The possible role of hyperhomocysteinemia on IVF outcome

Arianna Pacchiarotti · Mohamed A. Mohamed ·
Giulietta Micara · Antonella Linari ·
Daniela Tranquilli · Salomè B. Espinola ·
Cesare Aragona

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Abstract

Purpose To evaluate the effect of the hyperhomocysteinemia on pregnancy rate, implantation rate and abortion rate after IVF. **Method** Data from a total of 48 infertile couples with hyperhomocysteinemia were prospectively collected for this study. All patients underwent a standard down regulation protocol for ovarian stimulation. Oocytes recovery was performed at 36 h after hCG administration. Embryo transfer took place at 48 h after insemination. The patients were matched in two groups that received or did not receive therapy (group A and B respectively) to normalize homocysteine plasma level.

Results Pregnancy rate, implantation rate and abortion rate varied significantly ($p \leq 0.05$) between the two groups. The number and quality of embryos transferred did not differ between the groups.

Conclusion The results suggest that hyperhomocysteinemia could affect IVF outcome

Keywords Hyperhomocysteinemia · Implantation rate · Pregnancy rate · Abortion rate

Introduction

Successful pregnancy outcome is highly dependent on satisfactory placental development and sustained placen-

tal function that may be compromised by increased thrombosis caused by microthrombi in the vessel bed of the placenta [1]. These microthrombi may cause multiple placental infarctions and subsequently maternal complications of pregnancy [2]. Several studies suggest that recurrent pregnancy loss (RPL) is associated with an increased risk of thrombosis caused by heritable thrombophilic defects, or hyperhomocysteinemia, or combinations of these two pathologic conditions [3–5]. Indeed heritable thrombophilia consists of a group of genetic disorders of blood coagulation determining an increased risk of thrombosis; hyperhomocysteinemia is a risk factor for arterial and venous thrombosis and it could be a possible risk factor for habitual abortions or placental abruption [6]. Hyperhomocysteinemia and associated vascular diseases are probably caused by an interaction between nutritional and genetic factors: malnutrition and malabsorption of folate and vitamin B12 or an inherited enzymatic defect, such as methylenetetrahydrofolate reductase (MTHFR) deficiency [7]. Increased levels of homocysteine may lead to premature vascular disease, i.e., early damage to decidual or chorionic vessels that may cause disturbed implantation of the conceptus [6].

We worked with the hypothesis that hyperhomocysteinemia may be an important risk factor for implantation failure in patients undergoing IVF. The aim of the study was to evaluate the possible effect of hyperhomocysteinemia in IVF outcome.

Materials and methods

Patient selection

In a prospective randomized study, a total 48 infertile couples undergoing ICSI treatment were enrolled for this study from February 2005 to January 2007. Women aged 27–38 years were included if they fulfilled the following

A. Pacchiarotti (✉) · M. A. Mohamed · G. Micara · A. Linari ·
D. Tranquilli · S. B. Espinola · C. Aragona
Obstetric and Gynecology, Policlinico Umberto,
La Sapienza University of Rome,
Viale Regina Elena,
Rome, Italy
e-mail: arypac@aliceposta.it

A. Pacchiarotti · M. A. Mohamed · C. Aragona
Praxi Pro Vita Centro di Fertilità,
Via Magnagrecia 117,
Rome, Italy

criteria: (1) hyperhomocysteinemia ; (2) infertility attributable to tubal factor, male factor or idiopathic infertility; (3) serum hormonal profile (FSH, LH, prolactin) within the normal range; (4) regular ovulatory menstrual cycles; (5) presence of normal uterine cavity; (6) body mass index (BMI) ≥ 20 – ≤ 26 kg/m² and (7) first IVF treatment.

Patients were excluded if they had other inherited or acquired thrombophilia, previous poor response to gonadotropins, history of severe OHSS, or current polycystic ovarian syndrome or if the male partner had azoospermia or clinical signs of infection detected in semen analysis within 12 months before treatment.

Patients were counseled about the nature of the study and gave written informed consent. The study was approved by our local ethical committee.

The primary end points were clinical pregnancy (pregnancy in which the foetus can be shown on ultrasound at about seven weeks) rate and implantation rate (defined by the number of gestational sacs per 100 embryo's transferred). The secondary end point was abortion rate (between the ultrasound diagnosis of pregnancy and 20 completed weeks' gestational age).

Criteria for diagnosis of hyperhomocysteinemia

Plasma homocysteine levels were analyzed to identify subjects with hyperhomocysteinemia. The following values, corresponding to the 90th percentile of the value distribution in normal subjects, was used as a cutoff level: 17 μ mol/L.

Study design

This is a prospective randomized study designed to compare the effect of hyperhomocysteinemia on pregnancy and implantation rates. The patients were randomized in two groups: 24 patients in group A (study group) who received therapy consisting in folic acid 400 mcg daily (Fertifol, Effik, Italy), cyanocobalamin 5000 mcg per week (Dobetin, Angelini, Italia), pyridoxine chlorhydrate 600 mcg per week (Benadon, Bayer) until the complete normalization of homocysteine plasmatic levels < 17 μ mol/L (14 days ± 2 SD) before IVF attempt; group B included 24 patients (control group) without therapy. None of these patients was on vitamins prior to this study and none of the control group patients has taken vitamins during IVF cycle. Randomization was computerized; this randomized trial was registered. The study was not blinded.

All patients underwent a short down-regulation protocol with GnRH analogue hormone (triptorelina, Decapeptyl 0.1 mg/die, Ipsen). Recombinant FSH 225 IU (Gonal F, Serono, Rome, Italy) was started on day 3 of the treatment cycle until HCG day.

Patients with a previous poor response to gonadotropin treatment were withdrawn from the study. Patients with excessive response were counseled about the risk for OHSS and were advised to interrupt the stimulation cycle.

HCG 10000 IU (Gonasi HP Amsa, Roma, Italy) was administered i.m when the leading follicle was 18–19 mm and there were at least two follicles of 16–17 mm. Oocyte retrieval was performed 36 h after hCG administration and cumulus oocyte complex (COC) was assessed according to the oocyte maturation score established criteria. The oocytes were then inseminated in-vitro by ICSI. Of note, in Italy only three oocytes are permitted to be inseminated, therefore we performed ICSI as an in vitro fertilization technique of choice in order to select good quality oocytes for insemination.

The resultant embryos were scored according to established criteria. Briefly, embryos were scored on the basis of morphologic appearance and fragmentation. Grade 1 embryos had equal-sized blastomeres and no fragmentation; grade 2 embryos had equal-sized blastomeres with minor cytoplasmic fragmentation; grade 3 embryos had unequal-sized blastomeres with variable fragmentation; grade 4 embryos had equal- or unequal-sized blastomeres with $> 10\%$ fragmentation. Ultrasound guided embryo transfer took place 48 h following insemination by the same provider. The luteal phase was supported with the administration of 50 mg i.m. of progesterone.

Statistical analysis

Statistical analysis was performed using the JMP software (version 4.0.4; SAS Corp., Cary, NC). The parameters were compared using the Student's *t*-test for independent data and χ^2 -test, setting the significance level at $p \leq 0.05$. ANOVA test was also used to analyze continuous variables, including primary and secondary outcome parameters. The difference between treatments was evaluated using a one-sided, 95% confidence interval. No adjustment for multiplicity was performed. The difference had greater significance when the linear mixed model, which controls for intrasubject variation was used to compare data across all time points ($P \leq 0.001$). Statistical Power calculation was based on an α level of 0.05 (two-tailed test) with 80% power to detect a 20% difference with 23 evaluable patients per group.

Results

Two patients were cancelled: one woman in group A and one woman group B, because of excessive ovarian response leading to high risk for OHSS. This difference was not statistically significant. Of the 48 studied patients, 46

Table 1 Demographic data and stimulation outcome

	Study group	Control group	<i>P</i> value
Patients (<i>n</i>)	24	24	NS
Mean age (ys)	35.1±3.5	34.1±3.1	NS
BMI	23.6±1.8	22.6±1.7	NS
Duration of sterility (ys)	2.1±1.2	3.1±1.4	NS
Tubal factor % (<i>n</i>)	54.1% (13)	58.3% (14)	NS
Male factor % (<i>n</i>)	33.3% (8)	33.3% (8)	NS
Unexplained infertility % (<i>n</i>)	12.5% (3)	8.3% (2)	NS
Duration of stimulation (days)	12.4±1.6	13.1±2.1	NS
Estradiol level on HCG day (pg/ml)	2,126±560	2,065±699	NS
Endometrial thickness on HCG day (mm)	9.8±1.1	10.2±1.2	NS
Bias	4.1% (1)	4.1% (1)	NS

NS = not significant

underwent oocyte retrieval, 23 patients in group A and 23 in group B and all of these patients underwent embryo transfer.

As shown in Table 1, the two groups were comparable regarding demographic data, infertility factor distribution, duration of stimulation, estradiol level and endometrial thickness on the day of hCG administration. There was no significant difference observed between the two groups regarding the mean number of oocytes retrieved per patient and their maturation.

Significant differences ($p<0.05$) were found in favour of group A compared to group B in terms of implantation, pregnancy and abortion rates. For implantation rate, the comparative values for groups A and B were 12 gestational sacs (GS) out of 56 total embryos transferred vs 5 out of 54 (21.4% vs 9.2%) respectively; for pregnancy rate these

were 11 clinical pregnancies (CP) per 23 embryo transfer (ET) vs 4 CP per 23 ET (47.8% vs 17.3%) for groups A and B respectively; 2 abortions out of 11 CP vs 2 abortion out of 4 CP (18.2% vs 50%) for abortion rate for groups A and B, respectively (Table 2).

Discussion

Mild-to-moderate hyperhomocysteinemia is, in general, a risk factor for arterial and venous thrombosis. It has been suggested as a possible risk factor in women suffering from placental vascular diseases (placental infarction, placental abruption and habitual abortions) [8]. Increased levels of homocysteine may lead to premature vascular disease, i.e.,

Table 2 Embryological characteristics and clinical outcome

	Group A	Group B	
No of patients underwent embryo transfer	23	23	NS
Mean number of retrieved oocytes /patient±SD	11.6±1.4	13.2±2.9	NS
Mature oocytes (MII) %	53.1%	52.5%	NS
Mature oocytes (MI) %	33.5%	32%	NS
Immature oocytes (GV) %	13.4%	15.5%	NS
Mean number of embryos transferred	2.5±1.0	2.6±0.9	NS
Grade I embryos	60.2%	55.2%	NS
Grade II embryos	29.6%	31.7%	NS
Grade III embryos	9.0%	11.0%	NS
Grade IV embryos	1.2%	2.1%	NS
Pregnancy rate: CP/ET (%)	11/23 (47.8%)	4/23 (17.3%)	<0.05
Implantation rate: GS/No ET (%)	12/56 (21.4%)	5/54 (9.2%)	<0.05
Abortion rate: A/CP (%)	2/11 (18.2%)	2/4 (50%)	<0.05

NS = Not Significant

CP = Clinical Pregnancy

ET = Embryo Transfer

GS = Gestational Sacs

No. ET = Total number of Embryos Transferred

A = Abortions

early damage to decidual or chorionic vessels that may cause disturbed implantation of the concepts [9].

Some authors show that hyperhomocysteinemia leads to a three-fold increase in risk for early pregnancy loss [10]. Heby [11] reported that elevated maternal plasma homocysteine levels were associated with impaired DNA methylation and gene expression that lead to defective chorionic villous vascularization with subsequent early embryonic death. Moreover, investigating the chorionic villous vascularization by both histopathology and an image analysis system in spontaneous miscarriage tissues from 19 women with recurrent early pregnancy losses, Nelen et al. [12] observed a significant smaller median area, perimeters and diameters per chorionic vascular element in women with elevated total homocysteine levels.

Our data seem to show that the IVF outcome in terms of pregnancy rate and implantation rate was significantly reduced in the group of patients without treatment.

A statistically significant difference was found in the abortion rate that was reduced in patients who underwent IVF cycle after treatment to reduce homocysteine plasma levels.

In our study the two groups were similar for demographic data. No differences were found in oocytes number, oocyte maturity, number of embryos transferred and their quality. Patients were excluded from the study if they had any complications that could affect embryo implantation.

Hyperhomocysteinemia determine coagulation dysfunction that could cause microthrombi in the uterine vessels, compromise embryo implantation and determine early pregnancy loss.

Subject to further study will be to confirm whether vitamins administration in patient with normal homocysteinemia have no effect in IVF outcome. This should be possible by blinded trial with a placebo.

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