Prevalent genotypes of methylenetetrahydrofolate reductase (MTHFR) in recurrent miscarriage and recurrent implantation failure

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Received: 19 January 2018 / Accepted: 9 May 2018 / Published online: 21 May 2018
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Abstract

Objective To evaluate the association of two common methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms with recurrent miscarriage (RM) and repeated implantation failure (RIF)

Methods The study comprised of 521 patients, with a history of RM ($n = 370$) or RIF ($n = 151$). One hundred forty-four women with fallopian tube blockages who had successfully conceived after the first in vitro fertilization embryo transfer treatment served as the control group. The MTHFR alleles, genotypes, and haplotypes were assessed in different groups.

Results There was no difference in allele frequency and distribution of MTHFR polymorphisms between case and control patients. The 1298AA genotype was represented in a higher frequency, and 1298AC genotype was significantly lower in subfertile group when compared to the control group. A significant relationship was found between the 1298AC genotype and the RIF subgroup. The haplotype 677CC/1298AA was overrepresented in the RM subgroup (> 2 times) and haplotype 677CC/1298AC was underrepresented in the RIF subgroup ($P < 0.05$). Nevertheless, these two haplotypes were not connected to fertilization and embryo cleavage rates.

Conclusion Our findings indicate that the MTHFR gene polymorphism might play a role in the etiology of patients with RM or RIF. No adverse effects of different MTHFR haplotypes on embryo development were detected. Further studies on the biological role are needed to better understand the susceptibility to pregnancy complications.

Keywords MTHFR · Polymorphism · Genotype · Repeated implantation failure · Recurrent miscarriage

Introduction

Recurrent miscarriage (RM) is defined as two or more consecutive pregnancies failing before 20 weeks of gestation or fetal weight less than 500 g, and this condition affects about 1–3% of reproductively active couples [1, 2]. Previous studies

have reported that RM is caused by genetics, uterine anomalies, hormonal or metabolic disorders, antiphospholipid syndrome, thrombophilias, autoimmunity, infection, sperm quality, and lifestyle issues [3]. There are no definitive conclusions with respect to the causes of RM yet. Pregnancy loss has thus far focused on sporadic miscarriage and not strictly RM criteria. No apparent causative factor can be determined in 50% of couples with RM [4].

Repeated implantation failure (RIF) is defined as repeated implantation failure after several in vitro fertilization embryo transfers (IVF-ET). Initially, RIF was defined as failure to achieve pregnancy following 2–6 IVF cycles, when more than 10 high-quality embryos were transferred into the uterus [5]; more recently, researchers redefined it as three or more consecutive implantation failures, with one to two high-quality embryos being transferred in each cycle [6]. Even though RIF is of interest to clinicians and researchers, there are no

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uniform criteria defining the number of failed cycles or the total number of embryos to be transferred during IVF attempts, and different IVF centers may use different diagnostic standards for RIF. In this study, RIF was defined as three or more consecutive implantation failures.

Commonly, embryo quality and endometrial receptivity are two vital factors that are believed to be the key points in embryo implantation [7]. According to the statistics, about 10% of females have suffered this event after several IVF attempts, even though they have high-quality embryos and good endometrial receptivity.

The human methylenetetrahydrofolate reductase (MTHFR) gene is located on the short arm of chromosome 1 (1p36.22) and is composed of 11 exons. The MTHFR enzyme plays an essential role in folate metabolism, and it is essential for cell division, embryo development, and early pregnancy [8]. The two most common polymorphisms of the MTHFR gene are MTHFR A1298C (rs1801131) and MTHFR C677T (rs1801133) [9]. Reduction in MTHFR activity is associated with an increased blood level of homocysteine and cardiovascular disease [10, 11]. Associations between MTHFR gene polymorphisms (C677T and A1298C) and the risk of RM have been previously demonstrated [12, 13]. Underlying maternal thrombophilia also has a negative impact on IVF implantation rate or success rate [14]. Treatment with low molecular weight heparin improves IVF outcomes in women with thrombophilia [15]. The relationship between thrombophilia and recurrent pregnancy loss or poor pregnancy outcomes is relatively well known and it is possible that similar mechanisms are in play in RIF and RM [16]. However, the relationship between RIF, RM, and MTHFR gene polymorphisms is not clear. In our research, we intend to explore the following two questions: firstly, is a specific MTHFR gene polymorphism associated with abnormal reproductive histories (RIF and RM)? Secondly, do MTHFR gene polymorphisms have an effect on the developmental competence of embryos?

Materials and methods

Patients

The study is comprised of 521 patients undergoing assisted reproductive treatment (ART). All pregnancy losses met the following descriptions: (1) gestational sac was confirmed by ultrasound after embryo transferred at 5 weeks; (2) gestational age was between 7 and 11 weeks; (3) chorionic villus sampling was found after complete curettage of uterine cavity. The case cohort was divided into the following three subgroups:

(1) RM1, two clinical pregnancy failures ($n = 262$); (2) RM2, three or more clinical pregnancy failures ($n = 108$); (3) RIF, three or more IVF-embryo implantations failed with one or two high-quality embryos transferred each cycle ($n = 151$). A total of 144 patients were enrolled in the control groups with successful pregnancy more than 12 weeks after the first IVF-ET treatment and no history of miscarriage or implantation failure. Women with endometriosis, hydrosalpinx, and abnormal uterine cavity on a hysterosalpingogram and those who were receiving hormonal treatment were not included in the study. All patients underwent the same clinical treatment during the IVF cycles. A fresh embryo transfer cycle was conducted in all groups and one or two high-quality embryos were transferred. All patients were warned against smoking, alcohol, and some other bad habits before entering ART treatment, and the majority of patients were willing to correct bad habits due to as they were eager to conceive. Infertility caused by male factors was excluded in this study. This study was approved by the Research Ethics Committee of the Shenzhen Zhongshan Urology Hospital, and informed consent was obtained from all couples prior to enrollment in the study.

MTHFR genotyping

Peripheral blood specimens were collected in EDTA vacuum tubes and genomic DNA was extracted from whole blood using a whole blood DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. Amplification of the C677T region of the MTHFR gene was performed using the primers 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' (forward primer) and 5'-AGGACGGTGCGGTGAGAGTG-3' (reverse primer). The A1298C primers were 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' (forward primer) and 5'-CACTTTGTGACCGTTCCGGTTTG-3' (reverse primer). Amplification of the correct fragment was confirmed by agarose gel electrophoresis.

Prior to the mini-sequencing reaction, 0.5 μ l of the PCR product was purified using ExoSAP-IT (Affymetrix, High Wycombe, UK) following the manufacturer's instructions. Mini-sequencing primers were the following: 5'-3'((T)₃₆AGAAGGTGTCTGCGGGAG) for MTHFR C677T and 5'-3'((T)₂₇AGGAGCTGACCAGTGAAG) for A1298C. Mini-sequencing products were analyzed by capillary electrophoresis on an ABI 3500 Genetic Analyzer (Applied Biosystems, UK). The reaction mix consisted of 8.5 μ l Hi-Di Formamide (Applied Biosystems, UK), 0.5 μ l GeneScan-120LIZ Size standard (Applied Biosystems, UK), and 1 μ l of purified mini-sequencing product. Incubation at 95 °C for 5 min was performed prior to electrophoresis to denature the mini-sequencing product. Data were subsequently visualized

Table 1 *MTHFR* allele frequencies for control and subfertile groups

MTHFR allele	Allele frequency		χ^2	P value	Odds ratio	95% confidence interval
	Control group (n = 144), n (%)	Subfertile group (n = 521)				
1298A	222 (77.08)	845 (81.09)	2.288	0.130	0.784	0.572–1.075
1298C	66 (22.92)	197 (18.91)				
677C	191 (66.32)	681 (65.36)	0.093	0.760	1.044	0.792–1.375
677T	97 (33.68)	361 (36.64)				

and analyzed using the GeneMapper software (Applied Biosystems, UK).

MTHFR genotyping statistical analyses

MTHFR C677T and A1298C allele and genotype frequencies were compared and analyzed using a chi-squared test and *t* test. Genotype frequencies in all groups were assessed for compliance with the Hardy-Weinberg equilibrium using the Genemapping v.4.2 software. Statistical significance was defined as $P < 0.05$ and all analyses were performed using SPSS software.

Availability of data and material Yes

Results

Distribution and linkage disequilibrium analysis of MTHFR gene between studied groups

Some parameters, such as maternal age and maternal obesity, were similar among the groups (data are not shown). Some

basic parameters, such as age, BMI, hormone analysis, and oocyte number, were compared and analyzed. These data were similar between the two groups. The allele and genotype frequencies of MTHFR C677T and A1298C polymorphisms in case and control groups are shown in Table 1 and Fig. 1, respectively. No significant differences were observed in the distribution of the allele frequencies of the MTHFR gene C677T ($P = 0.760$, odds ratio = 1.044, 95% confidence interval = 0.792–1.375) and A1298C ($P = 0.130$, odds ratio = 0.784, 95% confidence interval = 0.572–1.075) between the case and control groups. The most frequent genotype of A1298C was 1298AA in both the cases and controls, but the incidence was higher in case patients. The distribution of 677CC and 677AC were similar between the two groups ($P > 0.05$). Furthermore, no significant difference was found in any of the examined haplotypes between the two groups ($P > 0.05$) (Table 2). A1298C and C677T are the two most common SNP loci in MTHFR gene. After analysis, the A1298C ($\chi^2 = 1.618$, $P > 0.05$) and C677T ($\chi^2 = 1.314$, $P > 0.05$) alleles in the control group conformed to Hardy-Weinberg equilibrium. Further linkage disequilibrium (LD) analysis demonstrated that these two alleles are correlated ($LD = 0.314$, $D' = 0.649$, $R^2 = 0.128$).

Fig. 1 Distribution of *MTHFR* C677T and A1298C genotypes in subfertile and control groups. **a** *MTHFR* A1298C genotypes in subfertile and control groups. **b** *MTHFR* C677T genotypes in subfertile and control groups. χ^2 test was used to estimate whether the genotypic frequencies differed significantly among the study groups. Significantly different groups ($P < 0.05$) are highlighted with an asterisk

Table 2 MTHFR C677T and A1298C genotypes in control and study group

MTHFR haplotype	Control group (<i>n</i> = 144) <i>n</i> , %	Subfertile group (<i>n</i> = 521) <i>n</i> , %	χ^2	<i>P</i> value	OR (95% CI)
A1298C/C677T					
AA+CC	28 (19.44)	119 (22.84)			1.0 (reference)
AA+CT	36 (25.00)	165 (31.66)	0.073	0.787	1.078 (0.624–1.864)
AA+TT	20 (13.88)	62 (11.90)	0.907	0.341	0.729 (0.380–1.398)
AC+CC	33 (22.91)	84 (16.12)	3.075	0.080	0.599 (0.337–1.065)
AC+CT	23 (15.97)	66 (12.66)	1.511	0.219	0.675 (0.360–1.266)
AC+TT	0	3 (0.57)	1.254	0.625	0.810 (0.748–0.876)
CC+CC	5 (3.47)	22 (4.22)	0.004	0.949	1.035 (0.361–2.972)

MTHFR genotypes and haplotypes increased the risk of RM and RIF

We divided the case group into three subgroups: RIF subgroup (*n* = 151), RM1 (*n* = 262), and RM2 (*n* = 108) subgroups, according to implantation failure and frequency of miscarriages. The incidence of 1298AC genotype is 25.82% in RIF subgroup and 38.88% in the control group (Table 3). However, the other genotypes were comparable in frequency among the different subgroups. The haplotype 1298AA/677CC can be found in 30.55% (33/108) in the RM2 subgroup and in 19.44% (28/144) in the control group (Table 4). The haplotype 1298AC/677CC was significantly lower in both RIF subgroup (11.25%, 17/151) when compared to the control group (22.91%, 33/144).

MTHFR haplotypes and embryo development

To assess the influence of MTHFR haplotypes on embryo development, fertilization and cleavage rates were explored. The fertilization rate was 79.5% (198/249) and embryo

cleavage rate was 96.9% (192/198) for 1298AC/677CC haplotype in the RIF subgroup. For the 1298AA/677CC haplotype in RM2 patients, the fertilization rate was 74.6% (345/462) and the embryo cleavage rate was 97.3% (336/345), and there was no significant difference compared to the other haplotypes (*P* > 0.05) (Table 5).

Discussion

Recurrent miscarriage and recurrent implantation failure are two pathologies that are complex diseases, determined by multiple factors, including genetic aspects. Several genes were found to be related to the pathology, and they are involved in the regulation of the endometrial receptivity [17], inflammation [18], and the vascular system. In recent years, increasing amounts of evidence have shown a relationship between the thrombophilia factor and low fertility in women. MTHFR is a key enzyme for homocysteine metabolism; mutations (homozygous and heterozygous) in the MTHFR (C677T and A1298C) gene result in a decrease in enzyme activity and poor

Table 3 MTHFR C677T and A1298C genotype frequencies for control group and subgroups

Genotypes	Frequency (<i>n</i> , %)			
	Control group (<i>n</i> = 144) <i>n</i> , %	RIF group (<i>n</i> = 151) <i>n</i> , %	RM (= 2 times) (<i>n</i> = 262) <i>n</i> , %	RM (> 3 times) (<i>n</i> = 108) <i>n</i> , %
1298AA	83 (57.63)	103 (68.21)	169 (64.50)	74 (68.51)
1298AC	56 (38.88)	39 (25.82)*	84 (32.06)	30 (27.77)
1298CC	5 (3.47)	9 (5.96)	9 (3.43)	4 (3.70)
677CC	66 (45.83)	59 (39.07)	111 (42.36)	55 (50.92)
677CT	59 (40.97)	74 (49.00)	115 (43.89)	42 (38.88)
677TT	19 (13.19)	18 (11.92)	36 (13.74)	11 (10.18)

χ^2 test was used to estimate whether the genotypic frequencies differed significantly among the study groups

RIF recurrent implantation failure, RM recurrent miscarriage

*Groups are significantly different to control group, χ^2 test, *P* < 0.05

Table 4 MTHFR C677T and A1298C genotypes in control and subgroups

MTHFR haplotype	Control group (<i>n</i> = 144) <i>n</i> , %	RIF group (<i>n</i> = 151) <i>n</i> , %	RM1 (= 2 times) (<i>n</i> = 262) <i>n</i> , %	RM2 (> 2 times) (<i>n</i> = 108) <i>n</i> , %
A1298C/C677T				
AA+CC	28 (19.44)	33 (21.85)	53 (20.22)	33 (30.55)*
AA+CT	36 (25.00)	52 (34.43)	83 (31.67)	30 (27.77)
AA+TT	20 (13.88)	19 (12.58)	33 (12.59)	11 (10.18)
AC+CC	33 (22.91)	17 (11.25)*	49 (18.70)	18 (16.66)
AC+CT	23 (15.97)	22 (14.56)	33 (12.59)	12 (11.11)
AC+TT	0	0	3 (1.14)	0
CC+CC	5 (3.47)	9 (5.96)	9 (3.43)	4 (3.70)

stability, impairing folic acid metabolism, leading to an accumulation of homocysteine, results in increase blood tendency. In the present study, the MTHFR alleles C677T and A1298C were observed at similar frequencies between the case and control groups; the genotype of 1298AC was significantly lower in RIF subgroup; the 1298AA/677CC and 1298AC/677CC haplotypes in RM2 and RIF were significantly different to the control group, but no adverse effects on the embryo development were observed. Previous studies have shown that RM and pregnancy complications, such as severe pre-eclampsia [13], fetal growth restriction, RM, RIF, and stillbirth, are higher among patients with either inherited or acquired thrombophilia defects [19, 20]. A possible mechanism for this could be thrombosis of maternal vessels, which might reduce the perfusion of the intervillous space leading to placental failure. It is possible that implantation failure of embryos during IVF may be caused by similar mechanisms. Thrombosis in the placental vessels leads to hypoperfusion of the intervillous space and may cause placental dysfunction. Our data demonstrate that the alleles of MTHFR C677T and A1298C were not associated with risk factors for implantation failure and recurrent miscarriage, which is in agreement with what was reported by Holmes et al. [21]. There are few

published reports on the association between the MTHFR genotypes and embryo development. In this study, the combined genotypes 1298AA/677CC were significantly higher in the RM2 subgroup, which suggests that this haplotype is a risk factor, and similar results were obtained by Barbosa et al. [22]. However, the combined genotypes 1298AC/677CC were lower in the RIF subgroup, which indicates that this haplotype is a protective factor in that group. A recent study reported that the 1298AA/677CC haplotype can increase the risk of embryo aneuploidy and result in a high risk of miscarriage and low implantation failure [23, 24].

Conclusion

In summary, our results demonstrated that MTHFR gene polymorphisms play a vital role in embryo implantation. Importantly, no adverse effects on embryo development were found. MTHFR haplotypes may be considered as biomarkers for RM and RIF, but further studies on the biological role of MTHFR are needed to better understand the biology of recurrent implantation failures.

Table 5 Mean embryo fertilization and cleavage rate (mean ± SEM) of patients with different MTHFR C677T/A1298T haplotypes

MTHFR haplotype		Embryo fertilization and cleavage rate					
		1298AA/677CC haplotypes (<i>n</i> %)	Other haplotypes (<i>n</i> %)	<i>P</i> value	1298AC/677CC haplotypes (<i>n</i> %)	Other haplotypes (<i>n</i> %)	<i>P</i> value
Control group	Fertilization (%)	78.5 (275/350)	76.4 (1170/1531)	0.390	74.8 (170/227)	77.0 (1275/1654)	0.462
	Cleavage rate (%)	96.3 (265/275)	95.7 (1120/1170)	0.634	94.7 (161/170)	96.0 (1224/1275)	0.427
RIF group	Fertilization (%)	—	—	—	79.5 (198/249)	75.6 (1334/1764)	—
	Cleavage rate (%)	—	—	—	96.9 (192/198)	96.1 (1283/1334)	—
RM2 (> 2 times)	Fertilization (%)	74.6 (345/462)	75.4 (847/1123)	0.754	—	—	—
	Cleavage rate (%)	97.3 (336/345)	96.4 (817/847)	0.411	—	—	—

Acknowledgements We are very grateful to all participants in this study and we thank all the clinical staff. We also thank all the members of the Zhang and Zeng group for their interesting discussions, helpful suggestions, and technical support.

Authors' contributions YOZ and YZ: supervised and supported the study. YCZ: designed and wrote the manuscript. GGL: revised the manuscript. THW: data collection. LJY: PCR assay. All authors read and approved the final manuscript.

Funding This study was supported by the National Natural Science Foundation of China (No. 31371315, 2014; No. 81601279, 2016) and International Cooperation Grant of Shenzhen (No. GJHZ20140416153718941, 2014).

Compliance with ethical standards

Ethics approval and consent to participate The research project met our ethics criteria and was approved and performed strictly according to the protocols. Informed consent was obtained from all couples prior to the study.

Consent for publication The ethical committee reviewed the manuscript and consent for publication.

Competing interests The authors declare that they have no conflicts of interest.

Abbreviations MTHFR, methylenetetrahydrofolate reductase; RM, recurrent miscarriage; RIF, repeated implantation failure; IVF, in vitro fertilization; ET, embryo transfers

References

- Practice Committee of the American Society for Reproductive, M. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertil Steril*. 2012;98:1103–11.
- Rai R, Regan L. Recurrent miscarriage. *Lancet*. 2006;368:601–11.
- Stephenson M, Kutteh W. Evaluation and management of recurrent early pregnancy loss. *Clin Obstet Gynecol*. 2007;50:132–45.
- Tang AW, Quenby S. Recent thoughts on management and prevention of recurrent early pregnancy loss. *Curr Opin Obstet Gynecol*. 2010;22:446–51.
- Simon A, Laufer N. Repeated implantation failure: clinical approach. *Fertil Steril*. 2012;97:1039–43.
- Ghosh S, Chattopadhyay R, Goswami S, Ganesh A, Chaudhury K, Chakravarty B. Recurrent implantation success in consecutive embryo transfer cycles: a unique case report. *Syst Biol Reprod Med*. 2013;59:285–6.
- RoyChoudhury S, Singh A, Gupta NJ, Srivastava S, Joshi MV, Chakravarty B, et al. Repeated implantation failure versus repeated implantation success: discrimination at a metabolomic level. *Hum Reprod*. 2016;31:1265–74.
- Obican SG, Finnell RH, Mills JL, Shaw GM, Scialli AR. Folic acid in early pregnancy: a public health success story. *FASEB J*. 2010;24:4167–74.
- Yang B, Liu Y, Li Y, Fan S, Zhi X, Lu X, et al. Geographical distribution of MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. *PLoS One*. 2013;8:e57917.
- Abd El-Aziz TA, Mohamed RH. Influence of MTHFR C677T gene polymorphism in the development of cardiovascular disease in Egyptian patients with rheumatoid arthritis. *Gene*. 2017;610:127–32.
- Zappacosta B, Graziano M, Persichilli S, Di Castelnuovo A, Mastroiacovo P, Iacoviello L. 5,10-Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms: genotype frequency and association with homocysteine and folate levels in middle-southern Italian adults. *Cell Biochem Funct*. 2014;32:1–4.
- Fatini C, Conti L, Turillazzi V, Sticchi E, Romagnuolo I, Milanini MN, et al. Unexplained infertility: association with inherited thrombophilia. *Thromb Res*. 2012;129:e185–8.
- Ge J, Wang J, Zhang F, Diao B, Song ZF, Shan LL, et al. Correlation between MTHFR gene methylation and pre-eclampsia, and its clinical significance. *Genet Mol Res*. 2015;14:8021–8.
- Azem F, Many A, Ben Ami I, Yovel I, Amit A, Lessing JB, et al. Increased rates of thrombophilia in women with repeated IVF failures. *Hum Reprod*. 2004;19:368–70.
- Skeith L, Carrier M, Kaaja R, Martinelli I, Petroff D, Schleussner E, et al. A meta-analysis of low-molecular-weight heparin to prevent pregnancy loss in women with inherited thrombophilia. *Blood*. 2016;127:1650–5.
- Christiansen OB, Nielsen HS, Kolte AM. Future directions of failed implantation and recurrent miscarriage research. *Reprod BioMed Online*. 2006;13:71–83.
- Celik O, Unlu C, Otlu B, Celik N, Caliskan E. Laparoscopic endometrioma resection increases peri-implantation endometrial HOXA-10 and HOXA-11 mRNA expression. *Fertil Steril*. 2015;104:356–65.
- Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. *Hum Reprod Update*. 2016;22:104–15.
- Cao Y, Xu J, Zhang Z, Huang X, Zhang A, Wang J, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. *Gene*. 2013;514:105–11.
- Yang Y, Luo Y, Yuan J, Tang Y, Xiong L, Xu M, et al. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. *Arch Gynecol Obstet*. 2016;293:1197–211.
- Holmes ZR, Regan L, Chilcott I, Cohen H. The C677T MTHFR gene mutation is not predictive of risk for recurrent fetal loss. *Br J Haematol*. 1999;105:98–101.
- Barbosa PR, Stabler SP, Machado AL, Braga RC, Hirata RD, Hirata MH, et al. Association between decreased vitamin levels and MTHFR, MTR and MTRR gene polymorphisms as determinants for elevated total homocysteine concentrations in pregnant women. *Eur J Clin Nutr*. 2008;62:1010–21.
- Chatzidimitriou M, Chatzidimitriou D, Mavridou M, Anetakis C, Chatzopoulou F, Lialiaris T, et al. Thrombophilic gene polymorphisms and recurrent pregnancy loss in Greek women. *Int J Lab Hematol*. 2017;39:590–5.
- Enciso M, Sarasa J, Xanthopoulou L, Bristow S, Bowles M, Fragouli E, et al. Polymorphisms in the MTHFR gene influence embryo viability and the incidence of aneuploidy. *Hum Genet*. 2016;135:555–68.