Pregnancy-related venous thrombosis: comparison between spontaneous and ART conception in an Italian cohort

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ABSTRACT
Objective: To evaluate in an Italian cohort the incidence of venous thromboembolic events (VTE) in pregnancies after assisted reproductive technologies (ART).
Participants: A prospective cohort of 998 women advised to undergo ART was referred by local fertility clinics from April 2002 to July 2011. Follow-up information was obtained during the check-up and/or by phone interviews. In a cohort of women who consecutively gave birth (n=3339) after spontaneous conception, information was obtained during the check-up and/or by patient administrative register.
Primary and secondary outcome measures: We calculated the incidence of VTE and superficial venous thrombosis in successful ART cycles and compared it with that of the general population conceiving spontaneously.
Results: Overall, 684 ART cycles were carried out by 234 women, who achieved a clinical pregnancy; in case of more than one successful cycle, only the first pregnancy was considered. Three vein thromboses (two VTE and one superficial vein thrombosis) were recorded. An antithrombotic prophylaxis with LMWH alone or combined with low-dose aspirin was prescribed in 23/234 (9.8%) women. In the reference cohort of 3339 women, a total of 11 vein thromboses were observed: six VTE and five SVT. The two-tailed Fisher exact test showed a trend towards statistical significance (p: 0.06, OR: 3.9, 95% CI 0.87 to 15.3). After the exclusion of three women who had undergone ART was 2/234 pregnancies (8.5‰), whereas that in our reference population was 6/3339 (1.8‰) (p: 0.09).
Conclusions: Our data show a slightly higher incidence of vein thromboses in pregnancies after ART than in those after natural conception.

INTRODUCTION
It is well known that pro-coagulant changes occurring during normal pregnancy cause an increased risk of venous thromboembolism (VTE). Previous studies have found that VTE after a spontaneous conception is slightly more than 1 in 1000 pregnant women.1 2 In women undergoing assisted reproductive technologies (ART), medical ovarian stimulation is thought to increase thrombotic risk. However, available data about the magnitude and the duration of VTE risk in ART are conflicting. A review of several published case reports concluded that in in-vitro fertilisation (IVF) the VTE risk is comparable to that of spontaneous conceptions.3 Similarly, a more recent Danish register-based cohort study did not find an increased risk after IVF treatment in unsuccessful cycles.4 Nevertheless, an increased risk in successful IVF cycles was very recently reported, and the risk seems to be significantly higher during the first trimester.4 5 An important role as a risk factor for VTE is played by ovarian hyperstimulation syndrome (OHSS) occurring during and

Strengths and limitations of this study
- This study was carried out in a single Institution of the Apulia region. Cases were referred by Fertility Clinics of the same region.
- The reference cohort of pregnant women who delivered at the same Institution was representative of the general population from the same geographical area, as maternal age and the number of live births were comparable in the two populations.
- In the reference cohort, the number of vein thromboses may be under-represented in respect of the general pregnant population, as some cases may not be admitted to the hospital.
- We were not able to evaluate in the reference cohort the influence of other risk factors and, in the study group, that of change in assisted reproductive technologies practice during a rather long period (2002–2011).
after an ART procedure, with supraphysiological oestri-
diol levels that sometimes can result. During ovarian
stimulation, the coagulation and fibrinolytic systems are
activated; this activation appears to be exaggerated
and prolonged with the development of OHSS.8 This
syndrome is a systemic disorder resulting from vaso-
active products released by hyperstimulated ovaries.
Haemoconcentration, altered coagulation system and
reduced venous return secondary to enlarged ovaries,
ascites and immobility are likely to be important deter-
minants for the increased risk of venous thrombosis in
women with OHSS. Thrombotic events have also been
reported to occur weeks after OHSS has resolved.9 It has
been calculated that OHSS increases until 100-fold the
risk of VTE.6 Inherited or acquired thrombophilias,
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risk of VTE.6 Inherited or acquired thrombophilias,
have been identified as a risk factor for VTE during
ART.8 In the present study, we identiﬁed all women who
had undergone at least one ART cycle and achieved at
least one clinical pregnancy after the procedure. However,
for final analysis, in case of more than one successful cycle,
only the ﬁrst pregnancy was considered. Similarly, in the reference
cohort, when the same woman delivered more than one
once, only the ﬁrst pregnancy was computed.

Between January 2010 and December 2012, 3,527
women gave birth at our hospital; after the exclusion of
155 pregnancies of those who delivered in this period
more than once and 33 pregnancies obtained by means
of ART, 3,339 pregnancies were considered as the
reference group. From 2010, an electronic database is
available in our Institution that includes validated
information on the pregnancy, delivery and neonatal
periods. We obtained information on the diagnoses of
pregnancy-related VTE by linkage to our Institutional
patient register, an administrative database containing
information on the dates of admission and discharge
and the main diagnosis. The choice to consider also
VTE depends on the reported association with deep-vein
thrombosis, as a high rate of pulmonary embolism in
patients with saphenous vein thrombosis and no obvious
deep vein involvement has been described.13

Laboratory tests

Blood samples were collected in 3.8% trisodium citrate
and centrifuged at 2000 g for 15 min to obtain platelet-
poor plasma, which was frozen and stored in small
 aliquots at –70°C until tested. Antiphospholipid anti-
obodies—LA and IgG, IgM aCL (QUANTA Lite, INOV A
Diagnostics, San Diego, California, USA)—antithrombin
and protein C (Berichrom Antithrombin and Protein C
amidolytic assays, Behring, Germany) and free protein S
(IMUCLOSE Protein S ELISA, American Diagnostica,
Stanford, Connecticut, USA) were determined in all
patients. A conﬁrmed presence of antiphospholipid anti-
obodies was deﬁned according to SSC criteria,15 or the
concurrent presence of more than one. DNA was

PATIENTS AND METHODS

We identiﬁed 998 women candidate to ART consecu-
tively referred by local Fertility Clinics to our Unit from
April 2002 to July 2011. At that time, detailed informa-
tion on maternal age, BMI, smoking status, causes of
infertility, personal history of obstetric complications
and VTE was collected. All the women were investigated
for the presence of inherited (FVL, PTm and de
ficiency in protein S and C and antithrombin) and
acquired (lupus anticoagulant (LA), anticardiolipin
antibodies (aCL)) thrombophilias. Follow-up informa-
tion was obtained during the check-up and/or by
phone interviews. The prescription of aspirin and/or
LMWHs was veriﬁed by reviewing medical records.
For each woman, the following information was collected:
cycles number, type of ART procedure (IVF and intra-
plasmatic sperm injection (ICSI), or intrauterine
injection (IUI)), occurrence of clinical pregnancy
(deﬁned as the ultrasonographic visualisation of one or
more gestational sacs and fetal heart beat),12 pregnancy
loss (deﬁned as a loss occurring before/at 20 weeks of
pregnancy), preterm delivery (deﬁned as delivery before
20 weeks of gestation), live birth (the delivery of a live
newborn), presence of OHSS, obstetric and thrombo-
embolic complications. Diagnosis of VTE or superficial
vein thrombosis (SVT) was objectively conﬁrmed by
Doppler ultrasonography, ventilation-perfusion lung
scanning or pulmonary angiography.8 Only cycles start-
ing with ovarian stimulation and ending with embryo-
transfer/IUI were considered.

We identiﬁed all women who had undergone at least
one ART cycle and achieved at least one clinical preg-
nancy after the procedure. However, for ﬁnal analysis, in
case of more than one successful cycle, only the ﬁrst
pregnancy was considered. Similarly, in the reference
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patients. A conﬁrmed presence of antiphospholipid anti-
obodies was deﬁned according to SSC criteria,15 or the
concurrent presence of more than one. DNA was
extracted from peripheral blood leucocytes according to standard protocols.

FVL and PTm genotyping was performed by a TaqMan (Applied Biosystems, Foster City, California, USA) probe-based real-time PCR technique.16

**Statistical analysis**

All the analyses were performed using SPSS V.11.0 (SPSS Inc, Chicago, Illinois, USA). The significance of any difference in means was evaluated by non-parametric tests, whereas the significance of any difference in proportions was tested using the Fisher exact test or by $\chi^2$ statistics as appropriate. OR and 95% CIs were calculated.

**RESULTS**

A flow chart of the study cohort is shown in figure 1. Overall, we identified 234 women who achieved a clinical pregnancy after an ART cycle. Median BMI was 22.4 kg/m$^2$, most women did not smoke (79.5%) and 10.3% carried congenital or acquired thrombophilias (table 1). As far as severe thrombophilias are concerned, two women showed protein S deficiency (one combined with FV Leiden) and one a confirmed presence of anti-phospholipid antibodies. The frequencies we observed were not significantly different from those found in a general population from the same ethnic background.17

Overall, these women were exposed to a total of 684 ART cycles, 75 IUI and 609 IVF/ICSI with a clinical pregnancy rate of 33.3% (n=25) and 41% (n=250), respectively. Thirty-five women had more than one clinical pregnancy; in this case, we considered only the first pregnancy; thus, 234 clinical pregnancies were included in the final analysis. One hundred and forty-seven (62.8%) pregnancies resulted in live births. Table 2 shows clinical features of women who suffered from VTE: two of three women tested negative for thrombophilia, while one had a PTm in heterozygosis; the latter had age and BMI as additional risk factors. None presented a family history of VTE. In one case, a SVT in the left leg was recorded during prophylaxis with LMWH in a patient suffering from erythema nodosum; later on, this patient also had a recurrence of SVT in the right leg in a following pregnancy during prophylaxis with LMWH. Both the events occurred during the first trimester.

An antithrombotic prophylaxis with LMWH alone or combined with low-dose aspirin was prescribed in 23/234 (9.8%) women; among them, 8/23 (34.8%) were thrombophilic (three FVL heterozygotes, three PTm heterozygotes, one carried a protein S deficiency combined with FV and one a confirmed presence of anti-phospholipid antibodies). Among non-treated women (n=211), 16 carried thrombophilia: 8 were FVL heterozygotes, 7 PTm heterozygotes and 1 carried a protein S deficiency. When we compared the incidence of thrombotic events in the presence or absence of prophylaxis with LMWH, we found no significant difference between the groups (thromboses: 1/23 vs 2/211, p: ns). The median age of the whole group of cases was 34 years (23–46) and was not significantly different from those who were

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**Table 1** Baseline characteristics of the study sample (N=234)

<table>
<thead>
<tr>
<th>Age years, median (range)</th>
<th>34 (23–46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, median (range)</td>
<td>22.4 (16.7–35.9)</td>
</tr>
<tr>
<td>Smoking habits, n/N (%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (3)</td>
</tr>
<tr>
<td>No smokers</td>
<td>186 (79.5)</td>
</tr>
<tr>
<td>1–10 cigarettes per day</td>
<td>31 (13.2)</td>
</tr>
<tr>
<td>10–20 cigarettes per day</td>
<td>7 (3)</td>
</tr>
<tr>
<td>&gt;20 cigarettes per day</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Not provided</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>Infertility factors, n/N (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92 (39.3)</td>
</tr>
<tr>
<td>Female</td>
<td>50 (21.3)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>79 (33.8)</td>
</tr>
<tr>
<td>Mixed</td>
<td>10 (4.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>FVL, n/N (%)</td>
<td>11 (4.7)</td>
</tr>
<tr>
<td>PTm, n/N (%)</td>
<td>10 (4.3)</td>
</tr>
<tr>
<td>Severe thrombophilias*, n/N (%)</td>
<td>3 (1.3)</td>
</tr>
</tbody>
</table>

*Either homozygosis for FVL or PTm; double mutation, FVL or PTm and/or natural anticoagulants deficiency.

BMI, body mass index; FVL, factor V Leiden; PTm, prothrombin mutation.
prescribed LMWH with/without low-dose aspirin (35 years, range 27–42 years) and the remaining ones (34 years, range 25–46 years).

Among 234 successful cycles, 10 (4.3%) were complicated by OHSS. An antithrombotic treatment with LMWH was prescribed because of a diagnosis of OHSS in 3 of 23 (13%) treated cases; in the remaining cases, the indication of an antithrombotic treatment was not indicated by Fertility Centres. On the other hand, an OHSS was recorded in 7 of 211 (3.3%) untreated pregnancies.

A cohort of 3339 women (median age 31 years, range: 14–46, no significant difference vs cases) who consecutively gave birth at our Hospital after spontaneous conception from January 2010 to December 2012, used as a reference population, showed no significant difference in maternal age and number of live births in respect of the general population of women who delivered between January 2008 and December 2010 in the same geographical area (Apulia region) (table 3). In this reference cohort, a total of 11 vein thromboses were observed: 6 deep vein thrombosis and 5 SVT (table 4).

The two-tailed Fisher exact test showed a trend towards statistical significance (p: 0.06, OR: 3.9, 95% CI 0.87 to 15.3). After the exclusion of superficial thromboses in both the groups, we found that the incidence of VTE in our population of women who had undergone ART was 2/234 pregnancies (8.5 %), whereas that in our reference population delivering in our hospital was 6/3339 (1.8 %) (p: 0.09). Furthermore, when we excluded those women who had a previous VTE (patients 2 and 9 of the reference cohort, table 4), the two-tailed Fisher exact test approached statistical significance (p: 0.054; OR: 7.2, 95% CI 0.91 to 45.6).

### DISCUSSION

To the best of our knowledge, this is the first attempt in Italy to calculate VTE incidence in pregnancies after ART as compared to the risk of pregnancies after natural conception. We found a slightly higher VTE incidence in pregnancies after ART than in those after natural conception. Our findings, although showing only a trend towards statistical significance, most likely due to the small sample size, are in agreement with those recently obtained in other Countries. However, the higher first trimester VTE incidence recorded in previous studies was not confirmed by the present findings; in fact, two of three VTE occurred after the end of the 14th gestational week (table 2).

During the ovulation induction, a haemoconcentration occurs, which with high oestrogen levels and an increased number of small ovarian follicles can predispose to the development of OHSS, which is an important trigger for VTE.

In our study, the role of other potential risk factors, such as the type of drugs (ie, gonadotropins) used for ovulation induction, was not evaluated. The role of inherited and acquired thrombophilias, well established conditions predisposing to thrombosis, seems to be rather weak for pregnant women after ART, as only one of them showed a common cause of thrombophilia (PTm). On the other hand, among the remaining 23 thrombophilic women, none developed a VTE; no VTE was recorded among women carrying the most severe thrombophilias (n=3). We cannot conclude about a potential benefit of antithrombotic prophylaxis in the group carrying thrombophilia, due to the small sample size.

### Table 2 Characteristics of women from the study cohort experiencing venous thrombosis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age at event</th>
<th>BMI</th>
<th>Thrombophilia or OHSS</th>
<th>Additional risk factors</th>
<th>Cycle</th>
<th>Type of event</th>
<th>Antithrombotic prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>22.3</td>
<td>No</td>
<td>No</td>
<td>1</td>
<td>SVT in the left leg at 12 weeks of pregnancy</td>
<td>LMWH*</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>20.4</td>
<td>No</td>
<td>No</td>
<td>3</td>
<td>PE during twin pregnancy ended with IUFD (22 weeks)</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>35.9</td>
<td>PTm heterozygous</td>
<td>No</td>
<td>3</td>
<td>DVT in the right leg at 18 weeks of pregnancy</td>
<td>None</td>
</tr>
</tbody>
</table>

*Started when pregnancy test was positive.

BMI, body mass index; DVT, deep vein thrombosis; LMWH, low-molecular-weight heparin; IUFD, intrauterine fetal death; PE, pulmonary embolism; OHSS, ovarian hyperstimulation syndrome; PTm, prothrombin mutation; SVT, superficial vein thrombosis.

### Table 3 Age and live births in the reference cohort and general population from the same geographical area

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>Reference cohort (n=3339), % 2010–2012</th>
<th>General population from the same geographical area (n=106 265), % 2008–2010</th>
<th>Live births number</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 years</td>
<td>3.7</td>
<td>2.3</td>
<td>3451</td>
</tr>
<tr>
<td>20–29 years</td>
<td>34.4</td>
<td>32.4</td>
<td></td>
</tr>
<tr>
<td>30–39 years</td>
<td>55.1</td>
<td>59.5</td>
<td></td>
</tr>
<tr>
<td>40 years</td>
<td>6.8</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>107 461</td>
</tr>
</tbody>
</table>
Overall, 10 (4.3%) OHSS were recorded; this percentage is not significantly different from that reported in other series (15); 3 of 10 OHSS were treated with LMWH, as suggested by the current guidelines, while the remaining seven cases were not treated; we are not able to investigate the reason for the lack of treatment, but we can hypothesise that they were moderate or mild forms of OHSS. Some limitations of our study need to be considered. First, there is a lack of detailed data on lifestyle factors such as smoking, obesity and thrombophilic status in the reference cohort; it was not possible to obtain these data, as they were extracted from an administrative database. Therefore, we were not able to compare for all possible variables the two groups of women. However, the reference cohort was found to be representative of the general population from the same geographical area, as maternal age and the number of live births were comparable in the two populations. Second, we cannot exclude that in our reference cohort the number of vein thromboses may be under-represented in respect of the general pregnant population, as some cases may not be admitted to the hospital. However, this possible bias is also present in our group of outpatients; in addition, the incidence we recorded in our reference cohort was found to be similar to that expected in the general population of pregnant women (1: 1000). Third, we cannot exclude that during the quite long period of observation (2002–2011) the ART practice has changed; however, it is likely that the number of adverse events is reduced, as improvements were seen in maternal and neonatal outcomes. On the other hand, the improvement of standard care allowed for a substantial fall in the rate of VTE in general pregnant population. The lack of confounders’ control is another limitation and depends on the observational nature of the study and the sample size.

In conclusion, an increased, although not significant, absolute risk of vein thrombosis during pregnancies after ART was found. Further studies aimed at identifying women who could potentially benefit from an antithrombotic prophylaxis are needed.

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**Contributors** EG, FD and MM designed the study. MV and GLT enrolled the cases and analysed the data. TP and FP produced data regarding the reference cohort. WA, MM and EG critically revised the manuscript. PV obtained biochemical and molecular results. TP and FP produced data on lifestyle factors such as smoking, obesity and thrombophilic status in the reference cohort. WA, MM and EG critically revised the manuscript.

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**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** S. Giovanni Rotondo Ethis Commettee.

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