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ORIGINAL RESEARCH

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The influence of paternal MTHFR C677T polymorphism on *in vitro* fertilization outcomes in male Han population

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Abstract

The methylenetetrahydrofolate reductase (MTHFR) regulates the metabolism of homocysteine in the human body, and *MTHFR* C677T polymorphism is correlated with male infertility among Asian populations. The relationship between paternal *MTHFR* C677T polymorphism and clinical outcomes is unclear due to conflicting study findings. In the current retrospective study, we enrolled 849 infertile couples from the First Affiliated Hospital of USTC, categorizing them into three subgroups based on their paternal *MTHFR* 677 genotype: CC, CT and TT. The clinical pregnancy (CC: 60.8%, CT: 62.5%, TT: 63.7%; $p = 0.83$), implantation (CC: 36.6%, CT: 42.2%, TT: 40.5%; $p = 0.15$), blastocyst formation (CC: 49%, CT: 48.4%, TT: 50.6%; $p = 0.49$), good-quality embryo (CC: 48.3%, CT: 49.8%, TT: 51.3%; $p = 0.19$), and normal fertilization (embryo development) (CC: 67.1%, CT: 66.2%, TT: 67.5%; $p = 0.51$) rates were comparable among all groups. Similarly, the live birth (CC: 54.2%, CT: 53.2%, TT: 53.7%; $p = 0.97$) and miscarriage (CC: 10.9%, CT: 14.9%, TT: 15.7%; $p = 0.45$) rates were comparable among the three cohorts. Regarding neonatal outcomes, the Apgar score, gestational age at delivery, neonatal sex, birth weight, birth height and preterm birth rates were non-significant among all groups. Finally, the rates of birth defects were also comparable among individuals of all groups (CC: 0%, CT: 0.3%, TT: 1.9%; $p = 0.18$). These findings suggest that paternal *MTHFR* C677T polymorphism does not exert any discernible effect on embryo quality, neonatal outcomes or birth defects *in vitro* fertilization (IVF) treatment. Therefore, in our population, paternal *MTHFR* C677T polymorphism is not informative in explaining IVF failure. Further studies, however examining the other enzymes in the folic acid pathway are warranted.

Keywords

MTHFR polymorphism; *In vitro* fertilization (IVF); Neonatal outcome; Male infertility

1. Introduction

One in six couples may encounter infertility at some point in their reproductive life, and male factor accounts for nearly 50% of infertility cases [1, 2]. One of the most successful therapies for male factor infertility is *in vitro* fertilization (IVF) [1]. However, the pregnancy outcome following IVF procedures has been unpredictable over the past decades [3]. One of the key reasons for IVF failure is sperm quality [4]. Sperm quality is commonly assessed by traditional semen analysis, comprising of parameters such as sperm number, concentration, motility and morphology. These parameters provide limited insight into the sperm's contribution to results of IVF such as embryo quality, live birth rate and birth defects [4].

Methylation abnormalities have a profoundly deleterious effect on sperm quality [5–7]. Homocysteine is converted to methionine by the methylenetetrahydrofolate reductase (MTHFR), yielding S-adenosylmethionine (SAM) for DNA methylation [8]. *MTHFR*, located on chromosome 1's short arm, plays critical roles in subcellular metabolic events, including carbon metabolism. Its importance as a drug target is evident, and some compounds that inhibit MTHFR have been reported, such as Vilanterol (β2-adrenergic agonist) [9]. There are three commonly known polymorphisms of the *MTHFR* gene, namely *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131) and MTHFR G1793A (rs2274976) [10]. The *MTHFR* C667T polymorphism is significantly associated with a higher risk of male infertility in the Asian population, while the MTHFR A1298C polymorphism is not considered a risk factor for male infertility [11]. *MTHFR* C677T polymorphism, which is located in exon 4, changes an alanine (Ala) to a valine (Val), reducing the enzyme's thermal stability [11]. Homozygous TT mutants have about 30% of the enzyme activity compared to homozygous CC individuals, while heterozygous CT individuals have approximately 65% of the enzyme activity observed in homozygous CC individuals [11]. Multiple meta-studies indicate *MTHFR*

C677T polymorphism to possibly impact the infertility among male Asian populations [11, 12]. However, controversial studies exist regarding the correlation between paternal *MTHFR* polymorphism and pregnancy outcome. Numerous meta-analyses have suggested a positive relationship between paternal *MTHFR* polymorphism and an increased risk of early spontaneous abortion and miscarriage [13–17]. On the other hand, several studies don't indicate any such correlation between embryo quality, clinical pregnancy outcomes and paternal *MTHFR* C677T. For example, clinical pregnancy, positive pregnancy test, and pregnancy rates in IVF therapy were found to have no role in paternal *MTHFR* C677T [18]. Meanwhile, the prevalence of the methylation *MTHFR* epigenotype among individuals with Recurrent Pregnancy Losses (RPL) and non-RPL individuals was not remarkably different, as indicated by Poorang *et al.* [19]. Also, according to the research by Enciso *et al.* [20], the paternal *MTHFR* genotypes did not have a significant effect on embryo aneuploidy. Therefore, the present retrospective study was conducted to investigate the effects of paternal *MTHFR* C677T polymorphisms on seminal parameters, sperm DNA fragmentation, and IVF outcome in a population being examined for infertility.

2. Materials and methods

2.1 Participants

Infertile couples (numbering 849) were recruited in our research between January 2020 and May 2022 for IVF therapy, at the First Affiliated Hospital of USTC. The male factor infertility was the cause of the infertility experienced by the study's recruited couples. In the beginning, there were no criteria for elimination. All women were strongly encouraged to have a daily prenatal vitamin (folic acid) with $\geq$400 μg at their first prenatal counseling appointment.

2.2 Semen parameters analysis

After 2–7 days of ejaculatory abstinence, sperm samples of male patients were collected and analyzed by liquefying them (37 °C; 30 min). The World Health Organization (WHO) semen analysis guidelines (5th edition, 2010) were used to calculate semen parameters. Computer-assisted sperm analysis (CASA) were employed to measure total and progressive motility of sperm. The sperm concentration was evaluated by using SAS-II system (SAS Medical, Beijing, China) and a phase contrast microscope (CX43, Olympus Corporation, Tokyo, Japan). Sperm morphology was observed under a light microscope (UB100i, UOP, Chongqing, China) at 100$\times$ magnification, through diff-Quick staining (Ankebio, Hefei, China). Benzidine was used to stain leukocytes for the peroxidase test (Ankebio). Anti-sperm antibodies (AsA) were detected by the mixed antiglobulin reaction (MAR) technique (Ankebio). Flow cytometry was used to measure the degree of DNA fragmentation in sperms according to the manufacturer's instructions (Cellpro, Ningbo, China).

2.3 *MTHFR* C677T genotype detection

Whole genome DNA from the blood samples of male patients was isolated with QIAquick polymerase chain reaction (PCR) purification kits (28104, QIAGEN, Hilden, Germany). The *MTHFR* C677T genotype was determined through fluorescence PCR detection kit (PCR-fluorescent probe) manufactured by Tailor Medical (P1302310009, Shenzhen, Guangdong, China). The prime pairs and probes used have been described previously (forward primer 5′-CCGAAGCAGGGAGCTTTG-3′, reverse primer 5′-CGGTGCATGCCTTCACAA-3′, probe 1 (VIC-labeled) AAATCG[G]CTCCCGC, probe 2 (FAM-labeled) AAATCG[A]CTCCCGC) [21]. Total genome DNA in a 10 μL-PCR apparatus was used for fluorescence PCR detection. Next, 45 cycles of this reaction were repeated (60 °C annealing/extension for 60 seconds and 95 °C denaturation for 15 seconds) following the methods reported earlier. The ABI 7500 fluorescence quantitative PCR instrument (7500, Applied Biosystems, Foster City, CA, USA) was utilized to effectively measure the post-PCR samples' endpoint fluorescence, and the ABI 3730 Genetic Analyzer was used to determine the MTHFR genotyping results.

2.4 *In vitro* fertilization (IVF) procedure

The women had ovarian hyperstimulation according to the conventional extended procedure. Fertilization was achieved through a 36-hour incubation in a MOPS (G-morpholinepropanesulfonic acid) medium (Vitrolife, Kungsbacka, Sweden) for oocyte extraction using 250 μg recombinant human chorionic gonadotophin (hCG) (Ovitrelle, Merck Serono, Switzerland). Hyaluronidase was used in G-IVF PLUS medium (Vitrolife) to break down the cumulus-oocyte complexes. Desktop incubators (COOK Medical, Bloomington, IN, USA) were used to cultivate embryos in G-1 medium (Vitrolife) for at least three days. The luteal phase was supported by progesterone.

2.5 Pregnancy determination

Two blood hCG tests were performed 14 days after the transplant confirmed the clinical pregnancy. An ultrasound was used 30 days following the transplant to observe the obvious sacs and assess their growth.

2.6 Statistical analysis

The compliance of genotype frequencies with the Hardy-Weinberg equilibrium was assessed using GENEPOP v.4.2 (http://genepop.curtin.edu.au/). The mean and standard deviation (SD) were utilized for quantitative variables having a normal distribution. For variables that did not exhibit normal distribution, the data were presented as medians with an accompanying interquartile range (IQR). Quantitative variables with a normal distribution were analyzed by analysis of variance (ANOVA), and those having a non-normal distribution were analyzed by the Kruskal-Wallis test. Qualitative variables were characterized by frequency ratios and percentages. For qualitative variables, either the Pearson's chi-square test or the Fisher's exact test were used.

The exact test was employed to check the observed frequency of each genotype for departure from the Hardy-Weinberg equilibrium. The statistical significance level was $p < 0.05$ for all two-tailed tests. R version 3.5.3 (R Core Team, Vienna, Austria) was used for all statistical analysis.

3. Result

3.1 The frequencies of genotypes CC, CT and TT for the *MTHFR* C677T polymorphism in male patients

The *MTHFR* C677T genotyping results of male infertility subjects of current investigation are shown in Fig. 1. Overall, 240 (28.3%), 419 (49.3%) and 190 (22.4%) individuals with *MTHFR* 677CC, *MTHFR* 677CT and *MTHFR* 677TT genotypes were included. The genotypes frequencies of *MTHFR* C677T was in Hardy-Weinberg equilibrium ($p > 0.05$). Based on the *MTHFR* loci 677 genotype, all subjects with male infertility were classified into three groups, including the 677CC, 677CT and 677TT groups.

3.2 Basic information of participants

The basic features of the included subjects in our study are listed in Table 1. The mean age of male participants was 33.3 (±5.4), 32.7 (±5.6) and 33.1 (±6.0) years in the 677CC, 677CT and 677TT groups, respectively. The mean body mass index (BMI) was 24.7 (±3.2), 24.6 (±3.3) and 24.7 (±3.1) kg/m^2 and the mean duration of infertility was 2.9 (±2.4), 2.9 (±2.5) and 3.1 (±2.4) years in the three groups, respectively. Moreover, the duration of infertility, clinical conditions (BMI), and age were not different between the three groups ($p = 0.54$, 0.90 and 0.35, respectively) (Table 1). Moreover, the basic characteristics of female spouse, including BMI, age, maternal baseline follicle stimulating hormone (FSH) concentration, female induction duration, and female endometrial thickness on hCG day ($p = 0.88$, 0.89, 0.49, 0.91 and 0.96, respectively) were also similar among the three groups' participants (Table 1).

3.3 Semen parameters of male patients across the three groups

The average sperm concentration in the 677CC, 677CT and 677TT groups was 85.0, 76.0 and 79.4 × 10^6/mL, respectively. The total sperm motility rate was 47.1%, 46.9% and 47.9%, while the normal sperm morphology percentage was 5.0%, 5.0% and 6.0% in the three groups, respectively. The mean sperm concentration, motility and morphology rates did not significantly differ among these groups. Similarly, other relevant sperm parameters (including, sperm DNA fragmentation index (DFI), leukocyte count, high DNA stainability (HDS) and anti-sperm antibody (AsA) level) were also comparable between the three groups (Table 2).

3.4 Embryonic quality, pregnancy outcome and neonatal outcome

Descriptive data of the embryonic quality and pregnancy outcome following IVF are shown in Table 3. The clinical pregnancy (60.8% *vs.* 62.5% *vs.* 63.7%; $p = 0.83$), implantation (36.6% *vs.* 42.2% *vs.* 40.5%; $p = 0.15$), blastocyst formation (49% *vs.* 48.4% *vs.* 50.6%; $p = 0.49$), good-

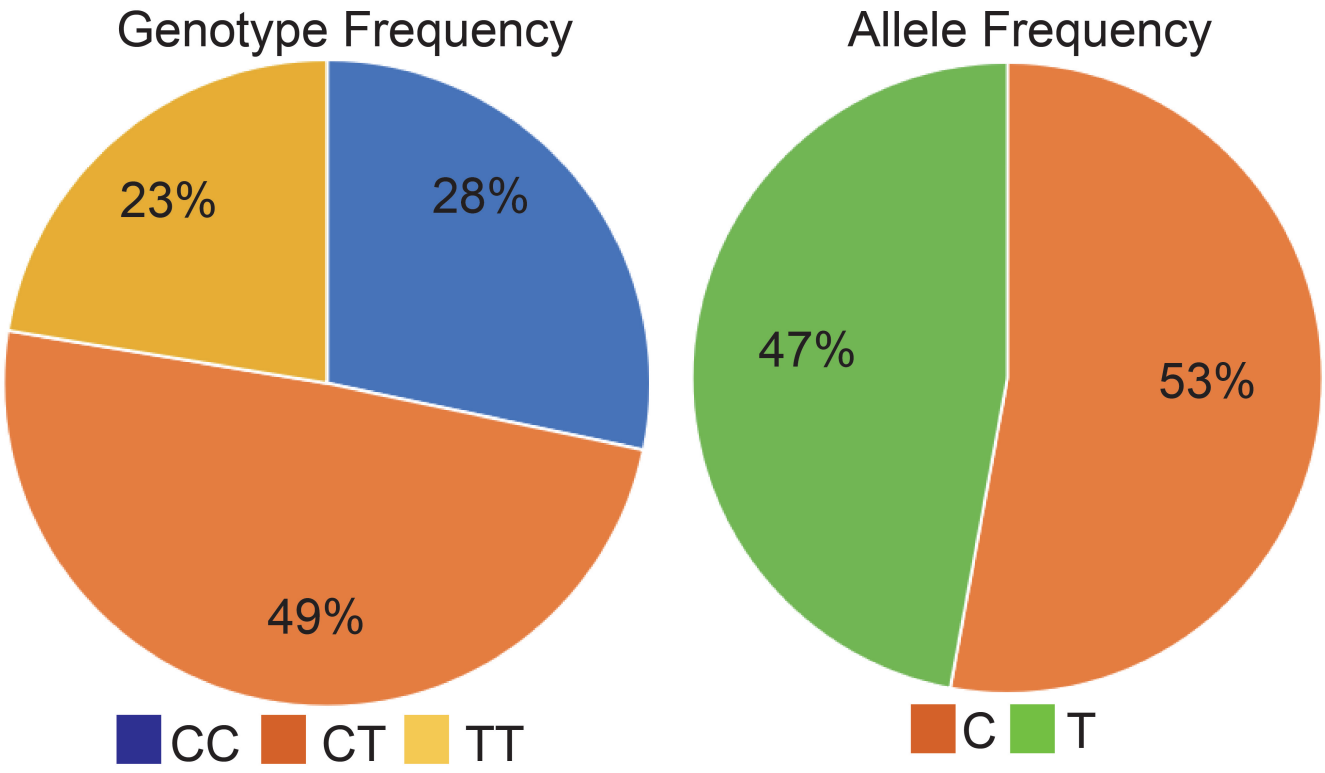

FIGURE 1. The genotype distributions of *MTHFR* C677T in all study subjects were tested according to the Hardy-Weinberg law of genetic equilibrium ($p = 0.95$).

TABLE 1. General clinical features of infertile patients.

Fundamental clinical characteristics	CC (n = 240)	CT (n = 419)	TT (n = 190)	*p*
Male age (yr), mean ± SD	33.3 ± 5.4	32.7 ± 5.6	33.1 ± 6.0	0.35
Female spouse age (yr), mean ± SD	32.1 ± 4.9	31.9 ± 5.1	31.9 ± 5.9	0.89
Male BMI (kg/m^2), mean ± SD	24.7 ± 3.2	24.6 ± 3.3	24.7 ± 3.1	0.90
Female spouse BMI (kg/m^2), mean ± SD	22.8 ± 3.3	22.7 ± 3.3	22.7 ± 3.2	0.88
Infertility duration (yr), mean ± SD	2.9 ± 2.4	2.9 ± 2.5	3.1 ± 2.4	0.54
Primary infertility, n (%)	113 (47.1)	189 (45.1)	87 (45.8)	0.89
Secondary infertility, n (%)	127 (52.9)	230 (54.9)	103 (54.2)	
Basal FSH levels in female spouse (mIU/mL), mean ± SD	7.7 ± 2.7	7.5 ± 2.9	7.5 ± 2.4	0.49
Ovulation induction time (d), mean ± SD	9.9 ± 4.3	9.9 ± 3.9	9.8 ± 4.1	0.91
Endometrial thickness on hCG day (mm), mean ± SD	10.6 ± 2.6	10.5 ± 2.7	10.6 ± 2.7	0.96

BMI: body mass index; FSH: follicle stimulating hormone; hCG: Human chorionic gonadotropin; SD: standard deviation.

TABLE 2. Semen parameters of the male patients.

Semen parameters	CC (n = 240)	CT (n = 419)	TT (n = 190)	*p*
Abstinence time (d), mean ± SD	4.0 (3.0, 7.0)	4.0 (3.0, 7.0)	4.0 (3.0, 6.0)	0.77
Semen volume (mL), median (IQR)	3.0 (2.0, 4.0)	3.0 (2.2, 4.1)	3.0 (2.1, 4.0)	0.38
Semen volume <1.5 (mL), n (%)	17 (7.1)	32 (7.6)	20 (10.5)	0.38
Sperm concentration ($\times 10^6$/mL), median (IQR)	85.0 (51.9, 129.5)	76.0 (43.7, 119.8)	79.4 (47.9, 134.3)	0.28
Sperm concentration <15 × 10^6/mL, n (%)	8 (3.3)	6 (1.4)	7 (3.7)	0.14*
Progressive motility (%), median (IQR)	39.9 (29.3, 50.8)	39.6 (28.6, 52.4)	40.3 (30.9, 50.0)	0.94
Progressive motility <32%, n (%)	71 (29.6)	132 (31.5)	54 (28.4)	0.72
Total motility (%), median (IQR)	47.1 (35.5, 58.9)	46.9 (43.1, 60.1)	47.9 (36.8, 59.0)	0.76
Total motility <40%, n (%)	84 (35.0)	152 (36.3)	61 (32.1)	0.61
Normal forms (%), median (IQR)	5.0 (4.0, 7.0)	5.0 (4.0, 7.0)	6.0 (4.0, 8.0)	0.12
Normal forms <4%, n (%)	38 (15.8)	79 (18.8)	23 (12.1)	0.11
Leukocyte count ($\times 10^6$/mL), median (IQR)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)	0.59
Leukocyte count >1 × 10^6/mL, n (%)	16 (6.7)	41 (9.8)	16 (8.4)	0.39
AsA (%), median (IQR)	2.0 (1.0, 4.8)	2.0 (0, 4.0)	2.0 (1.0, 5.0)	0.09
AsA >10, n (%)	17 (7.1)	33 (7.9)	12 (6.3)	0.89
DFI (%), median (IQR)	14.5 (8.9, 19.9) (n = 102)	12.9 (9.6, 17.4) (n = 169)	12.4 (8.3, 19.7) (n = 71)	0.85
DFI >15, n (%)	49 (48.0)	63 (37.3)	28 (39.4)	0.21
HDS (%), median (IQR)	5.8 (4.5, 8.4) (n = 102)	5.9 (4.6, 7.4) (n = 169)	5.6 (4.4, 8.1) (n = 71)	0.49
HDS >15, n (%)	3 (2.9)	3 (1.8)	2 (2.8)	0.72*

*AsA: antisperm antibody; DFI: DNA fragmentation index; HDS: high DNA stainability; IQR: (25th percentile, 75th percentile); *Fisher's exact test. SD: standard deviation.*

quality embryo (48.3% *vs.* 49.8% *vs.* 51.3%; $p = 0.19$), and normal fertilization (embryo development) (67.1% *vs.* 66.2% *vs.* 67.5%; $p = 0.51$) rates were similar among the three groups. Similarly, the live birth (54.2% *vs.* 53.2% *vs.* 53.7%; $p = 0.97$) and miscarriage (10.9% *vs.* 14.9% *vs.* 15.7%; $p = 0.45$) rates were consistent among all cohorts.

To evaluate neonatal outcome differences among the three groups, the Apgar score, birth height and weight, neonatal sex, preterm birth, and gestational age at delivery was analyzed. The Apgar score, gestational age at delivery, neonatal sex, birth weight, birth height, and preterm birth rate were non-significant in the three groups ($p = 0.84$, 0.61, 0.22, 0.32, 0.14 and 0.43, respectively). Finally, the rates of birth defects were also comparable among individuals of the three groups (0% *vs.* 0.3% *vs.* 1.9%; $p = 0.18$) (Table 4).

TABLE 3. Association between MTHFR polymorphism and embryo quality as well as pregnancy outcomes in infertile couples.

Embryo quality and pregnancy outcomes	CC (n = 240)	CT (n = 419)	TT (n = 190)	*p*
Number of retrieved oocytes, mean ± SD	10.7 ± 6.9	11.2 ± 7.2	11.8 ± 8.2	0.32
Normal fertilization rate, n (%)	1724 (67.1)	3106 (66.2)	1511 (67.5)	0.51
Good quality embryo rate, n (%)	941 (48.3)	1742 (49.8)	866 (51.3)	0.19
Blastocyst formation rate, n (%)	573 (49.0)	991 (48.4)	523 (50.6)	0.49
Embryo implantation rate, n (%)	171 (36.6)	310 (42.2)	143 (40.5)	0.15
Clinical pregnancy rate, n (%)	146 (60.8)	262 (62.5)	121 (63.7)	0.83
Miscarriage rate, n (%)	16 (10.9)	39 (14.9)	19 (15.7)	0.45
Live birth rate, n (%)	130 (54.2)	223 (53.2)	102 (53.7)	0.97

SD: standard deviation.

TABLE 4. Association between MTHFR polymorphism and neonatal birth outcomes.

Neonatal outcomes	CC (n = 240)	CT (n = 419)	TT (n = 190)	*p*
Gestational age (wk), mean ± SD	38.5 ± 1.7	38.4 ± 2.3	38.2 ± 2.4	0.61
Preterm birth rate, n (%)	20 (15.4)	37 (16.6)	22 (21.6)	0.43
Cesarean section rate, n (%)	87 (66.9)	142 (63.7)	64 (62.8)	0.77
Vaginal birth rate, n (%)	43 (33.1)	81 (36.3)	38 (37.2)	
Singleton pregnancies rate, n (%)	110 (84.6)	191 (85.7)	87 (85.3)	0.97
Twin pregnancies rate, n (%)	20 (15.4)	32 (14.3)	15 (14.7)	
Female baby birth rate, n (%)	85 (56.7)	123 (48.2)	64 (54.2)	0.22
Male baby birth rate, n (%)	65 (43.3)	132 (51.8)	54 (45.8)	
Apgar score, mean ± SD	9.8 ± 0.5	9.8 ± 0.6	9.8 ± 0.4	0.84
Birth height (cm), mean ± SD	49.5 ± 2.0	49.2 ± 2.6	48.8 ± 3.2	0.14
Birth weight (kg), mean ± SD	3.2 ± 0.7	3.1 ± 0.6	3.1 ± 0.7	0.32
Very low birth weight (g) (<1500 g), n (%)	0 (0)	6 (2.4)	3 (2.5)	0.11*
Low birth weight (g) (<2500 g), n (%)	23 (15.3)	43 (16.9)	23 (19.5)	0.66
High birth weight (g) (>4000 g), n (%)	7 (4.7)	8 (3.4)	3 (2.5)	0.64*
Abnormality rate of neonatal, n (%)	0 (0)	1 (0.3)	2 (1.9)	0.18*

**Fisher's exact test. SD: standard deviation.*

4. Discussion

The MTHFR enzyme is essential to produce 5-methyltetrahydrofolate (5-MTHF), for conversion of homocysteine into methionine. The resulting methionine generates S-adenosylmethionine (SAM), which is an essential methyl donor for DNA methylation processes [8]. DNA methylation is an important component of spermatogenesis due to its role in controlling gene expression [22]. Due to conflicting results and findings that vary with ethnicity, it is imperative to explore the impact of paternal *MTHFR* C677T polymorphism on IVF results.

This retrospective cohort study comprehensively analyzed the relationship between paternal *MTHFR* C677T polymorphism and IVF outcomes, including semen and embryonic quality, as well as pregnancy and neonatal outcomes of 849 infertile couples. No significant changes were observed in any of these outcomes across 677CC, 677CT and 677TT patients. It seems that the paternal *MTHFR* C677T polymorphism had no effect on the viability of the embryos or the outcome of the IVF treatment. Our results suggest that the paternal *MTHFR* C677T polymorphism is not a major contributor to the success or failure of IVF treatment in our study population.

The genotype frequency of *MTHFR* C677T varied by ethnicity and geographic location [11]. The *MTHFR* 677TT genotype frequency has been reported to be approximately 10% to 16% in populations from Canada, the United States, Brazil, Italy and Australia [11]. In China, the *MTHFR* genotype frequency is approximately 12%–17% in the general population [23, 24]. Several recent investigations suggest a correlation between *MTHFR* polymorphism and male infertility [11, 12]. Current results indicated a higher frequency of the C677T polymorphism in infertile patients than in the general population which is consistent with earlier studies. Similarly, sperm DFI

does not seem to be influenced by the *MTHFR* C677T polymorphism, implying that the effect of *MTHFR* polymorphism on male infertility may not be mediated by an increased in sperm DFI. According to the findings of Dominique Cornet *et al.* [25], MTHFR may contribute to sperm pathogenesis by increasing the SDI (sperm nucleus decondensation index) [25]. Patients with a high SDI should be tested undergo *MTHFR* isoform testing as part of healthcare policy. This underscores the importance of SDI in exploring the correlation between MTHFR polymorphism and male infertility.

In this experiment, women, particularly those with the *MTHFR* C677T polymorphism, consumed a daily intake of 400 or 800 μg of folic acid. The *MTHFR* polymorphism has been linked to male infertility, and few studies indicate that males, too, may benefit by consumption of folic acid supplements [26, 27]. However, some research does not support the notion of enhanced sperm quality in male patients by folic acid administration. A randomized clinical study indicates that folic acid and zinc supplementation does not substantially increase sperm quality or the rate at which couples had live births [28]. Also, Aarabi *et al.* [29] suggested that high dosage folic acid supplementation (5 mg/day) is not suitable, as this caused hypomethylation of sperm. Folic acid (FA) has poor metabolic capacity, especially in MTHFR carriers [30]. But another micronutrient, 5-Methyltetrahydrofolate (5-MTHF), the compound located downstream from the MTHFR enzyme should be recommended for hypofertile male patients with high homocysteine levels [31].

5. Conclusions

Current investigation contributes to the knowledge of the genetic variables that lead to male infertility by providing data on the prevalence of *MTHFR* C677T genotypes in a group of male infertility patients. This research not only adds to the growing literature and provides additional evidence for the possible involvement of *MTHFR* in male infertility, but it also highlights the correlation between paternal *MTHFR* polymorphism and IVF outcomes, which could have substantial consequences for clinical treatment of infertility. The intricate relationship between *MTHFR* and sperm DNA integrity, and its effects on reproductive outcomes and treatment, requires further investigation.

AVAILABILITY OF DATA AND MATERIALS

The raw evidence supporting this work are accessible from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

JH and YYW—design. XC, XNS and SYL—data collection. YYW, XC, XNS and SYL—analysis. JH—writing and revision. The study was reviewed and approved by all authors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The First Affiliated Hospital of USTC Ethical Committee authorized this study (Approval ID: 2022-RE-261). All individuals who took part signed a written informed consent form.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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