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GAMETE BIOLOGY

# MTHFR isoform carriers. 5-MTHF (5-methyl tetrahydrofolate) vs folic acid: a key to pregnancy outcome: a case series

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## Abstract

**Purpose** To evaluate the possibility of correcting metabolic defects in gametes and embryos due to methylene tetra hydrofolate reductase (MTHFR) isoforms C677T and A1298C, by supplementation with 5-methyl THF instead of synthetic folic acid. In these couples, high doses of folic acid lead to UMFA (un-metabolized folic acid) syndrome.

**Methods** Thirty couples with fertility problems lasting for at least 4 years, such as recurrent fetal loss, premature ovarian insufficiency, or abnormal sperm parameters, with two thirds of them having failed assisted reproductive technology (ART) attempts were included in this program. For all couples, at least one of the partners was a carrier of one of the two main MTHFR isoforms. Most of the women had been previously treated unsuccessfully with high doses of folic acid (5 mg/day), according to what is currently proposed in the literature. The couples carrying one of the isoforms were treated for 4 months with 5-MTHF, at a dose of 600 micrograms per day, before attempting conception or starting another attempt at ART. The duration of treatment corresponding to an entire cycle of spermatogenesis is approximately 74 days.

**Results** In this first series of 33 couples, one couple was not followed-up, and two are still currently under treatment. No adverse effects were observed. Thirteen of the couples conceived spontaneously, the rest needing ART treatment in order to achieve pregnancy. Only three couples have, so far, not succeeded.

**Conclusion** The conventional use of large doses of folic acid (5 mg/day) has become obsolete. Regular doses of folic acid (100–200 μg) can be tolerated in the general population but should be abandoned in the presence of MTHFR mutations, as the biochemical/genetic background of the patient precludes a correct supply of 5-MTHF, the active compound. A physiological dose of 5-MTHF (800 μg) bypasses the MTHFR block and is suggested to be an effective treatment for these couples. Moreover, it avoids potential adverse effects of the UMFA syndrome, which is suspected of causing immune dysfunction and other adverse pathological effects such as cancer (especially colorectal and prostate).

**Keywords** MTHFR · Gametes · Embryos · Miscarriages · Folic acid · 5-MTHF · UMFA

## Introduction

Methylation is a fundamental process in cell physiology. Methyl groups are added covalently to lipids, proteins, and DNA. Methylation is mandatory to all the parameters of cell physiology: DNA repair, neurotransmitter functions, and membrane transport. In the reproductive field, DNA and histone methylation are involved both in epigenesis and imprinting and also in gene and chromosome inactivation. Most of the DNA methyl tags are erased during early embryo development and then restored during prenatal life in males, and during post-natal follicle development in females. The DNA methyl transferases (DMTs) re-establish the methyl marks during oogenesis and spermatogenesis. The universal "fuel" necessary for methylation is SAM (S adenosyl Homocysteine). Once the target molecule has been methylated, SAH (S adenosyl homocysteine) and then homocysteine (Hcy) are formed. Hcy is a toxic compound: it can damage the vessels, induce inflammation, and affect liver detoxification.

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Moreover, it inhibits the methylation process [1] via negative feedback. It has to be recycled to methionine via the one carbon cycle (1-CC). The efficacy of the 1-CC relies strongly on the folic acid cycle (Fig. 1) and especially on 5MTHF as methyl donor. To be metabolically active, the synthetic folic acid must be transformed first in THF and then 5 MTHF (Fig. 1): MTHFR is a key "operator" in this aspect. Also it regulates the balance between folates for nucleotide synthesis and those for methionine synthesis. Support of the 1-CC has demonstrated to be efficient for both male and female gametes [2–5]. MTHFR isoforms are well-known genetic variants impairing the efficacy to form 5-MTHF and eventually the methylation process. The gene for MTHFR enzyme is located on chromosome 1 and although 35 different mutations have been identified, the most common are the C677T and A1298C. They are also the most deleterious as the capacity to generate 5-MTHF can decrease from 17 to 75% with these mutations [6, 7]; compound heterozygous mutation can also affect substantially the gene activity. There is now strong evidence indicating that MTHFR isoforms, especially T677T are detrimental for fertility in women [8] and men [9, 10]; men carrying the MTHFR single nucleotide polymorphism (SNP) increase the risks of repeat miscarriages (RM) in couples [11]. T677T isoform severely alters pre-implantation development, strongly inducing chromosomal abnormalities [12]. For patients carrying the mutations, excess of folic acid intake can lead to the so called UMFA syndrome which may have highly deleterious consequences [13, 14]. It may cause immune dysfunction and consequently a flare up of an active tumoral process. UMFA generated by high doses of synthetic folic acid may affect the binding of the methylfolate, brought by food, to the folate receptors and transporters and then lead to a pseudo MTHFR deficiency [14]; 5-MTHF supports plasma folate more actively than folic acid in the general population and in MTHFR SNP carriers [15]. A trial treatment with 5-MTHF was initiated in couples having repeat fetal losses, premature ovarian failure or a long history of infertility, for at least 4 months before allowing them to attempt natural conception or starting a new ART treatment.

## Material and methods

Following the work on the impact of MTHFR C677T and A1298C isoforms on male [16] and female gametes [2], it was decided during fall 2016 to study patients with normal caryotypes that had a long history (at least 4 years) of recurrent miscarriages or infertility.

The female partner had a complete gynecological screening including hysteroscopy and ultrasonography. To avoid any side effects linked to genital tract (anatomical, infectious) or ovarian pathologies, FSH was tested on D3 of the cycle and had to be below 9 IU/mL. Antiphospholipids and nuclear antigens were also controlled. All the men were tested for DNA fragmentation (SDF) and nucleus decondensation using a acridine orange flow cytometry ($SCSA^R$); all of them were below the critical threshold of 25% for the 2 parameters.

**Genetic testing** The presence of the MTHFR isoforms was determined using a venous blood sample; DNA analysis being performed by real-time PCR amplification followed by restriction analysis. The diagnostic sensitivity is > 99% for both isoforms.

In all couples where at least one of the members was homozygote (HMZ) or when the 2 members were heterozygote (HTZ), treatment with 5-MTHF was initiated and maintained for at least 3 months before moving to an ART treatment if indicated (see Table 1). The mean age of the women entering the program was 34 years (SD 4.7). Two of the couples had, prior to our consultation, been counseled for oocyte donation.

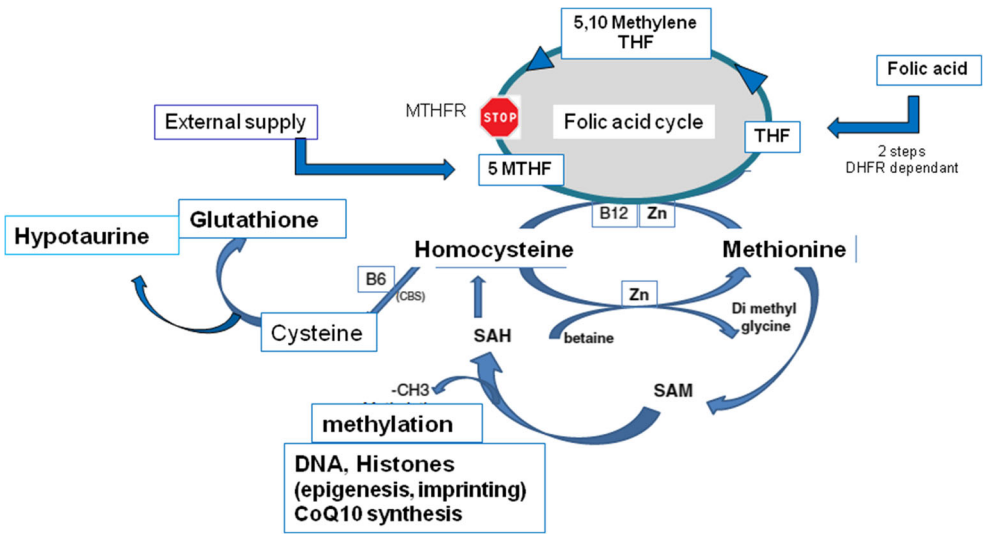


**Fig. 1** The folate cycle and the one carbon cycle. *5MTHF* 5 methyltetrahydrofolate, *MTHFR* methylenetetrahydrofotate reductase, *DHFR* dihydrofolate reductase, *THF* tetrahydrofolate, *SAM* S adenosyl methionine, *SAH* S adenosyl homocysteine

**Table 1** Description of the patients in the program, mean age: 33.6 years (4.9). *SPr* spontaneous pregnancy, *misc.* miscarriage, *Hcy* homocysteine

| Case (women age) | Genetic background | | Infertility/failures | TT duration (months) | Result |
|---|---|---|---|---|---|
| | Women | Man | | | |
| AS/EJS (31) | HMZ 1298CC | WT | 4 misc | 4 | SPr: normal delivery (male) |
| BE/EJS (29) | HMZ 677TT | HMZ 1298CC | Unexplained | 4 | SPr: pregnant 28 weeks |
| RK/EJS (31) | HMZ 677TT | WT | 4 years, 4 failed IUI | 4 | SPr: pregnant 21 weeks |
| BL/EJS (34)* | HMZ 677TT | WT | 2 misc./POF | 4 | SPr: pregnant 18 weeks |
| KD/EJS (28) | HMZ 1298CC | WT | Failed ovarian Stim | 4 | Ovarian stimulation: Pregnant 30 weeks |
| JP/LF (38) | HTZ677CT | HTZ677CT | 8 misc. (4IUI), 1 IVF | 4 | SPr: delivery (female) |
| ND/LF (37) | HTZ677CT | HTZ677CT | 6 misc | 4 | Pr post ovarian stimulation: delivery (female) |
| Ma/ LF (32) | HTZ677CT | HTZ677CT | 3 misc | 4 | SPr: delivery (female) |
| Va/LF* (37) | HTZ677CT | HTZ677CT | 2 misc., 1 ectopic | 4 | SPr: 30 weeks |
| HS/EJS (26) | HTZ677CT | HTZ677CT | 3 misc | 3 | SPr: 32 weeks |
| Ca/LF (40) | HMZ677TT | HTZ677CT | 6 misc | 4 | Twin Pregnancy post ovarian Stimulation: 20 weeks |
| Bou/LF (33) | HTZ677CT | HMZ677TT | 3 misc | 6 | One failed ovarian stimulation, then SPr: 14 weeks |
| BBM/DC (36) | HMZ677TT | HTZ677CT | 4 misc | 4 | Two failed IUI |
| DC/DC (38) | HMZ677TT | HTZ677CT | 2 failed IVF | 7 | Pregnant post IVF, 6 mths |
| Ib/MC (38) | HTZ677CT | HTZ677CT | 2 failed IVFs | 4 | Failed 3rd IVF |
| La/MC (33) | HTZ677CT | HTZ677CT | 2 failed IVFs | 4 | Failed 3rd IVF |
| Du/MC (42) | HMZ677TT | HTZ677CT | 2 failed IVFs | 4 | IUI: Twin pregnancy 6 months |
| Go/MC (28) | HTZ677CT | HTZ677CT | 5 failed IUI | 4 | Pr post IVF, 6.5 months |
| Ha/MC (38) | HTZ677CT | HTZ677CT | 2 misc | 4 | SPr: miscarriage |
| Be/MC (39) | HMZ677TT | HTZ677CT | Unexplained Primary | 4 | Spr: delivery (male) |
| Be/MC (39) | HMZ677TT | HMZ677TT | Unexplained | 4 | Pr post IVF: delivery female baby |
| Ba/MC (31) | HMZ677TT | HTZ677CT | PCOS, high Hcy | 5 | Pregnancy post IVF: delivery (female) |
| Bi/MC (26) | HMZ677TT | HMZ677TT | PCOS, endometriosis | 6 | Pr post IVF: delivery female baby |
| Sa/MC (37) | HMZ677TT | HTZ677CT | Unexplained primary | 4 | Pr post IVF: delivery female baby |
| Vi/MC (34) | HMZ677TT | HMZ677TT | Unexplained primary | 4 | Pr post IVF: delivery female baby |
| Yi/MC (26) | HMZ677TT | HTZ677CT | Unexplained primary | 6 | 3 failed IUI, Pr post IVF, delivery female baby |
| GSt/MC (35) | HMZ677TT | HMZ677TT | 8 misc | 4 | Spr 6.5 months |
| VM/EJS (35) | 677CT/1298AC | HTZ1298AC | Biochemical Pr | 4 | Spr: 10 weeks |
| AyM/MC (23) | HTZ677CT | HTZ677CT | 3 misc | 4 | Pr 6 months post IVF |
| MS/MC (32) | HMZ677TT | HTZ677CT | 7 misc | 5 | SPr 10 weeks |

*HMZ* homozygote, *HTZ* heterozygote, *WT* wild type (no mutation), *POF* premature ovarian failure

*Patients counseled for oocyte donation before entering the program

The daily dose for 5-MTHF was 800 micrograms/day, according to the folic acid requirements in healthy women [17]. Only the WT (wild type) members of the couples were not treated. Protected intercourse was recommended for the first 3 months, in order to achieve full therapeutic effect. After 3 months, the patients were allowed to attempt spontaneous conception for 2 months, without discontinuing the treatment before resuming ART when indicated. The women were advised to continue the treatment during pregnancy and subsequent breast feeding. Men were allowed to discontinue 5-MTHF when pregnancy was confirmed. B vitamin complex and chelated zinc were added to all the treatments (Impry®, Parthenogen, Switzerland or Tretrafolic,® Nurilia, France).

## Results (Table 1)

In our patient population for 2016 requesting infertility treatment, we observed the following C677T distribution for women: WT 38%, HTZ: 45%, HMZ:17%, which although not necessarily representative of the general population, is very close to what is generally observed in Europe [12, 18]. In the 30 patients treated here, 18 women were HMZ and one a compound heterozygote. Six men were found to be HMZ as

well. Thirteen spontaneous pregnancies were observed at the end of the treatment, and another one after a failed ART procedure (total 14/31: 45%). One woman had a miscarriage. Thirteen pregnancies were obtained after ART treatment (42%). For three (10%) of the couples the treatment was unsuccessful. So far a total of 11 deliveries (3 boys, 8 girls) have been recorded. In the group of 14 patients having undergone 61 miscarriages (4.36 per patients), we recorded 4 term deliveries and 8 ongoing pregnancies (> 3 months) the overal ongoing pregnancy rate is 86.7%. One couple was lost in the follow-up. Two couples are currently under treatment. Only one woman (677TT) but 3 men 677TT had a homocysteine level over 15 μmol/Liter. Twenty-five patients are initiating a new program.

## Discussion and conclusions

Our selected population shows a strong link between an impaired folate cycle, and consequently altered 1-CC, and the capacity to achieve conception and carry a pregnancy to term. Treatment with 5-MTHFF of the repeat miscarriages (RM) population carrying the MTHFR SNPs C677T and A1298C improved the chances of pregnancy to term. It also avoids an increase in the permanent risks related to UMFA. In a first approach, it is now clear that the MTHFR isoforms deregulate gametogenesis and embryogenesis and are manifest in the first pre-implantation embryo stages [12]. The chromosomal anomalies observed can be directly ascribed to them. Maternal DNA methylation also regulates early trophoblast development [18] and miscarriages may also be attributed to them. Zygotic genomic imprinting, i.e., methylation, will affect further placental development [19] In the male, the negative impact may be explained by the effect of DNA methylation on trophoblastic function in general [20]. A defective methylation process will affect totally trophoblast development and its physiological capacity to sustain implantation, and subsequently placental development and fetal growth [21, 22]. It seems obvious to test both partners in women affected with recurrent miscarriages, as the paternal genome, altered by SNPs, plays also a significant role in the RMs [11]. Since 5-MTHF increases the amount of "efficient" plasma folate more effectively than folic acid [15], external supplies are a rationale way to avoid the MTHFR-related early problems of conception and miscarriages. On the contrary, excess of synthetic folic acid intake will lead to the UMFA syndrome in this peculiar population; UMFA syndrome increase the cancer risks (colorectal, prostate still equivocal for the breast cancer) [23]. It has a disappointing low efficacy in reducing circulating homocysteine. In a cohort of patients with elevated Hcy and suffering cardiac pathologies, folic acid has been shown to be detrimental when compared to the placebo [24]. UMFA related or not to fortification is really a matter of concern [23, 25]. It has a perverse effect as it blocks the entry of folic acid in the folic acid cycle via an inhibition of DHFR (dihydrofolate reductase, see Fig. 1), reducing the weak remaining metabolic capacity of MTHFR in these patients. UMFA may also induce a pseudo MTHFR syndrome via a mechanism of substrate inhibition, inducing a reversal of the cycle and a resulting increase in Hcy [14]. Moreover the circulating UMFA competes for the binding and the transport of 5 MTHF in the cells, aggravating the shortage. In any case, patients with RMs need to be tested for the two major MTHFR isoforms. A high level of circulating homocysteine is not systematically linked to the MTHFR isoforms in a homozygous state; its determination is not necessary but may be useful. Testing for MTHFR isoforms should be mandatory in all oocyte donation programs not only for the donors but also for the male partner. Endocrine disruptors exert a negative pressure on the regulation on imprinting/epigenetics/methylation [2]. Pre-implantation genetic screening (PGS) has been commonly proposed as a technique to approach and to remedy unexplained multiple fetal losses. We show here that evaluating the presence of MTHFR mutations in couples prior to IVF, let alone the adjunct technique of PGS, is fundamental. Treatment of patients with 5-MTHF may avoid unnecessary expensive and invasive ART procedures.

**Authors' contributions** Edouard J Servy, MD, FACOG, followed clinically the patients and collected the data.

Laetitia Jacquesson-Fournols, MD, Endocrinologist,, followed clinically the patients and collected the data.

Marc Cohen MD, Obst Gyn, follows clinically the patients and collected the data.

Yves J R Menezo, PhD, Dr. Sci, FRSM, Senior Clinical Embryologist (ESHRE) (*corresponding author) controlled dosages designed the protocol and wrote the paper.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest except MC who is dealing with a company saling Neutraceuticals.

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