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Homocysteine pathway and in vitro fertilization outcome

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ABSTRACT

A prospective study investigating whether levels of the homocysteine pathway are associated with pregnancy rate in in vitro fertilization (IVF). Prior to initiate treatments, women gave a blood sample to test serum and red blood cell (RBC) folates, homocysteine and vitamin B12. The main outcome was cumulative clinical pregnancy rate according to basal levels of serum and RBC folates, homocysteine and vitamin B12. Two-hundred-nine women were selected, of whom 56 achieved a clinical pregnancy (27%). Median [interquartile range] levels of RBC and serum folate in women who did and did not become pregnant were 328 and 263 ng/ml (p = 0.018) and 13.6 and 9.4 ng/ml (p = 0.001), respectively. The adjusted ORs (95%CI) of pregnancy in women with RBC and serum folate concentrations in the upper tertile of the distributions were 2.6 (1.4–5.1) and 1.9 (1.0–3.7), respectively. Women undergoing IVF treatments with higher levels of folate have a higher chance of clinical pregnancy

1. Introduction

The advent of in vitro fertilization (IVF) has represented a fundamental breakthrough in the treatment of infertility. However, despite the improvement of the diagnostics tools and the development of new technologies, the majority of IVF cycles remains unsuccessful [1]. The reasons are not easily discernible since infertility is often an intricate multifactorial condition and factors influencing the rate of success of IVF are largely unknown. In order to improve results, researchers have focused on several aspects involving both the clinical characteristics of treated couples and the technology used in the IVF laboratories but results remain controversial and/or of scanty clinical relevance [2,3]. The identification of modifiable factors of success is recognized as a potential major advance in infertility treatment.

Dietary habits, with particular interest to folate and vitamin B12, have recently received some attention in this context [4,5]. These B vitamins have a key role in reproductive health since their shortage results in hyperhomocysteinemia which is related to important adverse obstetric/neonatal outcomes such as recurrent spontaneous abortion or abruption placentae and neural tube defects and other congenital malformations [6–8].

Of note, the use of folic acid supplementation represents a significant public health measure for the prevention of pregnancy-related disorders [9]. In Italy, the official recommendations state that all women planning a pregnancy and those who do not actively exclude the possibility of becoming pregnant should take a daily folic acid supplement of at least 0.4 mg from one month before conception and continue throughout the first trimester [10]. However, less than 50% of women seeking for a pregnancy do initiate folic acid supplementation before pregnancy [11]. Moreover, in Italy, food fortification is not mandatory and there is no public strategy to increase the antenatal use of folic acid in healthy women. Accordingly, we previously reported that most women undergoing infertility treatments do not reach a Red Blood Cell (RBC) folate concentration >400 ng/ml [12] which is considered the optimal threshold in women of reproductive age to achieve the greatest reduction in neural tube defects [9]. As a consequence we claimed the need to promote recommendations for adequate supplementation [12].

Of interest here is that higher concentrations of folate and vitamin B12 and higher folate intake have also been associated with improved live birth rates using IVF in the United States [13,14]. However, data are controversial. Previous European data, while suggesting that high folate status increases the chance of twin

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births in IVF pregnancies, failed to highlight an association between serum folate and vitamin B12 levels and likelihood of a pregnancy [15,16].

In order to shed light on this controversial background, we designed the present prospective study aimed at investigating whether levels of serum and red blood cell (RBC) folate, homocysteine and vitamin B12 are associated with clinical pregnancy rate among women undergoing IVF.

2. Material and methods

2.1. Study design

Between March and December 2015, Caucasian women aged 18–40 years referring to the Infertility Unit of the Fondazione Ca' Granda for IVF were consecutively considered for study entry. No exclusion criteria were foreseen. The study was approved by the local Ethical Committee and all recruited patients gave a specific written informed consent.

All patients selected for IVF in our Unit underwent a clinical assessment and a baseline transvaginal ultrasound during the menstrual cycle preceding the controlled ovarian hyperstimulation (COH). At this time, eligible women accepting to participate provided an additional amount of venous blood in order to measure serum folate, RBC folate, homocysteine and vitamin B12. Participation to the study did not influence clinical management and both patients and personnel (embryologists and physicians) were blinded to the results of these levels. Patients were managed according to a standardized clinical protocol as reported in details elsewhere [17]. Briefly, protocol of stimulation and drugs dosages were decided based on clinical characteristics and biomarkers of ovarian reserve. In case of hypo-response or abnormal follicular growth, the cycle could be canceled before ovum pick up. A freeze all strategy was conversely preferred in case of hyper-response. Oocyte retrieval was performed 36 h after ovulation triggering and embryo transfer was generally performed two to five days after oocyte insemination according to embryo quantity and quality. However, embryo transfer was postponed through embryo vitrification in the following conditions: 1) if the number of retrieved oocytes exceeded 15 or if serum estradiol level exceeded 4000 pg/ml in order to reduce the incidence of Ovarian Hyperstimulation Syndrome (OHSS); 2) if serum progesterone exceeded 1500 pg/ml at the time of ovulation triggering. Viable non-replaced embryos were vitrified mostly at the blastocyst stage. Women with frozen embryos were scheduled for natural cycle embryo transfer if they referred regular menstrual cycles and a mean cycle length between 24 and 35 days. Embryo transfer was performed 4–6 days after LH surge (detected with the use of urinary sticks) according to the embryo age. No luteal phase support was given. Hormone replacement treatment was prescribed if women had irregular menstrual cycles or if the monitoring of the natural cycle failed. Serum hCG assessment to detect pregnancy was performed +14/16 days after ovulation triggering or LH surge. Women with positive hCG values underwent a transvaginal sonography three weeks later. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac.

2.2. Blood samples and biochemical analyses

Fasting venous blood samples were taken to the nearby "Central Laboratory Unit" where they were processed within 2 h from collection (for measurement of vitamin B12 and homocysteine) or stored at −20 °C until assayed (for measurement of serum folate and RBC folate). Serum samples were used for folate and vitamin B12 while whole blood samples and plasma were used for RBC folate and homocysteine assays, respectively.

Folate, RBC folate and vitamin B12 were measured with electrochemiluminescence immunoassays (ECLIA) on a Cobas e 602 platform (Roche Diagnostics International, Rotkreuz Switzerlan). Homocysteine was measured through an enzymatic test (Sentinel Diagnostic, Milan, Italy) using a Cobas c 702 platform (Roche Diagnostic International).

The intra and inter-assay coefficients of variations were below 10% and 15%, respectively.

2.3. Statistical analysis

Data were analyzed using the software SPSS Statistics (version 23.0, IBM Corp, Armonk, NY, USA). Statistically significant differences were determined using Fisher's-Exact test, Chi-Square test, Student's *t*-test or the U Mann-Whitney test, as appropriate. Data are reported as absolute number (percentage), mean ± standard deviation (SD) or median and interquartile range (IQR) between square brackets. The primary outcome was the cumulative clinical pregnancy rate per started cycle, including fresh and subsequent frozen embryo transfers performed within 4 months from oocytes collection, according to RBC folate levels. For comparisons of serum concentrations of the analytes, non-parametric statistics was used. Moreover, women were grouped into tertiles according to the distribution of the analyzed analytes. For RBC folates, the clinical pregnancy rate of women belonging to the 3rd tertile was compared to the clinical pregnancy rate of women belonging to the 1 st and 2nd tertiles. For secondary outcomes, the analysis was repeated with serum folate, homocysteine and vitamin B12 levels. The clinical pregnancy rate of women belonging to the 3rd (for serum folate and vitamin B12) or 1 st (for homocysteine) tertile was compared to the clinical pregnancy rate of the remaining women. A binomial logistic regression model was used to adjust for variables known to be associated with pregnancy rate (age, current smoking and BMI) and for those that were found to differ at a significance level of $p < 0.10$ between the two groups at baseline univariate comparisons.

The sample size was calculated based on a 27% expected clinical pregnancy rate per started cycle in the whole cohort (based on data of our Clinic in the previous 6 months) and stating as clinically relevant a doubled chance of clinical pregnancy rate for patients belonging to the 3rd tertile of RBC folate compared to patients belonging to the 1 st and 2nd tertiles (41.0% and 20.5%, respec-

Table 1
Baseline characteristics of the selected women (n = 209).

Characteristics	Mean ± SD, Median [IQR] or Number (%)
Age (years)	36.1 ± 3.0
BMI (Kg/m^2)	22.1 ± 4.0
Current smoking	37 (18%)
Duration of infertility (years)	3.8 ± 2.5
Previous deliveries	31 (15%)
Number of previous IVF cycles	
0	124 (59%)
1–2	65 (31%)
3–5	20 (10%)
Day 3 serum FSH (IU/ml)	7.8 ± 3.2
AMH (ng/ml)	2.0 [0.9–3.7]
Indication to IVF	
Male factor	54 (26%)
Tubal Factor/endometriosis	57 (27%)
Ovulation disorders	6 (3%)
Mixed	48 (23%)
Unexplained	44 (21%)

Data is reported as Mean ± SD, Median [IQR] or Number (%) as appropriate.
BMI: Body Mass Index. IVF: In Vitro Fertilization. FSH: Follicle Stimulating Hormone. AMH: Anti-Mullerian Hormone.