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Predictive value of homocysteine levels in embryo culture media for embryo selection in infertile patients with endometriosis

Endometriozisli infertil hastalarda embriyo kültür ortamındaki homosistein düzeylerinin embriyo seçimi için prediktif değeri

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Abstract

Objective: To investigate the possible ability of homocysteine (Hcy) levels in embryo culture media for estimating better invitro fertilization outcomes in endometriosis patients.

Materials and Methods: Nineteen women with endometriosis who were admitted to Cerrahpaşa Medical Faculty, Department of Obstetrics and Gynecology, Infertility Outpatient Clinic with the diagnosis of infertility were included in the study. The results of intracytoplasmic sperm injection treatments were recorded and Hcy levels in the embryo culture were evaluated. The results were compared with those of the control patients without endometriosis, who had previously been admitted to our clinic for assisted reproductive technology.

Results: Mean Hcy levels in the culture media of the endometriosis group and non-endometriosis group were 4.31±0.48 µmol/L and 4.15±1.44 µmol/L, respectively (p>0.05). Pregnancy was achieved in 3 patients in the endometriosis group, while 13 pregnancies were obtained in the non-endometriosis group (p>0.05). When all cases were evaluated, the mean value of Hcy in the culture medium was found to be 3.60±0.84 µmol/L in the patients with a pregnancy and 4.21±0.84 µmol/L in the group that failed to achieve a pregnancy, and this difference was statistically significant (p<0.05).

Conclusion: Difference between mean Hcy levels in the culture media of the endometriosis group and non-endometriosis group was statistically non-significant. Further studies with larger groups are needed for evaluating the association of Hcy with infertility in endometriosis patients. Mean Hcy levels in the group of patients who succeeded in conceiving were statistically higher than the group of patients who failed to conceive. It may be suggested that Hcy levels in the embryo culture media can predict the achievement of a pregnancy independently from some conditions which may adversely affect the embryo quality, such as endometriosis.

Keywords: Infertility, embryo, culture, homocysteine, endometriosis

Öz

Amaç: Endometriozis hastalarında embriyo kültür ortamındaki homosistein (Hcy) düzeylerinin infertilite hastalarında embriyo seçiminde belirleyiciliğini araştırmaktır.

Gereç ve Yöntemler: Cerrahpaşa Tıp Fakültesi, Kadın Hastalıkları ve Doğum Anabilim Dalı İnfertilite Polikliniği'ne infertilite tanısıyla başvuran 19 endometriozis hastası kadın çalışmaya dahil edildi. İntrastoplazmik sperm enjeksiyonu tedavilerinin sonuçları kaydedildi ve embriyo kültüründeki

PRECIS: Homocysteine levels in the embryo culture media can serve as an independent predictor of pregnancy success and embryo quality, regardless of conditions like endometriosis that may negatively impact infertility treatment outcomes.

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homosistein düzeyleri değerlendirildi. Sonuçlar, daha önce kliniğimize başvuran ve endometriozis tanısı olmayan kontrol hastalarının sonuçlarıyla karşılaştırıldı.

Bulgular: Endometriozis grubu ve endometriozis olmayan grubun kültür ortamındaki ortalama Hcy düzeyleri sırasıyla 4,31±0,48 µmol/L ve 4,15±1,44 µmol/L idi. Bu değerler arasındaki fark istatistiksel olarak anlamlı değildi (p>0,05). Endometriozis grubunda 3 hastada gebelik elde edilirken, endometriozis olmayan grupta 13 hastada gebelik elde edildi (p>0,05). Tüm olgular değerlendirildiğinde; gebelik oluşan grupta kültür ortamındaki Hcy değerinin ortalaması 3,60±0,84 µmol/L, gebelik oluşmayan grupta ise 4,21±0,84 µmol/L olarak bulundu ve bu fark istatistiksel olarak anlamlıydı (p<0,05).

Sonuç: Endometriozis grubu ve endometriozis olmayan grubun kültür ortamındaki ortalama Hcy düzeyleri arasındaki fark istatistiksel olarak anlamlı değildi. Endometriozis hastalarında Hcy'nin infertilite ile ilişkisini değerlendirmek için daha büyük gruplarla yapılacak çalışmalara kesinlikle ihtiyaç vardır. Gebe kalmayı başaran hastaların grubundaki ortalama Hcy düzeyleri gebe kalmayı başaramayan hastaların grubuna kıyasla istatistiksel olarak daha yüksekti. Embriyo kültür ortamındaki Hcy düzeylerinin, endometriozis gibi embriyo kalitesini olumsuz etkileyebilecek bazı durumlardan bağımsız olarak gebelik oluşumunu öngörebileceği ileri sürülebilir.

Anahtar Kelimeler: İnfertilite, embriyo, kültür, homosistein, endometriosis

Introduction

Endometriosis is estimated to affect around 10-15% of reproductive-aged women and is a well-known factor in the etiology of infertility[1]. It is marked by the presence of tissue resembling the endometrial epithelium and/or stroma outside the endometrium and myometrium, often accompanied by an inflammatory response[2]. A wide spectrum of mechanisms has been thought to be involved in infertility related to endometriosis, including impaired oocyte quality and oxidative stress[3].

Enhancing the current embryo assessment methods is imperative, prompting numerous researchers to concentrate on developing the most effective methodology for gauging an individual embryo's reproductive potential. Lately, there has been significant interest in evaluating the metabolic parameters of developing embryos and analyzing the residual embryo culture media. Metabolomics refers to the comprehensive detection and measurement, without specific targeting, of all small molecular weight byproducts, known as metabolites, resulting from metabolic processes[4]. Metabolomics offers an overview of the levels of all metabolites present in cell's metabolic or environmental conditions. Thus, the metabolome, representing the array of small molecule metabolites within a biological sample, serves as a reliable indicator of cellular activity[5]. Metabolomics finds utility in reproductive medicine due to the potential association between subfertility causes and disruptions in typical metabolism. Consequently, there is a hypothesis that gaining deeper insights into the metabolic implications of different infertility causes could enhance reproductive outcomes. Moreover, it is expected that advancements in this domain would facilitate the discovery of non-invasive biomarkers for diagnostic and prognostic applications[6].

In a state of normalcy, where the body isn't experiencing heightened oxidative stress, a delicate equilibrium exists at the cellular level, regulating reactive oxygen species (ROS) to low levels through diverse antioxidant mechanisms[7]. Homocysteine (Hcy) is an amino acid formed during the metabolism of methionine, yet it doesn't become part of protein structures. Furthermore, Hcy plays a role in producing the thiol glutathione, which serves as a crucial endogenous antioxidant, vital for preserving the balance between pro-oxidants and antioxidants in human tissues[8].

Hyperhomocysteinemia can significantly impact reproductive processes in multiple ways[9]. The negative effects of hyperhomocysteinemia on reproductive processes at various levels are well-documented in numerous studies. These effects include poor oocyte quality, male infertility due to abnormal morphology, low sperm concentrations, and loss of motility, as well as congenital malformations, miscarriages, preeclampsia, and low birth weight[10,11].

Elevated endogenous oxidative stress, which is characterized by increased production of ROS and nitric oxide, along with changes in ROS detoxification pathways, is well documented[12]. In addition, high Hcy levels are shown in the follicular fluid of patients with endometriosis. However, endometriosis-related infertility, and Hcy stand as a field that warrants further investigation.

In this study, we aimed to evaluate the levels of Hcy in the embryo culture media in patients with endometriosis undergoing infertility treatment and its possible predictive value in predicting embryo quality and treatment outcomes.

Materials and Methods

Total number of 57 women with infertility who were seen at the in vitro fertilization (IVF) unit of a tertiary care center between May 2011-September 2014 were included. The study was approved by the Clinical Research Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (date: 07.03.2013, number: 83045809/5579). Informed consent was obtained from all participants. The study group comprised 19 patients with documented endometriosis with surgical pathology results. The control group included 38 patients without an endometriosis diagnosis. The initial gynecological exam included infertility tests and a detailed reproductive history. Hormone profile included follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), anti-Müllerian hormone (AMH) and estradiol (E2) levels (Roche Diagnostics Corporation, Indianapolis, IN, USA). Exclusion criteria were as follows: Age ≥42, presence of hydrosalpinx, serum levels of FSH ≥15 and LH ≥15.

Conventional long agonist protocol was initiated for all patients. On the 21st day of the last cycle, gonadotropin-releasing hormone (GnRH) analogues were started. rec-FSH 75 unit,: Gonal-f® (Merck Serono, Türkiye), or Puregon® (Schering-Plough, Türkiye) was initiated on the 3rd day of the cycle, following the confirmation of the absence of any corpus luteum or an ovarian cyst larger than 1.5 cm with transvaginal ultrasound (5MHz sector probe, Siemens Adara). The step down protocol was used and the gonadotropin dose was adjusted according to the body mass index (kg/m^2) values of the patients as either 275, 325, or 375 international unit (IU) ultrasonographic evaluations were initiated on the 8th day of the cycle and conducted daily. 10,000 IU of human chorionic gonadotropin (HCG, Pregnyl®, Schering-Plough) was administered when the largest follicle was seen to be at 18 mm in diameter and with the presence of two follicles >16 mm.

Oocyte retrieval was conducted 36-38 hours post, HCG. Day 3 transfer was performed in each case and intravaginal 600 mg/day micronized progesterone (Progestan® tb, Kocak, Türkiye) was started on the night of procedure and continued for 12 days when the pregnancy test was performed. Pregnancy was diagnosed by the visualization of a gestational sac on ultrasound examination five weeks after embryo transfer.

Follicular fluids from mature follicles (>17 mm) were aspirated, and clear samples were pooled for each patient. Any aspirates that were not clear or contaminated with blood were discarded. Retrieved oocytes underwent rinsing, grading, and placement in bicarbonate-buffered human tubal fluid (Lonza, Verviers, Belgium, with a 10% protein solution, Sanquin, Amsterdam, The Netherlands) at 37 °C under 5% CO_2 in air. Oocyte insemination commenced approximately 40 hours after HCG injection using standard IVF or intracytoplasmic sperm injection (ICSI) procedures. Following the 40th hour of the ICSI, the dividing embryos were observed, and embryo grading was performed. This process was managed by the same embryologist for all cases. Embryos were categorized based on blastomere size and cytoplasmic fragmentation: “grade 1” for equally-sized blastomeres without cytoplasmic fragmentation, “grade 2” for equally-sized blastomeres with minor cytoplasmic fragmentations, “grade 3” for embryos without equally-sized blastomeres and cytoplasmic fragmentations, “grade 4” for embryos with or without equally-sized blastomeres and major cytoplasmic fragmentations, and “grade 5” for embryos with blastomeres that cannot be distinguished and major cytoplasmic fragmentations .

Embryo transfer took place at the two-cell stage or later on the third day after oocyte collection. After removing the embryos for transfer, 65 µL of Vitrolife G-2 v5 (Vitrolife Sweden AB, Göteborg, Sweden) medium was added to 35 µL of spent medium for each embryo. Each sample was brought up to 100 µL for laboratory evaluation. Samples, including a control sample incubated under the same conditions without an embryo, were immediately sent to the laboratory. Hcy levels in the collected samples were estimated using the enzyme cycling method with the Diazyme enzymatic Hcy assay kit (Diazyme Laboratories, CA, USA) on Beckman CX (Beckman Coulter Inc., CA, USA) automated chemistry analyzer . The intra, and inter-assay coefficients of variation values were <5.9%.

Statistical Analysis

Analyses were performed using the statistical package for the social sciences (SPSS) version 20.0 (Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. Using the independent samples t-test, we compared the variables with normal distributions; data were presented as mean ± standard deviation. Continuous variables in more than two groups were analyzed using either the Kruskal-Wallis test or analysis of variance and were represented as median and interquartile range or mean ± standard deviation, respectively. Spearman’s rank correlation coefficient was used to calculate correlations between continuous variables. A two-tailed p-value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics, hormone values, and embryologic parameters of the patients enrolled in this study are presented in Table 1. Mean age in the endometriosis group was 32±8.31, whereas it was found to be 31.21±3.72 in the control group. The average infertility duration for the endometriosis group was 5.89±4.3 years. For the control group, it was found to be 5.93±3.42 years. In terms of total administered gonadotropin dose, the mean dose of the endometriosis group was 2673.6±722.3 IU, and in the control group, it was 2284.3±785.7 IU. Hormonal parameters on the 3rd day of menstruation were compared between the groups. The average FSH value was found to be 7.34±2.57 ng/mL in the endometriosis group and 5.4±1.59 ng/mL in the control group. A statistically significant difference was found ($p<0.05$). LH, E2, TSH, and PRL values were 5.93±2.42 ng/mL, 51.67±24.44 ng/mL, 2.42±1.15 ng/mL, and 15.04±6.18 ng/mL in the endometriosis group. In the control group, the concentrations were found to be 4.96±4.64 ng/mL, 54.97±49.58 ng/mL, 2.19±1.34 ng/mL, and 17.58±8.30 ng/mL. No significant difference was detected among all these parameters ($p>0.05$). AMH levels were found to be 2.98±2.79 ng/mL in the endometriosis group and 3.90±4.33 ng/mL in the control group, with no significant difference between the groups ($p>0.05$).

The average number of the total oocytes obtained on the day of aspiration, the number of oocytes subjected to ICSI, and the number of fertilized oocytes were 7.32±3.11, 5.16±2.29, and 4.32±24 in the endometriosis group, respectively. In the control group, the values were found to be 8.5±3.94, 5.42±2.18, and 3.06±2.15. No statistical significance was observed ($p>0.05$).

In the group with endometriosis, the average culture medium Hcy value was 4.31±0.48 µmol/L; in the control group, it was 4.15±1.44 µmol/L (Table 1). The difference was not found to be

statistically significant. (p>0.05). In terms of embryos obtained, a significant difference was found only in the number of grade 2 embryos on the 3rd day, but no significant difference could be detected in terms of other grades.

While 5 pregnancies were achieved in the endometriosis group, 14 pregnancies were achieved in the control group, and no statistically significant difference was found (p>0.05).

In the 2nd stage of the statistical analysis, patients were

Table 1. Clinical characteristics and serum hormone values of all patients

	Endometriosis		Control		
	n	Mean ± SD	n	Mean ± SD	p-value
Age (years)	19	32±8.31 32	38	31.21±3.72 31	0.486
Total gonadotropin dose (IU)	19	2673.6±722.3 2775	38	2284.3±785.7 2100	0.026
Duration of infertility (years)	19	5.89±4.3 5	38	5.93±3.42 5	0.970
FSH (mIU/mL)	19	7.34.1±2.57 6.8	38	5.4±1.59 5.17	**0.001**
LH (mIU/mL)	19	5.93±2.42 5.69	38	4.96±4.64 4.27	0.022
E2 (mIU/mL)	19	51.67±24.44 50	38	54.97±49.58 39.5	0.531
TSH (mIU/mL)	19	2.42±1.15 2.3	38	2.19±1.34 1.8	0.275
AMH (ng/mL)	19	2.98±2.79 2.1	38	3.90±4.33 2.56	0.472
Prolactin (mIU/mL)	19	15.04±6.18 13.09	38	17.58±8.30 15.6	0.400
Total oocystes	19	7.32±3.11 7	38	8.5±3.94 9	0.287
ICSI oocytes	19	5.16±2.29 5	38	5.42±2.18 5	0.601
Fertilized oocytes	19	4.32±24 4	19	3.06±2.15 3	0.475
Day 2nd embryo grade 1	12	2.50±1.83 3	26	2.54±1.52 2	0.946
Day 2nd embryo grade 2	7	1.43±1.39 1	23	2.30±1.22 2	0.098
Day 2nd embryo grade 3	3	1.33±0.57 1	7	1.57±0.78 1	0.696
Day 3rd embryo grade 1	13	3±2.58 3	24	2.88±1.62 3	0.871
Day 3rd embryo grade 2	11	1.64±0.67 2	22	2.55±1.22 2	**0.031**
Day 3rd embryo grade 3	6	1.50±1.22 1	12	1.67±0.65 2	0.227
Homocysteine (µmol/L)	19	4.31±0.48 4.47	38	4.15±1.44 3.08	0.123
Pregnancy (pregnant/total)	19	5/14	38	14/24	0.326

p<0.05 statistically significant
FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation

distributed into three separate groups: all patients, patients with endometriosis, and patients without an endometriosis diagnosis. All the above-mentioned parameters were compared again each group based on pregnancy diagnosis.

The patient data in the endometriosis group (n=19) according to pregnancy diagnosis are shown in Table 2. Among all parameters, a significant difference was found only in terms of the number of grade 2 embryos on the 3rd day (p<0.05). No

Table 2. Clinical characteristics of patients with endometriosis based on pregnancy outcomes

	Pregnancy (+)		Pregnancy (-)		
	n	Mean ± SD median	n	Mean ± SD median	p-value
Age (years)	5	33.40±3.20 32	14	31.50±3.7 32	0.512
Total gonadotropin dose (IU)	5	2650±871.06 2775	14	2682.14±699.13 2765.50	0.765
Duration of infertility (years)	5	3.80±1.78 4	14	6.64±4.73 5	0.258
FSH (mIU/mL)	5	7.46±2.03 7.9	14	7.30±2.81 6.32	0.817
LH (mIU/mL)	5	5.61±1.38 5.8	14	6.04±2.74 5.49	0.488
E2 (mIU/mL)	5	49.43±12.42 57	14	52.47±27.87 49	0.711
TSH (mIU/mL)	5	2.91±1.52 3	14	2.25±1.01 2.25	0.459
AMH (ng/mL)	5	2.73±2.56 2.5	14	3.07±2.97 2.01	0.588
Prolactin (mIU/mL)	5	16.90±9.33 15.95	14	14.51±5.33 13.09	0.750
Total oocystes	5	8.4 ± 4.39 7	14	6.93±2.61 6.5	0.637
ICSI oocytes	5	6.40 ± 3.43 5	14	4.71±1.68 5	0.397
Fertilized oocytes	5	6 ± 3.53 5	14	3.71±1.63 3.5	0.158
Day 2nd embryo grade 1	2	3.50±0.70 3.5	10	2.3±1.94 2.5	0.269
Day 2nd embryo grade 2	1	2 3.5	6	1.33±1.5 1	0.441
Day 2nd embryo grade 3	0	0 0	3	1.33±0.57 1	0.696
Day 3rd embryo grade 1	4	4.50±3.78 3	9	2.33±1.73 3	0.389
Day 3rd embryo grade 2	4	2 2	7	1.43±0.787 1	**0.009**
Day 3rd embryo grade 3	1	1 1	5	1.60±1.34 1	0.677
Homocysteine (µmol/L)	5	4.23±0.68 4.47	14	4.29±0.57 4.43	0.853

p<0.05 statistically significant
FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation

significant difference was detected in Hcy levels between the groups or conditions studied.

The patients' data in the group without endometriosis diagnosis (n=38) are shown in Table 3. A significant difference was detected between Hcy levels in the two groups, with levels significantly lower in the pregnancy group ($p<0.05$).

When all cases were evaluated, the mean value of Hcy in the culture medium was found to be 3.60±0.84 μmol/L in the patients with a pregnancy and 4.21±0.84 μmol/L in the group in which failed to achieve a pregnancy (Table 4) and this difference was statistically significant ($p<0.05$).

Table 3. Clinical characteristics of control patients based on pregnancy outcomes

	Pregnancy (+)		Pregnancy (-)		
	n	Mean ± SD median	n	Mean ± SD median	p-value
Age (years)	15	30.67±3.26 30	23	31.57±4.02 32	0.376
Total gonadotropin dose (IU)	15	2220±871.07 2100	23	2326.30±742.27 2250	0.580
Duration of infertility (years)	15	6.96±4.17 6	23	5.26±2.71 5	0.291
FSH (mIU/mL)	15	5.96±1.54 6	23	5.03±1.54 5.06	0.100
LH (mIU/mL)	15	3.78±1.76 3.39	23	5.73±5.71 4.4	0.210
E2 (mIU/mL)	15	48.73±25.44 40	23	59.04±60.65 39	0.858
TSH (mIU/mL)	15	2.19±1.16 1.79	23	2.19±1.47 1.81	0.709
AMH (ng/mL)	15	4.55±5.63 2.6	23	3.48±3.30 2.52	0.881
Prolactin (mIU/mL)	15	17.36±8.20 16	23	17.73±8.54 15.3	0.917
Total oocystes	15	8.87±3.72 9	23	8.26±4.14 9	0.787
ICSI oocytes	15	5.87±2.20 6	23	5.13±2.18 5	0.342
Fertilized oocytes	8	5.07±1.87 5	10	4.17±1.82 4	0.141
Day 2nd embryo grade 1	11	3.09±1.57 3	15	2.13±1.40 2	0.095
Day 2nd embryo grade 2	9	2.44±1.50 2	14	2.21±1.05 2	0.895
Day 2nd embryo grade 3	5	1.60±0.89 1	2	1.50±0.70 1.5	0.898
Day 3rd embryo grade 1	10	3±1.49 3	14	2.79±1.76 2.5	0.632
Day 3rd embryo grade 2	8	2.63±1.30 2	14	2.50±1.22 2	0.859
Day 3rd embryo grade 3	4	1.50±0.57 1.5	8	1.75±0.70 2	0.571
Homocysteine (μmol/L)	15	3.39±0.94 3.13	23	4.16±0.97 4.24	**0.022***

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation, *: Statistically significant

When receiver operating characteristic (ROC) analysis of Hcy level in culture medium is used to predict inability to conceive, the area under the curve was found to be 0.675. Since the ROC curve did not intersect the threshold of 0.5, the relationship was found to be significant ($p<0.05$) (Figure 1). For 3.145 μmol/L, which was chosen as the most appropriate cut-off value, the sensitivity was determined as 81.8%, the specificity as 76.1%, the positive predictive value as 0.45, and the negative predictive value as 0.94.

Discussion

Endometriosis and its possible effects on embryo quality have been investigated in many studies. An experimental study by Da Broi et al.[16], demonstrated impaired oocyte quality in bovine oocytes that were subjected to follicular fluid obtained from patients with endometriosis. Similar results were confirmed by Giorgi et al.[17] In a subsequent study, Da Broi et al.[18] were successful to identify elevated levels of oxidative stress markers in serum and follicular fluid of endometriosis patients. Yanushpolsky et al.[19] compared the IVF results of 45 endometriosis patients and 55 normal patients. They revealed that early pregnancy losses were significantly higher in the endometriosis group, and the number of embryos reaching the four-cell stage 48 hours after the IVF procedure was significantly lower. They suggested that there was a negative relationship between endometriosis and embryo quality. Studies on patients who have undergone oocyte donation are particularly noteworthy. Hauzman et al.[20] compiled five studies on patients who underwent oocyte donation and were diagnosed with endometriosis. In this review, it was concluded that relatively negative pregnancy outcomes were obtained if embryos from oocytes taken from patients with endometriosis were transferred to patients with endometrial receptivity, which was determined to be appropriate by morphological and molecular analyses.

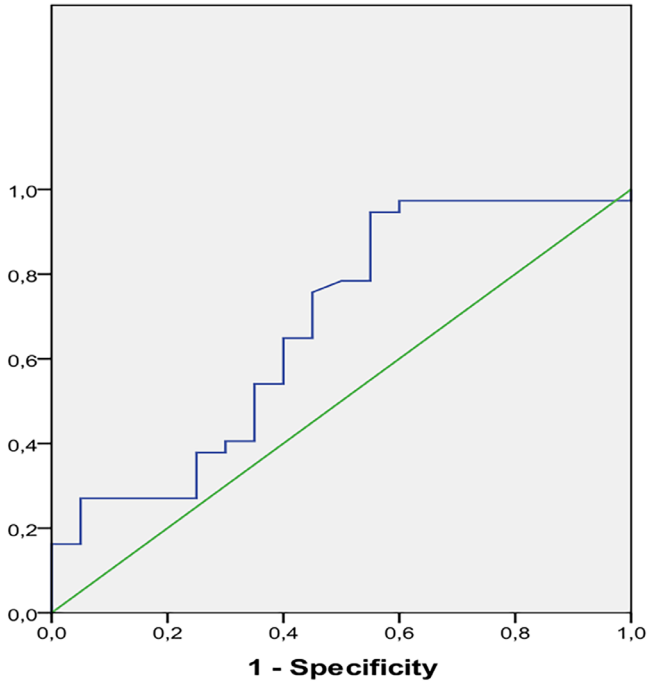

Figure 1. Receiver operative characteristic curve analysis for Homocysteine to predict inability to conceive

Our results revealed an inverse association between Hcy levels in embryo culture and pregnancy. Hcy levels were significantly lower in the group of patients with a positive pregnancy test, regardless of endometriosis diagnosis. The same association was found in the patients without endometriosis; no clear association was found in patients with endometriosis.

When we look at previous studies on Hcy, most studies are on serum, seminal plasma, and follicular fluid. These studies indirectly support our results by emphasizing the inverse relationship between high Hcy values and quality embryos, and pregnancy. In an old study conducted on mice, which is similar to our study, it was shown that L-Hcy was embryotoxic and that the rate of embryos reaching the blastocyst stage in these mice decreased in inverse proportion to Hcy levels. In the same study, it was stated that the other form of Hcy, D-Hcy, and its oxidation product, L-Hcy, were not embryotoxic[21]. Based on this, it can be suggested that the impaired embryo cannot progress to the blastocyst stage because it cannot clear Hcy from the environment. Hansen et al.[22] found that D,L-Hcy added to the culture medium during the early organogenesis stage was not embryotoxic in mouse embryos at an average concentration of 1.5 mM, and argued that these mouse embryos metabolized Hcy. This is most likely because Hcy enters the transsulfuration pathway and is metabolized. From this, it suggests that there is a certain non-toxic value range for Hcy and that the embryo metabolizes Hcy within this range. Berker et al.[23] and Aitken et al.[24] have emphasized the significance of Hcy levels in follicular fluid. Their findings indicated that elevated Hcy levels in follicular fluid were associated with reduced cell division, increased fragmentation in embryo cultures, and subsequently, diminished oocyte and embryo quality. Hcy and its levels in embryo culture media were evaluated by Aydin et al.[25] for the 1st time and their study had shown lower Hcy levels in patients with successful pregnancies, in accordance with the results of this study. They were also able to show a relationship between embryo grades and lower Hcy levels. The results of all these studies, in addition to ours, support the hypothesis that the viability and well-being of the embryo is revealed by examining the metabolic activity of that embryo, including its metabolic components, and cell residues in the culture medium.

Study Limitations

A small number of patients in the population poses a limitation, and necessitates further studies with a larger population to validate these results.

Table 4. Clinical characteristics of all patients based on pregnancy outcomes

	Pregnancy (+)		Pregnancy (-)		
	n	Mean ± SD median	n	Mean ±SD median	p-value
Age (years)	19	31.35±3.39 31	37	31.54±3.86 32	0.669
Total gonadotropin dose (IU)	19	2327.50±869.09 2112.25	37	2460.95±737.44 2475	0.389
Duration of infertility (years)	19	6.17±3.93 5	37	5.78±3.61 5	0.781
FSH (mIU/mL)	19	6.33±1.75 6.3	37	5.89±2.35 5.30	0.285
LH (mIU/mL)	19	4.24±1.83 4.12	37	5.85±4.76 4.75	0.213
E2 (mIU/mL)	19	48.90±22.57 40	37	56.55±50.39 47	0.913
TSH (mIU/mL)	19	2.37±1.26 1.95	37	2.21±1.30 2.09	0.634
AMH (ng/mL)	19	4.10±5.04 2.55	37	3.33±3.15 2.48	0.939
Prolactin (mIU/mL)	19	17.26±8.18 16	37	16.51±7.57 15.26	0.809
Total oocystes	19	8.75±3.78 8.5	37	7.76±3.66 8	0.400
ICSI oocytes	19	6±2.47 5.5	37	4.97±1.99 5	0.173
Fertilized oocytes	19	5.25±2.35 4	37	4±1.74 3	**0.037**
Day 2nd embryo grade 1	13	3.15±1.46 3	25	2.20±1.60 2	0.069
Day 2nd embryo grade 2	10	2.40±1.43 2	20	1.95±1.23 2	0.465
Day 2nd embryo grade 3	5	1.60±0.89 1	5	1.40±0.54 1	0.811
Day 3rd embryo grade 1	14	3.43±2.31 3	23	2.61±1.72 3	0.356
Day 3rd embryo grade 2	12	2.42±1.08 2	21	2.14±1.19 2	0.396
Day 3rd embryo grade 3	5	1.40±0.54 1	13	1.69±0.94 1	0.658
Homocysteine (µmol/L)	19	3.60±0.94 3.47	38	4.21±0.84 4.29	**0.030***

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation, *: Statistically significant

Conclusion

Lower Hcy levels in embryo culture media are associated with successful pregnancy outcomes, suggesting Hcy as a potential predictor of conception, independent of endometriosis. Larger studies are needed to confirm these findings and explore correlations with embryo morphology.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (date: 07.03.2013, number: 83045809/5579).
Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Concept: M.İ., L.M.Ş., Design: M.İ., L.M.Ş., Data Collection or Processing: M.İ., Analysis or Interpretation: M.Ö., A.T., Literature Search: M.İ., Writing: M.İ., L.M.Ş.
Conflict of Interest: No conflict of interest was declared by the authors.
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