Original Article

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Association of MTHFR 677C>T polymorphism with pregnancy outcomes in IVF/ICSI-ET recipients with adequate synthetic folic acid supplementation

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SUMMARY Methylenetetrahydrofolate reductase (*MTHFR*) genetic polymorphism rs1801133 (677C>T) will decrease the utilization of folate. Folate deficiency and its resulting homocysteine (HCY) accumulation can impair female fertility. Folic acid (FA) supplementation is necessary in pregnant women who are undergoing in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) - embryo transfer (ET), and especially in women with *MTHFR* rs1801133 C-to-T mutations. At present, affordable and accessible synthetic FA is mainly used. However, some studies have suggested that 5-methylenetetrahydrofolate (5-MTHF), a type of active FA, may be more suitable for women with the *MTHFR* 677C>T polymorphism, since it is safer and more effective. This retrospective study aimed to evaluate whether the *MTHFR* rs1801133 gene polymorphism is related to the pregnancy outcomes of IVF/ICSI-ET recipients after sufficient supplementation with FA instead of 5-MTHF. Data on 692 women undergoing IVF/ICSI-ET and taking adequate FA were collected. Participant characteristics were compared using the Kruskal-Wallis test and Pearson chi-square test. Logistic regressions were used to calculate the odds ratio (OR) and 95% confidence interval (95% CI), after adjusting for age, BMI, method of fertilization, method of embryo transfer and number of embryos transferred. An additive model (T/T *vs.* C/C), dominant model (C/T + T/T *vs.* C/C), and recessive model (T/T *vs.* C/T + C/C) were evaluated. Analysis revealed that *MTHFR* rs1801133 in IVF/ICSI-ET women with adequate FA supplementation was not associated with the pregnancy rate but with age (OR = 0.91, 95% CI = 0.88, 0.94, $P < 0.001$) and BMI (OR = 0.95, 95% CI = 0.90, 0.997, $P = 0.037$). In 349 clinically pregnant women, no association of the *MTHFR* 677C>T with pregnancy outcomes was found in the additive model, dominant model, or recessive model. Of the 273 women with positive pregnancy outcomes, 34 had a preterm delivery. *MTHFR* 677C>T was not associated with a preterm delivery after adjusting for age and BMI. The current results indicated that *MTHFR* polymorphism rs1801133 was not related to the pregnancy rate or pregnancy outcomes of women undergoing IVF/ICSI-ET with adequate synthetic FA supplementation, suggesting that simple supplementation with less expensive and readily available FA, rather than expensive 5-MTHF, appeared to be appropriate.

Keywords methylenetetrahydrofolate reductase, IVF/ICSI-ET, folic acid supplementation, active folic acid, 5-MTHF, adverse pregnancy, preterm delivery

1. Introduction

Folic acid (FA) is an important type of vitamin B, which is involved in various biological transmethylation reactions that include many physiological and pathological processes. Folate deficiency elevates the frequency of uracil misincorporation into DNA, disrupts nucleic acid integrity, slows DNA replication, and increases the risk of chromosomal breakage, thereby negatively affecting female fertility and fetal viability (*1*). Severe folate deficiency before and during pregnancy can lead to oocyte and follicle development disorder, reduced endometrial receptivity, and impair the implantation process and fetal development (*2*). In addition, folate deficiency can lead to the accumulation of homocysteine (HCY), which may damage the

vascular endothelium and disrupt the coagulation and fibrinolytic system, subsequently causing hypercoagulability and eventually leading to recurrent abortion, fetal growth restriction, and stillbirth (*3-5*).

The methylenetetrahydrofolate reductase (*MTHFR*) gene is located at the end of the short arm of chromosome 1 (chr1:11796321), and is 2.2 kb long. One of the most common single nucleotide polymorphisms (SNPs) in the *MTHFR* gene is rs1801133 (677C>T). A systematic review and meta-analysis indicated that the overall T allele frequency of *MTHFR* rs1801133 was 36.9% in Chinese (*6*), and 78.4% of Chinese people have homozygous or heterozygous mutations (*7*); this figure exceeds that in many other countries. The C-to-T transition results in a missense mutation that changes alanine (Ala) to valine (Val), which reduces the thermal stability and activity of MTHFR (*8*). The enzyme activity of individuals with homozygous TT mutation was about 30% of that in individuals with the wild-type (CC) genotype, whereas individuals with the heterozygous genotype (CT) had about 65% of the wild-type enzyme activity (*9*). Subsequently, the mutated genotype reduced the capacity to convert 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methylenetetrahydrofolate (5-MTHF), the predominant circulating form of folate, thus decreasing the utilization of folate (*10*) (Figure 1). Several studies have suggested that the *MTHFR* gene is a major genetic factor for adverse pregnancy outcomes (APOs) (*11-14*). Moreover, intake of FA from supplements has been found to reduce the risk of spontaneous abortion and pregnancy complications (*15-17*).

In-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and embryo transfer (ET) are assisted reproductive techniques (ARTs). Several studies have indicated that women after IVF/ICSI-ET have a higher spontaneous miscarriage rate than those after natural conception (*18,19*). In addition, IVF/ICSI-ET was found to be associated with preterm delivery (*20*). With the implementation of the three-child policy and the increase in women of advanced maternal age in China, the need for and use of ART is also estimated to be increasing.

FA supplementation is necessary in pregnant women who are undergoing ART, and especially in women with *MTHFR* rs1801133 C-to-T mutations. Therefore, the choice of the type of FA supplementation is not only a family-related but also a social issue that needs to be addressed. At present, synthetic FA is mainly used. Synthetic FA is a form of FA extracted and synthesized from the chemical raw material L-N-p-aminophenylmethylcool glutamic acid or 2,4,5- triamino -6- hydroxypyrimidine sulfate, and it has the advantages of good stability and affordability. China has been implementing a folate fortification policy since 2009, providing free supplements of synthetic FA to women of childbearing age.

Nevertheless, studies have pointed out that the conversion of synthetic FA to 5-MTHF is limited in women with the *MTHFR* rs1801133 CT or TT genotype (*21,22*), and FA is inactive until reduced into the bioactive folate derivative, 5-MTHF (*23*). Active FA, 5-MTHF, is the main circulating form of folate in the body, and it can bypass the MTHFR block. However, whether active FA is better than synthetic FA in preventing APOs remains a subject of controversy (*24,25*).

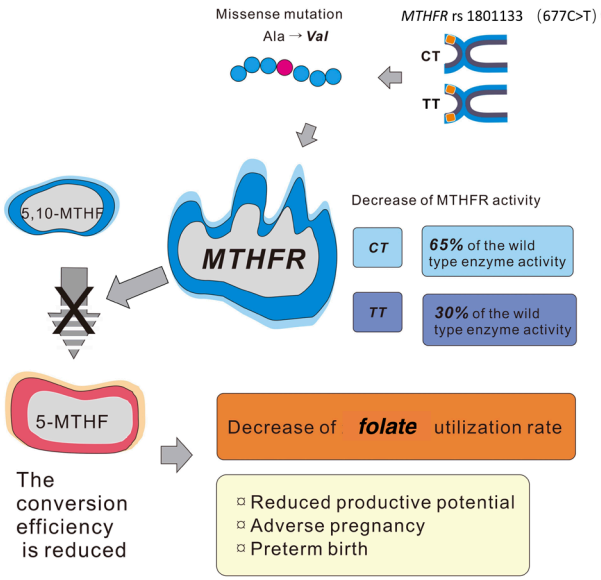

Figure 1. Possible mechanism of *MTHFR* rs1801133 affecting folic acid and pregnancy outcomes. MTHFR rs1801133 will lead to a missense mutation that changes Ala to Val, which can decrease the thermal stability and reduce the activity of MTHFR. The enzyme activity of individuals with homozygous TT mutation is about 30% of that in individuals with the wild-type (CC) genotype, whereas individuals with the heterozygous genotype (CT) have about 65% of the wild-type enzyme activity. Subsequently, the mutated genotype reduces the capacity to convert 5,10-MTHF to 5-MTHF, the predominant circulating form of folic acid, thus decreasing the utilization of folic acid.

Folate levels in women are still low even in countries with mandatory folate fortification (*21*). Since China is a developing country with a large population, affordable and accessible synthetic FA is more readily available than 5-MTHF, which is more expensive. Therefore, the aim of the current study was to evaluate whether *MTHFR* rs1801133 gene polymorphism was related to the pregnancy outcomes of IVF/ICSI-ET subjects after taking sufficient synthetic FA supplements.

2. Materials and Methods

2.1. Study population

The study population was 692 women who underwent IVF/ICSI-ET at Zhoushan Women's and Children's Health Hospital from 2016 to 2020. All of the women had adequate synthetic FA supplementation depending on their genotype, and they sought the treatment due to

fallopian tube or ovulation disorders or rare, weak, or abnormal sperm of their husbands.

2.2. Genotype determination

The extraction of whole-genome DNA from blood samples was performed using QIAquick PCR purification kits (QIAGEN, Germany). The *MTHFR* rs1801133 genotype was determined using a fluorescence PCR detection kit (PCR-fluorescence probe) designed by Osama Medicine (Shenzhen, Guangdong, China). Genotyping was performed under the following conditions: a 25-μL whole blood sample, a 16-μL PCR reaction system, reaction conditions of 45 cycles, 95°C denaturation for 15 s, and 60°C annealing/extension for 1 min, as recommended by the manufacturer. After the reaction was completed, the end point fluorescence in sample wells was read on the ABI 7900 fluorescence quantitative PCR instrument (Applied Biosystems, Foster City, CA), and the genotyping results for each sample were determined using the ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.3. Ovarian stimulation and oocyte retrieval

A controlled ovarian hyperstimulation protocol was tailored to the individual. According to comprehensive factors such as age of the woman, ovarian preparation, and outcomes of previous ovulation induction protocols, a long luteal phase protocol, a long early follicular phase protocol, an overlength protocol, a short protocol, an antagonist protocol, a natural cycle protocol, or a microstimulation protocol was adopted. When the diameter of two dominant follicles was ≥ 18 mm and that of three follicles ≥ 16mm, human chorionic gonadotropin (HCG) (a recombinant HCG alfa solution for injection, Merck Serono Sweden; chorionic gonadotrophin for injection, Livzon China, 6500/8500 IU) was injected at 9 PM on the same night. If the follicle diameter was ≥ 14 mm and urine luteinizing hormone (LH) was positive or blood LH > 10 mIU/ml, the oocyte was retrieved 24 hours after the immediate injection of HCG. Oocyte retrieval was performed under ultrasound guidance using a K-OPSD oocyte retrieval needle (Cook, Australia).

2.4. IVF/ ICSI-ET

The oocytes and sperm are then fertilized using IVF or ICSI and cultured *in vitro* on cleavage stage/blastocyst culture medium (Vitrolife Sweden). Embryo transfer was performed using a K-JETS catheter (Cook, Australia) 3-5 days after oocyte retrieval. Women younger than 35 years of age or who had received IVF/ ICSI-ET for the first time underwent single embryo transfer, while women older than 35 or who had failed IVF/ ICSI-ET several times received 2-3 embryos.

2.5. Determination of pregnancy

Twelve days after transplantation, a urine pregnancy test or a blood HCG quantitative test was conducted. For HCG-positive patients, the first vaginal ultrasound was performed 21 days after transplantation to exclude ectopic pregnancy. Ultrasound was performed again after 28 days to determine the number of embryos and their development.

2.6. FA supplementation

Patients were encouraged to take FA (Silian, China), instead of 5-MTHF, depending on their genotype. For the CC genotype: 400 μg/day FA was taken three months before pregnancy, 400 μg/day FA was taken in early pregnancy (0-12 weeks), and food supplementation was considered in middle/late pregnancy (13-40 weeks), but no extra supplementation was needed. For the CT genotype: FA was supplemented 400 μg/day three months before pregnancy, 800 μg/day in early pregnancy (0-12 weeks), and 400 μg/day in middle/late pregnancy (13-40 weeks). For the TT genotype: FA was supplemented 800 μg/day three months before pregnancy, 800 μg/day in the early pregnancy (0-12 weeks), and 400 μg/day in middle/late pregnancy (13-40 weeks).

2.7. Statistical analysis

Characteristics of participants with different genotypes were compared using the Kruskal–Wallis test for continuous variables and the chi-square test or Fisher's exact test for discrete variables. Genotype and allele frequencies were calculated. Observed genotype frequencies in different genotypes were separately tested for deviation from the Hardy–Weinberg equilibrium (HWE) using the exact test. Logistic regressions were used to calculate the odds ratio (OR) and 95% confidence interval (95% CI), after adjusting for age, body mass index (BMI), method of fertilization, method of embryo transfer and number of embryos transferred. An additive model (T/T *vs.* C/C), dominant model (C/T + T/T *vs.* C/C), and recessive model (T/T *vs.* C/T + C/C) were evaluated to assess the strength of association between *MTHFR* polymorphism rs1801133 and pregnancy outcomes. All significance tests were two-sided; a P value of < 0.05 was considered to be statistically significant. Data analyses were performed using the software platform SPSS v.26.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Participant characteristics

The age range of the 692 women was between 22 and 49 years of age, and the range of their BMI was

Table 1. Participant characteristics

Characteristics	*MTHFR* rs1801133 genotype			*p*
	CC (*n* = 316)	CT (*n* = 226)	TT (*n* = 150)	
Age, years (mean ± SD)	33.13 ± 4.64	32.51 ± 4.65	32.91 ± 4.33	0.34[a]
BMI, kg/m^2 (mean ± SD)	22.11 ± 2.95	22.32 ± 3.01	22.47 ± 3.17	0.53[a]
Method of fertilization (*n*, %)				
IVF	235 (74.37)	173 (76.55)	114 (76.00)	0.83[b]
ICSI	81 (25.63)	53 (23.45)	36 (24.00)	
Method of embryo transfer (*n*, %)				
FET	251 (79.43)	171 (75.66)	121 (80.67)	0.44[b]
ET	65 (20.57)	55 (24.34)	29 (19.33)	
Number of embryos transferred (*n*, %)				
1	99 (31.33)	71 (31.42)	43 (28.67)	0.82[b]
≥ 2	217 (68.67)	155 (68.58)	107 (71.33)	

BMI: body mass index; ET: fresh embryo transfer; FET: frozen embryo transfer; ICSI: intracytoplasmic sperm injection; IVF: *in-vitro* fertilization; [a]Kruskal-Wallis test. [b]Pearson chi-square test.

Table 2. Pregnancy outcomes

Pregnancy outcome	*MTHFR* rs1801133 genotype			*p*
	CC (*n* = 316)	CT (*n* = 226)	TT (*n* = 150)	
Not pregnant (*n*, %)	149 (47.15)	89 (39.38)	69 (46.00)	0.18[a*]
Pregnancy	167 (52.85)	137 (60.62)	81 (54.00)	
Biochemical pregnancy (*n*, %)	21 (12.57)	12 (8.76)	3 (3.70)	
Positive pregnancy outcome (*n*, %)	114 (68.26)	95 (69.34)	64 (79.01)	
Adverse pregnancy (*n*, %)	32 (19.16)	30 (21.90)	14 (17.28)	
Miscarriage (*n*, %)	22 (68.75)	21 (7.00)	9 (64.29)	
Ectopic pregnancy (*n*, %)	9 (28.13)	6 (2.00)	4 (28.57)	
Preterm delivery (*n*, %)	13 (11.40)	10 (10.53)	11 (17.19)	0.42[a]

[a]Pearson chi-square test. [*]Between pregnant patients and non-pregnant patients.

between 32.66 kg/m^2 and 16.02 kg/m^2. Based on the genotype of *MTHFR* rs1801133, 316 (45.66%) women were classified as wildtype homozygotes (CC), 226 (32.66%) women were classified as heterozygotes (CT), and 150 (21.68%) women were classified as mutated homozygotes (TT). There were no differences found regarding age (P = 0.34), BMI (P = 0.53), method of fertilization (P = 0.83), method of embryo transfer (P = 0.44), or number of embryos transferred (P = 0.82) among the three genotypes (Table 1).

3.2. Pregnancy outcomes

The pregnancy outcomes were compared among the different genotypes. Results revealed no significant differences in pregnancy rates of wildtype homozygotes (CC, 47.15%), heterozygotes (CT, 39.38%), or mutated homozygotes (TT, 46.00%). Among pregnant women, 12.75% of wildtype homozygotes (CC), 8.76% of heterozygotes (CT) and 3.7% of mutated homozygotes (TT) had a biochemical pregnancy. One hundred and fourteen pregnant women with the CC genotype (114/167, 68.26%), 95 with the CT genotype (95/137, 69.34%), and 64 with the TT genotype (64/81, 79.01%) had a positive pregnancy outcome (live birth ≥ 1), while 32 with the CC genotype (32/167, 19.16%), 30 with the CT genotype (30/137, 21.90%), and 14 with the TT genotype (14/81, 17.28%) had adverse pregnancy outcomes including miscarriage, ectopic pregnancy, and other pathological pregnancies (Table 2). The proportion of preterm deliveries by women with a positive pregnancy outcome is also shown in Table 2.

3.3. Association of the *MTHFR* rs1801133 genotype with a successful pregnancy

All DNA samples were successfully genotyped for rs1801133, and this SNP deviated from HWE (P < 0.01) in women receiving IVF/ICSI-ET. The proportions of each genotype among 385 pregnant and 307 non-

Table 3. Association of *MTHFR* rs1801133 with pregnancy or no pregnancy

Variables	*N* (%)		OR (95% CI)	*p*
	Pregnancy (*n* = 385)	No pregnancy (*n* = 307)		
Genotype/Allele				
CC	167 (43.38)	167 (43.38)	reference	
CT	137 (35.58)	137 (35.58)	1.35 (0.94, 1.95)	0.10
TT	81 (21.04)	81 (21.04)	1.03 (0.68, 1.55)	0.89
Age			0.91 (0.88, 0.94)	< 0.001
BMI			0.95 (0.90, 0.997)	0.04
Additive model				
CC	167 (43.38)	167 (43.38)	reference	
TT	81 (21.04)	81 (21.04)	1.02 (0.68, 1.54)	0.92
Dominant model				
CC	167 (43.38)	167 (43.38)	reference	
CT+TT	218 (56.62)	218 (56.62)	1.21 (0.88, 1.66)	0.23
Recessive model				
TT	81 (21.04)	81 (21.04)	reference	
CT+CC	304 (78.96)	304 (78.96)	1.11 (0.76, 1.62)	0.60

pregnant women are shown in Table 3. Logistic regression was used to analyze the association between the*MTHFR* rs1801133 genotype and a successful pregnancy, after adjusting for age, BMI, method of fertilization, method of embryo transfer, and number of embryos transferred. An additive model, dominant model, and recessive model were all assessed (Table 3). Results indicated that *MTHFR* genetic polymorphism rs1801133 was not associated with a successful pregnancy but with age (OR = 0.91, 95% CI = 0.88-0.94, $P < 0.001$) and BMI (OR = 0.95, 95% CI = 0.90, 0.997, $P = 0.04$) (Table 3) (Figure 2).

3.4. Association of the *MTHFR* rs1801133 genotype with pregnancy outcomes

Of 385 pregnant women, 349 were clinically pregnant, and 36 were biochemically pregnant. The proportions of genotypes in positive and adverse pregnancy outcomes are shown in Table 4. Results indicated that after adjusting for covariates, *MTHFR* rs1801133 was not associated with pregnancy outcomes in the additive model, dominant model, or recessive model. However, a younger age (OR = 0.92, 95% CI = 0.86-0.98, $P = 0.01$) was positively associated with pregnancy outcomes (Table 4) (Figure 2).

3.5. Association of the *MTHFR* rs1801133 genotype with preterm delivery

The association of *MTHFR* rs1801133 with a preterm delivery was further analyzed in 273 women with positive pregnancy outcomes. The genotype proportions in preterm deliveries and normal deliveries are shown in Table 5. *MTHFR* rs1801133 was not associated with a preterm delivery after adjusting for age and BMI (Table 5) (Figure 2).

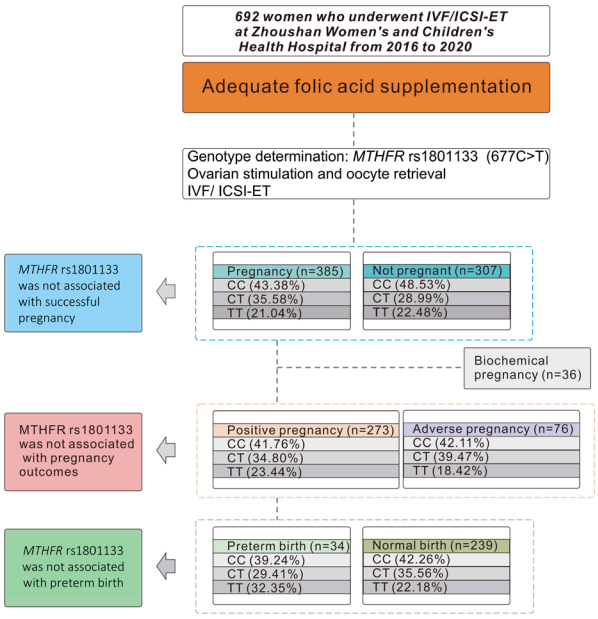

Figure 2. Flow diagram of the study population and results. A total of 692 women were included. They all received sufficient folic acid supplementation, and their genotype of *MTHFR* rs1801133 was determined. These participants were divided into two groups depending on whether they were pregnant or not. Then, the participants who were pregnant were divided into two groups, those with a positive pregnancy and those with an adverse pregnancy. The participants with a positive pregnancy were divided into two groups, those with a preterm delivery and those with a normal delivery.

Table 4. Association of the *MTHFR* rs1801133 genotype with pregnancy outcomes

Variables	*N* (%)		OR (95% CI)	*p*
	Positive pregnancy (*n* = 273)	Adverse pregnancy (*n* = 76)		
Genotype/Allele				
CC	114 (41.76)	32 (42.11)	reference	
CT	95 (34.80)	30 (39.47)	0.85 (0.47, 1.51)	0.57
TT	64 (23.44)	14 (18.42)	1.29 (0.63, 2.61)	0.49
Age			0.92 (0.86, 0.98)	0.01
BMI			0.99 (0.90, 1.07)	0.73
Additive model				
CC	114 (41.76)	32 (42.11)	reference	
TT	64 (23.44)	14 (18.42)	1.28 (0.62, 2.65)	0.50
Dominant model				
CC	114 (41.76)	32 (42.11)	reference	
CT+TT	159 (58.24)	44 (57.89)	0.99 (0.59, 1.67)	0.96
Recessive model				
TT	64 (23.44)	14 (18.42)	reference	
CT+CC	209 (76.56)	62 (81.58)	0.72 (0.37, 1.39)	0.33

Table 5. Association of the *MTHFR* rs1801133 genotype with a preterm delivery

Variables	*N* (%)		OR (95% CI)	*p*
	Preterm delivery (*n* = 34)	Normal delivery (*n* = 239)		
Genotype/Allele				
CC	13 (38.24)	101 (42.26)	reference	
CT	10 (29.41)	85 (35.56)	0.89 (0.37, 2.16)	0.80
TT	11 (32.35)	53 (22.18)	1.63 (0.68, 3.94)	0.28
Age			0.93 (0.84, 1.03)	0.15
BMI			0.97 (0.86, 1.10)	0.66
Additive model				
CC	13 (38.24)	101 (42.26)	reference	
TT	11 (32.35)	53 (22.18)	1.65 (0.68, 3.98)	0.27
Dominant model				
CC	13 (38.24)	101 (42.26)	reference	
CT+TT	21 (61.76)	138 (57.74)	1.16 (0.55, 2.45)	0.70
Recessive model				
TT	11 (32.35)	53 (22.18)	reference	
CT+CC	23 (67.65)	186 (77.82)	0.58 (0.26, 1.29)	0.18

4. Discussion

This study found no association between *MTHFR* polymorphism rs1801133 and pregnancy outcomes of women undergoing IVF/ICSI-ET with adequate synthetic FA supplementation. *MTHFR* rs1801133 fits the additive model, but results were also analyzed in a dominant model and a recessive model. Age was found to be related to the pregnancy rate (OR = 0.91, 95% CI = 0.88-0.94, $P < 0.001$) and pregnancy outcome (OR = 0.92, 95% CI = 0.86-0.98, $P = 0.01$), which was consistent with the results of previous studies (*18,26*). Older women may have an increased risk of impaired oocyte quality and chromosomal abnormalities and decreased endometrial receptivity (*27-29*). In addition, BMI also had an impact on the pregnancy rate (OR = 0.95, 95% CI = 0.90, 0.997, $P = 0.04$). Obese women often have impaired folliculogenesis, ovulation, and conception, resulting in decreased reproductive potential (*30*).

The conversion of 5,10-MTHF to 5-MTHF, a co-substrate for the re-methylation of HCY to methionine, requires the protein encoded by the *MTHFR* gene. The T allele of rs1801133 will lead to decreased activity of MTHFR, thus affecting the conversion of 5,10-MTHFR to 5-MTHF, and reduce the amount of folate circulating in the blood. In addition, the T allele of rs1801133 can also reduce the rate of FA utilization (*8*). Studies have been conducted to evaluate the association between *MTHFR* genetic polymorphism rs1801133 and pregnancy outcomes. Some have found that the mutated T allele of *MTHFR* rs1801133 was associated with a higher risk of adverse outcomes, including spontaneous abortion, premature birth, and stillbirth (*2,11,31-33*).

Folate deficiency and its resulting HCY accumulation can impair female fertility; possible mechanisms for this include reduced cell division, increased apoptosis, overproduction of inflammatory cytokines, impaired nitric oxide (NO) metabolism, oxidative stress, and defective methylation reaction (*34*). Moreover, maternal demand for folate increases during pregnancy, and a maternal folate deficiency often leads to APOs (*35*). Previous *in vivo* experiments have found that in the absence of maternal folate, placental mTOR signaling and amino acid transporter activity are inhibited (*36*) as well as the uterine decidualization (*37*) and decidual angiogenesis in pregnant mice (*38*), subsequently causing placental dysplasia and dysfunction and ultimately resulting in fetal growth restriction. A case-control study in Venezuela confirmed the association between maternal folate deficiency and an increased risk of a preterm delivery at the end of the third trimester and in labor (*39*). Another case-control study in Sweden suggested that the increased risk of early spontaneous abortion was also associated with low plasma folate levels (*40*).

Taking high doses of FA can prevent developmental delay and placental abnormalities (*41*) that may reduce the risk of low birth weight and premature birth (*36*). FA supplementation is especially necessary in patients undergoing IVF/ICSI-ET because of the inherent risk of an adverse pregnancy. Patients taking FA supplements were found to have significantly reduced HCY levels in follicle fluid, indicating that the recovered oocytes would be more mature and of better embryo quality (*42*).

However, studies in recent years have indicated that 5-MTHF is safer than FA. 5-MTHF is comparable to FA in reducing HCY, and it is comparable to or more effective than FA in maintaining serum and plasma folate levels (*43*). Compared to FA, 5-MTHF is less likely to cause unmetabolized folic acid (UMFA) syndrome or mask a B12 deficiency (*43*).

A Chinese study analyzed *MTHFR* polymorphism in normal pregnant patients and found that active FA (5-MTHF) had a significant therapeutic effect for patients with *MTHFR* rs1801133 C-to-T mutations. In patients without such a mutation, the therapeutic effect of 5-MTHF did not differ significantly from that of FA (*25*). A possible explanation for this is that 5-MTHF does not require the already reduced activity of MTHFR in individuals with the *MTHFR* 677C>T polymorphism to convert 5,10-MTHFR to 5-MTHF to become effective, but it can function directly.

5-MTHF appears to be more advantageous in women with the *MTHFR* 677C>T polymorphism than FA. However, a randomized, double-blind, placebo-controlled trial found that there was no difference in the abortion rate between women with *MTHFR* 677C>T polymorphism taking 5-MTHF and those taking FA; that is, there was no beneficial effect of 5-MTHF compared to FA supplementation (*44*). The current study also found that for women undergoing IVF/ICSI-ET, taking a sufficient amount of FA, rather than 5-MTHF, starting 3 months before conception can lead to a result where the *MTHFR* 677C>T genotype is irrelevant to the pregnancy outcome.

Although 5-MTHF has a slight advantage in increasing serum folate levels for the population with the *MTHFR* 677C>T polymorphism, previous studies found no difference in the effect on HCY in groups taking FA or 5-MTHF (*44,45*). Pharmacokinetic studies have also attempted to explain the possible reason why 5-MTHF did not appear superior to FA in improving pregnancy outcomes. On each of the four mornings following the start of dosing (7.5 mg/day), the serum total folate level of 5-MTHF was 23 to 55% higher than that of FA. Interestingly, 12 days later, when both groups continued to take a dose of 0.4 mg/day, serum total folate levels in the 5-MTHF and FA groups were indistinguishable (*21*). One of the advantages of 5-MTHF is that it can replenish the body's reserves more quickly in women with a folate deficiency. However, the quick replenishment for women undergoing IVF/ICSI-ET seems unnecessary since they usually start taking a full, genotypic dose of FA three months before conception.

For developing countries like China, affordable and more accessible synthetic FA seems to be cost-effective for most women than expensive 5-MTHF at the current stage. Because, according to the current results, the use of synthetic FA alone, in a sufficient amount and with enough time, can nullify the association between the *MTHFR* 677C>T genotype and pregnancy outcomes in IVF/ICSI-ET recipients. However, the related mechanism and whether FA alone is enough to eliminate the effects of the *MTHFR* 677C>T polymorphism still needs to be investigated further.

In summary, the current study did not find that *MTHFR* polymorphism rs1801133 was related to the pregnancy rate or pregnancy outcomes of women undergoing IVF/ICSI-ET with adequate synthetic FA supplementation, suggesting that simple supplementation with less expensive and readily available synthetic FA, rather than expensive 5-MTHF, appeared to be appropriate.

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