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Association of methylene tetrahydrofolate reductase polymorphisms with cycle day two hormonal characteristics.

Rabab Z. Al-yasiry [1], Mufeeda A. Jawad [2], Muayad S. Abbood [2]

[1] Department of Anatomy, and Histology, College of Medicine / Babylon University, Babylon / Iraq

[2] High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

rabab.mousa@uobabylon.edu.iq

ABSTRACT

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is a key methionine and folate metabolism enzyme that is found in oocytes and embryos in preimplantation stages and plays a vital role in female reproduction.To assess the impact of MTHFR genetic polymorphisms (C677T and A1298C) on follicular stimulating hormone (FSH), luteinizing hormone(LH), Estradiol (E2), progesterone and antimullerian hormone (AMH) in female receiving intracytoplasmic sperm injection (ICSI), 85 infertile women undergoing ICSI treatment at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies in Baghdad, Iraq, were recruited. These patients had their MTHFR polymorphism genotypes examined, FSH, LH, E2, progesterone, and AMH levels were compared between groups. Furthermore, all demographic characteristics as well as ICSI outcomes were compared between pregnant and non-pregnant women. The research revealed no discernible variation between the means of FSH, LH, E2 and AMH with respect to the 219A>C(rs1801131) (A1298C) genotypes ($p > 0.05$). Progesterone level was significantly differed with high level found in CC followed by AA and AC. There was no statistically significant variation in hormonal features between CC, CT, and TT for A1298C, 219A>C(rs1801133). The demographic features (age, duration, type of infertility, cause of infertility and previous trial) were not notably different between women who were pregnant and those who were not. In women undergoing ICSI, the maternal MTHFR polymorphisms is not related to FSH, LH, E2 and AMH. MTHFR polymorphisms therefore don't offer useful information regarding the hormonal levels in our groups.

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KEYWORD

Methylenetetrahydrofolate reductase, Intracytoplasmic, C677T, A1298C.

1. Introduction

Global rates of infertility are rising; it is estimated that approximately 70 million couples, or 15% of couples, are suffering, with approximately half undergoing in vitro fertilization (IVF) (Lu et al., 2023 [1]). Healthy reproduction may be impacted by poor lifestyle, environmental factors, maternal and/or paternal aging, anatomical or genetic abnormalities, neurological or systemic illnesses, infection, trauma, and antibody production. As a result, infertility can be brought on by both genetic and nongenetic reasons, and it is frequently polygenic, multifactorial causes, or both (Cariati et al., 2019 [2]). It is anticipated that many genes would exactly cooperate to produce healthy children during sex determination, gametogenesis, complicated hormone actions/interactions, embryo implantation, and early development. In fact, recognized genetic causes of infertility include single gene modifications, chromosomal defects, and phenotypes with inheritance that is multifactorial (Panera et al., 2022 [3]). Although a lot of genes could influence reproduction, MTHFR polymorphism is one genetic variation that has received significant attention from researchers. The MTHFR enzyme mediates the process of conversion of 5,10 methylene tetrahydrofolate to 5-methyl tetrahydrofolate and is a major modulator of folate and homocysteine metabolism (Simkanin, 2023 [4]). There is growing evidence that the metabolism of folate affects human reproductive health, including the success of ART. Changes in folate status have an effect on the one-carbon cycle and the synthesis of S-adenosylmethionine (SAM), that is involved in the methylation of DNA. The methylation of DNA is crucial for both sperm production and female reproduction (Lu et al., 2023 [1]).

Methylation is a critical biochemical process in the regulation of

metabolism. It influences gene expression through imprinting and epigenesis. Lipids, proteins, and DNA are the targets of this process. It controls the quantity of homocysteine, a hazardous amino acid that has been linked to autoimmune illnesses, mental problems, diabetes, and other renal and liver metabolic pathologies. The ability to reproduce is strongly "methylation dependent." Methylation mistakes will have a significant impact on gametogenesis, early embryogenesis, trophoblast development, and implantation (Clement et al., 2020 [5]). Many research investigations have demonstrated that DNA methylation is dramatically and extensively reprogrammed in oocytes and early embryos. MTHFR polymorphisms, an essential enzyme in methyl-donor metabolism, can impede this process and result in potential genetic errors in developing oocytes and embryos (Yan et al., 2021 [6]).

Several research papers have indicated that variations at the MTHFR gene are linked to reduced oocyte retrieval, decreased maturation rate of the oocyte, lower rate of fertilization, increased miscarriage rate, and pregnancies that are smaller than average for gestational age (D'Elia et al., 2014 [7]; Xu et al., 2019 [8]; Bulloch et al., 2020 [9]). The most prominent and recognized MTHFR polymorphisms are C677T (rs1801133) and A1298C (rs1801131). A frequent A>C variation in the MTHFR gene at nucleotide location 1298 leads to glutamate to be replaced for alanine in MTHFR protein and a decrease in the activity of the enzyme by about forty percent of the normal form people (He et al.,2014 [10]). The C677T polymorphism, on the other hand, results in an alanine to valine substitution and an overall decrease in the enzyme's activity in the 677CT and 677TT polymorphisms that is approximately sixty-five percent and thirty percent of the 677CC genotype, accordingly (Lu et al., 2023 [1]).

Compared to the CT and the CC genotypes, genotype TT of the 677C>T polymorphism is related to higher serum homocystine (Hcy) and lower serum folate concentrations. However, it has not been demonstrated that the gene variant 1298A>C affects blood levels of Hcy or folate (Murto et al., 2014 [11]). Because it plays a role in a range of physiological functions, such as folliculogenesis and the preservation of genomic stability in oocytes and ovarian reserve (Rosen et al., 2007 [12]; Enciso et al., 2016 [13]), it is reasonable to explore the relationship between MTHFR and hormone levels.

2. Patients and Methods

At Baghdad, Iraq's Al-Nahrain University's High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, a prospective cohort research was conducted. The recruitment period runs from January 2022 to September 2023. The study has been approved by the Local Medical Ethical Committee. All infertile ladies who agreed to participate in the study gave their informed consent. Eighty-five females with good ovarian function who were referred to an infertility clinic because of tubal, unexplained, or male-factor infertility were included in the study's sample. For this study, additional selection criteria included the following: 20 to 43 years old, 3 to 15 years of infertility, a regular menstrual cycle, and no signs of endocrine abnormalities. Some of these infertile ladies were going through their first IVF/ICSI treatments, while others had already completed at least one cycle. Participants were eliminated from the study for a number of reasons, including endometriosis, ovarian surgery history, endocrine abnormalities, and systemic diseases. Medical and gynecological history, physical examination, hormonal analysis (E2, LH, FSH, Prolactin, TSH, and AMH), and TVUS for ovarian reserve and endometrial thickness were performed on all females on the second

day of their menstrual cycle. For a later MTHFR genetic examination, 5 ml of blood in an EDTA tube were drawn from participant women. At the same time, their male partners were evaluated using seminal fluid analysis (WHO, 2010). The antagonist protocol was employed, and transvaginal oocyte pickup was done 34-46 hours after the human chorionic gonadotropin (hCG) trigger. The oocyte and embryo quality were assessed microscopically using unique grading systems (Pierson et al., 2023 [14]). According to the number and quality of embryos, embryo transfer was done two to three days following ovum pickup. For luteal phase enhancement, progesterone supplementation started on the evening of the oocyte retrieval day and lasted two weeks. In order to evaluate chemical pregnancy, the serum beta-hCG level was assessed 14 days following embryo transfer.

Regarding the MTHFR genetic analysis, The DNA was extracted from the female participants' whole blood using the Promega Wizard® Genomic DNA Purification Kit in compliance with the manufacturer's guidelines. The isolated DNA was detected using agarose gel electrophoresis. The amount and purity of DNA were determined using nano drops and the diode array's ability to scan between 200 and 320 Nm in wavelength. The absorbance profile was then processed and evaluated to identify the amount and quality of DNA by computing the 260/280 and 260/230 ratios. The genotypes of the MTHFR gene A1298C were determined by DNA sequencing of PCR amplicons used the forward 5'-GTGGAGGTCTCCCAACTTACC-3' and reverse 5'AGAACTCAGCCTCAGGGGTG-3' primers, while C677T was determined by using forward 5'-AACTCAGCGAACTCAGCACT-3' and reverse 5'-TCACTATCTTGGACCCCTGA-3'

primers (both designed in the current study).

The reaction mixture for PCR contained 25µl of nuclease-free water, 20-50 ng template DNA, Promega Go taq Green master mix 1X, downstream primers 0.1-1.0 pmol, and upstream primers 0.1-1.0 pmol. As a preliminary stage, PCR optimization was carried out utilizing a gradient temperature spanning from 50 Co to 65 Co with variations in PCR wells. The GTC Class thermocycler (Cleaver Scientific/UK) was utilized to carry out the amplification process. Amplified DNA fragments were electrophoresed for an hour at 75 volts in a 1.5% agarose (0.5x) TBE buffer. The bands were then revealed under ultraviolet light after being treated with ethidium bromide. The sizes of the fragments were estimated using a size marker of a 100 base-pair ladder. The resolved PCR samples were subsequently sequenced from both the forward and reverse ends according to the sequencing provider's protocols (Macrogen Inc. Geumchen, Seoul, South Korea). Only accurate chromatographs from ABI sequence data were examined further to guarantee that the annotation and variances were not the result of PCR or sequencing errors. The virtual locations and other details of the obtained PCR fragments were determined by comparing the detected DNA sequences of the investigated samples to the recovered adjacent DNA sequences of the NCBI Blastn engine. The BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI) was used to edit, align, and analyze the findings of PCR product sequencing. The patients have been grouped into three groups according to the genotypes of A1298C (AA, AC, CC) and C677T(CC, CT, TT), and hormonal levels were compared among the studied groups. Because they only possessed immature oocytes, seven of the participating females were later eliminated from the study.

Statistical analysis

Microsoft Office Excel 2010 and the Statistical Program for Social Sciences (SPSS) version 23 were used to gather, summarize, analyze, and present data. The Kolmogorov-Smirnov test was used to determine if quantitative (numeric) variables had a normal distribution before the mean (an index of central tendency) and standard deviation (an index of dispersion) were used to express normally distributed numeric variables. The association between any two category variables was examined using the Chi-square test. The means of the numerical variables were compared using the independent samples t-test. The mean difference between more than two groups was calculated using a one-way analysis of variance (ANOVA). P-values equal to or lower than 0.05 were used to determine the significance.

3. Results

1. Demographic features of infertile women involved in the current research

Demographic features of infertile women participating in the current research are shown in Table (1). The mean age of all enrolling women was 35.42 ±4.24 years, and no notable difference was observed in mean age between women with a positive pregnancy test and women with a negative pregnancy test (p = 0.117). The mean duration of infertility was 6.27 ±2.01 years, and it ranged from 3 to 13 years. There was not a statistically significant distinction in the mean duration of infertility between women who had a positive pregnancy test and those who did not (p = 0.696) .

According to causes of infertility, women were grouped into those with male factor infertility (46.2 %), those with tubal factor (39.7 %), and those with unexplained infertility (14.1 %); there was no significant variation in

causes of infertility between women with positive pregnancy and women with negative pregnancy (p = 0.475). With respect to type of infertility, there were 44 (56.4 %) women with primary infertility and 34 (43.6 %) women with secondary infertility; there was no significant variation in types of infertility between women with positive pregnancy and women with negative pregnancy (p = 0.129).

With respect to previous trials, women were categorized into 61 (78.2 %) with single trials and 14 (17.9 %) with 2 or 3 previous trials; there was no significant variation in trail numbers between women with positive pregnancy and women with negative pregnancy (p = 0.150).

2. The study's infertile participants' hormone levels

Hormonal levels of infertile females participating in this study are shown in Table (2). Mean serum FSH level of all enrolled women was 7.80 ±1.16 IU/L, and there was no significant difference in mean serum FSH between women with positive pregnancy tests and women with negative pregnancy tests (p = 0.859) . Mean serum LH level of all enrolled women was 5.51 ±1.13 IU/L, and there was no significant difference in mean serum LH between women with positive pregnancy tests and women with negative pregnancy tests (p = 0.240) .Mean serum estradiol (E2) level of all enrolled women was 22.80 ±5.50 pg/ml, and the mean serum E2 did not differ significantly between women with positive pregnancy tests and women with negative pregnancy tests (p = 0.870).

Mean serum progesterone level of all enrolled women was 20.36 ±4.66 ng/dl, and the mean serum progesterone did not significantly differ. Between women with a positive pregnancy test and women with a negative pregnancy test (p = 0.976).

Mean serum prolactin level of all enrolled women was 21.63 ±7.25 ng/ml, and there was no significant difference

in mean serum prolactin between women with a positive pregnancy test and women with a negative pregnancy test (p = 0.872) .Mean serum AMH level of all enrolled women was 1.67 ±0.42 ng/ml, and there was no significant difference in mean serum AMH between women with positive pregnancy tests and women with negative pregnancy tests (p = 0.728).

3. Oocyte and embryo characteristics of infertile women involved in the current study

Table (3) shows the oocyte and embryo characteristics of infertile women who participated in this study. Mean total oocyte count of all enrolled women was 9.37 ±2.46. The mean did not significantly differ between women who tested positive for pregnancy and those who tested negative for pregnancy(p = 0.147). Mean immature oocyte count of all enrolled women was 3.37 ±1.45, and no significant difference in the mean between women with positive pregnancy tests and women with negative pregnancy test (p = 0.059) were found. Mean mature oocyte count of all enrolled women was 5.99 ±2.20, and the mean was higher significantly in women with positive pregnancy tests in comparison with women with negative pregnancy tests (p = 0.003) .Mean fertilization rate of all enrolled women was 73.96 ±11.48 %, and there was no statistically significant difference in mean between women who had positive pregnancy tests and those who had negative pregnancy tests (p = 0.200). Mean total embryo count of all enrolled women was 4.72 ±1.93, and the mean was higher significantly in women with positive pregnancy tests in comparison with women with negative pregnancy tests (p = 0.011).

Mean good embryo count of all enrolled women was 3.15 ±1.82, and the mean was higher significantly in women with positive pregnancy tests in comparison with women with negative pregnancy tests (p < 0.001). Mean bad

embryo count of all enrolled women was 1.62 ±1.02, and there was no significant difference in the mean between women with positive pregnancy tests and women with negative pregnancy tests (p = 0.425). Mean transferred embryo count of all enrolled women was 2.47 ±0.62, and there was no significant difference between both groups (p = 0.739).

4. Comparison of cycle day two hormonal characteristics based on MTHFR genotypes

Regarding cycle day two, hormonal characteristics based on 219A>C (rs1801131) genotypes are shown in Table (4). The means of all characteristics showed no significant variation with respect to 219A>C(rs1801131) genotypes (p > 0.05) except for progesterone. No significant difference was found between hormonal levels and 219C>T(rs1801133) genotypes (p > 0.05), as revealed in Table (5).

5 .Pregnancy outcome

25 women succeeded to have a positive pregnancy, while 53 women failed to do so, and subsequently, the pregnancy rate was 32.1 %, as demonstrated in Figure 1

Table (1): Demographic features of infertile women involved in the current research

Characteristics	Total *n* = 78	Positive pregnancy test *n* = 25	Negative pregnancy test *n* = 53	*P*
Age (years)				
Mean ±SD	35.42 ±4.24	36.52 ±4.19	34.91 ±4.19	0.117 I NS
Range	27-42	29 -42	27 -41	
Duration (years)				
Mean ±SD	6.27 ±2.01	6.40 ±2.04	6.21 ±2.01	0.696 I NS
Range	3 -13	3 -11	3 -13	
Cause of infertility				
Male factor, *n* (%)	36 (46.2 %)	12 (48.0 %)	24 (45.3 %)	0.475 C NS
Tubal factor, *n* (%)	31 (39.7 %)	8 (32.0 %)	23 (43.4 %)	
Unexplained, *n* (%)	11 (14.1 %)	5 (20.0 %)	6 (11.3 %)	
Type of infertility				
Primary, *n* (%)	44 (56.4 %)	11 (44.0 %)	33 (62.3 %)	0.129 C NS
Secondary, *n* (%)	34 (43.6 %)	14 (56.0 %)	20 (37.7 %)	
Trial number				
1, *n* (%)	61 (78.2 %)	22 (88.0 %)	39 (73.6 %)	0.150 C NS
2 or 3, *n* (%)	14 (17.9 %)	3 (12.0 %)	14 (26.4 %)	

n: number of cases; SD: standard deviation; I: independent samples t-test; C: chi-square test; NS: not significant

Table (2): The study's infertile participants' hormone levels

Characteristics	Total *n* = 78	Positive pregnancy test *n* = 25	Negative pregnancy test *n* = 53	*P*
FSH (IU/L)				
Mean ±SD	7.80 ±1.16	7.76 ±1.02	7.81 ±1.23	0.859 I NS
Range	4.5	4.5 -9.4	5.45 -10.9	
LH (IU/L)				
Mean ±SD	5.51 ±1.13	5.73 ±0.96	5.40 ±1.20	0.240 I NS
Range	3.2 -8.8	3.9 -8	3.2 -8.8	
E_2 (pg/ml)				
Mean ±SD	22.80 ±5.50	22.65 ±6.47	22.87 ±5.04	0.870 I NS
Range	14.9 -39	14.9 -39	15 -33.7	
Progesterone (ng/dl)				
Mean ±SD	20.36 ±4.66	20.34 ±5.06	20.37 ±4.51	0.976 I NS
Range	10.23 -33	12.9 -32	10.23 -33	
Prolactin (ng/ml)				
Mean ± SD	21.63 ±7.25	21.82 ±7.70	21.53 ±7.11	0.872 I NS
Range	12.6 -46	12.9 -46	12.6 -43	
AMH (ng/ml)				
Mean ±SD	1.67 ±0.42	1.70 ±0.44	1.66 ±0.42	0.728 I NS
Range	1 -3	1.2 -3	1 -3	

Table (3): Oocyte and embryo features of infertile women enrolled in this study

Characteristics	Total *n* = 78	Positive pregnancy test *n* = 25	Negative pregnancy test *n* = 53	*P*
Total oocyte				
Mean ±SD	9.37 ±2.46	9.96 ±2.28	9.09 ±2.51	0.147 I NS
Range	4 -14	7 -14	4 -13	
Immature oocytes (GV and MI)				
Mean ±SD	3.37 ±1.45	2.92 ±1.32	3.58 ±1.47	0.059 I NS
Range	0 -7	0 -7	1 -7	
Mature oocytes				
Mean ±SD	5.99 ±2.20	7.04 ±2.11	5.49 ±2.07	0.003 I **
Range	2 -11	4 -11	2 -10	
Fertilization rate				
Mean ±SD	73.96 ±11.48	76.40 ±8.16	72.81 ±12.66	0.200 I NS
Range	7 -90	60 -88	7 -90	
Total embryos				
Mean ± SD	4.72 ±1.93	5.52 ±1.92	4.34 ±1.84	0.011 I *
Range	1 -9	3 -9	1 -9	
Good embryo (grade I and grade II)				
Mean ±SD	3.15 ±1.82	4.20 ±1.68	2.66 ±1.68	< 0.001 I ***
Range	0 -8	2 -8	0 -7	
Bad embryo (grade III)				
Mean ±SD	1.62 ±1.02	1.48 ±1.16	1.68 ±0.96	0.425 I NS
Range	0 -4	0 -4	0 -4	
Transferred embryo				
Mean ±SD	2.47 ±0.62	2.44 ±0.65	2.49 ±0.61	0.739 I NS
Range	1 -4	1 -3	1 -4	

Table (4): Comparison of cycle day two hormonal characteristics based on 219A>C(rs1801131) genotypes.

Characteristic	AA *n* = 24	AC *n* = 51	CC *n* = 3	*P*
AMH	1.74±0.51	1.64±0.37	1.50±0.33	0.51 O NS
FSH	7.63±1.10	7.79±1.17	9.08±0.64	0.14 O NS
LH	5.78±0.88	5.33±1.19	6.33±1.25	0.12 O NS
E2	20.91±3.42	23.80±6.14	20.73±3.32	0.08 O NS
Progesterone	22.79±4.80	18.96±3.78	24.63±8.02	0.001 O S

Table (5): Ultrasound findings, biophysical profile, and serum leukemia factor levels at the day of triggering of ovulation in all enrolled infertile women

Characteristic	CC *n* = 37	CT *n* = 37	TT *n* = 4	*P*
AMH	1.60±0.39	1.78±0.43	1.42±0.37	0.1 O NS
FSH	7.83±1.21	7.86±1.16	6.94±0.42	0.5 O NS
LH	5.86±1.13	5.28±1.09	4.73±0.98	0.06 O NS
E2	22.92±6.72	22.66±4.34	23.92±5.45	0.9 O NS
Progesterone	20.85±4.77	20.13±4.83	17.79±1.92	0.6 O NS

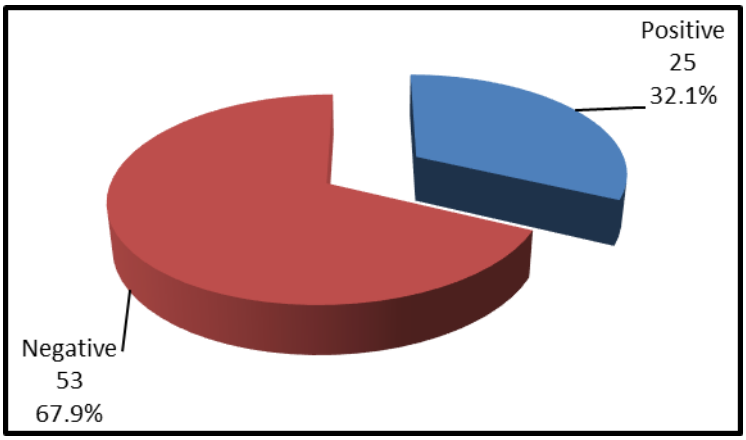

Figure (1): Pie chart showing positive pregnancy rate in infertile women enrolled in this study

4. Discussion

Genetic polymorphisms have been highlighted as a possible factor affecting fertility, and despite the fact that a variety of genes may regulate reproduction, one specific genetic variation that researchers have explored is the methylenetetrahydrofolate reductase polymorphism (Ledowsky, Steel and Schloss, 2021 [15]). MTHFR is an important regulating enzyme in folate metabolism. The folate metabolic pathway is crucial in cellular physiology because it involves in nucleotide synthesis, repair of DNA, methylation, and genome maintenance and stability. MTHFR polymorphisms could influence homocysteine levels, which might interfere with the methylation process, increase oxidative stress, and ultimately affect reproductive function (Guo, Tian and Wang, 2016 [16]). The potential impact of MTHFR polymorphism on reproductive hormones has been studied previously; however, the results were inconclusive.

Regarding cycle day two hormonal characteristics, the current study showed no significant difference with respect to 219A>C(rs1801131) genotypes except for progesterone.

This finding is consistent with other study that found the MTHFR mutant allele had a small but non-significant influence on AMH and no effect at all on certain hormone levels like FSH and LH (Shahrokhi et al., 2021 [17]). Others found that the MTHFR A1298C polymorphism was linked to increased baseline FSH concentrations and a reduced response to ovarian stimulation, suggesting that it could be affecting granulosa cell activity in developing follicles, which would then affect folliculogenesis (Rosen et al., 2007 [12] ;Shahrokhi et al., 2021 [17]). The granulosa cell secretes and controls the hormone inhibin, which controls FSH secretion and prompts the cell to undergo mitosis. The A1298C polymorphism may reduce the amount of methylene-THF available for the production of thymidine and purine, affecting the rates of DNA replication and apoptosis in granulosa cells. This could lead to fewer healthy follicles producing less E and inhibin, which would cause a compensatory rise in basal FSH (Rosen et al., 2007 [12]). Given the rarity of the genotype and the fact that the A1298C polymorphism reduces MTHFR activity by 10% in heterozygous form (AC variant) and by 40% in homozygous form (CC variant), it's probable that the homozygous mutant group's lack of a noticeable effect is connected to this (Undas et al., 2019 [18] ; Shahrokhi et al., 2021 [17]). Based on 219 C>T (rs1801133) genotypes, cycle day two hormonal parameters were examined. The means of all characteristics revealed no significant differences between the studied groups. The study done by (Rosen et al., 2007 [12]) revealed that MTHFR C677T polymorphisms were not associated with FSH and E2. However, other researchers discovered that homozygotes with the TT genotype of MTHFR 677 had higher basal levels of FSH and AMH than those with the CC or CT genotypes (Shahrokhi et al., 2017 [19]; Zeng et al., 2019 [20]). The findings in the current study might have

happened by chance or could be the result of the limited sample size and uncommon TT genotype. However, large-sample and long-term prospective studies are required to determine the link between MTHFR gene variants and reproductive hormones.

Regarding the oocyte and embryo features, our study revealed no discernible difference in the total number of oocytes between women who were pregnant and those who were not. Similar findings were published by another study, which demonstrated that the quantity of oocytes retrieved was unrelated to the success of IVF in achieving pregnancy (Cai et al., 2013 [21]). In contrast to these findings, additional studies showed that more oocytes had been associated with a higher likelihood of conception (Van Loendersloot et al., 2010 [22] ; Edan, Al-Katib, AlGhazali, 2015 [23] ; Hsu et al., 2016 [24]). This result could be explained by the fact that the ovarian reserve of the study participants was relatively equal and the presence of infertility factors unrelated to ovarian function .The current results showed that there was no statistically significant relationship between an immature oocyte, a poor embryo, fertilization rate, and pregnancy rate; however, pregnant women had a higher means number of metaphase II oocytes, a total of embryos, and a good quality embryo than non-pregnant women. This conclusion was validated by numerous studies that showed that a high number of recovered oocytes, MII oocytes, and grade I embryos increased the likelihood of getting pregnant (Berger et al., 2014 [25]; Kumar and Adiga, 2014 [26] ; Edan, Al-Katib, AlGhazali, 2015 [23]). The number and quality of embryos have been positively related to the number and maturity of oocytes, resulting in higher pregnancy and implantation rates (Cai et al.,2013 [21]; Shim et al., 2020 [27]).

6- Conclusion

In women undergoing ICSI, the maternal MTHFR polymorphisms are not related to FSH, LH, E2, and AMH. MTHFR polymorphisms, therefore, don't offer useful information regarding the hormonal levels in our groups.

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Author Contribution

Sahar S. Raheem performed the study, and Mufeeda A. Alammar, Muayad S. abbood, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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