A J M S

Online ISSN (2789-3219)

Research Article

Association of Methylenetetrahydrofolate Reductase Gene Polymorphism C677T with Oocyte Number and Embryo Quality in Iraqi Infertile Women Undergoing Intracytoplasmic Sperm Injection

Rabab Zahir Al-Yasiry[1]* , Mufeeda Ali Jawad[2] , Muayad Sraibet Abbood[3]

[1]Department of Anatomy, Embryology and Histology, College of Medicine, Babylon University, Babylon, Iraq; [2]Department of Physiology, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq; [3]Department of Embryology, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

Abstract

Background: Methylenetetrahydrofolate reductase (MTHFR) C677T is a single nucleotide polymorphism (SNP) that affects the production of 5-methyltetrahydrofolate (5-MTHF), the active folate that enables the recycling of homocysteine (Hcy) to methionine. *Objective*: to investigate the association between the MTHFR (C677T) polymorphism and the outcomes of intracytoplasmic sperm injection (ICSI). *Methods*: A prospective cohort study included 85 infertile women undergoing ICSI treatment at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies in Baghdad, Iraq. The study period extended from January 2022 to September 2023. The MTHFR C677T polymorphism genotyping was evaluated in these patients, and they were classified into three groups according to genotyping results: normal (CC), heterozygote mutated (CT), and mutated homozygote (TT). In addition, we conducted a comparative analysis of oocyte, embryo and pregnancy rates among these three groups. *Results*: In comparison to the CT and TT genotypes, the total number of oocytes, total embryos, mature oocytes, good-quality embryos, and pregnancy rate were all found to be significantly higher ($p<0.05$) in the CC genotype. Compared to the CC group, the proportion of immature oocytes and poor-quality embryos was significantly higher in the TT and CT groups ($p<0.05$). The rate of fertilization was comparable among the study groups. *Conclusions*: The maternal MTHFR C677T polymorphism is linked to oocyte number, maturity, total embryo, embryo quality, and pregnancy in ICSI. In light of this, MTHFR polymorphism in our community offers useful data regarding the success of ICSI.

Keywords: Infertility, Intracytoplasmic sperm injection, Methylene tetrahydrofolate reductase, Single nucleotide polymorphism.

ارتباط تعدد الأشكال الجيني المختزل مثيل تتراهيدروفولات C677T مع عدد البويضات وجودة الجنين لدى النساء العراقيات المصابات بالعقم اللائي يخضعن للحقن المجهري

الخلاصة

الخلفية: اختزال الميثيلين تتراهيدروفولات (MTHFR) C677T هو تعدد أشكال النوكليوتيدات المفردة (SNP) الذي يؤثر على إنتاج 5-ميثيل تتراهيدروفولات (5-MTHF)، وهو حمض الفوليك النشط الذي يتيح إعادة تدوير الهوموسيستين (Hcy) إلى ميثيونين. **الهدف**: التحقيق في العلاقة بين تعدد الأشكال MTHFR (C677T) ونتائج الحقن المجهري. **الطريقة**: تضمنت الدراسة 85 امرأة مصابة بالعقم يخضعن لعلاج الحقن المجهري في المعهد العالي لتشخيص العقم والتقنيات المساعدة على الإنجاب في بغداد، العراق، بين يناير 2022 وسبتمبر 2023. تم تقييم التنميط الجيني لتعدد الأشكال MTHFR C677T في هؤلاء المرضى، وتم تصنيفهم إلى ثلاث مجموعات وفقا لنتائج التنميط الجيني: طبيعي (CC)، متحور متغاير الزيجوت (CT) ، ومتماثل الزيجوت المتحور (TT). بالإضافة إلى ذلك، أجرينا تحليلا مقارنا لمعدلات البويضات والأجنة والحمل بين المجموعات الثلاثة. **النتائج**: بالمقارنة مع الأنماط الجينية CT و TT ، تم العثور على العدد الإجمالي للبويضات، وإجمالي الأجنة، والبويضات الناضجة، والأجنة ذات النوعية الجيدة، ومعدل الحمل أعلى بكثير في النمط الجيني (CC). بالمقارنة مع مجموعة CC ، كانت نسبة البويضات غير الناضجة والأجنة ذات النوعية الرديئة أعلى بكثير في مجموعات TT و CT. كان معدل الإخصاب قابلا للمقارنة بين المجموعات الثلاثة. **الاستنتاجات**: يرتبط تعدد الأشكال MTHFR C677T للأم بعدد البويضات والنضج والجنين الكلي وجودة الجنين والحمل في الحقن المجهري. في ضوء ذلك ، يقدم تعدد الأشكال MTHFR في مجتمعنا بيانات مفيدة فيما يتعلق بنجاح الحقن المجهري.

* *Corresponding author*: Rabab Z. Al-Yasiry, Department of Anatomy, Embryology and Histology, College of Medicine, Babylon University, Babylon, Iraq; Email: rabab.mousa@uobabylon.edu.iq

INTRODUCTION

Assisted reproductive technologies (ART) have gained widespread acceptance as a tried-and-true treatment for infertility. Nonetheless, despite recent advances in IVF and ICSI, only roughly one-third of these treatments result in live newborns. Understanding the factors that influence IVF/ICSI pregnancy outcomes is critical for developing safer and more successful infertility therapies [1,2]. According to growing evidence, folate metabolism affects human reproductive health, including the results of ART [3]. Folate-mediated carbon metabolism is necessary for the synthesis of DNA, RNA, and proteins as well as for the control of Hcy levels and the availability of methyl donors for methylation [4]. The MTHFR gene, which is located at position p36.3 on human chromosome 1, codes for the methylenetetrahydrofolate reductase enzyme. The primary form of folate found in blood, 5-methyltetrahydrofolate, is produced through the reduction of 5,10-methylenetetrahydrofolate by MTHFR, an enzyme that is essential to the metabolism of homocysteine and folate [5]. Genetic polymorphisms are increasingly being acknowledged as potential determinants of fertility. Although reproduction may be regulated by numerous genes, MTHFR is a particular genetic variant that has garnered attention from scientists [6]. Each individual has two MTHFR genes, one from each parent. Mutations in either one (heterozygous) or both (homozygous) of these genes can have an impact. Health problems are less likely to result from heterozygosity. Some scientists think that having two mutations may cause more severe issues. According to the SNP database (dbSNP), the MTHFR gene has at least 247 different SNPs. MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) SNPs are the two most prevalent polymorphisms of the MTHFR gene's DNA sequence variations [7]. The most frequent form of mild MTHFR insufficiency is the CT sequence alteration at nucleotide 677. At codon 222, this alteration causes an alanine-to-valine substitution, which is known as a missense change [5]. The second prevalent MTHFR variant is the A>C transition at nucleotide position 1298. At codon 429, a glutamine-to-alanine substitution occurs as a result of this alteration [7,8]. Enzyme activity in homozygous TT mutant individuals is around 30% lower than that in homozygous CC genotype individuals, whereas enzyme activity in heterozygous genotype (CT) individuals is roughly 65% lower than that in homozygous CC genotype individuals [9]. The 677 C>T MTHFR polymorphism causes a decrease in MTHFR activity, which causes an increase in Hcy concentration in body fluids. Excess Hcy can affect developing gametes and embryos by causing DNA damage and improper methylation [9,10]. MTHFR isoforms, particularly T677T, have been linked to decreased fertility in both men and women, and repeat miscarriage are more likely in couples with males who carry the MTHFR SNP than without. The T677T isoform, which also strongly induces chromosomal abnormalities, dramatically alters pre-

implantation development [11]. Evidence suggests that MTHFR 677C>T is connected to premature ovarian failure, recurrent miscarriage, and recurrent implantation failure, as well as ovarian reserve, oocyte maturation, and embryonic aneuploidy [12]. The high rate of ART failure, as well as the financial and emotional hardship, necessitate further investigation of the influence of MTHFR genetic variations in couples seeking reproductive therapy. Understanding the relationship between MTHFR polymorphisms and ICSI outcomes may aid practitioners in providing a better outcome for couples and a greater ART success rate.

METHODS

Study design and participants

During January 2022 to September 2023, a prospective cohort study comprising 85 subfertile females visited the consultation clinic of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq and underwent the ICSI program.

Inclusion criteria

The investigated participants included 85 women with a normal ovarian function who referred to an infertility clinic due to male factor, unexplained and tubal infertility. Further selection criteria for this study were as follows: 20 to 43 years of age, infertility duration ranging from 3 to 15 years, a normal menstrual cycle, and no evidence of endocrine disorders. Some of these infertile women were undergoing their initial IVF/ICSI procedures, while others had already done at least one cycle.

Exclusion criteria

Endometriosis, ovarian surgery, endocrine disorders, and systemic diseases were all reasons for excluding participants from the study.

Sample Collection

5.0 ml of blood were drawn into sterile ethylene diamine tetra-acetic acid (EDTA) tubes for hormone testing and detection of the C677T allele of the MTHFR gene. A hormonal assay was performed using the standard routine laboratory process for ELISA with specific antibodies for determining FSH, LH, estradiol, progesterone, prolactin and AMH levels.

Genotyping study

By using the Promega Wizard® Genomic DNA Purification Kit and adhering to the manufacturer's instructions, the DNA was isolated from the whole blood

of female participants. Agarose gel electrophoresis was used to identify the isolated DNA. Using the diode array's scanning capability from 200 to 320 nm in wave length, nanodrops measured the DNA quantity and quality. The absorbance profile was then processed and analyzed to identify the DNA quantity and quality by computing the 260/280 and 260/230 ratios. To find the MTHFR gene C677T genotype, DNA sequencing of PCR amplicons was done with primers designed for this study: forward 5'-AACTCAGCGAACTCAGCACT-3ˆ and reverse 5'-TCACTATCTTGGACCCCTGA-3ˆ. The PCR reaction mixture contained 25 µl of nuclease-free water, 20–50 ng template DNA, Go Taq Green Master Mix 1X (Promega), downstream primers 0.1–1.0 pmol, and upstream primers 0.1–1.0 pmol. PCR optimization was carried out as a preliminary step, utilizing a gradient temperature spanning from 50 °C to 65 °C with variations in PCR wells (Figure 1).
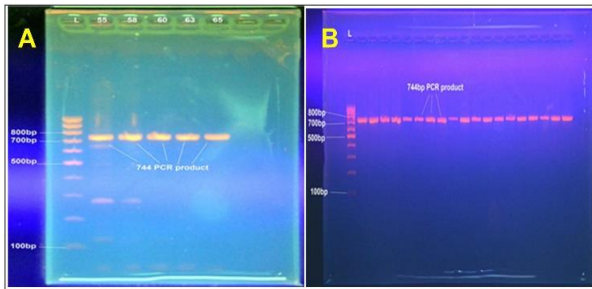

Figure 1: PCR optimization of MTHFR PCR amplification of MTHFR lane L 100bp DNA ladder (A), other lanes represent different annealing temperature (B).

Using apparatus from the GTC Series thermocycler (Cleaver Scientific, UK), amplification reactions were conducted. Amplified DNA fragments were electrophoresed on 1.5% agarose (0.5x) TBE buffer for 1 hour at 75 volts. The bands were then seen under ultraviolet light after being stained with ethidium bromide. A 100-base-pair ladder was used as a size marker to estimate the sizes of the fragments. The resolved PCR amplicons were commercially sequenced from both forward and reverse termini according to the instruction manuals of the sequencing company (Macrogen Inc., Geumchen, Seoul, South Korea) (Figure 2).
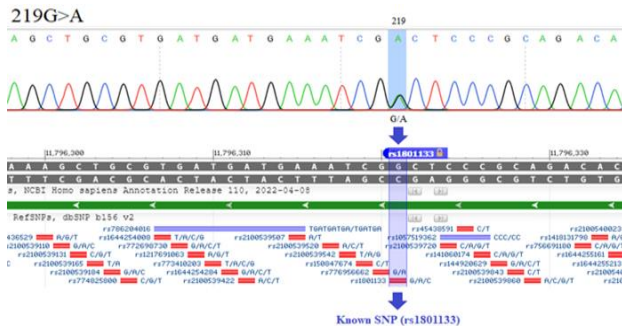

Figure 2: The SNP's annotations checking of MTHFR SNP using the dbSNP server. The identified known SNPs were marked with a blue color. GenBank acc. no. NC_000001.11 is used in the positioning of the identified SNPs in the MTHFR.

Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. The virtual positions and other details of the retrieved PCR fragments were found by comparing the DNA sequences of the samples that were being studied with neighboring DNA sequences that were retrieved by the NCBI Blastn engine. The sequencing results of the PCR products were edited, aligned, and analyzed as long as they matched the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA) (Figure 3).
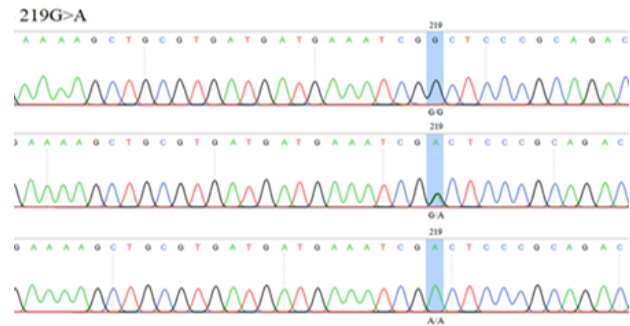

Figure 3: The pattern of the detected SNPs within the DNA chromatogram of the MTHFR gene.

ICSI Procedure

The women were subjected to controlled ovarian hyperstimulation using the antagonist protocol. Human menopausal gonadotropin in the form of in vitro fertilization-Menotropin (IVF-M), LG Chem Ltd., Korea, 75–150 IU, or recombinant FSH in the form of Gonal-F®, Merck KGaA Darmstadt, Germany 75–300 IU was used for controlled ovarian stimulation. When follicular recruitments began and a significant number of follicles reached a size of 14, the pituitary was down-regulated by using the gonadotropin-releasing hormone (GnRH) antagonist Cetrotide, Merck KGaA Darmstadt, Germany, 0,25 mg. When the leading follicles' diameter reaches 18 mm and there is sufficient E2 concentration in the serum (> 1500 p/ml), ovulation is triggered by the administration of 500 micrograms of recombinant human chorionic gonadotropin (Ovitrelle®, Merck Global, Germany). Following the ovulation trigger, a follicular puncture was used to retrieve oocytes while the patient was under general anesthesia. Chemical pregnancy was verified 14 days after embryo transfer by blood human chorionic gonadotropin (hCG) testing. Based on the MTHFR gene C677T polymorphism test results, 85 patients were then put into one of three groups: CC, CT, or TT. The outcomes of ICSI were then compared between these groups. Because they only had immature oocytes (germinal vesicle (GV)), seven females were excluded from the study. Later, those women were revealed to have an uncommon genotype.

Ethical consideration

As part of a Doctor of Philosophy degree in infertility and clinical reproduction, the study received approval from the Ethical Committee of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq (0701-PF-2021R7/September 2021). Each participant in the study gave informed consent.

Statistical analysis

Data were collected, summarized, analyzed and presented using the statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. We used number and percentages to show qualitative (categorical) variables and the Kolmogorov-Smirnov test to see if quantitative (numeric) variables had a normal distribution. If they did, we used mean (an indicator of central tendency) and standard deviation (an indicator of dispersion) to show normally distributed numerical variables. The following statistical tests were used: A chi-square test was used to evaluate the association between any two categorical variables, provided that less than 20% of cells had an expected count of less than 5. Independent samples A *t*-test is used to evaluate the difference in mean of numeric variables between any two groups, provided that these variables are normally distributed. One-way analysis of variance (ANOVA) was used to evaluate the difference in mean of numeric variables among more than

two groups, provided that these numeric variables were normally distributed. A one-way ANOVA was followed by a post hoc LSD test to evaluate individual differences in mean values between any two groups. Hardy-Weinberg equilibrium was used to evaluate the difference between observed counts and expected counts of genotypes. Risk was estimated using the odds ratio and corresponding 95% confidence interval. The level of significance was considered to be a *p*-value less than 0.05.

RESULTS

The Hardy-Weinberg equilibrium frequency distribution of 219C>T (rs1801133) SNP is shown in Table 1.

Table 1: Hardy Weinberg equilibrium frequency distribution of 219C>T (rs1801133) SNP

219C>T (rs1801133)	Observed count	Expected count
CC	37	39.5
CT	37	32.0
TT	4	6.5
X	1.887	
p-value	0.169	

There was no significant difference between observed counts and expected counts (*p*=0.169). A comparison of 219C>T (rs1801133) genotypes and alleles according to pregnancy outcome is shown in Table 2.

Table 2: Comparison of 219C>T (rs1801133) genotypes and alleles according to pregnancy outcome

219C>T(rs1801133)	Positive pregnancy test *n*=25	Negative pregnancy test *n*=53	*p*	OR	95% CI
Genotypes					
CC	18(72.0)	19(35.8)	0.003	4.60	1.63-12.99
CT	7(28.0)	30(56.6)	0.018	0.30	0.11-0.83
TT	0(0.0)	4(7.5)	0.158	0.22	
Alleles	Positive pregnancy test *n*=50	Negative pregnancy test *n*=106	*p*	OR	95% CI
C	43(86.0)	68(64.2)	0.005	3.43	1.41- 8.38
T	7(14.0)	38(35.8)		0.29	0.12-0.71

Values were expressed as number and percentage. *n*: number of cases; OR: odds ratio; CI: confidence interval.

Genotype CC was significantly higher in women with positive pregnancy tests in comparison to women with negative pregnancy tests (72% versus 35.8%, respectively) (*p*=0.003); therefore, the CC genotype is a positive predictor of pregnancy, with an odds ratio of 4.6, indicating that women with this particular genotype have a 4.6 chance of getting pregnant in comparison with women lacking that particular genotype. Genotype CT was lower significantly in women with positive pregnancy tests in comparison to women with negative pregnancy tests (28% versus 56.6%, respectively) (*p*=0.018), therefore, the CT genotype is a negative predictor of pregnancy, with an odds ratio of 0.3 indicating that women with this particular genotype have a reduced chance of getting pregnant by 70% in comparison with women lacking that particular genotype. There was no

significant difference in the proportion of TT genotype between women with a positive pregnancy test and women with a negative pregnancy test (*p*=0.158). Allele C was significantly higher in women with positive pregnancy tests in comparison to women with negative pregnancy tests (86% versus 64.2%, respectively) (*p*=0.005); therefore, the C allele is a positive predictor of pregnancy with an odds ratio of 3.43, indicating that women with this particular allele have a 3.43 chance of getting pregnant in comparison with women lacking that particular allele. Allele T was significantly lower in women with positive pregnancy tests in comparison to women with negative pregnancy tests (14% versus 35.8%, respectively) (*p*=0.005), therefore, the T allele is a negative predictor of pregnancy with an odds ratio of 0.29, indicating that women with this particular allele have a reduced chance of getting pregnant by 71% in comparison

with women lacking that particular allele. A comparison of oocyte and embryo characteristics based on 219C>T (rs1801133) genotypes is shown in Table 3. The mean total oocyte was significantly highest in the CC and CT genotypes, followed by the TT genotypes (*p*=0.047). The mean immature oocyte (GV) and metaphase I (MI) were

significantly highest in CT genotypes, followed by CC and TT genotypes (*p*<0.001). The mean mature oocyte (metaphase II (MII)) was significantly highest in the CC genotype, followed by the CT genotype, and then the TT genotype (*p*=0.001).

Table 3: Comparison of oocyte and embryo characteristics based on 219C>T (rs1801133) genotypes

Characteristic	CC *n*=37	CT *n*=37	TT *n*=4	*p*-value (ANOVA)
Total oocyte	9.68±2.25[a]	9.382.56[a]	6.50±1.73[b]	0.047
Immature (GV, MI)	2.78±0.95[b]	4.05±1.6[a]	2.5±1.29[b]	<0.001
Mature (MII)	6.89±2.17[a]	5.30±1.91[b]	4.00±1.41[c]	0.001
Fertilization rate	76.65±7.52	72.24±13.91	65.00±12.25	0.069
Total embryos	5.41±1.92[a]	4.24±1.74[b]	2.75±1.26[c]	0.003
Good embryo (G I, G II)	4.0±1.72[a]	2.62±1.46[b]	0.25±0.5[c]	<0.001
Bad embryo (G III, G IV)	1.41±0.99[b]	1.70±1.02[b]	2.75±0.50[a]	0.032
Transferred embryo	2.38±0.64	2.54±0.61	2.75±0.50	0.353

Values are expressed as mean±SD. Values with non-identical superscripts (a,b,c) within the same character are significantly different according to *post hoc* analysis (*p*<0.05).

Fertilization rates were comparable among the CC, CT and TT genotypes (*p*=0.069). The mean total embryo count was significantly highest in the CC genotype, followed by the CT genotype, and then by the TT genotype (*p*= 0.003). The mean good embryo (grade I (G I) and grade II (GII)) count was significantly highest in the CC genotype, followed by the CT genotype, and then by the TT genotype (*p*<0.001). The mean bad embryo (grade III (GIII) and grade IV (G IV)) count was significantly highest in the TT genotype, followed by the CT and CC genotypes (*p*=0.032). The mean transferred embryo count was comparable among the CC, CT and TT genotypes (*p*=0.353).

DISSCUSION

The MTHFR gene is crucial for the metabolism of folate and is required for human reproduction. The MTHFR genetic mutation has been linked to a number of disorders that affect fertility. MTHFR C677T polymorphism testing may therefore have significant clinical significance. However, there is no definitive evidence linking the MTHFR C677T polymorphism to ICSI outcomes. The current study revealed that genotype CC was considerably greater among women who had positive pregnancy tests compared to those who had negative pregnancy tests. Additionally, genotype CT was significantly lower in women with a positive pregnancy compared to those with a negative pregnancy. Furthermore, there was no discernible difference in the proportion of TT genotype between both groups. This finding is consistent with research indicating that women with the MTHFR C677T polymorphism were at increased risk for unsuccessful conception and implantation, especially in those with high serum homocysteine [13]. Other research findings indicated that the MTHFR C677T variation had no effect on the success of pregnancies [10,14,15]. There are several possible explanations for this, including: First, accurate blastocyst implantation into the endometrium is essential throughout pregnancy. This is a complex process

that involves trophoblastic and endometrial cell molecular interactions, as well as coagulation and fibrinolysis reactions at the embryo-maternal interface [16]. Changes in the blood coagulation factors' functional activity as a result of decreased MTHFR activity may have an impact on the likelihood of implantation. Numerous studies have linked inherited thrombophilic mutations and polymorphisms to recurrently failed IVF cycles [17,18]. Second, implantation failure is assumed to be predominantly caused by aneuploidy, which is a common defect in human preimplantation embryos in both spontaneous and assisted reproduction [18,19]. The third explanation for the mutant allele's poor pregnancy rate could be greater homocysteine concentration as a result of lower enzymatic activity; elevated homocysteine has a negative correlation with pregnancy and implantation rate [13]. Based on a comparison of oocyte and embryo features, the present study discovered that total oocytes, mature oocytes, total embryos and good-quality embryos were significantly higher in the CC genotype, followed by the CT genotype, and then the TT genotype. Concern for the immature oocyte was significantly highest in the CT genotype, followed by the CC and TT genotypes. CC, CT, and TT genotypes all had comparable rates of fertilization. Finally, the TT genotype had a considerably higher, poorer embryo count than the CT and CC genotypes. These findings were similar to the results obtained by other studies showing that the MTHFR 677 mutant allele had a negative effect on oocyte number and embryo quality [18,20]. Pavlik *et al*. found that women with MTHFR677TT produced a lower number of oocytes than were expected [21]. In addition, women older than 35 who carried the MTHFR 677C major allele had higher levels of estradiol in their blood, produced more oocytes, and needed much less FSH during ovarian stimulation than women older than 35 who carried the 677T allele [22]. Another study demonstrated that women with homozygous MTHFR677TT produced fewer embryos [12]. Contrary to these findings, other research suggests that the number and maturity of oocytes retrieved may be associated with the MTHFR polymorphism C677T [23].

Another study found that MTHFR 677 CT heterozygotes had a larger proportion of good-quality embryos and a higher chance of conception [24]. Research done by Zeng *et al*. showed that the MTHFR C677T polymorphism was not related to the number of oocytes retrieved, metaphase II oocyte or chemical pregnancy [15]. There are a number of factors that could account for the association between the MTHFR C677T polymorphism and the embryological and clinical results of IVF/ICSI. The decrease in MTHFR enzyme activity caused by the C677T and A1298C polymorphisms may affect folliculogenesis by inhibiting DNA and protein synthesis and thus inhibiting granulosa and theca cell proliferation [25], changing the DNA methylation pattern of oocytes and interfering with the expression of important folliculogenesis genes, and finally by increasing homocysteine and decreasing folate concentration in follicular fluid [23]. Because only one dominant follicle emerges during a normal pregnancy, and follicles with more metabolic errors caused by MTHFR polymorphism are less likely to be dominant, the detrimental effects of MTHFR variation may not be immediately apparent. Ovarian stimulation during the ICSI cycle, on the other hand, yields follicles with atresia, some of which may be the worse follicles impacted by the MTHFR mutation [26]. Additionally, the massive increase in metabolic demands brought on by the simultaneous activation of several follicles may worsen any metabolic defects present in these follicles, eventually decreasing the oocyte maturation rate [26,27]. Oocyte maturation is crucial for later embryonic development. From zygotes to day 3 embryos, maternal genes in oocytes control how the embryo grows and develops. In humans, the zygotic genome is activated during the 4–8 cell stage [28]. Some oocytes with low MTHFR enzyme activity may not be able to develop properly because of the bad effects of MTHFR polymorphisms [26]. Numerous studies have shown that oocytes and early embryos go through dramatic and extensive DNA methylation reprogramming. Polymorphisms of MTHFR, a crucial enzyme in methyl-donor metabolism, may interfere with this process and cause potential epigenetic abnormalities in oocytes and embryos in the early stages of life [29–31]. The decline of MTHFR activity causes a rise in blood total homocysteine, especially in cases of folate deficiency. Homocysteine level in the follicular fluid also appears to be negatively linked with in vitro embryonic development and oocyte maturation. According to the research done by Razi *et al*., homocysteine level is inversely linked with oocyte maturation and embryo quality [32].

Conclusion

During the ICSI procedure, the maternal MTHFR C677T polymorphism is associated with pregnancy, oocyte number and maturation, total embryo and quality. Therefore, MTHFR variations in our population provide important information on the ICSI success rate. In order to confirm our findings and increase the statistical power, future research should take into account a bigger sample size, blood homocysteine and folate levels, and paternal MTHFR. These investigations might yield more findings and help us comprehend how MTHFR ultimately affects ICSI results.

REFERENCES

1. Mani S, Ghosh J, Coutifaris C, Sapienza C, Mainigi M. Epigenetic changes and assisted reproductive technologies. *Epigenetics*. 2020;15(1-2):12-25. doi: 10.1080/15592294.2019.1646572.
2. Gleicher N, Barad DH. Assessing in-vitro fertilization at age 40 years. *Lancet*. 2019;393(10177):1181-1183. doi: 10.1016/S0140-6736(18)32070-1.
3. Gernand AD, Schulze KJ, Stewart CP, West Jr KP, Christian P. Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. *Nature Rev Endocrinol*. 2016;12(5):274-289. doi: 10.1038/nrendo.2016.37.
4. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. *Cell Metab*. 2017;25(1):27-42. doi: 10.1016/j.cmet.2016.08.009.
5. Simkanin E. Thesis. Effects that the methylenetetrahydrofolate gene mutation (both the C677T and A1298C polymorphisms) have on both men and women's fertility abilities and subsequent fetal development, as well as what nutritional changes can possibly do to aid in reversing these supposed negative effects. The University of Akron in Akron, Ohio, USA; 2023.
6. Guo J, Hao X, Wang R, Lian K, Jiang J, Chen N, Feng Z, Rao Y. MTHFR polymorphism's influence on the clinical features and therapeutic effects in patients with migraine: An observational study. *Front Neurol*. 2022;13:1074857. doi: 10.3389/fneur.2022.1074857.
7. Mallhi TH, Shahid M, Rehman K, Khan YH, Alanazi AS, Alotaibi NH, et al. Biochemical association of MTHFR C677T polymorphism with myocardial infarction in the presence of diabetes mellitus as a risk factor. *Metabolites*. 2023;13(2):251. doi: 10.3390/metabo13020251.
8. Abood ES, Naser MS, Naser RJ. MTHFR 677 C< T and 1298 A> C polymorphisms increases the risk of recurrent abortion in the Iraqi woman. *Research Square*. 2023. Pre-print. doi: 10.21203/rs.3.rs-480746/v1.
9. Ye F, Zhang S, Qi Q, Zhou J, Du Y, Wang L. Association of MTHFR 677C> T polymorphism with pregnancy outcomes in IVF/ICSI-ET recipients with adequate synthetic folic acid supplementation. *BioScience Trends*. 2022;16(4):282-290. doi: 10.5582/bst.2021.01306.
10. Liu L, Lin Z, Lin P, Jiang Z. Association between serum homocysteine level and unexplained infertility in in vitro

fertilization/intracytoplasmic sperm injection (IVF/ICSI): A retrospective, hospital-based, case-control study. *J Clin Lab Anal*. 2020;34(5):e23167. doi: 10.1002/jcla.23167.

11. Servy EJ, Jacquesson-Fournols L, Cohen M, Menezo YJ. MTHFR isoform carriers. 5-MTHF (5-methyl tetrahydrofolate) vs folic acid: a key to pregnancy outcome: a case series. *J Assist Reprod Genetics*. 2018;35:1431-1435. doi: 10.1007/s10815-018-1225-2.

12. Zeng H, Liu Z, Zhang L, Liu N. MTHFR 677TT is associated with decreased number of embryos and cumulative live birth rate in patients undergoing GnRHa short protocol: a retrospective study. *BMC Preg Childbirth*. 2022;22(1):170. doi: 10.1186/s12884-022-04506-4.

13. Chen L, Chen H, Wang X, Wei B, Wu Z, Chen S, et al. Association of homocysteine with IVF/ICSI outcomes stratified by MTHFR C677T polymorphisms: a prospective cohort study. *Reprod Biomed Online*. 2021;43(1):52-61. doi: 10.1016/j.rbmo.2021.04.009.

14. Dobson AT, Davis RM, Rosen MP, Shen S, Rinaudo PF, Chan J, et al. Methylenetetrahydrofolate reductase C677T and A1298C variants do not affect ongoing pregnancy rates following IVF. *Human Reprod*. 2007;22(2):450-456. doi: 10.1093/humrep/del396.

15. Zeng S, Wang X, Wang Y, Xu Z, Zhang J, Liu W, et al. MTHFR C677T polymorphism is associated with follicle-stimulating hormone levels and controlled ovarian hyperstimulation response: a retrospective study from the clinical database. *Fertil Steril*. 2019;111(5):982-990. doi: 10.1016/j.fertnstert.2019.01.016.

16. Deryabin PI, Ivanova JS, Borodkina AV. Senescence of stromal cells contributes to endometrium dysfunction and embryo implantation failure. *BioRxiv*. 2021;2021-07. doi: 10.1101/2021.07.19.452880.

17. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations are risk factors for implantation failure. *Reprod Biomed Online*. 2006;12(3):322-327. doi: 10.1016/S1472-6483(10)61004-8.

18. Enciso M, Sarasa J, Xanthopoulou L, Bristow S, Bowles M, Fragouli E, et al. Polymorphisms in the MTHFR gene influence embryo viability and the incidence of aneuploidy. *Human Genetics*. 2016;135:555-568. doi: 10.1007/s00439-016-1652-z.

19. Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. *Fertil Steril*. 2013;100(6):1695-1703. doi: 10.1016/j.fertnstert.2013.07.2002.

20. Shahrokhi SZ, Kazerouni F, Ghaffari F, Rahimipour A, Omrani MD, Arabipoor A, et al. The relationship between the MTHFR C677T genotypes to serum anti-Müllerian hormone concentrations and in vitro fertilization/intracytoplasmic sperm injection outcome. *Clin Lab*. 2017;63(5):927-934. doi: 10.7754/clin.lab.2016.161104.

21. Pavlik R, Hecht S, Ochsenkühn R, Noss U, Lohse P, Thaler CJ. Divergent effects of the 677C> T mutation of the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene on ovarian responsiveness and anti-Müllerian hormone concentrations. *Fertil Steril*. 2011;95(7):2257-2262. doi: 10.1016/j.fertnstert.2011.03.023.

22. Thaler CJ, Budiman H, Ruebsamen H, Nagel D, Lohse P. Effects of the common 677C> T mutation of the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene on ovarian responsiveness to recombinant follicle-stimulating hormone. *Am J Reprod Immunol*. 2006;55(4):251-258. doi: 10.1111/j.1600-0897.2005.00357.

23. D'Elia PQ, dos Santos AA, Bianco B, Barbosa CP, Christofolini DM, Aoki T. MTHFR polymorphisms C677T and A1298C and associations with IVF outcomes in Brazilian women. Reprod Biomed Online. 2014;28(6):733-738. doi: 10.1016/j.rbmo.2014.02.005.

24. Altmäe S, Hovatta O, Stavreus-Evers A, Salumets A. Genetic predictors of controlled ovarian hyperstimulation: where do we stand today? *Human Reprod Update*. 2011;17(6):813-828. doi: 10.1093/humupd/dmr034.

25. Rosen MP, Shen S, McCulloch CE, Rinaudo PF, Cedars MI, Dobson AT. Methylenetetrahydrofolate reductase (MTHFR) is associated with ovarian follicular activity. *Fertil Steril*. 2007;88(3):632-638. doi: 10.1016/j.fertnstert.2006.11.165.

26. Lu YJ, Li Q, Chen LX, Tian T, Kang J, Hao YX, et al. Association between maternal MTHFR C677T/A1298C combination polymorphisms and IVF/ICSI outcomes: a retrospective cohort study. *Human Reprod Open*. 2023;2023(1):055. doi: 10.1093/hropen/hoac055.

27. Ledowsky CJ, Schloss J, Steel A. Variations in folate prescriptions for patients with the MTHFR genetic polymorphisms: A case series study. *Explor Res Clin Soc Pharm*. 2023;10:100277. doi: 10.1016/j.rcsop.2023.100277.

28. Jukam D, Shariati SA, Skotheim JM. Zygotic genome activation in vertebrates. *Devel Cell*. 2017;42(4):316-332. doi: 10.1016/j.devcel.2017.07.026.

29. Sendžikaitė G, Kelsey G. The role and mechanisms of DNA methylation in the oocyte. *Essays Biochem*. 2019;63(6):691-705. doi: 10.1042/EBC20190043.

30. Zhou F, Wang R, Yuan P, Ren Y, Mao Y, Li R, et al. Reconstituting the transcriptome and DNA methylome landscapes of human implantation. *Nature*. 2019;572(7771):660-664. doi: 10.1038/s41586-019-1500-0.

31. Yan R, Gu C, You D, Huang Z, Qian J, Yang Q, et al. Decoding dynamic epigenetic landscapes in human oocytes using single-cell multi-omics sequencing. *Cell Stem Cell*. 2021;28(9):1641-1656. doi: 10.1016/j.stem.2021.04.012.

32. Razi Y, Eftekhar M, Fesahat F, Dehghani Firouzabadi R, Razi N, et al. Concentrations of homocysteine in follicular fluid and embryo quality and oocyte maturity in infertile women: a prospective cohort. *J Obstet Gynecol*. 2021;41(4):588-593. doi: 10.1080/01443615.2020.1785409.