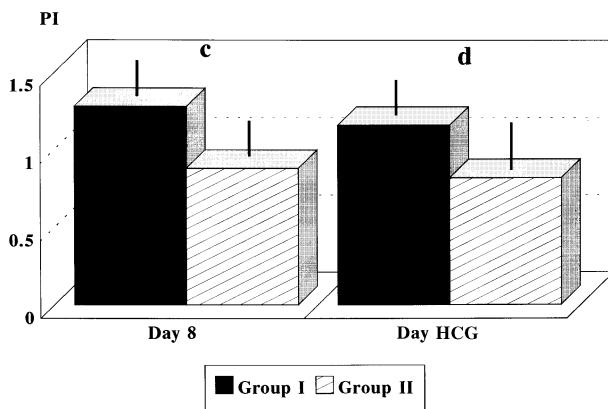


Figure 4. Uterine and perifollicular blood flow during ovarian stimulation. See Figure 1 for definition of groups. Significance: ^a $P = 0.047$; ^b $P = 0.001$; ^c $P = 0.005$; ^d $P = 0.012$.

ation of granulosa cells through various mechanisms (Erikson *et al.*, 1989; Adashi *et al.*, 1991). The intrafollicular milieu in L-arginine treated patients improved the follicular growth, and, probably, the oocyte quality and fertilization (Artini *et al.*, 1994).

Three pregnancies were observed in the L-arginine group. This result might be due to the increased number of transferred embryos, to better embryo quality, and/or to improved endometrial receptivity. No differences in embryo quality have been observed in the studied groups. There are no accepted standard criteria for evaluating endometrial receptivity, although attempts have been made to correlate ultrasound parameters and endometrial receptivity (Gonen and Casper, 1990; Dickey *et al.*, 1992; Khalifa *et al.*, 1992; Coulam *et al.*, 1994; Yaron *et al.*, 1994; Noyes *et al.*, 1995). In those patients who reached the stage of oocyte retrieval, we obtained similar results in terms of endometrial texture and thickness with or without L-arginine. These data are in accordance with those of others, who affirmed that ultrasound determination of these parameters was not helpful in evaluating endometrial receptivity (Sterzick *et al.*, 1991). Recently, 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 our study, a significant lower downstream impedance in uterine arteries resulted in L-arginine 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.*, 1989; Battaglia *et al.*, 1990; Steer *et al.*, 1992; Favre *et al.*, 1993; Battaglia *et al.*, 1997). A relationship has been found between vascularity and hormonal changes (Bassil *et al.*, 1995). The present study showed a significant inverse correlation between uterine artery Doppler PI and plasma oestradiol concentrations. Oestradiol has been demonstrated to have effects on the vascular bed and cardiac function probably by exerting a direct effect on arterial tone (Bourne *et al.*, 1990; de Ziegler *et al.*, 1991; Gangar *et al.*, 1991; Pines *et al.*, 1991; Battaglia *et al.*, 1995). Animal studies have demonstrated that oestrogen receptors are present in both the heart and large vessels. In addition, our data showed an inverse correlation between plasma $\text{NO}_2^-/\text{NO}_3^-$ concentrations and uterine artery Doppler PI. Hence, we suggest that relaxation of vascular smooth muscle of endometrial vessels may be partially mediated by NO. This is in accordance with previous studies (Ramsay *et al.*, 1994, 1995), in which it was found that human uterine blood flow, assessed using Doppler ultrasound, can be increased by administration of NO donor drug. In sheep, the administration of N-monomethylarginine (a specific inhibitor of NO) reduces by 60% the uterine artery blood flow improvements induced by oestrogens (Van Buren *et al.*, 1992). The above considerations support the hypothesis that modulation of endometrial vascularity by oral L-arginine supplementation may improve the pregnancy rate, especially in poor responder patients who normally present an impaired uterine perfusion.

Even though randomized studies are necessary to elucidate the factors that influence intra-ovarian regulation of ovarian function, the present data obtained in poor responder patients will allow better management of ovarian stimulation and may lead to more effective treatments.

Acknowledgements

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