Homocysteine Level Related To Age Is Associated With Embryo Quality In IVF Women With Diminished Ovarian Reserve

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Research Article

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Abstract

Background: Diminished ovarian reserve (DOR) contributes significantly to female infertility. Embryo quality affects pregnancy outcome. However, the factors that influence pregnancy outcomes in DOR patients are not well established. In this study, we analyzed the medical records of infertile women with DOR who underwent ART to reveal the possible correlation between clinical characteristics and embryo quality.

Methods: A retrospective cohort study was conducted using 3390 medical record data on IVF cycles from Dalian Municipal Women and Children's Medical Center (Group) from January 2015 to December 2020. The data was divided into poor-quality embryos group and high-quality embryos group according to the embryo status. We summarized the demographic and clinical characteristics (age, infertility years, infertility type, AFC, and BMI), medical history, reproductive hormone parameters (AMH, FSH, LH, PRL, and E_2), and carbohydrate and lipid metabolic indexes (CHO, TG, LP, LDL, HDL, FINS, Glucose, and Hcy). To further evaluate the Hcy levels in patients with different embryo quality, we performed subgroup analysis. In order to further evaluate the Hcy levels of patients with different embryo quality, we conducted a subgroup analysis, which was divided into three subgroups: $Hcy \geq 15$, $15 > Hcy > 8$ and $Hcy \leq 8$. A total of 1023 patients with DOR were included in this study.

Results: Of these, 483 cases were with high-quality embryos (the number of high quality embryo, number ≥ 1), and 540 cases with poor-quality embryos (no high quality embryo, number = 0). Demographic characteristics of the two groups were similar. However, the level of serum Hcy was significantly higher in the poor-quality embryo group compared with the high-quality embryo group ($P < 0.05$). In addition, the levels of serum AMH and PRL were statistically different between the two groups. Patients with serum $Hcy \geq 15 \mu\text{mol/L}$ were at higher risk of having poor-quality embryos (82.1%) compared to patients with serum Hcy less than $8 \mu\text{mol/L}$ (43.9%) and at $8 \sim 15 \mu\text{mol/L}$ (48.1%) ($\chi^2 = 15.859$, $P < 0.001$).

Conclusion: The presented study demonstrated that high serum Hcy level is linked to poor-quality embryos. Moreover, Hcy coupled with age might be a sensitive marker for embryo quality after IVF.

Background

Infertility has become a worldwide public health problem, affecting 12.5 to 15 in 100 couples of reproductive ages annually in China [1]. Diagnosis of diminished ovarian reserve (DOR), a predominant contributor to infertility, has increased from 19% in 2004 to 26% in 2011 in the United States, while similar trends have emerged in China [2]. Aging is directly associated with physiological DOR, whereas endometriosis and some diseases contribute to pathological DOR [3]. However, the pathophysiological mechanisms of DOR remain unclear.

Assisted reproductive technique (ART) have been broadly used to improve reproductive outcomes for women with infertility. Nevertheless, DOR is also a big challenge in ART, which addresses reductions in

oocyte quantity and quality, poor ovarian response (POR), high cancellation rate, and recurrent pregnancy loss (RPL)[4, 5].

The number of oocytes and high-quality embryos acquired was crucial across all in vitro fertilization (IVF) laboratories in ART. Progress is being made in exploring the pathophysiologic mechanisms of poor-quality embryos and discovering therapeutic strategies for improving the embryo quality of women with DOR[6, 7].

Recently, investigators have demonstrated some potential correlations between serum homocysteine (Hcy) level and embryo quality[8]. In brief, Hcy concentration correlates with oocyte and embryo qualities in patients with infertility and polycystic ovary syndrome (PCOS). Hyper-homocysteinemia (hHcy) has been identified as a risk factor for unexplained infertility [9, 10]. Furthermore, aging (≥ 39 years) and high levels of plasma Hcy are strongly associated with thrombotic events during IVF. Mild-to-moderate hHcy is an independent risk factor for thromboembolic disorders[11], implantation failure in patients undergoing IVF[9], and early pregnancy loss[12]. However, to our knowledge, no study has yet assessed the correlation between serum Hcy level and embryo quality in patients with DOR undergoing IVF.

In the presented study, we analyzed the medical records of infertile women with DOR who underwent ART at our center to reveal the possible correlation between clinical characteristics and embryo quality. Thus, our objective was to provide evidence for predicting embryo quality and suggesting potential therapeutic approaches by evaluating Hcy levels in patients undergoing IVF treatment.

Methods

Data source

A retrospective cohort study was conducted using 3390 medical record data on IVF cycles from Dalian Municipal Women and Children's Medical Center (Group) from January 2015 to December 2020. The inclusion criteria were (1) females aged 20~49 years old, (2) patients underwent IVF/ICSI for the first time, and (3) patients with serum basal follicle-stimulating hormone (FSH) ≥ 10 mIU/ml, anti-Müllerian hormone (AMH) < 1.1 ng/ml, or antral follicle count (AFC) < 7 (1 to 3 months of the IVF cycle).

Patients were excluded if any of the following conditions were present: (1) premature ovarian failure (POF) or primary ovarian insufficiency (POI), (2) polycystic ovary syndrome (PCOS), (3) acute cardiovascular disease, renal function damage, or malignant tumor, and (4) chromosome abnormality by preimplantation genetic screening.

Stimulation and IVF procedure

Stimulation protocols were selected based on the individual patient's conditions. Standard ovarian stimulation with gonadotrophins was performed using gonadotrophin-releasing hormone (GnRH) antagonist protocol, mini-stimulation protocol or natural protocol. Oocyte retrieval was performed 34~36

h after triggering with hCG, a GnRH agonist, or combined hCG and GnRH agonist under transvaginal ultrasound guidance.

Cases with no oocyte obtained or ovulation occurring at the time of egg collection were excluded. Oocytes were fertilized using either IVF or intracytoplasmic sperm injection (ICSI) depending on the semen parameters of the husbands per the standard protocols in the centers. The fertilization was confirmed by the presence of two pronuclei at 16~18 h after conventional insemination or ICSI. Three days after oocyte retrieval, an embryo with at least seven cells and Grades 1 and 2 was defined as good quality. Embryos with at least six cells and fragments <50% were frozen. All good embryos were frozen or vitrified using the Cryotop method as cleavage-stage embryos on Day 3 or as blastocysts on Day 5 or 6 according to the standard operating protocol.

Embryo quality assessment

DX1800 automatic chemiluminescence instrument (Beckman Coulter Company, USA) was used for hormone determination, and automatic biochemical analyzer (Hitachi Company, Japan) was used to detect blood lipid levels. The selection of fertilization method and embryo score, according to pronuclear stage score, development speed, number of blastomeres, size, morphology, cytoplasm, fragment ratio, embryo quality score of the cleavage stage, divided into four grades: Grade 1 embryo: blastomere equality, regular morphology, bright, no fragment. Grade 2 embryo: blastomere unequal in size and/or fragmentation <10%. Grade 3 embryo: 10-50% fragments. Grade 4 embryo: fragmentation >50%. Embryos with more than six cells at grades 1-2 on Day 3 after egg collection were defined as high-quality embryos. Embryos with less than 30% fragments are available and can be transplanted or frozen (Fig. 1).

Data collection and handle

A total of 1,023 data met the criteria. The data was divided into poor-quality embryos group and high-quality embryos group according to the embryo status.

We summarized the demographic and clinical characteristics (age, infertility years, infertility type, AFC, and BMI), medical history, reproductive hormone parameters (AMH, FSH, LH, PRL, and E₂), and carbohydrate and lipid metabolic indexes (CHO, TG, LP, LDL, HDL, FINS, Glucose, and Hcy).

Statistical analyses

Descriptive statistics were presented as mean $\pm$ standard deviation (SD) for continuous variables. Categorical variables were presented as frequency and percentage (%). Normally distributed data were analyzed using Student *T*-test. Non-parametric variables were compared using the Mann-Whitney *U* test. Categorical variables were compared using the chi-square or Fisher's exact test. Binary logistic regression analysis with backward stepwise elimination of candidate variables was used to measure the association between independent and outcome variables. Results were presented as Odds ratios (ORs) with 95% confidence intervals (CIs) and area under the curves (AUCs).

Data were analyzed using SPSS version 20 (IBM SPSS, Inc., Chicago, IL, USA). Comparisons were interpreted as significant when associated with $P < 0.05$. The ROC curves and AUC values were calculated using the pROC package of R statistical software (Version 4.1.0). The scatter box diagram was drawn by python software (Version 3.8.3).

Results

Patient characteristics

A total of 1023 patients with DOR were included in this study .Of these, 483 cases were with high-quality embryos (the number of high quality embryo, number ≥ 1), and 540 cases with poor-quality embryos (no high quality embryo, number=0). Demographic characteristics of the two groups were similar (Table 1). However, the level of serum Hcy was significantly higher in the poor-quality embryo group compared with the high-quality embryo group ($P < 0.05$). In addition, the levels of serum AMH and PRL were statistically different between the two groups.

Table 1
Characteristics of the patients with DOR

Characteristics	embryos quality		z/ χ^2	Pvalue
	poor-quality (0) (n = 540)	high-quality (≥ 1) (n = 483)		
Age, y	36.78 $\pm$ 4.79	36.19 $\pm$ 4.53	-1.68	0.093
Infertility Years, y	3.71 $\pm$ 3.26	3.48 $\pm$ 2.81	-0.49	0.621
Infertility Type				
Primary, n (%)	244 (50.5%)	258 (47.8%)	0.766	0.382
Secondary, n (%)	239 (49.5%)	282 (52.2%)		
AFC, number	3.44 $\pm$ 1.28	3.62 $\pm$ 1.46	-0.97	0.331
BMI, kg/m ²	23.15 $\pm$ 3.54	22.98 $\pm$ 3.28	-0.50	0.618
AMH, ng/mL	0.78 $\pm$ 0.94	0.81 $\pm$ 0.49	-2.38	0.017
FSH, mIU/mL	9.73 $\pm$ 4.43	9.07 $\pm$ 3.62	-1.83	0.067
LH, mIU/mL	5.10 $\pm$ 2.51	4.97 $\pm$ 2.30	-0.35	0.728
PRL, ng/ml	19.35 $\pm$ 30.34	19.3 $\pm$ 26.45	-2.15	0.031
E ₂ , pmol/ml	142.95 $\pm$ 72.59	147.68 $\pm$ 66.88	-1.13	0.258
CHO, mmol/L	4.74 $\pm$ 0.84	4.70 $\pm$ 0.87	-0.99	0.324
TG, mmol/L	1.36 $\pm$ 0.94	1.30 $\pm$ 0.84	-1.36	0.174
LP, g/L	192.26 $\pm$ 223.71	157.95 $\pm$ 214.27	-1.63	0.104
LDL, mmol/L	2.76 $\pm$ 0.67	2.76 $\pm$ 0.68	-0.09	0.931
HDL, mmol/L	1.49 $\pm$ 0.32	1.48 $\pm$ 0.31	-0.73	0.464
FINS, μ IU/mL	9.77 $\pm$ 3.89	10.14 $\pm$ 3.89	-1.45	0.148
Glucose, mmol/L	5.14 $\pm$ 0.71	5.12 $\pm$ 0.65	-0.15	0.885
Hcy, μ mol/L	9.22 $\pm$ 3.70	8.57 $\pm$ 2.41	-2.31	0.021
Note: Data presented as mean $\pm$ SD or n (%). T-test or Mann-Whitney U tests were used in the continuous data and the chi-square test or Fisher's exact tests were used in the categorical data.				

Relationship between Hcy and high-quality embryo number

Impressed with the higher Hcy level in the high-quality embryo group, we analyzed the relationship between the number of high-quality embryos and the level of serum Hcy. The level of serum Hcy

negatively correlated with the number of high-quality embryos (Fig. 2). A high level of serum Hcy might be one of the key factors that triggers the decline in embryo quality.

To further evaluate the Hcy levels in patients with different embryo quality, we performed subgroup analysis. Patients with serum Hcy > 15 $\mu\text{mol/L}$ were at higher risk of having poor-quality embryos (82.1%) compared to patients with serum Hcy less than 8 $\mu\text{mol/L}$ (43.9%) and at 8~15 $\mu\text{mol/L}$ (48.1%) (Table 2; $\chi^2 = 15.859$, $P < 0.001$).

Table 2 Comparison of high-quality embryo rate among different Hcy groups				
Hcy Level	Embryos quality		χ^2	p
	poor-quality (0) (n = 560)	High-quality(≥ 1) (n = 515)		
$\leq 8 \mu\text{mol/L}$, n (%)	193 (43.9%)	247 (56.1%)	15.859	<0.001
8~15 $\mu\text{mol/L}$, n (%)	244 (48.1%)	263 (51.9%)		
$\geq 15 \mu\text{mol/L}$, n (%)	23 (82.1%)	5 (17.9%)		
Note: P value of <0.05 was considered to be statistically significant.				

Logistic regression analyses

Since the above results showed a negative correlation between the serum Hcy and high-quality embryos, logistic regression analysis was performed to measure the association between independent variables and high-quality embryos. Patients who were older ($OR = 0.960$, 95% $CI = 0.926\sim 0.996$, $P < 0.05$) or with higher Hcy level ($OR = 0.901$, 95% $CI = 0.847\sim 0.959$, $P < 0.05$) had lower risk of having high-quality embryos (Table 3). The Logistic regression model was established, the formula followed:

Table 3 the results of logistic regression analysis				
Variable	β	SE	P	OR(95% CI)
Age	-0.041	0.019	0.028	.960 (0.926 to 0.996)
Hcy	-0.104	0.032	0.001	.901 (0.847 to 0.959)
β_0	2.641	0.952	0.001	14.028
Note: n = 975. Missing data have not been imputed. In variable screening process, “backward: LR” method is used.				

$$p = \frac{1}{1 + \exp[-(2.641 - 0.041x_1 - 0.104x_2)]}$$

Note: in the formula, p indicates the probability of having high quality embryo; x_1 and x_2 are explanatory variables, representing the values of age and Hcy, respectively.

The AUC for using this formula to differentiate age and Hcy was 0.53 and 0.543, respectively (Fig. 3).

Discussion

According to the latest national census of China, the fertility rate significantly decreased in 2020 (1.3 children per woman). One reason is that more Chinese young people tend to postpone marriage and childbearing to later reproductive years; the other reason is that the number of infertile couples has increased[13, 14]. Furthermore, the Chinese government released a two-child policy encouraging families to have a second child in January 2016. To sum up the above reasons, the reproductive need in women with advanced age (≥ 40) has been increasing dramatically and thus led to higher demands on receiving ART in recent years. Unfortunately, DOR has become a huge challenge of reproductive medicine. The published results showed that about 20% of infertile women suffered from DOR in China[15–17]. DOR induces not only infertility but also poor fertility outcomes even when in vitro fertilization-embryo transfer (IVF-ET) is used[18].

The goal of ART is to deliver a healthy baby. Therefore, it is essential to evaluate ovarian reserve, oocyte quality, and embryo quality. In clinical practice, there is a standard scheme to definite DOR, in which age, baseline AFC ($< 5-7$), baseline AMH ($< 0.5\sim 1.1$ ng/ml), and baseline FSH (> 10 mIU/ml) are considered[19, 20]. However, the existing research showed that these parameters are not good predictors of embryo quality or pregnancy in controlled ovarian stimulation cycles in ART processing[6, 21, 22]. Therefore, we designed a project to explore the potential correlation between embryo quality and potential parameters from medical records.

Our study showed that the level of serum AMH was significantly higher while PRL and Hcy were statistically lower in patients with high-quality embryos compared to those with poor-quality embryos. The role of AMH level as a biomarker of embryo quality remains controversial[23–26]. Most researchers argued that serum AMH concentrations fluctuate on three days after stimulating with standard hormone-based drugs. PRL, a 198 amino acid polypeptide hormone, is secreted from the anterior pituitary. Its secretion could be affected by stress, pain, trauma, ingesting food, and pregnancy.[27, 28]. In hence, some researchers do not believe it might affect the outcome of IVF[29]. Hcy, a sulfur-containing non-essential amino acid generated from the metabolism of the methionine and cysteine, plays a vital role in remethylation and trans-sulphuration. Previous studies have shown that a high serum Hcy level might lead to a reduction in the number of oocytes, suppress oocyte maturity, and reduce embryo quality[30–32]. In order to reveal the correlation between AMH, PRL, Hcy and embryo quality, a logistic regression analysis model was built for the analysis. The results demonstrated that only serum Hcy level correlated

with embryo quality. In particular, high Hcy level coupled with aging might partly reduce embryo quality. Our findings suggested that Hcy might be a sensitive marker for predicting embryo quality in DOR patients with DOR of advanced age who underwent IVF. In other words, high serum level of Hcy might reduce the embryo quality.

Hcy is an intermediate product of methionine metabolism via methylation catalyzed by various enzymes, including betaine-homocysteine *S*-methyltransferase (BHMT), glycine *N*-methyltransferase (GNMT), guanidinoacetate *N*-methyltransferase (GAMT), cystathionine β -synthase (CBS), γ -cystathionase[33, 34]. These reactions depend on folate and B-Vitamins, including Vitamin B-2, B-6, and B-12 as cofactors at different steps of the metabolic pathways[35, 36]. The main catabolic product of Hcy is cysteine, which is helped by CBS and γ -cystathionase. Thus, we can assume that a moderate supply of B-vitamin, folate, and cysteine in our diet might be beneficial to get high-quality embryos in IVF[37]. A high-methionine diet could induce hyperhomocysteinemia that is related to some pathological status, including infertility[28].

In conclusion, the presented study demonstrated that high serum Hcy level is linked to poor-quality embryos. Moreover, Hcy coupled with age might be a sensitive marker for embryo quality after IVF. In connection with this result, we assumed that improving the metabolism of Hcy will benefit the success of assisted reproduction.

Abbreviations

Hcy: homocysteine; DOR: Diminished ovarian reserve; IVF: In vitro fertilization; ICSI: Intracytoplasmic sperm injection; AMH: Anti-Müllerian hormone; FSH: Follicle stimulating hormone; AFC: Antral follicle count; POR: Poor ovarian response; POF: Premature ovarian failure; POI: Primary ovarian insufficiency; LH: Luteinizing hormone; PRL: Prolactin ; E₂: Estradiol; CHO: Cholesterol; TG: Triglyceride; LP: Lipoprotein; LDL: Low density lipoprotein; ART: Assisted reproductive technique; BMI: Body mass index; Gn: Gonadotrophin; 2PN: Two pronuclear; IQR: interquartile range; hHcy: Hyper-homocysteinemia

Declarations

Ethics Approval and consent to participate

This study was approved by the Ethics Committee of Dalian Municipal Women and Children's Medical Center, China. (#No. 2020010). All participants were informed and written consent prior to the collection of samples. All methods were performed in accordance with the relevant guidelines and regulation.

Consent for publication

Not applicable.

Availability of data and materials

All data analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the experiments: Haiyan Wang, Aiqing Han, Xiaoguang Shao, Jian Li; Performed the experiments: Dan Cao, Yangyu Jiang, Lin Sun, Na Zou, Shiyong Tao; Analyzed the data: Aiqing Han, Bowen Wei; Contributed reagents/materials/analysis tools: Xiaoguang Shao, Jian Li, Xiaoyu Xue, Shiyong Tao; Wrote the manuscript: Haiyan Wang, Aiqing Han, Bowen Wei, Jian Li. Haiyan Wang, Xiaoguang Shao, Bowen Wei, Jian Li conducted the final review and approval of the final version of this manuscript. The final manuscript was read and approved by all authors.

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Figures

Figure 1

Pictures of embryos of different grades

Note: Grade 1 embryo: blastomere equality, regular morphology, bright, no fragment. Grade 2 embryo: blastomere unequal in size and/or fragmentation <10%. Grade 3 embryo: 10-50% fragments. Grade 4 embryo: fragmentation >50%.

Figure 2

Serum Hcy levels negatively correlated with high-quality embryo number. Horizontal lines represent the median and interquartile range (IQR) of the data.

Joint comparison

Figure 3

The areas under the receiver operating characteristics curves for three different models.

Supplementary Files

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- [DORdata.xlsx](#)