Exploratory Research in Clinical and Social Pharmacy 10 (2023) 100277

Contents lists available at ScienceDirect

Exploratory Research in Clinical and Social Pharmacy

journal homepage: www.elsevier.com/locate/rcsop

# Variations in folate prescriptions for patients with the MTHFR genetic polymorphisms: A case series study

Carolyn Jane Ledowsky [a,*], Janet Schloss [b], Amie Steel [c]

[a] Endeavour College of Natural Health, now at University of Technology Sydney, Faculty of Health, Australia
[b] Southern Cross University, Natural Centre for Naturopathic Medicine, Lismore, NSW, Australia
[c] University of Technology Sydney, Faculty of Health, Australia

ARTICLE INFO

*Keywords:*
*MTHFR*
Polymorphism
Infertility
Folate
Folic acid
5-MTHF

ABSTRACT

*Background:* Over 48.5 million couples are reported with infertility worldwide. Health policy recommends folic acid in women of childbearing age, particularly in preconception and pregnancy which results in women purchasing over-the-counter prenatal multivitamins containing folic acid through pharmacies and other retail outlets. Emerging studies are investigating whether other forms of supplemental folate are more suitable, particularly for those with methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms. This case series aimed to document variations in forms and dosage of folate prescribed by Australian practitioners to patients with diagnosed infertility and *MTHFR* polymorphisms.
*Methods:* Australian practitioners were invited to complete a retrospective case report form for patients that presented with unexplained infertility. This case report form documented the form and dose of folate that practitioners were prescribing to their infertility patient with MTHFR polymorphisms, together with their fertility history.
*Results:* Six practitioners submitted case information for 12 patients with diagnosed infertility and *MTHFR* polymorphisms. All patients had been advised by their practitioner to remove folic acid in supplemental form and were prescribed 5-methyltetrahydrofolate (5-MTHF) or a combination of 5-MTHF and folinic acid, at higher doses than the Australian recommended dose (mean daily maximum prescribed dose: 2325μg). Eleven patients conceived within the treatment period (average treatment of one year) and ten were reported as having a live birth.
*Conclusion:* This case series has highlighted clinical practices that vary from the recommendations by Australian policy. Further research is required to verify the clinical importance of variations in folate prescriptions for women with *MTHFR* polymorphisms and how folate recommendations may need to change depending on these polymorphisms. This has direct relevance to those prescribing at the pharmacy and retail level, specifically pharmacists and pharmacy assistants.

## 1. Introduction

Infertility is defined as the failure to conceive a child after one year of unprotected intercourse[1] and is estimated to affect 48.5 million couples worldwide.[2–4] In Australia, one in six couples are expected to be diagnosed with infertility each year[5] and these couples will commonly consult initially with a General Practitioner (GP) and then be referred to fertility specialists or clinics.[6] Australian rates of couples seeking assisted reproductive technologies (ART) are on the incline – a 13.9% increase was noted in 2009 compared to 2008 levels and a 48% increase on 2005 levels – and almost all (92.4%) couples are treated through Australian fertility centres.[7,8] Only 17.2% of the 70,541 ART treatment cycles in 2009 resulted in live births. This low rate of success has emotional and psychological[9,10] as well as economic (each in vitro fertilisation (IVF) cycle costs approximately $9828 AUD per cycle[3,11]) impacts on couples. There are many identified possible causes of infertility, including recent research indicating a correlation between polymorphisms in folate-metabolising enzymes and individuals with infertility seeking ART.[12] Females who have been found to have deficiencies of folate or low plasma levels of folate have been linked to neural tube defects[13–17] and this led to Australian national health advice recommending at last 400μg of folate daily for one month before conception as a supplement or through food fortification[18]. Folic acid oral supplementation has been the traditional form of folate prescribed by Australian practitioners for preconception and pregnancy[19] and up until 2021 the

*Abbreviations:* 5-MTHF, 5-methyltetrahydrofolate; ANA, Antinuclear Antibodies; ART, Assisted Reproductive Technology; DHF, Dihydrofolate; DHFR, Dihydrofolate reductase; DMT's, DNA methyltransferase; FA, Folic Acid; FAD, Flavin adenine dinucleotide; GP, General Practitioner; HREC, Human research and ethics Committee of Endeavour College of Natural Health; ICSI, Intracytoplasmic sperm injection; IUI, Intrauterine Insemination; IVF, In vitro fertilisation; MTHFR, Methylenetetrahydrofolate reductase; NHMRC, National Health and Medical Research Council; NTD, Neural Tube Defects; RPL, Recurrent Pregnancy Loss; SAM, S-adenosylmethionine; THF, Tetrahydrofolate; UMFA, Unmetabolised folic acid.

* Corresponding author.
*E-mail address:* carolyn.j.ledowsky@student.uts.edu.au (C.J. Ledowsky).

http://dx.doi.org/10.1016/j.rcsop.2023.100277
Received 8 October 2022; Received in revised form 16 March 2023; Accepted 30 April 2023
Available online xxxx

only form of folate available in prenatal multivitamins at pharmacies and retail outlets was folic acid with the leading brand, Elevit containing 800μg of folic acid.

Fortification of foods with FA was introduced in Australia in September 2009 to reduce neural tube defects (NTD) and has, by all accounts, been successful.[20,21]

Folate-dependent pathways are influenced by various biochemical variations of folate and its bioavailability (see Fig. 1).[22] Folate is a generic term for vitamin B9 that includes several supplemental forms. All folate forms have a common structure but differ based on the whether the pteridine ring is reduced or oxidised.[23] *Folic acid* is the synthetic form of folate and requires the enzyme dihydrofolate reductase (DHFR) to be converted to its metabolically active form, 5-methyltetrahydrofolate (5-MTHF).[24] The DHFR enzyme has been found to be down-regulated by an oral intake of around 200-300μg of folic acid.[24] Other forms of folate naturally occur in a reduced form in foods such as leafy green vegetables.[25] This 'natural folate', exists in a polyglutamate form, known as *tetrahydrofolate* (THF), and needs to be further hydrolysed to a monoglutamate form by the intestinal lumen to be absorbed.[26] This intestinal hydrolysis and absorption are the responsibility of the brush border enzyme glutamate carboxypeptidase II[27] and occurs independent of the dihydrofolate reductase (DHFR) enzyme.[26,28] *Folinic acid*, a 5-formyl derivative of THF, is also converted to THF without requiring the DHFR enzyme.[28] *5-MTHF* is a reduced derivative of THF.[25] The most metabolically active form of folate found in the body is 5-MTHF (accounting for around 90% in the blood).[26,29] 5-MTHF plays a critical role in methylation as it donates its methyl group to the methionine cycle after which it is converted to THF which in turn provides moieties for the DNA bases.[27,30] This methylation process is required for synthesis of DNA, RNA, neurotransmitters, and proteins and for gene imprinting.[31–33] This conversion of folate to 5-MTHF is dependent upon the activity of the methylenetetrahydrofolate reductase enzyme (MTHFR) encoded by the *MTHFR* gene.[34,35]

The *MTHFR* gene is located on chromosome 1 and it is reported there are over 35 identified polymorphisms, with the most common of these the 'C677T' and 'A1298C' variants.[36] MTHFR is the enzyme required to activate all forms of folate to 5-MTHF. Specifically, MTHFR catalyses the conversion of 5,10 methylenetetrahydrofolate to 5-MTHF[37] which serves as the methyl donor for synthesis of homocysteine to methionine and is the precursor to S-adenosylmethionine (SAM).[38] MTHFR is a flavoprotein with a cofactor of flavin adenine dinucleotide (FAD) and reducing agent nicotinamide adenine dinucleotide phosphate NAD(*P*)H which produces the sole source of methyl folate in an irreversible reaction and therefore integral to the synthesis of SAM, the major methyl donor for numerous biosynthetic reactions.[39,40] MTHFR activity is regulated by SAM which acts as an inhibitor of the MTHFR activity.[40] DNA methylation that modulates gene expression and ensures genomic integrity is maintained by DNA methyltransferases. These DNA methyltransferases use SAM as the cofactor or donor of methyl groups. The polymorphisms in the MTHFR gene cause gene instability and reduce activity. Without an optimal functioning MTHFR enzyme, the synthesis of methyl folate is reduced and this in turn will reduce one carbon metabolism required for DNA synthesis and methylation of DNA, essential for fertility.[41]

Some individuals have variations, or polymorphisms, in the genetic code used for MTHFR production which differs from the 'wild type' or normal gene.[42,43] The *MTHFR* polymorphisms C677T and A1298C result in down regulation of enzymatic activity due to an amino acid change that influences the stability of the gene[44] and affects the conversion of 5,10-methylene tetrahydrofolate to 5-MTHF.[34,35] This is the only mechanism for intracellular generation of 5-MTHF.[40,45] A reduction in folate status due to the *MTHFR* polymorphisms can affect one-carbon metabolism[22] and this may directly affect the production of SAM which is responsible for DNA methylation.[46] The *MTHFR* C677T homozygous polymorphisms – defined as two identical copies of an allele from both parents[47]- occur in approximately 10–14% of Caucasians, 21% of Hispanics, 1–7% of Africans, and 11% of Asians.[48] These homozygous carriers have a 70% reduction in enzyme activity.[44] Heterozygous C677T frequency is approximately 20–25% in the general population[49] and reduces the activity of the enzyme by 30% whilst the homozygous A1298C carriers have a 40% reduction in enzyme activity.[44] Research has identified that *MTHFR* C677T homozygous individuals have lower DNA methylation than 'wild type' individuals.[41]

One aspect of infertility that can affect both male and female has been linked to a lack of DNA methylation which can influence key functions associated with the first phases of spermatogenesis[50] and in the growing oocytes with the transition from primary to secondary follicles.[51]

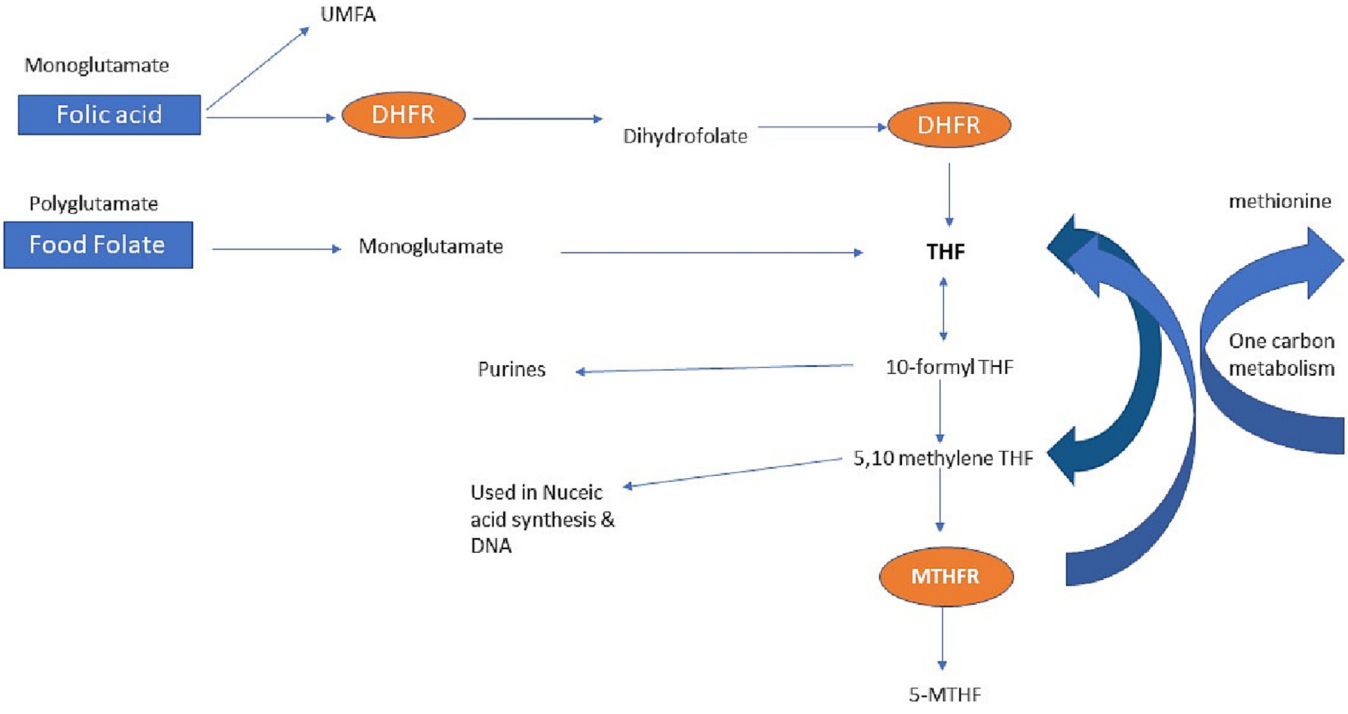


**Fig. 1.** Folate Metabolism: UMFA-Unmetabolised folic acid, DHFR-Dihydrofolate reductase, THF-Tetrahydrofolate, MTHFR-Methylenetetrahydrofolate Reductase, 5-MTHF-5-methyltetrahydrofolate.

One carbon metabolism is not only important systemically but also directly important for the ovary[30] and DNA methylation of imprinted genes. This plays important roles in embryo development and growth. A murine study showed that a deficiency in one carbon metabolism impairs both antral follicular development and oocyte maturation and has led to speculation that the cytoplasm of the oocyte may be an important reserve of one carbon metabolites.[30] Steegers-Theunissen et al. concluded that folate deficiency in women can impair oocyte quality, reduce the success of IVF/Intracytoplasmic sperm injection (ICSI) procedures, and is associated with reproductive failure.[30] Folate is essential to both folliculogenesis[52] and spermatogenesis[53] as it results in an uracil mis incorporation into DNA and may lead to disruption in DNA synthesis, DNA repair and chromosomal errors.[54] In female infertility, the effect of the *MTHFR* C677T polymorphism has been associated with hyperhomocysteinemia,[55] low folate[45] and reduced oocytes quantity.[56] In males, the effect of *MTHFR* polymorphisms is associated with spermatogenesis issues such as oligozoospermia and azoospermia.[34,57] The MTHFR genetic polymorphisms have been shown to impair both folliculogenesis and spermatogenesis and therefore the association with reduced methyl folate synthesis needs to be addressed by the prescribing practitioner.

Research has identified that people living in countries with folic acid fortification of food have an intake that exceeds the original intended dose.[29,31,58] Questions have been raised as to the implication of this excess folic acid in the blood, which is referred to as unmetabolised folic acid (UMFA).[25,28,29,59–61] As more research unfolds a question is being raised as to whether 5-MTHF is required for those people with *MTHFR* polymorphisms and infertility.[27,33,36,62,63] Studies are emerging that suggest that administration of 5-MTHF may be more effective than folic acid in improving folate status in people who have an *MTHFR* polymorphism[32] and it has also been suggested that 5-MTHF may bypass *MTHFR* polymorphisms.[36,64,65] There are only a few studies that address what dose of 5-MTHF is optimal for preconception and pregnancy in those with *MTHFR* polymorphisms. These studies quote 5-MTHF doses (e.g., 7.5 mg daily[32,33,36] well above the recommended daily dose of folate for women of childbearing age (400-500μg). Understanding the most effective form of folate to prescribe in infertility is essential for practitioners to assist in improving pregnancy outcomes and may be critical for public health policymakers going forward. So too, it is essential for pharmacists and pharmacy assistants to understand the nuance of folate prescriptions to be able to fulfill their critical role in providing support to consumers who may benefit from prenatal multivitamins.

As a result of this limited research on dose and form of folate in infertility patients, we endeavour to understand further what forms and dosage of folate, practitioners who test for *MTHFR* polymorphisms, may be prescribing to achieve improved fertility outcomes.

The pharmacy environment is such, that since the COVID pandemic the pharmacist is well placed to become the non-medical prescriber to support the current healthcare system.[66] What patients now want is a patient health centric model in pharmacies and the pharmacies that are thriving are those that offer every patient health solutions, recommendations, advice and services.[67] Research has shown that in most countries, including Australia, pharmacies supply complementary medicines including prenatal multivitamins most of the time[68] and that these complementary medicines prescribed in pregnancy and lactation are seen to offer benefits to their babies in utero or while being breastfed.[69]

## 2. Material and methods

### 2.1. Study aim

Describe the different forms and doses of folate that practitioners prescribe to individuals with infertility and a confirmed *MTHFR* polymorphism diagnosis.

### 2.2. Study design

#### 2.2.1. Case series report

An online case report form was sent to Australian practitioners via the MTHFR Support Australia Practitioner membership base. Invited practitioners were asked to submit cases for women aged 18–49 years of age where they had been given a diagnosis of infertility and had an MTHFR polymorphism. This case report form was a unique instrument designed by the research team to collect the relevant data regarding infertility history and supplement use. The objective was to achieve a minimum of five cases. Collecting data via Survey Gizmo allowed the researchers to achieve the aims and objectives of the study. The online case report form allowed collection of descriptive statistics relating to the cases nominated by the practitioners. It also allowed collection of clinical data and protocols which could then be described narratively. The incidence of MTHFR polymorphisms were recorded using measures of frequency and the collection of information relating to practitioner protocols, dosages and types of folate was documented and explored narratively.

This retrospective case series was a descriptive study which aimed to explore the pregnancy outcomes and different forms and dosage of folate prescribed in patients accessing fertility treatments who had the MTHFR genetic polymorphisms. This is in alignment with other studies that seek to form a basis on which further research may be conducted. This study design and format was carefully considered to reduce as many confounding factors as possible to address the research aims and objectives.

### 2.3. Participants

The target participants of the study were registered Australian based health professionals, with at least two years' clinical experience who identified as providing care to patients with infertility for at least one year and screened their patients for *MTHFR* genetic polymorphisms. We aimed to collect case reports from at least five practitioners to maximise diversity of treatment approach and patient populations. Recruitment of Practitioners took place via the MTHFR Support Australia Practitioner membership base which consists of approximately 1200 practitioners. This target sample of practitioners was selected as they were likely to be aware of, and regularly test for, the *MTHFR* genetic polymorphisms. All practitioner listed on the membership database were invited to complete an online screening questionnaire to verify their compliance with the inclusion criteria. This screening instrument was expected to take no more than two minutes and asked seven questions to the practitioner to answer based on the following: Practitioner details: gender, state, where they practice, type of practitioner and years in clinical practice, Information: number of fertility patients seen in the last year and whether they screen their patient for MTHFR polymorphisms. The online screening instrument included a consent form, participant information sheet and further information to ensure inclusion criteria were met. The practitioners who met the selection criteria were then invited to submit appropriate cases and complete a detailed online case report form to record the details of their cases that met the inclusion criteria.

#### 2.3.1. Selection criteria

Participants were able to be included in the study, and therefore provide cases, if they self-reported as Australian-based practitioner with at least two years' clinical experience who provided care to patients seeking assistance with fertility in the last year. They also needed to have screened their fertility patients for the *MTHFR* genetic polymorphisms, *MTHFR* C677T and A1298C. Only Australian-based practitioners were included in the study to minimise the number and quality issues of various supplements that may be prescribed by the practitioners in addition to assays used for identification. A screening instrument administered via an online survey platform was employed to screen Practitioners to ensure they met the inclusion criteria prior to receiving access to the case report form.

### 2.4. Data collection

#### 2.4.1. Case study selection criteria

Participants were asked to provide information from historical cases of female patients aged 20–49 years, who had been diagnosed with infertility and had an identified *MTHFR* genetic polymorphisms C677T and/or A1298C. A diagnosis of infertility was defined as unsuccessfully attempting to conceive for at least the last 12 months but not longer than 2 years and where pregnancy and/or birth outcomes were reported.

#### 2.4.2. Data collection instrument

Data were collected via an online case report form that was shared with participants via Qualtrics online survey platform. The case report form for the initial consultation consisted of 27 questions across four domains: (1) *Patient information;* (2) *Fertility history and fertility status of patient and partner;* (3) *MTHFR polymorphism*; and (4) *Prescriptions.* For each consecutive appointment the practitioner was required to respond to a further seven items across another three domains: (1) *Supplement's prescription;* (2) *Dietary prescription;* (3) *Pregnancy and/or birth outcome.* The full case report form is attached as Supplemental File 1.

#### 2.4.3. Data cleaning and analysis

Data obtained from the electronic case report forms (CRFs) was analysed using descriptive statistics (STATA 16 software).[70] The data was extracted into an excel document, cleaned, and coded from the CRF and tabulated in order to summarise the results and report descriptive data. A pseudonym was given to each case to allow easier reporting and description of findings. As the case report study design was not intended to infer associations, inferential statistical analysis was not employed.

## 3. Results

Six practitioners consented to be involved in the study and completed the screening instrument, all of whom met the eligibility criteria. The practitioners all identified as female ($n$ = 6,) and worked in clinical practice in NSW ($n$ = 5) and ACT ($n$ = 1). Four of the practitioners self-identified as naturopaths while the remaining two identified as a nutritionist and an acupuncturist, respectively. All practitioners reported being in clinical practice for greater than 20 years. Three of the practitioners indicated they 'always' investigate *MTHFR* polymorphisms in fertility patients, while the remainder said they screen for *MTHFR* polymorphisms 'when indicated'. The six practitioners submitted a combined total of 12 cases. One case was excluded from the analysis as the practitioner did not report the patient's *MTHFR* genotype. This patient had been diagnosed with infertility by her GP. She had one ovulation induction and IVF which did not result in an egg harvest. The cases submitted by each practitioner is presented in Table 1.

## 4. Case summaries

A cumulative summary of the patient characteristics is presented in Table 2. Patients' age ranged from 26 to more than 46 years old, with the majority of cases between 31 and 40 years of age ($n$ = 8). Of the twelve cases, 83.3% of these women presented with a history of miscarriage ($n$ = 10), 41.7% experienced failed IVF or ICSI ($n$ = 5), 41.7% experienced fertility related issues (n = 5), 33.3% with unexplained infertility ($n$ = 4).

**Table 1**
Cases submitted by Practitioner.

| Practitioner 1 | Case 1 & 2 |
|---|---|
| Practitioner 2 | Case 3 & 7 |
| Practitioner 3 | Case 4 & 6 |
| Practitioner 4 | Case 5 |
| Practitioner 5 | Case 8, 10, 11 & 12 |
| Practitioner 6 | Case 9 |

**Table 2**
Participant characteristics.

| Characteristic | N | % |
|---|---|---|
| Gender ($n$ = 12) | | |
| *Female* | 12 | 100 |
| *Male* | 0 | 0 |
| *Non-binary* | 0 | 0 |
| Age ($n$ = 12) | | |
| *20–25 years* | 0 | 0 |
| *26–30 years* | 1 | 8.33 |
| *31–35 years* | 5 | 41.67 |
| *36–40 years* | 3 | 25.00 |
| *41–45 years* | 2 | 16.67 |
| *46 years and over* | 1 | 8.33 |
| History of miscarriage ($n$ = 12) | | |
| *No* | 2 | 16.67 |
| *Yes* | 10 | 83.33 |
| MTHFR polymorphism ($n$ = 12) | | |
| *MTHFR C677T heterozygous* | 5 | 41.67 |
| *MTHFR C677T homozygous* | 1 | 8.33 |
| *MTHFR A1298C heterozygous* | 2 | 16.67 |
| *MTHFR A1298C homozygous* | 0 | 0 |
| *MTHFR Compound heterozygous* | 4 | 33.33 |
| Presenting symptoms | | |
| *Previous miscarriage* | 10 | 83.33 |
| *Unexplained infertility* | 4 | 33.33 |
| *Endometriosis* | 2 | 16.67 |
| *Failed IVF or ICSI* | 5 | 41.67 |
| *Partner related issues* | 2 | 16.67 |
| Other – including same sex partner, failed IUI, low ovarian reserve, | *5* | *41.67* |

Patients presented most commonly with an *MTHFR* C677T heterozygous polymorphism (n = 5, 41.67%) followed by *MTHFR* compound heterozygous (C677T heterozygous, A1298C heterozygous) (n = 4, 33.3%), *MTHFR* A1298A heterozygous ($n$ = 2, 16.67%) and *MTHFR* C677T homozygous ($n$ = 1, 8.33%). The approximate time between appointments was between one and two months. A descriptive summary of details for each of the included cases is presented below. While the data for all cases was provided to the research team in a de-identified form, all cases have been allocated a pseudonym for ease of presentation.

### 4.1. Case 1 – Karen– MTHFR-A1298C heterozygous

Karen (36–40 years) presented with specialist-diagnosed infertility. She had a previous miscarriage, endometriosis, low ovarian reserve and adenomyosis. Her partners sperm had been assessed by another health professional and classified as 'normal'. She had three previous IVF attempts of which one resulted in an egg harvest. One egg was retrieved and progressed to embryo and transfer. This did not result in a pregnancy. On first presentation to the practitioner, Karen reported using an oral MTHF-based prenatal supplement and additional MTHF oral supplement. Her naturopath then prescribed phosphatidylcholine, magnesium and adenosylcobalamin and recommended Karen avoid all sources of FA fortified foods.

### 4.2. Case 2 – Carol – MTHFR C677T homozygous

Carol (26–30 years of age) presented with specialist-diagnosed infertility and a history of more than five previous miscarriages. Carol was taking 2.5 mg of methyl folate on presentation to the clinic. Her partner's sperm was assessed and considered normal. Her naturopath prescribed a methyl folate and folinic based prenatal multivitamin.

### 4.3. Case 3 – Erica – MTHFR A1298C heterozygous

Erica (31–35 years) presented with a history of two previous miscarriages. Erica reported three previous rounds of IUI, two embryo transfers and a pregnancy loss at 6 weeks. Her partner's sperm was assessed and considered to be normal. The patient had a history of low serum iron and essential thrombocytosis. Erica was given a supplement containing 500μg of

methyl folate. The patient was advised to be mindful of FA fortified foods and avoid them where possible.

### 4.4. Case 4 – Susie – MTHFR C677T heterozygous

Susie (41–45 years) presented with specialist-diagnosed infertility. She has no family history of fertility issues and presented with three previous pregnancies: one live birth and two miscarriages. Over the previous nine years she had completed more than five IVF and ICSI procedures, with 6–10 eggs retrieved, resulting in one pregnancy and live birth. Susie had low serum vitamin B12, low serum folate and a positive ANA on blood tests. Her practitioner prescribed a methylated prenatal multivitamin, additional methyl folate to what was in the prenatal multivitamin, herbal medicines, phosphatidylcholine among others. Susie was advised to go on a gluten free and whole foods balanced diet and be mindful of FA fortified foods and avoid where possible.

### 4.5. Case 5 – Sonia – MTHFR Compound heterozygous

Sonia, (aged 31–35 years), presented with specialist diagnosed infertility. She had three previous pregnancies, with one live birth, previous miscarriages and a 12-week growth restriction trisomy miscarriage. She was on a homocysteine lowering formula when she presented to her practitioner. Her practitioner prescribed herbs, homeopathics and a methylated prenatal formula. She was advised to follow a gluten free, dairy free, unprocessed, whole foods diet and to avoid FA fortified foods where possible.

### 4.6. Case 6 – Senna- MTHFR C677T heterozygous

Senna (aged 36–40 years) presented with specialist diagnosed infertility. She presented to her practitioner with three recent miscarriages with one previous successful birth. She had low serum B12, low plasma zinc, low vitamin D and low protein. She was prescribed supplements including a methylated prenatal multivitamin, additional supplemental methyl folate to her prenatal multivitamin, herbal medicines for fertility. She was advised to follow a gluten free, whole foods balanced diet.

### 4.7. Case 7 – Emily- MTHFR C677T heterozygous

Emily (aged 36–40 years) had been recently diagnosed by her GP with infertility. History of two previous IVF treatments, one IVF attempt did not result in an egg harvest, the other IVF attempt resulted in an egg harvest of 1–5 eggs. Of these, two fertilized to embryo's that resulted in a pregnancy, but she miscarried. Emily was taking a B Vitamin containing 500μg of methyl folate.

### 4.8. Case 8 – Sheena – MTHFR Compound heterozygous

Sheena (aged 41–45 years) presented with GP diagnosed infertility. She presented with unexplained infertility, endometriosis, and failed IVF. Sheena had 4 IVF attempts, two resulting in an egg harvest with 11–15 eggs retrieved and five or more progressing to embryo stage. No transfers resulted in a pregnancy. On presenting to her practitioner, she had low serum vitamin B12, low active B12, low folate, and low iodine. Her practitioner prescribed a methylated folate and folinic acid based prenatal multivitamin, vitamin B12, zinc, phosphatidylcholine, and glutathione. Sheena was advised to follow a gluten free, dairy free, unprocessed whole foods diet and to avoid all sources of FA fortified foods.

### 4.9. Case 9 – Mary – MTHFR C677T heterozygous

Mary (aged 31–35 years) presented with GP diagnosed infertility. Mary had two previous miscarriages and has had one live birth. Her partner had a sperm analysis conducted and all markers were considered to be within range. Her practitioner prescribed herbs and advised the patient to follow a dairy free, unprocessed whole foods diet and be mindful of FA fortified foods and avoid where possible.

### 4.10. Case 10- Cassandra – MTHFR Compound heterozygous

Cassandra (an over 46-year female of Mediterranean background) presented to her practitioner with GP diagnosed infertility. She had 10 previous IVF procedures with none resulting in a pregnancy or live birth. Cassandra was on 5 mg of FA, had low zinc, low iodine, and high mercury. She was prescribed fertility-based herbs, supplements, including a methylated prenatal multivitamin and told to stop the FA-based supplement. She was asked to follow a whole food, balanced diet, avoid high mercury foods like tuna and avoid all sources of FA fortified foods.

### 4.11. Case 11- Donna – MTHFR C677T heterozygous

Donna (aged 31–35 years) presented to her practitioner with three previous pregnancies, two of which resulted in a live birth. She was prescribed a methylated prenatal multivitamin, zinc, magnesium, and phosphatidylcholine and advised to avoid all sources of FA fortified foods.

### 4.12. Case 12 – Rebecca – MTHFR Compound heterozygous

Rebecca (aged 31–35 years) presented to her practitioner with specialist-diagnosed infertility. She had previously been prescribed 5 mg of FA. Her partner had a sperm analysis conducted and all markers were considered to be within range. She had one previous pregnancy, with the aid of IVF that resulted in a miscarriage at 12 weeks. The IVF attempt resulted in one egg harvest of 6–10 eggs of which five fertilized to embryo status. All were transferred. The patient had low serum levels of vitamin B12, folate and vitamin D. Rebecca was prescribed nutritional supplements, a methylated prenatal multivitamin and advised to stop her FA-based supplement. She was put on a gluten free diet and told to avoid all sources of FA fortified foods.

## 5. Folate prescription and use

Six patients were taking folate-containing supplements at their initial appointment. Two of the four patients with a compound heterozygous polymorphism (Case 10 & 12) were taking high dose FA (5000 μg or greater), and their practitioner changed the form of supplemental folate to either 5-MTHF or folinic acid. See Table 3 for dosages and forms that patients were taking prior to their initial consultation. The other two patients with compound heterozygous polymorphism (Case 5 & 8)) and the patient who had a homozygous *MTHFR* C677T polymorphism (Case 2) were taking 5-MTHF prior to their initial consultation. Nine patients were prescribed a 5-MTHF product by their practitioner following the first consultation. In relation to dietary advice, 75% of patients ($n = 9$) were given advice in relation to FA-fortified foods; five patients were advised to avoid all sources of FA fortified foods while four were advised to 'be mindful' of FA fortified foods.

In this case series, the practitioners enrolled did not prescribe FA to any patients throughout treatment whereas, they all prescribed 5-MTHF. The mean maximum amount of 5-MTHF prescribed across the twelve cases was 2325μg per day. The minimum dose prescribed was 500μg and the maximum dose prescribed was 3800μg. Five patients were prescribed a combination of 5-MTHF and folinic acid and the average amount of folinic acid prescribed was 670 micrograms. The minimum dose of folinic acid was 450μg while the maximum dose was 800μg.

Patients with a A1298C heterozygous polymorphism were prescribed between 500μg and 1400μg of 5-MTHF. Individuals with a C677T heterozygous polymorphism were prescribed between 500μg to 3800μg of 5-MTHF. The mean daily amount of 5-MTHF prescribed to heterozygous C677T patients was 2850μg per day. The only patient with a homozygous C677T polymorphism was prescribed 3500μg of 5-MTHF and 700μg of folinic acid. There were 4 patients with compound heterozygous polymorphisms

**Table 3**
Forms of folate in micrograms per day prescribed by appointment and MTHFR polymorphism.

| | | | | | Total folate (mcg) amount the patient was taking at the conclusion of the appointment i.e.: accumulative amount if the practitioner increased the dose. n/a – there was no appointment follow up recorded by the practitioner | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MTHFR polymorphism | Case | Form of folate* | Baseline | | V1 First follow up | V2 Second follow up | V3 Third follow up | V4 Fourth follow up | V5 Fifth follow up | V6 Sixth follow up | V7 Seventh follow up | Maximum Total Folate per day over treatment period | Pregnancy outcome |
| | | | Folate on presentation | Initial Prescription (Initial appointment) | | | | | | | | | |
| MTHFR A1298C Heterozygous | Karen- Case 1 | Folic acid | – | – | – | n/a | n/a | n/a | n/a | n/a | n/a | | Pregnancy via IVF confirmed at visit 1. Live birth |
| | | Folinic acid | 300 | 300 | 600 | n/a | n/a | n/a | n/a | n/a | n/a | 600 | |
| | | MTHF | 700 | 700 | 1400 | n/a | n/a | n/a | n/a | n/a | n/a | 1400 | |
| | | FA FF | | Avoid all | Avoid all | n/a | n/a | n/a | n/a | n/a | n/a | | |
| | Erica- Case 3 | Folic acid | – | – | – | n/a | n/a | n/a | n/a | n/a | n/a | | Pregnancy at visit 1. Live birth |
| | | Folinic acid | – | – | – | n/a | n/a | n/a | n/a | n/a | n/a | | |
| | | MTHF | 500 | 500 | 500 | n/a | n/a | n/a | n/a | n/a | n/a | 500 | |
| | | Dietary folate | | Be Mindful of | | | | | | | | | |
| MTHFR C677T Heterozygous | Susie- Case 4 | Folic acid | – | | – | n/a | n/a | n/a | n/a | n/a | n/a | | Successful pregnancy at appt 1. Live birth |
| | | Folinic acid | – | | – | n/a | n/a | n/a | n/a | n/a | n/a | | |
| | | MTHF | 1500 | 3500 | 3500 | n/a | n/a | n/a | n/a | n/a | n/a | 3500 | |
| | | Dietary folate | | Be mindful of | | | | | | | | | |
| | Senna – Case 6 | Folic acid | – | | – | – | n/a | n/a | n/a | n/a | n/a | | Pregnancy after appt 1 and live birth |
| | | Folinic acid | | 300 | 450 | 450 | n/a | n/a | n/a | n/a | n/a | 450 | |
| | | MTHF | | 1700 | 2300 | 3800 | n/a | n/a | n/a | n/a | n/a | 3800 | |
| | | Dietary folate | | None | | | | | | | | | |
| | Emily – Case 7 | Folic acid | – | | – | – | – | – | – | – | – | | Pregnant at Initial, miscarried after visit 1, fell pregnant again appt 7. Miscarried v8. |
| | | Folinic acid | – | | – | – | – | – | – | – | – | | |
| | | MTHF | | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | |
| | | Dietary folate | | None | | | | | | | | | |
| | Mary – Case 9 | Folic Acid | – | – | – | – | – | – | n/a | n/a | n/a | | Pregnant at V4 (12 weeks). Live birth |
| | | Folinic Acid | – | – | – | 400 | 800 | 800 | n/a | n/a | n/a | 800 | |
| | | MTHF | – | – | 800 | 1200 | 1600 | 1600 | n/a | n/a | n/a | 1600 | |
| | | Dietary Folate | | Be Mindful of | | | | | n/a | n/a | n/a | | |
| | Donna- Case 11 | Folic Acid | – | – | – | – | n/a | n/a | n/a | n/a | n/a | | Pregnant at visit 1. Live birth |
| | | Folinic Acid | – | – | – | – | n/a | n/a | n/a | n/a | n/a | | |
| | | MTHF | | 500 | 2500 | 2500 | n/a | n/a | n/a | n/a | n/a | 2500 | |
| | | Dietary Folate | | Avoid All | | | | | | | | | |
| MTHFR C677T Homozygous | Carol – Case 2 | Folic acid | – | | – | – | – | – | n/a | n/a | n/a | | Pregnant at visit 4. 27 weeks at visit 4. Live birth |
| | | Folinic acid | | 300 | 300 | 300 | 300 | 500 | n/a | n/a | n/a | 700 | |
| | | MTHF | 2500 | 2700 | 2700 | 2700 | 2700 | 3500 | Na/ | n/a | n/a | 3500 | |
| | | Dietary folate | | Avoid All | | | | | | | | | |
| Compound Heterozygous | Sonia- Case 5 | Folic acid | – | – | – | n/a | n/a | n/a | n/a | n/a | n/a | | Pregnant at visit 1 and live birth |
| | | Folinic acid | – | – | – | n/a | n/a | n/a | n/a | n/a | n/a | | |
| | | MTHF | 1200 | 2000 | 3800 | n/a | n/a | n/a | n/a | n/a | n/a | 3800 | |
| | | Dietary folate | | Be Mindful of | | | | | | | | | |
| | Sheena – Case 8 | Folic acid | – | – | – | – | – | n/a | n/a | n/a | n/a | | Did not fall pregnant |
| | | Folinic acid | | 400 | 800 | 800 | 800 | n/a | n/a | n/a | n/a | 800 | |
| | | MTHF | 1000 | 1400 | 1800 | 1800 | 1800 | n/a | n/a | n/a | n/a | 1800 | |
| | | Dietary folate | | Avoid All | | | | | | | | | |
| | Cassandra- Case 10 | Folic acid | 5500 | – | – | – | – | – | – | – | n/a | | Pregnancy at visit 5. Twins born 23/7/20 via IVF |
| | | Folinic acid | – | – | – | – | – | – | – | – | n/a | | |
| | | MTHF | – | 500 | 3500 | 5500 | 2500 | 2500 | 2500 | 2500 | n/a | 2500–5500 | |
| | | Dietary folate | Avoid All | | | | | | | | | | |
| | Rebecca- Case 12 | Folic acid | 5000 | – | – | – | – | – | – | n/a | n/a | – | Pregnancy at visit 2. Successful live birth via IVF |
| | | Folinic acid | | 400 | 400 | 400 | – | – | – | n/a | n/a | – | |
| | | MTHF | | | 400 | 1400 | 1500 | 1500 | 1500 | n/a | n/a | 1500 | |
| | | Dietary folate | | Avoid all | | | | | | | | | |

who were prescribed amounts of 5-MTHF ranging from 1500μg to 3800μg with the mean amount of 2400μg 5-MTHF per day prescribed to this group.

## 6. Pregnancy and birth outcomes

The practitioners reported that 11 patients conceived during the course of the treatment, with one patient reporting a subsequent pregnancy loss. Three pregnancies were achieved via IVF. The practitioners reported nine live births and one ongoing pregnancy at the time of data collection, with all patients who became pregnant conceived within two and four months upon commencement of non-folic acid forms of folate. Both patients with the A1298C heterozygous polymorphism conceived and had a live birth. Five of the six patients with heterozygous C677T polymorphism became pregnant and had a live birth. The one patient with homozygous C677T

polymorphism successfully conceived and had a live birth. Three of the four patients with compound heterozygous polymorphism became pregnant and had live births. The fourth patient had no history of pregnancy prior to her initial appointment, nor did she fall pregnant during the treatment time. She had been prescribed 1800μg of 5-MTHF and 800μg of folinic acid. Another patient became pregnant twice in the treatment time and miscarried both times. This patient had been prescribed the lowest dose of 5 MTHF (500μg).

Adherence and tolerability of the 5 MTHF was good with only one patient reporting an increase in anxiety with the higher dose. She was subsequently told to reduce her dose, which she did and had no further issues. One patient miscarried during the treatment period and there were no other adverse events.

The patients did not share their perspective on the treatment as this was a retrospective case study and the researchers had no contact with the patients.

## 7. Discussion

This study provides some novel insights into clinical management of infertile patients with *MTHFR* polymorphisms that may warrant closer investigation. The findings from this study suggest that these practitioners are advising their patients to actively avoid dietary intakes of FA-fortified food. This approach to clinical care may be because the folate naturally occurring in foods such as leafy green vegetables are more readily converted to the metabolically-active 5-MTHF[29] and differs from FA, as the synthetic form of folate used in food fortification.[26,27,29,31] There is no debate that the national public health policies to mandatorily fortify bread and cereals with FA[71] has been effective in preventing neural tube defects.[72–78] Practitioners in this cohort, however, may be responding to more recent research that questions the amount of FA patients are consuming due to food fortification as it far exceeds the expected intake of 100 to 200μg.[28,29,58,79–82]

It is postulated that pregnant women are possibly ingesting well above 2000 nmol/l from both their diet and their prenatal supplements.[83] FA had been found to be readily absorbed, and its conversion to tetrahydrofolate is a two-step process utilizing the enzyme DHFR.[24] Human studies propose that when FA cannot be converted to THF the enzyme is saturated and instead UMFA is produced and transported around in the blood.[25,26,29,82,84,85] In vitro studies suggest that UMFA may act as a folate antagonist which may not only inhibit purine synthesis but the formation of 5-MTHF required for DNA synthesis and methylation.[28] This folate antagonism may contribute to a loss of DNA methylation in sperm among men with *MTHFR* polymorphisms[86,87] and affect embryo development.[88] A murine study found that when FA is low due to no fortification of foods, 5-MTHF and folinic acid levels are significantly higher in the brain which suggests that a possible diversion from 5-MTHF to THF and folinic acid,.[89]

In vitro studies indicate that high doses of FA have resulted in reduced MTHF and methylation capacity.[90] Although the existence of UMFA has been extensively studied[25,28,29,59–61,82–85,91–97] its clinical impact is not clear and requires additional researcher attention. Human studies have identified that 96% of breastfeeding women had high levels of UMFA and a corresponding reduction in 5-MTHF levels, possibly due to down regulation of folate-binding protein, which is thought to regulate folate secretion.[83] Outside of fertility, in human studies, UMFA has also been linked to natural killer cell cytotoxicity in post-menopausal women,[28] cobalamin deficiency[91] and some cancers.[28]

This case series also suggests practitioners from this cohort are prescribing different forms of supplemental folate to their infertility patients with *MTHFR* polymorphisms, than the folic acid recommended by Australian health policy. The health recommendation in Australia is that women preparing for pregnancy should take 400-500μg of supplemental FA.[21] The practitioners in this study advised their patients to discontinue their supplements containing FA and instead prescribed folinic acid and/or 5-MTHF. 5-MTHF was the most frequently prescribed form of folate and in cases where folinic acid and methyl folate were prescribed concurrently, 5-MTHF was much higher. This deviation from accepted policy guidelines regarding folic acid supplementation in pregnancy may be due to concerns that when the policy guidelines were formulated the only research on supplemental folate was examining FA.[32,33,36,98] The main form of folate that has been found in cord blood is 5-MTHF and is, therefore, likely to be the form of folate that is transported to the foetus[27] and generally increases mean plasma and red cell folate concentration significantly.[32,62] In vitro studies have found that impairment of the folate pathway can affect one carbon metabolism, and this is critical to methylation of DNA, histones and proteins[26] directly linked to the viability of the embryo and sperm.[36,50,88] As these patients have *MTHFR* polymorphisms, practitioners may prefer 5-MTHF based on the results of human studies indicating methyl folate may bypass the MTHFR enzyme.[27,32,33,36]

It is important to note that of the twelve cases presented in this case series, all were told by their practitioner to discontinue their FA supplement and were prescribed 5-MTHF or a combination of 5-MTHF and folinic acid in its place. Furthermore, eleven of the women fell pregnant within two to four months, ten had successful live births. Two of the three patients that became pregnant via IVF presented to their initial appointment on high dose FA (5000μg) which is the accepted protocol for those with a high risk of miscarriage or neural tube defects.[81,99] All three of these cases became pregnant and had a successful live birth after starting treatment, which included replacing their FA with an alternative form of supplemental folate. The practitioners who contributed cases to this study also prescribed much higher doses of folate (mean: 2325μg per day) than the Australian recommendation, which is currently 400-500μg of FA.[100] Our study does not uncover the reasons the practitioners were using doses much higher than the recommendations, but it may be in response to emerging research indicating that MTHF may be more effective in raising red blood cell and serum folate levels,[27,32] is safe[101] and may be effective in the prevention and treatment of perinatal depression.[102] Furthermore, recent research regarding doses of folate suggests approximately 800μg or more of 5-MTHF may bypass the *MTHFR* gene polymorphism and raise plasma folate levels rapidly, resulting in repletion of folate stores within days without creating UMFA.[33,36,98] However, this possible interpretation would need to be further studied to be confirmed.

### 7.1. Implications for research and practice

These findings warrant closer attention from researchers to clarify whether different forms of folate have an impact on the clinical outcome for women with infertility. In particular, further research is needed to investigate if these alternative forms of folate given at higher than recommended doses improve outcomes for women with infertility and *MTHFR* polymorphisms. The absence of substantive clinical research in humans limits the transferability of this evidence to clinical practice. Furthermore, future research that examines the reasons and implications of this gap between existing policy recommendations and clinical practice behaviour is needed. Every case presented in this study included women who had been diagnosed with infertility, had *MTHFR* polymorphisms and had previous miscarriages or infertility. Human research has shown that *MTHFR* polymorphisms may contribute to infertility in women and describes the *MTHFR* gene polymorphisms contributing to low folate status, elevation in homocysteine which affects folliculogenesis,[11,46,52,85,94] embryo viability,[54] recurrent implantation failure[22] and may predispose those patients with *MTHFR* polymorphisms to recurrent pregnancy loss.[103–105] Although this research cannot be extrapolated to the wider community, the successful pregnancy rate for this case series, gives rise to the need for further research to investigate whether individuals with infertility and *MTHFR* polymorphism may require a more nuanced approach to achieve pregnancy and live births. Research has identified that *MTHFR* polymorphisms affect male fertility as well and men who have polymorphisms may influence recurrent pregnancy loss irrespective of the female having the *MTHFR* polymorphism.[106,107] In males, *MTHFR* polymorphisms are associated with oligozoospermia[34,108] and male infertility in general.[53,109] *MTHFR* increases the risk in pregnancy for preeclampsia[110,111] and outside fertility have been linked to thrombosis and coronary artery disease,[112]

cancer,[113,114] vascular disease, depression,[115] schizophrenia,[116] renal failure and several drugs that interfere with folate metabolism such as methotrexate and 5-fluorouracil.[43,116] Future research should explore the potential importance of alternative forms of folate for both men and women with infertility. It should also investigate any clinical implications associated with UMFA in individuals with *MTHFR* polymorphisms and highlights the need for pharmacists to be aware of advancements in the research as folic acid based supplements are primarily purchased over the counter in pharmacies by consumers who may be unaware of UMFA implications particularly if they have MTHFR polymorphisms. The increasing role of the pharmasist in every day health care means that they will be required to recommend different forms and dosages of folate for preconception and pregnancy, particularly as the rate of infertility increases and consumers become more aware of genetic susceptibility to health conditions, including the MTHFR gene polymorphisms and the metabolic differences between folic acid and 5-MTHF.

## 8. Limitations of the study

Case reports and case series are considered to be the lowest level of evidence within the evidence-based medicine hierarchy, yet they can offer valuable information from clinical practice to inform more robust clinical research.[117] By their nature, case series are limited by their small sample sizes as case studies/series generally have small numbers of people or only one person which is studied[118–120]. There may also be bias because the primary researcher is in a group that is exposed to information about clinical management of *MTHFR* polymorphisms. The practitioners recruited for this study were all from the *MTHFR* Support Australia database because we required patients who had been screened for the *MTHFR* gene polymorphisms. This most likely will bias results in relation to the amount and type of folate prescribed to the patients compared to other practitioners who are not in this group. As such, these results should not be interpreted as representative of common clinical care for infertility in Australia or elsewhere. So too, some practitioners may not have participated in the study due to unsatisfactory clinical outcomes from their treatments even though recruitment material encouraged participants to share any relevant cases, whether clinical outcomes were positive or not. The type of *MTHFR* polymorphism testing was also not specified. Either blood or saliva testing was accepted, and no specific test was required. This may have inferred a limitation due to the variability in the specificity and sensitivity between tests. Despite these identified limitations, this study seeks not to draw causality but rather describe and view trends that practitioners may be observing when treating infertility patients who have *MTHFR* polymorphisms. As such this study is suited to the research aim and objectives.

## 9. Conclusion

In conclusion, this case series has highlighted clinical practices that are different from the recommendations from the Australian policy for fertility in relation to the dose and form of folate and identifies areas for future research. Individuals with *MTHFR* polymorphisms may benefit from tailored prescribing practices but further studies are required. The predominant form of folate prescribed in this cohort is 5-MTHF and not FA as recommended. The dose of 5-MTHF that was prescribed far exceeded the recommended 400μg of FA. The practitioners that participated in this study had been recruited from an *MTHFR* educational site and therefore may be more aware of potential health implications of a *MTHFR* polymorphism. The fertility outcomes noted from these individuals with diagnosed infertility were extremely positive, with all but two women having successful pregnancies and live births. This research identifies novel insights from clinical practice that requires further investigation and raises public health implications for recommending folic acid to all women particularly in preconception and pregnancy. Given the leading prenatal multivitamin in Australia is a retail brand and contains 800μg of folic acid it is imperative for pharmacists and their pharmacy assistants to understand that different prescriptions may be required for women with MTHFR polymorphisms, and that one form of folate may not be appropriate for all women.

## Funding

The researcher received funding from Endeavour College of Natural Health for this project.

## Availability of data and material

The data that supports the findings of this study are not openly available due to privacy reasons and are available from the corresponding author upon reasonable request.

## Code availability

Not applicable.

## Authors contributions

C.L was primarily responsible for the authorship of this paper, providing the majority of contribution to: development of the article topic or research question; synthesis and/or analysis of data; and drafting of the manuscript and subsequent revision. A.S and J.S provided guidance to the project, type of methods required for this study and the study itself. A.S and J.S provided feedback on all drafts of the manuscript. A.S and J.S aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

## Ethics approval

The research project was approved by the Human research and ethics Committee (HREC) of Endeavour College of Natural Health (HREC #20200406-CL-1).

## Consent to participate

In accordance with NHMRC guidelines participation in this research study was voluntary and as such a consent form was presented to each practitioner that met the eligibility criteria. As only select and de-identified data was presented to the researcher it was not necessary for the practitioner to gain consent from the patients.

## Consent for publication

Not applicable.

## Declaration of Competing Interest

In accordance with my ethical obligation as a researcher, Carolyn Ledowsky is reporting that she is the founder of MTHFR Support Australia and as such has access to the practitioner database of whom she contacted for the purpose of this research. A. Steel and J. Schloss declare no conflicts of interest.

## Acknowledgements

The authors would like to thank the Endeavour College of Natural Health for their support for this research.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rcsop.2023.100277.

## References


1. Rai V, Kumar P. Methylenetetrahydrofolate reductase C677T polymorphism and risk for male infertility in Asian population. Indian J Clin Biochem 2017;32(3):253–260.
2. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37.
3. Elliott PA, Hoffman J, Abad-Santos M, Herndon C, Katz PP, Smith JF. Out-of-pocket costs for men undergoing infertility care and associated financial strain. Urol Pract 2016;3(4):256–261.
4. Meka A, Reddy BmJIJoHG. Recurrent spontaneous abortions: an overview of genetic and non-genetic backgrounds. 2006;6(2):109–117.
5. Commonwealth of Australia. In: *Health Do, ed.* Reproductive Health; 2020.
6. Ried K, Alfred A. Quality of life, coping strategies and support needs of women seeking traditional Chinese medicine for infertility and viable pregnancy in Australia: a mixed methods approach. BMC Womens Health 2013;13(1):17.
7. Macaldowie A.W.Y., Chambers G.M., Sullivan E.A. In: Welfare AIoHa, ed. Assisted reproductive technology in Australia and New Zealand 2010. 2010. Canberra. Assisted Reproductive Series 16.
8. Macaldowie A. Assisted reproductive technology in Australia and New Zealand 2010. Aust Inst Health Welfare 2012.
9. Alesi R. Infertility and its treatment–an emotional roller coaster. Aust Fam Physician 2005;34(3):135–138.
10. Rozen G, Stern K, Lensen S, Polyakov A. Barriers to reproductive treatments in Australia. Aust J Gen Pract 2023;52(3):109–112.
11. I.V.F Australia. IVF Treatment Costs. https://www.ivf.com.au/ivf-cost/ivf-costs 2020.
12. Ledowsky C, Steel A, Schloss J. Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and the risk of infertility in couples accessing assisted reproductive technologies. Adv Integr Med 2021;8(9521).
13. Centers for Disease Control and Prevention. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Morb Mortal Wkly Rep 1992;1992(41):1–7.
14. Food And Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Fed Regist 1996;61(44):8781–8797.
15. Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid.Final rule. 21 CFR parts 136, 137 and 139. Fed Regist 1996;1996(61):8781–8807.
16. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council vitamin study. Lancet 1991;338(8760):131–137.
17. Smithells R, Sheppard S, Schorah C. Vitamin dificiencies and neural tube defects. Arch Dis Child 1976;51(12):944–950.
18. Institute of Medicine (US). *Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline. Washington (DC)*. 1998.
19. Gomes S, Lopes C, Pinto E. Folate and folic acid in the periconceptional period: recommendations from official health organizations in thirty-six countries worldwide and WHO. Public Health Nutr 2016;19(1):176–189.
20. Bell KN, Oakley Jr GP. Update on prevention of folic acid-preventable spina bifida and anencephaly. Birth Defects Res A Clin Mol Teratol 2009;85(1):102–107.
21. Department of Health. *Folate*. 2010.
22. Choi Y, Kim JO, Shim SH, et al. Genetic variation of methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) genes is associated with idiopathic recurrent implantation failure. PloS One 2016;11(8), e0160884.
23. Cochrane KM, Mayer C, Devlin AM, Elango R, Hutcheon JA, Karakochuk CD. Is natural (6 S)-5-methyltetrahydrofolic acid as effective as synthetic folic acid in increasing serum and red blood cell folate concentrations during pregnancy? A proof-of-concept pilot study Trials. 2020;21:1-12.
24. Bailey SW, Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. Proc Natl Acad Sci 2009;106(36):15424–15429.
25. Maruvada P, Stover PJ, Mason JB, et al. Knowledge gaps in understanding the metabolic and clinical effects of excess folates/folic acid: a summary, and perspectives, from an NIH workshop. Am J Clin Nutr 2020 Nov 11;112(5):1390–1403.
26. Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 2014;44(5):480–488.
27. Obeid R, Holzgreve W, Pietrzik K. Is 5-methyltetrahydrofolate an alternative to folic acid for the prevention of neural tube defects? J Perinat Med 2013;41(5):469–483.
28. Smith AD, Kim Y-I, Refsum H. Is folic acid good for everyone? Am J Clin Nutr 2008;87 (3):517–533.
29. Obeid R, Herrmann W. The emerging role of unmetabolized folic acid in human diseases: myth or reality? Curr Drug Metab 2012;13(8):1184–1195.
30. Steegers-Theunissen RPM, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. Hum Reprod Update 2013;19(6):640–655.
31. Bjelakovic G, Stojanovic I, Jevtovic-Stoimenov T, et al. Is folic acid supplementation to food benefit or risk for human health? Pteridines 2013;24(3–4):165–181.
32. Lamers Y, Prinz-Langenohl R, Brämswig S, Pietrzik K. Red blood cell folate concentrations increase more after supplementation with [6 S]-5-methyltetrahydrofolate than with folic acid in women of childbearing age. Am J Clin Nutr 2006;84(1):156–161.
33. Tafuri L, Servy E, Menezo Y. *The Hazards of Excessive Folic Acid Intake in MTHFR Gene Mutation Carriers: An Obstetric and Gynecological Perspective.* Clinical Obstetrics, Gynecology and Reproductive Medicine. 2018:4.
34. Eloualid A, Abidi O, Charif M, et al. Association of the MTHFR A1298C variant with unexplained severe male infertility. PloS One 2012;7(3), e34111.
35. Gupta N, Sarkar S, David A, et al. Significant impact of the MTHFR polymorphisms and haplotypes on male infertility risk. PloS One 2013;8(7), e69180.
36. Servy EJ, Jacquesson-Fournols L, Cohen M, Menezo YJ. MTHFR isoform carriers. 5-MTHF (5-methyl tetrahydrofolate) vs folic acid: a key to pregnancy outcome: a case series. J Assist Reprod Genet 2018;35(8):1431–1435.
37. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci U S A 2001;98(26):14853–14858.
38. Fodinger M, Horl WH, Sunder-Plassmann G. Molecular biology of 5,10-methylenetetrahydrofolate reductase. J Nephrol 2000;13(1):20–33.
39. Trimmer E, E. Methylenetetrahydrofolate reductase: biochemical characterization and medical significance. Curr Pharm Des 2013;19(14):2574–2593.
40. Matthews RG, Sheppard C, Goulding C. Methylenetetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. Eur J Pediatr 1998;157(2). (S54–9).
41. Friso S, Choi S-W, Girelli D, et al. A common mutation in the 5, 10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci 2002;99(8):5606–5611.
42. Mahmud N, Molloy A, McPartlin J, et al. Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and its clinical implications. Gut 1999;45(3):389–394.
43. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 2001;22(4):195–201.
44. Morovvati S, Khaleghparast A, Noormohammadi Z. Evaluation of the association between the C677T and A1298C polymorphisms of MTHFR gene and recurrent miscarriage. J Med Council Islamic Republic of Iran 2010;28(4):465.
45. Khaleghparast A, Khaleqparast S, Khaleghparast H. Association between the A1298C polymorphism of the methylenetetrahydrofolate reductase gene and recurrent spontaneous abortion. Iran J Neonatol 2014;5(2):7-11.
46. Altmae S, Stavreus-Evers A, Ruiz JR, et al. Variations in folate pathway genes are associated with unexplained female infertility. Fertil Steril 2010;94(1):130–137.
47. Thompson GR, Miller JP, Breslow JL. Improved survival of patients with homozygous familial hypercholesterolaemia treated with plasma exchange. Br Med J (Clin Res Ed) 1985;291(6510):1671–1673.
48. Botto LD, Yang Q. 5,10-methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 2000;151(9):862–877.
49. Cornet D, Cohen M, Clement A, et al. Association between the MTHFR-C677T isoform and structure of sperm DNA. J Assist Reprod Genet 2017;34(10):1283–1288.
50. Cisneros FJ. DNA methylation and male infertility. Front Biosci 2004;9(1189):1189–1200.
51. Tomizawa S-I, Nowacka-Woszuk J, Kelsey G. DNA methylation establishment during oocyte growth: mechanisms and significance. Int J Dev Biol 2013;56(10-11–12):867–875.
52. Rosen MP, Shen S, McCulloch CE, Rinaudo PF, Cedars MI, Dobson AT. Methylenetetrahydrofolate reductase (MTHFR) is associated with ovarian follicular activity. Fertil Steril 2007;88(3):632–638.
53. Hong HH, Hu Y, Yu XQ, et al. Associations of C677T polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene with male infertility risk: a meta-analysis. Eur J Obstet Gynecol Reprod Biol 2017;212:101–109.
54. Enciso M, Sarasa J, Xanthopoulou L, et al. Polymorphisms in the MTHFR gene influence embryo viability and the incidence of aneuploidy. Hum Genet 2016;135(5):555–568.
55. D'Uva M, Di Micco P, Strina I, et al. Hyperhomocysteinemia in women with unexplained sterility or recurrent early pregnancy loss from southern Italy: a preliminary report. Thromb J 2007;5:10.
56. Shahrokhi SZ, Kazerouni F, Ghaffari F, et al. The relationship between the MTHFR C677T genotypes to serum anti-Mullerian hormone concentrations and in vitro fertilization/intracytoplasmic sperm injection outcome. Clin Lab 2017;63(5):927–934.
57. Mfady DS, Sadiq MF, Khabour OF, Fararjeh AS, Abu-Awad A, Khader Y. Associations of variants in MTHFR and MTRR genes with male infertility in the Jordanian population. Gene 2014;536(1):40–44.
58. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 2004;80(5):1123–1128.
59. Sweeney MR, McPartlin J, Scott J. Folic acid fortification and public health: report on threshold doses above which unmetabolised folic acid appear in serum. BMC Public Health 2007;7(1):41.
60. Sweeney MR, Staines A, Daly L, et al. Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. Implications for further mandatory fortification? BMC Public Health 2009;9(1):295.
61. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136(1):189–194.
62. Fohr IP, Prinz-Langenohl R, Brönstrup A, et al. 5,10-methylenetetrahydrofolate reductase genotype determines the plasma homocysteine-lowering effect of supplementation with 5-methyltetrahydrofolate or folic acid in healthy young women. Am J Clin Nutr 2002;75(2):275–282.
63. Hekmatdoost A, Vahid F, Yari Z, et al. Methyltetrahydrofolate vs folic acid supplementation in idiopathic recurrent miscarriage with respect to methylenetetrahydrofolate reductase C677T and A1298C polymorphisms: A randomized controlled trial. PloS One 2015;10(12).
64. Clément A, Menezo Y, Cohen M, Cornet D, Clément P. 5-Methyltetrahydrofolate reduces blood homocysteine level significantly in C677T methyltetrahydrofolate reductase single-nucleotide polymorphism carriers consulting for infertility. J Gynecol Obstetrics Human Reprod 2020;49(1), 101622.

65. Servy E, Menezo Y. The methylene tetrahydrofolate reductase (MTHFR) isoform challenge. High doses of folic acid are not a suitable option compared to 5 Methyltetrahydrofolate treatment. Clin Obstet Gynecol Reprod Med 2017;3:1–5.
66. Ogilvie M, Nissen L, Kyle G, Hale A. An evaluation of a collaborative pharmacist prescribing model compared to the usual medical prescribing model in the emergency department. Res Social Adm Pharm 2022;18(10):3744–3750.
67. Annabel B, Scrymgeour M. Retail matters: survivor bias. Aust J Pharm 2022;103(1216): 72–73.
68. Tiralongo E, Braun LA, Wilkinson JM, et al. Exploring the integration of complementary medicines into Australian pharmacy practice with a focus on different practice settings and background knowledge. J Complemen Integr Med 2010;7(1).
69. Barnes LAJ, Rolfe MI, Barclay L, McCaffery K, Aslani P. Women's reasons for taking complementary medicine products in pregnancy and lactation: results from a national Australian survey. Complement Ther Clin Pract 2022;49, 101673.
70. Whitaker HJ, Farrington CP, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. Stat Med 2006;25(10):1768–1797.
71. Government AF. *Australia New Zealand Food Standards Code - Standard 2.1.1 - Cereals and Cereal Products*. 2009.
72. Božović IB, Vraneković J. Folate and folic acid: current knowledge and gaps. Med Fluminensis 2014;50(2):169–175.
73. Czeizel AE, Dudás I, Vereczkey A, Bánhidy F. Folic acid and pregnancy: properties, medical uses and health benefits. Folic Acid: Properties, Medical Uses and Health Benefits; 2012. p. 1-37.
74. Fitzpatrick A. Folate (folic acid): implications for health and disease. Agro Food Ind Hi Tech 2003;14(3):45–48.
75. Haggarty P, Campbell DM, Duthie S, et al. Folic acid use in pregnancy and embryo selection. BJOG 2008;115(7):851–856.
76. Imbard A, Benoist JF, Blom HJ. Neural tube defects, folic acid and methylation. Int J Environ Res Public Health 2013;10(9):4352–4389.
77. Minns RA. Folic acid and neural tube defects. Spinal Cord 1996;34(8):460–465.
78. Rossokha Z, Gorovenko N. Assessment of the individual folic acid doses requirement for patients with reproductive disorders. J Perinat Med 2017;45:493.
79. Brown RD, Langshaw MR, Uhr EJ, Gibson JN, Joshua DE. The impact of mandatory fortification of flour with folic acid on the blood folate levels of an Australian population. Med J Aust 2011;194(2):65–67.
80. Herrmann W, Obeid R. The mandatory fortification of staple foods with folic acid: a current controversy in Germany. Deutsches Arzteblatt international 2011;108(15):249–254.
81. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340(19):1449–1454.
82. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr 1997;65(6):1790–1795.
83. Houghton LA, Yang J, O'Connor DL. Unmetabolized folic acid and total folate concentrations in breast milk are unaffected by low-dose folate supplements. Am J Clin Nutr 2009;89(1):216–220.
84. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. Am J Clin Nutr 2010;91(6):1733–1744.
85. Obeid R, Kasoha M, Kirsch SH, Munz W, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. Am J Clin Nutr 2010;92(6):1416–1422.
86. Aarabi M, Christensen KE, Chan D, et al. Testicular MTHFR deficiency may explain sperm DNA hypomethylation associated with high dose folic acid supplementation. Hum Mol Genet 2018;27(7):1123–1135.
87. Aarabi M, San Gabrie MC, Chan D, et al. High-dose folic acid supplementation alters the human sperm methylome and is influenced by the MTHFR C677T polymorphism. Hum Mol Genet 2015;24(22):6301–6313.
88. Canovas S, Ross PJ, Kelsey G, Coy P. DNA methylation in embryo development: epigenetic impact of ART (assisted reproductive technologies). Bioessays 2017;39(11): 1700106.
89. Harlan De Crescenzo A, Panoutsopoulos AA, Tat L, et al. Deficient or excess folic acid supply during pregnancy Alter cortical neurodevelopment in mouse offspring. Cereb Cortex 2021 Jan;31(1):635–649.
90. Christensen KE, Mikael LG, Leung K-Y, et al. High folic acid consumption leads to pseudo-MTHFR deficiency, altered lipid metabolism, and liver injury in mice. Am J Clin Nutr 2015;101(3):646–658.
91. Obeid R, Kirsch SH, Kasoha M, Eckert R, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in plasma after folic acid treatment in older adults. Metabolism 2011;60(5):673–680.
92. Page R, Robichaud A, Arbuckle TE, Fraser WD, MacFarlane AJ. Total folate and unmetabolized folic acid in the breast milk of a cross-section of Canadian women. Am J Clin Nutr 2017;105(5):1101–1109.
93. Page R, Wong A, Arbuckle TE, MacFarlane AJ. The MTHFR 677C>T polymorphism is associated with unmetabolized folic acid in breast milk in a cohort of Canadian women. Am J Clin Nutr 2019;110(2):401–409.
94. Sweeney MR, McPartlin J, Weir DG, Daly L, Scott JM. Postprandial serum folic acid response to multiple doses of folic acid in fortified bread. Br J Nutr 2006;95(1):145–151.
95. Sweeney MR, McPartlin J, Weir DG, Scott JM. Measurements of sub-nanomolar concentrations of unmetabolised folic acid in serum. J Chromatogr B Analyt Technol Biomed Life Sci 2003;788(1):187–191.
96. Tam C, O'Connor D, Koren G. Circulating unmetabolized folic acid: relationship to folate status and effect of supplementation. Obstet Gynecol Int 2010;2012.
97. Tam C, O'Connor D, Koren G. Circulating unmetabolized folic acid: relationship to folate status and effect of supplementation. Obstet Gynecol Int 2012;2012, 485179.
98. Bailey SW, Ayling JE. The pharmacokinetic advantage of 5-methyltetrahydrofolate for minimization of the risk for birth defects. Sci Rep 2018;8(1):4096.
99. South Australian Health Safety & Quality Strategic Governance committee. *Clinical Guideline - Vitamin and Mineral Supplementation in Pregnancy*. 2015.
100. Royal Australian College of General Practitioners. *Guidelines for preventative activities in general practice*, 19. 2016.
101. Niederberger KE, Dahms I, Broschard TH, Boehni R, Moser R. Safety evaluation of calcium L-methylfolate. Toxicol Rep 2019;6:1018–1030.
102. Freeman MP, Savella GM, Church TR, et al. A prenatal supplement with methylfolate for the treatment and prevention of depression in women trying to conceive and during pregnancy. Ann Clin Psychiatry 2019;31(1):4-16.
103. Hwang KR, Choi YM, Kim JJ, et al. Methylenetetrahydrofolate reductase polymorphisms and risk of recurrent pregnancy loss: a case-control study. J Korean Med Sci 2017;32(12):2029–2034.
104. Kos BJP, Leemaqz SY, McCormack CD, et al. The association of parental methylenetetrahydrofolate reductase polymorphisms (MTHFR 677C > T and 1298A > C) and fetal loss: a case-control study in South Australia. J Matern Fetal Neonatal Med 2020;33 (5):752–757.
105. Lissak A, Sharon A, Fruchter O, Kassel A, Sanderovitz J, Abramovici H. Polymorphism for mutation of cytosine to thymine at location 677 in the methylenetetrahydrofolate reductase gene is associated with recurrent early fetal loss. Am J Obstet Gynecol 1999;181 (1):126–130.
106. Sah AK, Shrestha N, Joshi P, et al. Association of parental methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism in couples with unexplained recurrent pregnancy loss. BMC Res Notes 2018;11(1).
107. Vanilla S, Dayanand CD, Kotur PF, Kutty MA, Vegi PK. Evidence of paternal N5, N10 - methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism in couples with recurrent spontaneous abortions (RSAs) in Kolar District- a south west of India. J Clin Diagn Res 2015;9(2).(BC15-BC8).
108. Gava MM, Kayaki EA, Bianco B, et al. Polymorphisms in folate-related enzyme genes in idiopathic infertile Brazilian men. Reprod Sci 2011;18(12):1267–1272.
109. Karimian M, Colagar AH. Association of C677T transition of the human methylenetetrahydrofolate reductase (MTHFR) gene with male infertility. Reprod Fertil Dev 2016;28 (6):785–794.
110. Salimi M, Mozdarani H. Different aspects of cytochalasin B blocked micronucleus cytome (CBMN cyt) assay as a comprehensive measurement tool for radiobiological studies, biological dosimetry and genome instability. Int J Radiat Res 2015;13(2): 101–126.
111. Wu X, Yang K, Tang X, et al. Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for preeclampsia: a meta-analysis. J Assist Reprod Genet 2015;32(5):797–805.
112. Varga EA, Sturm AC, Misita CP, Moll S, Cardiology patient pages. Homocysteine and MTHFR mutations: relation to thrombosis and coronary artery disease. Circulation 2005;111(19):e289–e293.
113. Gallegos-Arreola MP, Garcia-Ortiz JE, Figuera LE, Puebla-Perez AM, Morgan-Villela G, Zuniga-Gonzalez GM. Association of the 677C –>T polymorphism in the MTHFR gene with colorectal cancer in Mexican patients. Cancer Genomics Proteomics 2009;6(3): 183–188.
114. Hosseini M, Houshmand M, Ebrahimi A. MTHFR polymorphisms and breast cancer risk. Arch Med Sci 2011;7(1):134–137.
115. Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B12, homocysteine, and the MTHFR 677C->T polymorphism in anxiety and depression: the Hordaland homocysteine study. Arch Gen Psychiatry 2003;60(6):618–626.
116. Marginean C, Procopciuc L, Fodoreanu L. Implications of MTHFR polymorphisms in the development of treatment-resistant schizophrenia. Human Vet Med 2017;9(4):124–129.
117. Merlin T, Weston A, Tooher R. Extending an evidence hierarchy to include topics other than treatment: revising the Australian "levels of evidence". BMC Med Res Methodol 2009;9(1):34.
118. Lu CY. Observational studies: a review of study designs, challenges and strategies to reduce confounding. Int J Clin Pract 2009;63(5):691–697.
119. Reio TG. Nonexperimental research: strengths, weaknesses and issues of precision. Eur J Train Dev 2016;40(8/9):676–690.
120. Tellis WM. Introduction to case study. Qual Rep 1997;3(2):1-14.