

# Increased rates of thrombophilia in women with repeated IVF failures

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**BACKGROUND:** We investigated whether hereditary thrombophilia is more prevalent in women with recurrent IVF-embryo transfer failures. **METHODS:** This case-control study was conducted in an academic tertiary care hospital and compared 45 women with a history of four or more failed IVF cycles (group A) with 44 apparently healthy women matched for age and ethnic origin (group B). All participants were tested for inherited thrombophilias: mutations of prothrombin, factor V Leiden and methylene tetrahydrofolate reductase (MTHFR), and protein C, protein S and antithrombin III deficiencies. **RESULTS:** Excluding homozygotic MTHFR, the incidence of thrombophilia in group A, was 26.7% compared with 9.1% in group B ( $P = 0.003$ ; odds ratio 2.9; 95% confidence interval 1.02–8.4). The incidence of thrombophilia in women with unexplained infertility in group A was 42.9% (9/21), compared with 18.2% in group B ( $P < 0.002$ ). **CONCLUSIONS:** These data suggest that inherited thrombophilia may play a role in the aetiology of repeated IVF failures, particularly in the subgroup with unexplained fertility.

*Key words:* failure/implantation/IVF-embryo transfer/thrombophilia

## Introduction

Repeated IVF-embryo transfer failures, defined as at least four IVF cycles without pregnancy, have been attributed to either embryo quality or endometrial receptivity. Inherited thrombophilia has been associated with thromboembolism, recurrent pregnancy loss and pregnancy complications (Blumenfeld and Brenner, 1999; Grandone *et al.*, 1999; Kupferminc *et al.*, 1999). IVF implantation failure has recently been associated with inherited thrombophilias (Grandone *et al.*, 2001). The current case-control study was conducted to investigate whether hereditary thrombophilia is more prevalent in women with repeated IVF-embryo transfer failures.

## Materials and methods

Group A comprised consecutive 45 women, treated in the IVF unit, aged 24–45 years (mean  $36.7 \pm 5.9$  SD). All the women in group A had a history of four or more failed IVF cycles (mean  $5.9 \pm 2.6$  SD), in which at least three good quality (grades 1 and 2) embryos were transferred (mean number of embryos  $3.9 \pm 0.8$  SD). The indications for IVF-embryo transfer are given in Table I. The subgroup of unexplained infertility included women with ovulation dysfunction who failed to conceive following ovulation induction with at least four cycles of HMG/HCG. Patients with male factor were treated by ICSI-embryo transfer.

Group B (controls) was comprised of 44 consecutive apparently healthy women, with at least one uneventful pregnancy, who delivered

in our hospital during the research period and were matched for age and ethnic origin to group A. The results of 15 consecutive women [mean age  $26 \pm 2.3$  (SD) years] who conceived in the first IVF-embryo transfer cycle are also reported (group C). They were matched to the IVF failure patients with regard to ethnic origin. No patient from any group refused to participate in the study. All patients provided informed consent.

The prevalence of thrombophilia was assessed in all 104 women. Determination of mutations in prothrombin genes, factor V Leiden and methylene tetrahydrofolate reductase (MTHFR), as well as deficiencies of protein C, protein S and antithrombin III (AT-III) were performed as described elsewhere (Kupferminc *et al.*, 1999). Both total and free protein S assays were performed and repeated after 2–4 months in cases of abnormal findings. No false-positive results were found.

Since estrogen levels may alter protein S and AT-III, all thrombophilia testing was performed at least 2 months after hormonal therapy or delivery.

We routinely assess women for the presence of polycystic ovary syndrome (PCOS). The incidence of PCOS was 13%, in group A, comparable to the incidence in other patients in our unit.

None of the study participants had a history of thromboembolism.

## Statistics

Statistical analysis was performed using the Pearson test, Fisher's exact test and Student's *t*-test. All test were two tailed.  $P < 0.05$  was considered statistically significant. For sample size estimation, assuming the incidence of thrombophilias in the normal population

**Table I.** Indications for IVF-embryo transfer in group A

Indications for IVF-embryo transfer	Group A (n = 45) [n (%)]
Male factor	17 (37.8)
Tubal factor	7 (15.6)
Unexplained infertility	21 (46.7)

is 16%, and assuming the rate in IVF failure patients is 40% (relative risk 2.5), with alpha of 0.05 and 80% power, 45 women are needed in each group. The data are given as mean values  $\pm$  SD.

## Results

The incidence of inherited thrombophilias is displayed in Table II. Twenty women in group A (20/45, 44.4%) had at least one inherited thrombophilia compared with eight women in group B (8/44, 18%) [ $P = 0.012$ ; odds ratio (OR) 3.6; 95% confidence interval (CI) 1.25–10.6]. MTHFR, protein S deficiency and prothrombin mutations were found to be the most common inherited thrombophilias. The incidence of these mutations in group A was 17.8, 8.9 and 8.9%, respectively.

Since folic acid intake was not known in most women, we analysed the thrombophilia rate again, without MTHFR mutation. The incidence of thrombophilias was 26.7% in group A compared with 9.1% in group B ( $P = 0.03$ , OR 2.9; 95% CI 1.02–8.4).

There were 13 cases of thrombophilia in the 21 (61.9%) women who comprised the subgroup of unexplained infertility (including four cases of homozygotic MTHFR). When comparing the prevalence of the thrombophilia in these 21 women with diagnosis of unexplained infertility without MTHFR (9/21, 42.8%) with group B (4/44), the difference was statistically significant ( $P = 0.002$ , Fisher's exact test).

## Discussion

The results of the current study revealed a high prevalence of thrombophilia in women with repeated IVF-embryo transfer failure (group A) compared with normal women (group B). There was a tendency for a lower prevalence of thrombophilia in group C compared with group A, but we abstained from carrying out any formal statistical analysis due to the small sample size and the significantly lower average age in group C, which may be the main reason for the good IVF outcome in this group.

Several studies have shown an association between inherited thrombophilias and recurrent early pregnancy loss (Blumenfeld and Brenner, 1999; Reznikoff-Etievant *et al.*, 2001; Alonso *et al.*, 2002; Sarig *et al.*, 2002). There is, however, no consensus on the type of inherited thrombophilia associated with recurrent pregnancy loss. It was hypothesized Rey *et al.* (2003) that the mechanism involved in recurrent pregnancy loss may be hypercoagulation at the implantation site, where successful connection between placenta and maternal blood should be established, thus resulting in miscarriage.

**Table II.** Summary of the incidence of inherited thrombophilias in the study population

Thrombophilia	Group A (n = 45) [n (%)]	Group B (n = 44) [n (%)]	Group C (n = 15) [n (%)]
All thrombophilias	20 (44.4)	8 (18.2) <sup>a</sup>	3 (20)
All thrombophilias without MTHFR	12 (26.7)	4 (9.1) <sup>b</sup>	1 (6.7)
MTHFR homozygote	8 (17.8)	4 (9.1)	2 (13.3)
Prothrombin mutation	4 (8.9)	1 (2.3)	1 (6.7)
Protein S deficiency	4 (8.9)	0	0
Protein C deficiency	0	0	0
Factor V Leiden	3 (6.7)	3 (6.8)	0
AT-III	1 (2.2)	0	0

<sup>a</sup> $P = 0.012$ ; OR 3.6; 95% CI 1.25–10.6.

<sup>b</sup> $P = 0.03$ ; OR 2.9; 95% CI 1.02–8.4.

The current findings are in agreement with those of Grandone *et al.* (2001), who reported an association between IVF-embryo transfer failure and an increased incidence of thrombophilia. The same authors also reported that mutations of the factor V Leiden and prothrombin genes might play a role in implantation failure or in fetal loss after IVF (Grandone *et al.*, 2001). Bare *et al.* (2000) described a higher risk of miscarriages or infertility problems in carriers of factor V Leiden. The risk of one miscarriage was 1.5-fold greater, and the risk of two or more miscarriages or infertility problems was 2.5-fold greater, for Leiden mutation carriers than for normal controls.

Finally, protein S deficiency and hypofibrinolysis were also implicated in recurrent loss and implantation failure (Glueck *et al.*, 2000).

The precise mechanism by which thrombophilias affect recurrent pregnancy loss and implantation failure is as yet undetermined. Several studies have reported an association between hereditary thrombophilias and increased complications of pregnancy, such as severe preeclampsia, fetal growth restriction, stillbirth and abruptio placenta (Kupferminc *et al.*, 1999; Grandone *et al.*, 2001). It has been suggested that thrombosis of maternal vessels and reduced perfusion to the intervillous space may contribute to these complications in association with thrombophilias (Kupferminc *et al.*, 1999). Invasion of maternal vessels by the syncytiotrophoblast may be affected by local microthrombosis at the site of implantation leading to implantation failure or pregnancy loss/miscarriage. It should be mentioned, however, that development of the intervillous space occurs only at 11–12 weeks of gestation, making it somewhat difficult to explain implantation failure solely by thrombosis. Furthermore, Gopel *et al.* (2001) showed higher ICSI success in mother–child pairs with a factor V Leiden, suggesting that a thrombotic tendency of carriers of the Leiden mutation has some advantage in fetal implantation. These latter data, however, are contrary to the recent meta analysis by Rey *et al.* (2003), which showed an association between recurrent first trimester loss and factor V Leiden.

It is unlikely that the difference in age ( $36.7 \pm 5.9$  versus  $26 \pm 2.3$  years) between groups A and C attributed to the higher protein S deficiency rate in group A. Although protein S

levels increase with age (Dykes *et al.*, 2001), protein S deficiency was increased in the elderly patients (group A).

We analysed the data with and without MTHFR mutation, and the difference between women with IVF failure and controls was statistically significant in both cases. Although recent meta-analysis suggests that MTHFR may not be important in recurrent miscarriage (Rey *et al.*, 2003), Nelen *et al.* (2000) performed a meta-analysis to evaluate the relationship between recurrent early pregnancy loss and hyperhomocysteinaemia and the MTHFR C677T mutation. Overall, the pooled ORs for elevated homocysteine were 2.7 (95% CI 1.5–5.2), for afterload homocysteine 4.2 (95% CI 2.0–8.8) and for MTHFR 1.4 (95% CI 1.0–2.0). These data support hyperhomocysteinaemia as a risk factor for recurrent early pregnancy loss. Homozygosity for the MTHFR mutation represents a small increase in women's risk for recurrent pregnancy loss. Until more data emerge, the role of MTHFR is debatable. As far as AT-III, although Rey *et al.* (2003) did not find an association between AT-III and fetal loss, this, as mentioned, may be due to a lack of data.

The main implication of our results, if confirmed, is that antithrombotic therapy should be evaluated in patients with IVF failure and thrombophilia.

Since thrombophilias are inherited, it is possible that fetal thrombophilia may also affect IVF-embryo transfer results. It is known that fetal thrombophilia may affect placental infarcts at a later stage of pregnancy (Dizon-Townson *et al.*, 1997). However, with the current knowledge it is not known whether fetal thrombophilia may also affect implantation.

Our findings suggest that inherited thrombophilias may play a role in the aetiology of repeated IVF-embryo transfer failure, particularly in a subgroup of women with infertility of unknown aetiology.

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### References

- Alonso A, Soto I, Urgelles MF, Corte JR, Rodriguez MJ and Pinto CR (2002) Acquired and inherited thrombophilia in women with unexplained fetal losses. *Am J Obstet Gynecol* 187,1337–1342.
- Bare SN, Poka R, Balogh I and Ajzner E (2000) Factor V Leiden as a risk factor for miscarriage and reduced fertility. *Aust N Z J Obstet Gynaecol* 40,186–190.
- Blumenfeld Z and Brenner B (1999) Thrombophilia-associated pregnancy wastage. *Fertil Steril* 72,765–774.
- Dizon-Townson DS, Meline L, Nelson LM, Varner M and Ward K (1997) Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction. *Am J Obstet Gynecol* 177,402–425.
- Dykes AC, Walker ID, McMahon AD, Islam SI and Tait RC (2001) A study of Protein S antigen levels in 3788 healthy volunteers: influence of age, sex and hormone use, and estimate for prevalence of deficiency state. *Br J Haematol* 113,636–641.
- Glueck CJ, Awadalla SG, Phillips H, Cameron D, Wang P and Fontaine RN (2000) Polycystic ovary syndrome, infertility, familial thrombophilia, familial hypofibrinolysis, recurrent loss of in vitro fertilized embryos, and miscarriage. *Fertil Steril* 74,394–397.
- Gopel W, Ludwig M, Junge AK, Kohlmann T, Diedrich K and Moller J (2001) Selection pressure for the factor-V-Leiden mutation and embryo implantation. *Lancet* 358,1238–1239.
- Grandone E, Colaizzo D, Lo Bue A, Checchia MG, Cittadini E and Margaglione M (2001) Inherited thrombophilia and in vitro fertilization implantation failure. *Fertil Steril* 76,201–202.
- Grandone E, Margaglione M, Colaizzo D, Cappucci G, Sciannone N, Montanaro S, Paladini D, Martinelli P and Di Minno G (1999) Prothrombotic genetic risk factors and the occurrence of gestational hypertension with or without proteinuria. *Thromb Haemost* 81,349–352.
- Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G and Lessing JB (1999) Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med* 340,1584–1589.
- Nelen WL, Blom HJ, Steegers EA, den Heijer M and Eskes TK (2000) Hyperhomocysteinemia and recurrent early pregnancy loss: a meta-analysis. *Fertil Steril* 74,1196–1199.
- Rey E, Kahn SR, David M and Shrier I (2003) Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet* 361,901–908.
- Reznikoff-Etievan MF, Cayol V, Carbonne B, Robert A, Coulet F and Milliez J (2001) Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage. *Br J Obstet Gynecol* 108, 1251–1254.
- Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z and Brenner B (2002) Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. *Fertil Steril* 77,342–347.

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