Comparison of different types of twin pregnancies in terms of obstetric and perinatal outcomes: association of vanished twins with methylenetetrahydrofolate reductase (MTHFR) polymorphism(s)

Murat Aykut Ozek^{1,2} · Ergun Karaagaoglu³ · Gokcen Orgul¹ · Fatma Gumruk⁴ · Murat Yurdakök⁵ · M. Sinan Beksac¹

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Abstract

Purpose Vanished twin (VT) has been associated with poor perinatal outcomes. Our research aimed to investigate the outcomes of pregnancies with vanished twin and its possible association with methylenetetrahydrofolate reductase (MTHFR) polymorphisms.

Methods This study consisted of 30 of 38 VT pregnancies (group 1, VT group), 109 singletons (group 2), 70 spontaneous twins (group 3), and 101 in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) twins (group 4).

Results Most patients in group 1 (28/30) were tested for MTHFR genes (C677T or A1298C polymorphisms). Eight of the 38 pregnancies with VT (21.1%) resulted in miscarriage. The prevalence of “2 or more pregnancy losses” in the “obstetric history” in group 1 was higher (23.3%) than those in the other groups ($p = 0.007$, $\chi^2 = 17.8$). The allelic frequencies of MTHFR 677 and MTHFR 1298 in group 1 were 0.268 and 0.429, respectively (higher than those in healthy population). The median birthweights in groups 1, 2, 3, and 4 were 2940, 3200, 2300, and 2095 g, respectively. The prevalence of respiratory distress syndrome was significantly higher in the IVF/ICSI twin pregnancy group ($p < 0.001$, $\chi^2 = 21.2$). Early pregnancy loss and the presence of “2 or more miscarriages” in the obstetric history of pregnancies with VT were more frequent.

Conclusion The coincidence of VT and MTHFR polymorphisms might play an incidental or factual role in this connection.

Keywords Multiple pregnancies · Vanished twin · Methylenetetrahydrofolate reductase polymorphism · Miscarriage

Introduction

The vanished twin (VT) phenomenon is defined by the disappearance of an entire gestational sac or one of the fetuses after detection of cardiac activity during multiple pregnancies [1, 2].

Previous studies showed that the prevalence of this phenomenon is approximately 30% among pregnancies that started with two sacs/embryos [3–5]. Evidence exists about VTs and triplets, and that these pregnancies have poorer obstetric and perinatal outcomes than other pregnancies with the same number of gestations without this complication [3]. The pathophysiology behind VT is thought to be the same as that of miscarriages, which are mainly due to genetic disorders that lead to formation of blighted ovum and early pregnancy loss (EPL) [2, 6]. A relationship between “methylation pathway disorders” (MTHFR polymorphisms) and poor pregnancy outcomes (repeated miscarriages, intrauterine growth retardation, preterm delivery, preeclampsia, and ablation placenta), as well as chromosomal abnormalities and congenital malformations, has also been reported [7–11].

Methylenetetrahydrofolate reductase (MTHFR) reduces “5,10-methylenetetrahydrofolate” (5,10-methylene-THF) to “5-methyltetrahydrofolate” (5-methyl-THF), which is responsible for folate and vitamin B₁₂-dependent remethylation of homocysteine to methionine through the activation of methionine synthase [12]. Thus, MTHFR polymorphism has been

✉ Murat Aykut Ozek
aykut.spm@gmail.com

¹ Division of Perinatal Medicine, Department of Obstetrics and Gynecology, Hacettepe University Medical Faculty, Ankara, Turkey

² Gazi University School of Medicine,
06500 Besevler-Ankara, Turkey

³ Department of Biostatistics, Hacettepe University Medical Faculty, Ankara, Turkey

⁴ Division of Hematology Department of Pediatrics, Hacettepe University Medical Faculty, Ankara, Turkey

⁵ Division of Neonatology, Department of Pediatrics, Hacettepe University Medical Faculty, Ankara, Turkey

reported to be associated with the reduction of the enzyme activity, which leads to increased levels of homocysteine, impaired methionine metabolism, and abnormal methylation processes of nucleotides/DNA, which are most probably responsible for chromosome damage, congenital abnormalities, and miscarriage [10, 13–16]. We believe that multiple pathophysiological processes are responsible from the formation of VT, and MTHFR polymorphisms might be one of the rationales behind these pathological conditions. MTHFR polymorphisms are also risk factors of thrombotic events, and special care is necessary in VT cases with this type of risk.

In this study, we evaluated the outcomes of “VT gestations” and compared them with those of singleton pregnancies, spontaneous twins, and twins conceived through assisted reproductive techniques (ART). We also investigated the possible relationship between the VT phenomenon and maternal polymorphisms of MTHFR genes.

Materials and methods

This retrospective study was composed of 30 VT pregnancies (group 1), whereas the control groups were composed of 109 singleton pregnancies (group 2), 70 spontaneous twin pregnancies (group 3), and 101 in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) twin pregnancies (group 4). The patients in group 2 were randomly selected among all singletons eligible during the study period. The perinatal medicine registry and the University Hospital Electronic Database of Hacettepe University, Ankara, Turkey, were used for data withdrawal between January 2010 and December 2012. Next, we retrospectively evaluated the neonatal outcomes of 30 VT pregnancies (after the exclusion of eight early pregnancy losses among the 38 VT cases) and compared these with those of 109 singleton pregnancies (group 2), 70 spontaneous twins (group 3), and 101 IVF/ICSI twins (group 4). The demographic characteristics and medical and obstetric histories of the pregnant women were also recorded for further evaluation. Study groups were evaluated in terms of gestational age, birthweight, admission to the neonatal intensive care unit (NICU), retinopathy of prematurity (RoP), respiratory distress syndrome (RDS), intracranial hemorrhage (ICH), necrotizing enterocolitis (NEC), indirect hyperbilirubinemia (IHB), and sepsis.

At the beginning of the study, 38 patients had VT. Two of these cases were obtained from the Primary Antenatal Care Medicine Registry (PACMR; without MTHFR polymorphism results), and 36 were from the Perinatal Medicine Registry (PMR) and had full laboratory results. As described earlier, group 1 included 30 VT cases (2 PACMR and 28 from the PMR) after the exclusion of eight pregnancies with EPL.

Twenty-eight of the 30 VT cases were withdrawn from the PMR, which consisted of high-risk pregnancies and pregnancies with bad obstetric history (with full laboratory results). Searching

for some genetic polymorphisms, including MTHFR mutation(s), is part of our routine practice for pregnancies with bad obstetric history (repeated miscarriages and obstetric complications) and in pregnant women with risk factors of thrombotic events. Inherently, follow-up protocols of perinatal medicine patients are different from the antenatal care program protocols of the obstetrics department. The number of ultrasonographic examinations and the type of laboratory tests also differed between the two different patient populations.

Almost all the patients (28/30) in group 1 had a laboratory workup for MTHFR polymorphism(s). Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) methods were used for the analyses of MTHFR polymorphisms (Applied Biosystems 3130/3130xl-Hitachi, 2007) [17–19]. Unfortunately, information regarding MTHFR polymorphisms was not available in the other groups. In addition, the necessary laboratory test results (factor V Leiden and prothrombin 20210A mutations, and antithrombin III activity, active protein C resistance, vitamin B₁₂, folate, thyroid function tests, [auto]-antibodies [ANA, anti-cardiolipins, anti-phospholipids, APA, ASMA, Anti dsDNA, AMA, etc.], blood counts, liver enzymes, and blood groups) were also recorded in the VT group. All those tests were performed in the same laboratory.

The VT cohort consisted of patients with MTHFR polymorphisms who were carefully assigned to a specific treatment/management protocol (methionine-restricted diet, 100 mg/day salicylic acid, vitamins B₁, B₂, B₃, B₆, B₉, and B₁₂ intakes) at least 3 months before getting pregnant, and low-dose low-molecular-weight heparin (LMWH; enoxaparine 1 × 2000 Anti-XA IU/0.2 ml/day) was started early in the subsequent pregnancies. Pre-pregnancy preparation and management were missing only in two patients (2/38, 5.3%) who were referred from the antenatal care program.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. For comparison of the four groups in terms of qualitative data (e.g., NICU admission rate, and RDS rate), chi-square analysis was performed. A *p* value of < 0.05 was considered statistically significant. When a significant difference among the groups was found, the largest chi-square value was calculated to define the group, which made the difference. For quantitative data (e.g., birthweight), a between-group comparison was performed by using the Kruskal–Wallis test. The distributions of gestational ages and birthweights were not normal among the groups, so the median, minimum, and maximum values of these variables were used.

This study was approved by the institutional review board of Hacettepe University (GO 13/152-15).

Results

Eight of the 38 pregnancies with VT (21.1%) resulted in EPL, and the remaining 30 pregnant women delivered at our

hospital (group 1). The VT group (group 1) was composed of ten IVF/ICSI pregnancies (10/30, 33.3%) and 20 spontaneous pregnancies (20/30, 66.6%).

After the exclusion of two patients without MTHFR polymorphism test results, we found that 85.7% (24/28) of the VT pregnancies delivered at our hospital had homozygous, compound heterozygous, and heterozygous MTHFR polymorphisms. The allelic frequency of MTHFR 677 and MTHFR 1298 in group 1 were 0.268 and 0.429, respectively. Moreover, 10.7% (3/28) and 14.3% (4/28) of the VT cases were found to be homozygous for the MTHFR C677T and A1298C polymorphisms, respectively.

In this study, we demonstrated higher rate (23.3%) of “2 or more pregnancy losses” in the obstetric history of group 1 (VT pregnancies) than in those of the other groups ($p = 0.007$, $\chi^2 = 17.8$). Table 1 shows the EPL rates of the study and control groups.

We found that the median gestational ages at birth were 36.0, 38.0, 35.0, and 34.0 weeks for groups 1, 2, 3, and 4, respectively. The number of neonates were 30, 109, 136, and 194 in the groups, respectively, while the stillbirth rates were 0% (0/30), 0% (0/109), 2.9% (4/140), and 4% (6/202) in the groups, respectively. Our results also show that the median birth weights in groups 1, 2, 3, and 4 were 2940, 3200, 2300, and 2095 g, respectively.

The neonatal outcome findings are presented in Table 2. The rate of admission to the NICU was significantly different between the groups ($p < 0.001$, $\chi^2 = 112.2$). The singletons had the lowest rate of admission to the NICU (15.6%), so this group caused the difference ($\chi^2 = 75.6$). The prevalence of RDS was significantly higher in the IVF/ICSI twin pregnancy group ($p < 0.001$, $\chi^2 = 21.2$). The prevalences of ICH and NEC were not significantly different between the groups ($p = 0.416$ and $p = 0.461$, respectively). Singletons had the lowest prevalence rate of IHB, and this was statistically

significant ($p = 0.001$, $\chi^2 = 19.2$). The prevalence of sepsis was not significantly different between the groups ($p = 0.465$). None of the newborns in this study had RoP, so statistical analysis could not be conducted.

The MTHFR polymorphisms of 28 of the 30 VT pregnancies are presented in Table 3. Unexpectedly, only 14.3% (4/28) of the VT pregnancies had no MTHFR polymorphisms. Of the VT cases, 53.6% were found to be homozygous for MTHFR C677T or A1298C polymorphisms, or to be compound heterozygous (Table 3). The mean levels of homocysteine, folic acid, and cobalamine in the VT group were $10.24 \pm 2.67 \mu\text{mol/L}$, $15.86 \pm 4.64 \text{ ng/mL}$, $305.96 \pm 92.81 \text{ pmol/L}$, respectively.

We found that the newborns of the patients homozygous for the MTHFR C677T or A1298C polymorphisms or compound heterozygous had significantly higher rates of IHB ($p = 0.044$; Table 4).

Discussion

The biological facts behind VT are not clear, although this pathological condition is known for a long time [1, 20]. The result of VT due to some factors (genetic, inflammatory, metabolic, etc.) is the presence of necrotic tissue in utero. The presence of necrotic tissue is generally concurrent with inflammatory processes and thrombotic events, and may trigger various obstetric complications. Thus, the clinical importance of VT is the concern of physicians due to the nature of the problem.

In this study, we compared the obstetric histories and pregnancy outcomes of VT gestations with singletons and twin gestations (spontaneous and IVF/ICSI) to define a biological model for the occurrence of this pathological condition. During the organization of the study, we noticed that VT pregnancies came from a special type of patient population (patients with bad obstetric history). For this reason, we searched for VT pregnancies from “the presence of MTHFR polymorphisms” point of view by thinking that both VT and MTHFR polymorphisms may be risk factors of thrombotic events and bad obstetric outcome.

An association has been described between VT and preterm delivery [3, 4, 20]. Meanwhile, an association between MTHFR polymorphisms and bad pregnancy outcomes (miscarriages and various obstetric complications including IUGR, preterm delivery, and preeclampsia) has also been reported [10, 11]. In this study, we demonstrated that the allelic frequencies of MTHFR 677 and MTHFR 1298 were 0.268 and 0.429, respectively, which were higher than those in the general populations [21, 22]. We have previously published the prevalence of MTHFR polymorphism(s) in our hospital population, which was consisted of 10,449 cases with various thrombotic risk factors (coronary artery diseases, thrombotic

Table 1 Comparison of the groups in terms of obstetric history of early pregnancy loss (EPL)

Groups		Number of EPLs in the obstetric history			Total
		0	1	≥ 2	
Group 1	N	20	3	7	30
	% in the group	66.7	10	23.3	100
Group 2	N	91	14	4	109
	% in the group	83.5	12.8	3.7	100
Group 3	N	55	11	4	70
	% in the group	78.6	15.7	5.7	100
Group 4	N	82	15	4	101
	% in the group	81.2	14.9	3.9	100
Total	N	261	43	26	330
	% in the group	79.1	13	7.9	100

Table 2 Obstetric and perinatal outcomes of the patient groups

	Group 1	Group 2	Group 3	Group 4	<i>p</i> value
Number of alive neonates/pregnancies	30/30	109/109	136/70	194/101	
NICU admission (%)	11 (36.7)	17 (15.6)	98 (71)	144 (72.7)	<i>p</i> < 0.001
RDS (%)	0 (0)	4 (3.7)	16 (11.8)	38 (19.6)	<i>p</i> < 0.001
ICH (%)	0 (0)	0 (0)	0 (0)	2 (1)	<i>p</i> = 0.416
NEC (%)	0 (0)	0 (0)	1 (0.7)	7 (3.6)	<i>p</i> = 0.061
IHB (%)	5 (16.7)	9 (8.3)	37 (27.2)	56 (28.9)	<i>p</i> < 0.001
Sepsis (%)	2 (6.7)	3 (2.8)	4 (2.9)	11 (5.7)	<i>p</i> = 0.465
RoP (%)	0 (0)	0 (0)	0 (0)	0 (0)	N/A

Group 1: VT group, group 2: singletons, group 3: spontaneous twin gestations, group 4: IVF/ICSI twin gestations

events, repeated miscarriages, etc.) [23]. The allelic frequencies of MTHFR 677 and MTHFR 1298 were 0.296 and 0.283, respectively [23]. There seems to be an association between VT occurrence and MTHFR polymorphisms, especially with MTHFR 1298 mutation. We also showed that 85.7% of VT pregnancies had homozygous, compound heterozygous, or heterozygous MTHFR polymorphisms.

In this study, we showed that 21.1% of VT pregnancies resulted in miscarriage. We also demonstrated a higher rate (23.3%) of “2 or more pregnancy losses” (repeated miscarriage) in the “obstetric history” of VT pregnancies than in the other groups (*p* = 0.007, $\chi^2 = 17.8$). Some publications reported an association between MTHFR polymorphisms and miscarriages [9, 24]. The high rate of miscarriage in the VT cases might be due the presence of MTHFR polymorphisms in these cases.

Previous studies reported that VT is a risk factor of lower birthweight than those of singleton pregnancies [4, 25]. In our study, the birthweights of the neonates in the VT group were lower than those of the singletons but better than those of other twin pregnancies as reported in other studies [20]. The reason of the lower birthweights in twin pregnancies has been reported to be most probably early placental crowding [25]. In this study, the outcomes of the VT pregnancies were not statistically significantly different from those of the singletons, most probably because almost all these cases were registered pre-conceptionally and were under specific care because of their MTHFR polymorphisms.

Table 3 MTHFR polymorphisms in the VT group

MTHFR polymorphisms	<i>N</i>	%
Normal	4	14.3
Heterozygous A1298C	8	28.6
Heterozygous C677T	1	3.6
Heterozygous 677 + 1298 (compound heterozygous)	8	28.6
Homozygous A1298C	4	14.3
Homozygous C677T	3	10.7
Total	28	100

MTHFR is a critical enzyme that plays a role in folate metabolism and participates in “methylation-related enzyme pathways.” It converts dietary folate (methylentetrahydrofolate) to active folate, which is the coenzyme of methionine synthase together with vitamin B₁₂ [26, 27]. This pathway is also critical in the methylation of nucleotide/DNA, which is important in regular DNA synthesis.

MTHFR polymorphisms may cause various medical disorders through different mechanisms [28–30]. One of the mechanisms is “hyperhomocysteinemia,” which goes together with endothelial injury of vascular structures of different organs. Homocysteine is an endothelial-toxic amino acid, which is metabolized through methylation and trans-sulfurylation processes. It is accepted that hyperhomocysteinemia induces cardiac and cerebrovascular diseases. Those bloodstream problems associated with endothelial injury are also valid for placental vascular bed. Imbalance in homocysteine metabolism which is associated with reduced levels of *S*-adenosyl methionine (SAM) also leads to methylation problems in DNA by means of impaired one carbon metabolism associated with fetal malformations and aneuploidy [31].

MTHFR polymorphisms also affect DNA synthesis by various routes, one of which is impaired DNA methylation, as mentioned earlier. The other mechanism is the accumulation of

Table 4 Comparison of obstetric and perinatal outcomes in the VT group

	Normal/ heterozygous (%)	Homozygous or compound heterozygous (%)	<i>p</i> value
Number of patients (28)	13 (46.4)	15 (53.6%)	
NICU admission	4 (30.8)	7 (46.7)	0.39
RDS	0 (0)	0 (0)	N/A
ICH	0 (0)	0 (0)	N/A
NEC	0 (0)	0 (0)	N/A
IHB	0 (0)	5 (33.3)	0.044
Sepsis	0 (0)	2 (13.3)	0.484
RoP	0 (0)	0 (0)	N/A

dietary folate (methylenetetrahydrofolate), which may cause adverse effects on dihydrofolate dehydrogenase- and thymidylate synthase-related pathways and DNA synthesis [26, 32, 33]. Chromosomal abnormalities have been reported to be responsible for first-trimester miscarriages in singleton pregnancies [34]. It was shown that MTHFR polymorphisms are associated with production of aneuploid embryos, implantation failures and may affect viability of embryos [16]. This might also be valid for VT pregnancies with MTHFR polymorphisms. Defective methylation associated with MTHFR polymorphisms contributes fertility problems also by means of defective spermatogenesis and impaired embryonic development [35, 36].

DNA methylation is involved in epigenetic control of gene expression [37]. Impaired methylation of DNA interferes expression of genes involved in trophoblastic functions including early trophoblastic differentiation and invasion leading to compromised placental development [38, 39]. Impaired invasion of interstitial cytotrophoblasts and defective remodeling of maternal spiral arteries were also thought to lead pregnancy loss [40].

The rate of admission to the NICU is increased in twin pregnancies [41, 42]. In this study, we found similar results. We also found that the rate of respiratory distress syndrome (RDS) was significantly higher in the pregnancies with assisted reproductive technologies (ART). The only adverse finding in VT pregnancies was the “higher rate of IHB” in the patients with homozygous MTHFR polymorphisms.

In our study population, most VT pregnancies were under specific care because of their known “bad obstetric histories” and MTHFR polymorphisms, and this might be the reason of the favorable outcomes in these patients. The coincidence of VT and MTHFR polymorphisms might be incidental or factual natural connection.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Jauniaux E, Elkazen N, Leroy F, Wilkin P, Rodesch F, Hustin J. Clinical and morphologic aspects of the vanishing twin phenomenon. *Obstet Gynecol*. 1988;72(4):577–81.
- Landy H, Keith L. The vanishing twin: a review. *Hum Reprod Update*. 1998;4(2):177–83.
- Dickey RP, Taylor SN, Lu PY, Sartor BM, Stoment JM, Rye PH, et al. Spontaneous reduction of multiple pregnancy: incidence and effect on outcome. *Am J Obstet Gynecol*. 2002;186(1):77–83.
- Pinborg A, Lidegaard Ø, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod*. 2005;20(10):2821–9.
- Van Oppenraaij R, Jauniaux E, Christiansen O, Horcajadas J, Farquharson R, Exalto N. Predicting adverse obstetric outcome after early pregnancy events and complications: a review. *Hum Reprod Update*. 2009;15(4):409–21.
- Jauniaux E, Van Oppenraaij RH, Burton GJ. Obstetric outcome after early placental complications. *Curr Opin Obstet Gynecol*. 2010;22(6):452–7.
- Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. *Eur J Obstet Gynecol Reprod Biol*. 2002;101(1):6–14.
- van Beynum IM, Kapusta L, den Heijer M, Vermeulen SH, Kouwenberg M, Daniëls O, et al. Maternal MTHFR 677C>T is a risk factor for congenital heart defects: effect modification by periconceptional folate supplementation. *Eur Heart J*. 2006;27(8):981–7.
- Wu X, Zhao L, Zhu H, He D, Tang W, Luo Y. Association between the MTHFR C677T polymorphism and recurrent pregnancy loss: a meta-analysis. *Genet Test Mol Biomarkers*. 2012;16(7):806–11.
- Nair RR, Khanna A, Singh R, Singh K. Association of maternal and fetal MTHFR A1298C polymorphism with the risk of pregnancy loss: a study of an Indian population and a meta-analysis. *Fertil Steril*. 2013;99(5):1311–8. e4.
- Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland homocysteine study. *Am J Med*. 2004;117(1):26–31.
- Bhargava S, Tyagi S. Nutriepigenetic regulation by folate-homocysteine-methionine axis: a review. *Mol Cell Biochem*. 2014;387(1–2):55–61.
- Kim SY, Park SY, Choi JW, Kim DJ, Lee SY, Lim JH, et al. Association between MTHFR 1298A>C polymorphism and spontaneous abortion with fetal chromosomal aneuploidy. *Am J Reprod Immunol*. 2011;66(4):252–8.
- Coppedè F, Migheli F, Bargagna S, Siciliano G, Antonucci I, Stuppia L, et al. Association of maternal polymorphisms in folate metabolizing genes with chromosome damage and risk of down syndrome offspring. *Neurosci Lett*. 2009;449(1):15–9.
- Rodríguez-Guillén MR, Torres-Sánchez L, Chen J, Galván-Portillo M, Blanco-Muñoz J, Anaya MA, et al. Maternal MTHFR polymorphisms and risk of spontaneous abortion. *Salud Publica Mex*. 2009;51(1):19–25.
- 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(5):555–68. <https://doi.org/10.1007/s00439-016-1652-z>.
- Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10(1):111–3. <https://doi.org/10.1038/ng0595-111>.
- Machnik G, Zapala M, Pelc E, Gasecka-Czapla M, Kaczmarczyk G, Okopien B. A new and improved method based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for the determination of A1298C mutation in the methylenetetrahydrofolate reductase (MTHFR) gene. *Ann Clin Lab Sci*. 2013;43(4):436–40.
- Turgal M, Gumruk F, Karaagaoglu E, Beksac MS. Methylenetetrahydrofolate reductase polymorphisms and pregnancy outcome. *Geburtshilfe Frauenheilkd*. 2018;78(09):871–8.
- Almog B, Levin I, Wagman I, Kapustiansky R, Lessing JB, Amit A, et al. Adverse obstetric outcome for the vanishing twin syndrome. *Reprod BioMed Online*. 2010;20(2):256–60.
- Sazci A, Ergul E, Kaya G, Kara I. Genotype and allele frequencies of the polymorphic methylenetetrahydrofolate reductase gene in Turkey. *Cell Biochem Funct*. 2005;23(1):51–4.
- Djordjevic V, Rakicevic L, Mikovic D, Kovac M, Miljic P, Radojkovic D, et al. Prevalence of factor V leiden, factor V Cambridge, factor II G20210A and methylenetetrahydrofolate reductase C677T mutations in healthy and thrombophilic Serbian populations. *Acta Haematol*. 2004;112(4):227–9.

23. Gumruk F, Orgul G, Dogan OA, Tanacan A, Karaagaoglu E, Beksac MS. The prevalence of homozygous MTHFR polymorphism (s) in a Turkish university hospital population that necessitated MTHFR polymorphism investigation. *Electron J Gen Med*. 2018;15(4).
24. Aracic N, Roje D, Drmic Hofman I, Capkun V, Stefanovic V. Low molecular weight heparin treatment and impact of inherited thrombophilia type in pregnancies with previous adverse outcome. *J Matern Fetal Neonatal Med*. 2015;28(3):306–10.
25. Pinborg A, Lidegaard Ø, la Cour Freiesleben N, Andersen AN. Vanishing twins: a predictor of small-for-gestational age in IVF singletons. *Hum Reprod*. 2007;22(10):2707–14.
26. Hoffer LJ. Homocysteine remethylation and trans-sulfuration. *Metabolism*. 2004;53(11):1480–3.
27. Şahin TG, Sayal B, Coşgun E, Besler T, Beksac S. Methylenetetrahydrofolate reductase enzyme mutations and relationship of homocysteine Vitamin B12 and folate blood levels. *Gynecol Obstet Reprod Med*. 2016;19(1).
28. Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Hine RJ, et al. Polymorphisms in genes involved in folate metabolism as maternal risk factors for down syndrome. *Am J Hum Genet*. 2000;67(3):623–30.
29. Crott JW, Mashiyama ST, Ames BN, Fenech M. The effect of folic acid deficiency and MTHFR C677T polymorphism on chromosome damage in human lymphocytes in vitro. *Cancer Epidemiol Biomarkers Prev*. 2001;10(10):1089–96.
30. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. 1995.
31. Skovierova H, Vidomanova E, Mahmood S, Sopkova J, Drgova A, Cervenova T, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. *Int J Mol Sci*. 2016;17(10). <https://doi.org/10.3390/ijms17101733>.
32. Harpel PC, Zhang X, Borth W. Homocysteine and hemostasis: pathogenic mechanisms predisposing to thrombosis. *J Nutr*. 1996;126(4S):1285S–9S.
33. Stover PJ. One-carbon metabolism–genome interactions in folate-associated pathologies. *J Nutr*. 2009;139(12):2402–5.
34. Ljunger E, Cnattingius S, Lundin C, Annerén G. Chromosomal anomalies in first-trimester miscarriages. *Acta Obstet Gynecol Scand*. 2005;84(11):1103–7.
35. Cornet D, Cohen M, Clement A, Amar E, Fournols L, Clement P, et al. Association between the MTHFR-C677T isoform and structure of sperm DNA. *J Assist Reprod Genet*. 2017;34(10):1283–8. <https://doi.org/10.1007/s10815-017-1015-2>.
36. Kobayashi H, Sato A, Otsu E, Hiura H, Tomatsu C, Utsunomiya T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. *Hum Mol Genet*. 2007;16(21):2542–51. <https://doi.org/10.1093/hmg/ddm187>.
37. Serman L, Dodig D. Impact of DNA methylation on trophoblast function. *Clin Epigenetics*. 2011;3:7. <https://doi.org/10.1186/1868-7083-3-7>.
38. Georgiades P, Watkins M, Burton GJ, Ferguson-Smith AC. Roles for genomic imprinting and the zygotic genome in placental development. *Proc Natl Acad Sci U S A*. 2001;98(8):4522–7. <https://doi.org/10.1073/pnas.081540898>.
39. Branco MR, King M, Perez-Garcia V, Bogutz AB, Caley M, Fineberg E, et al. Maternal DNA methylation regulates early trophoblast development. *Dev Cell*. 2016;36(2):152–63. <https://doi.org/10.1016/j.devcel.2015.12.027>.
40. Hustin J, Jauniaux E, Schaaps JP. Histological study of the materno-embryonic interface in spontaneous abortion. *Placenta*. 1990;11(6):477–86.
41. Petit N, Cammu H, Martens G, Papiemik E. Perinatal outcome of twins compared to singletons of the same gestational age: a case-control study. *Twin Res Hum Genet*. 2011;14(01):88–93.
42. Rydhstroem H, Heraib F. Gestational duration, and fetal and infant mortality for twins vs singletons. *Twin Res*. 2001;4(04):227–31.