Fetal loss in women with hereditary thrombophilic defects and concomitance of other thrombophilic defects: a retrospective family study

FJ Korteweg, a N Folkeringa, b J-LP Brouwer, b JJHM Erwich, a JP Holm, a J van der Meer, b NJGM Veeger c

a Department of Obstetrics b Division of Haemostasis, Thrombosis and Rheology, Department of Haematology and c Department of Epidemiology, University Medical Centre Groningen, Groningen, the Netherlands

Correspondence: Dr FJ Korteweg, Department of Obstetrics and Gynaecology, University Medical Centre Groningen, Hanzeplein 1, 9700 RB Groningen, the Netherlands. Email f.j.korteweg@og.umcg.nl

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Objective To assess the absolute risk of fetal loss associated with hereditary deficiencies of antithrombin (AT), protein C (PC) and protein S (PS), and the contribution of additional thrombophilic defects to this risk.

Design A retrospective family cohort study.

Setting A tertiary referral teaching hospital.

Population Women from families with hereditary deficiencies of AT, PC and PS, and their non-deficient relatives.

Methods We assessed the absolute risk of fetal loss, comparing deficient women with non-deficient female relatives.

Main outcome measures Early, late and total fetal loss rates; odds ratios of fetal loss.

Results We evaluated 289 women, who had 860 pregnancies. The total fetal loss rates were 23% (AT deficient), 26% (PC deficient), 11% (type-I PS deficient) and 15% (type-III PS deficient), compared with 11, 18, 12 and 13% in non-deficient women, respectively. Odds ratios were 2.3 (95% CI 0.9–6.1), 2.1 (95% CI 0.9–4.7), 0.7 (95% CI 0.2–1.8) and 1.1 (95% CI 0.6–2.0), none of which reached statistical significance. Differences were mainly the result of higher late fetal loss rates in women deficient in AT (OR 11.3, 95% CI 3.0–42.0) and PC (OR 4.7, 95% CI 1.3–17.4). The concomitance of factor-V Leiden and prothrombin G20210A was observed in 19% of women, and did not increase the risk of fetal loss.

Conclusions Although absolute risks of fetal loss were high, odds ratios of total fetal loss were not statistically significant in deficient versus non-deficient women. However the higher absolute risks appeared to reflect higher late fetal loss rates as opposed to early fetal loss rates. An additional effect of concomitance of factor-V Leiden and prothrombin G20210A was not demonstrated, which may result from the exclusion of women at highest risk of venous thromboembolism, or from the small numbers sampled in the study.

Keywords Antithrombin deficiency, concomitance, fetal loss, protein-C deficiency, protein-S deficiency.


Introduction

Hereditary deficiencies of antithrombin (AT), protein C (PC) and protein S (PS) are strong risk factors for venous thromboembolism (VTE).1–3 Women with these deficiencies are at higher risk of VTE during pregnancy and the puerperium, because of the acquired hypercoagulable state associated with this condition.4–6 It is likely that not only maternal veins but also maternal–placental vessels are more prone to the development of thrombosis, as has been demonstrated in women with mild thrombophilic defects.7–10 Consequently, women with strong thrombophilic defects, i.e. deficiencies of AT, PC or PS, may be at higher risk of fetal loss, because of placental insufficiency as a result of placental infarction. Thus far, only a few family studies addressed fetal loss in women with these rare deficiencies.11–14 Although the reported risk of fetal loss was increased compared with controls,11,12 this result was not consistent in meta-analyses.13,14

Recently, it was demonstrated that the concomitance of other thrombophilic defects increases the risk of VTE in
subjects with hereditary deficiencies of AT, PC or PS. Moreover, concomitance was frequently observed in families with these deficiencies.3,15–17 Similarly, concomitance might also increase the risk of fetal loss.

We performed a retrospective family cohort study to assess the absolute risk of fetal loss associated with hereditary deficiencies of AT, PC and PS, and the contribution of additional thrombophilic defects to this risk.

Methods

We studied women from a retrospective study of families with hereditary deficiencies of AT, PC, type-I PS or type-III PS.2,3,9 The probands of the study were consecutive patients with documented VTE, in whom one of these deficiencies was demonstrated. They were referred with clinically suspected VTE to our thrombosis outpatient clinic over a period of 12 years. First-degree relatives older than 15 years of age were identified. As the number of probands deficient in AT was small, second-degree relatives from a deficient parent were also identified. The study was approved by the institutional review board of our hospital. Informed consent was obtained from all participants. In the present study, women were eligible if they had been pregnant at least once resulting in live birth or fetal loss, excluding ectopic and terminated pregnancies. Considering the high risk of VTE in women with hereditary deficiencies and the probability that they had received thromboprophylaxis during pregnancies after prior VTE, we excluded pregnancies after a prior episode of VTE from analysis, because thromboprophylaxis might have influenced the outcome of these pregnancies. As a consequence, women without a pregnancy before the first VTE were excluded. Detailed information about episodes of venous thromboembolism, and the course and outcome of previous pregnancies, from 15 years of age up to enrolment was collected using a standardised questionnaire and by reviewing medical records. We defined early fetal loss as loss up to 22 weeks of gestation and late fetal loss as loss after 22 completed weeks of gestation, according to the criteria of the World Health Organization.18 Recurrent fetal loss was defined as two or more losses. Blood samples for testing on thrombophilic defects were taken after clinical data had been collected. These tests included factor-V Leiden and the prothrombin G20210A mutation, in addition to all aforementioned deficiencies.

Laboratory studies

The following methods described were used in a previous study.6 AT activity (Coatest; Chromogenix, Mölndal, Sweden) and PC activity (Berichrom Protein C; Dade Behring, Marburg, Germany) were measured by chromogenic substrate assay, and the Enzyme Linked Immuno Sorbent Assay (DAKO, Glostrup, Denmark) was used to measure PC-antigen and total and free PS-antigen levels. Normal ranges were determined in 393 healthy blood donors that had no (family) history of venous or arterial thromboembolism, and were neither pregnant nor had used oral contraceptives for at least 3 months. We defined AT deficiency by levels below the lower limit of its normal range (<74 IU/dl), and type-I and type-II PC deficiency by lowered levels of PC antigen (<63 IU/dl) and/or activity (<64 IU/dl). We defined type-I PS deficiency by total (<67 IU/dl) and free PS levels (<65 IU/dl) below the lower limit of their normal ranges, and type-III deficiency by lowered free PS levels and normal total PS levels. We considered deficiencies to be inherited if they were confirmed at repeated measurements of samples demonstrated in at least two family members and collected at a 3-month interval, whereas acquired conditions were excluded. A deficiency was considered to be acquired, and to have resulted from oral contraception or pregnancy, unless it was confirmed at least 3 months after the discontinuation of oral contraception and delivery, respectively. Factor-V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions.19,20

In the probands and relatives who experienced VTE, blood samples were collected at least 3 months after the VTE had occurred. If they were undergoing treatment with a vitamin-K antagonist, samples were taken after the therapy had been interrupted and nadroparin had been administered subcutaneously.

Statistical analysis

Continuous variables were expressed as median values and ranges, and categorical data were expressed as counts and percentages. Differences between groups for continuous data were evaluated using the Student’s t-test or Mann–Whitney U-test, depending on the normality of data, and by using the Fisher’s exact test or chi-square test for categorical data. The absolute fetal loss risks were expressed as percentages of pregnancies resulting in fetal loss prior to enrolment, i.e. prior to their classification as deficient or non-deficient (see also Table 2). In addition, the percentage of women with at least one fetal loss was calculated (see also Table 1). For the estimation of the relative risks (i.e. odds ratios), probands and deficient relatives were compared with all non-deficient relatives. The odds ratios were adjusted for clustering of pregnancies in women using the generalised equation estimation methodology: a logistic regression analysis with individual women as clustering variables was performed to estimate odds ratios, with confidence limits based on the Wald statistic. The effect of concomitant thrombophilic defects was assessed for factor-V Leiden and the prothrombin G20210A mutation, comparing women that are deficient or non-deficient. A two-tailed P < 0.05 was considered to indicate statistical significance.
Statistical analyses were performed using **SAS 9.1** (SAS Institute Inc., Cary, NC, USA).

**Results**

Overall, of 89 female probands and 541 female relatives, 175 women were excluded because of age <15 years, death, geographic distance and refused consent. A total of 455 women were eligible, of whom 136 were never pregnant and two had only ectopic or terminated pregnancies. Another 28 women were excluded because they had their first pregnancy after VTE. The remaining 289 women were analysed, of which 162 were deficient and 127 were non-deficient (Figure 1). Their clinical characteristics are summarised in Table 1. Overall, the median age at first pregnancy in women who were deficient or non-deficient was comparable. At least one fetal loss was observed in 33% of women who were deficient, whereas in women who were non-deficient the loss was 28% \((P = 0.37)\). These were comparable in probands and deficient relatives \((P = 0.86)\). Of women who were deficient (including probands), 35% had VTE at a fertile age, compared with 6% of women who were non-deficient \((P < 0.001)\). This difference was less pronounced in the cohort of PS type-III deficient families \((23 \text{ versus } 10\%; \ P = 0.10)\). The concomitance of factor-V Leiden and/or the prothrombin G20210A mutation was demonstrated in 23% of women who were deficient, and in 14% of women who were non-deficient \((P = 0.12)\). The distribution of fetal loss related to gestational age in women that are deficient or non-deficient for the separate cohorts of families deficient in AT, PC, type-I PS and type-III PS showed that early fetal loss mainly occurred between 6 and 12 weeks of gestation, and that late fetal loss occurred between 36 and 42 weeks of gestation.

Table 2 shows the absolute risks of fetal loss as the total number of fetal losses out of the total number of pregnancies. The total fetal loss rates were 23% in women deficient in AT, 26% in women deficient in PC, 11% in women deficient in type-I PS and 15% in women deficient in type-III PS, compared with 11, 18, 12 and 13% in non-deficient women, respectively. Adjusted for the clustering of pregnancies among the women, and compared with all non-deficient women, odds ratios were 2.3 (95% CI 0.9–6.1) in women deficient in AT, 2.1 (95% CI 0.9–4.7) in women deficient in PC, 0.7 (95% CI 0.2–1.8) in women deficient in type-1 PS and 1.1 (95% CI 0.6–2.0) in women deficient in type-III PS, none of which reached statistical significance. Early fetal loss rates showed no statistically significant differences between women who are deficient and women who are non-deficient. Late fetal loss rates were 13 and 6% in women deficient in AT and women deficient in PC, respectively; odds ratios were 11.3 (95% CI 3.0–42.0) and 4.7 (95% CI 1.3–17.4). In women deficient in type-I and type-III PS, the late fetal loss rates were 1 and 2%, respectively; odds ratios were 0.9 (95% CI 0.1–7.8) and 1.9 (95% CI 0.6–6.4), and did not reach statistical significance.

Of 289 women who were included in our analysis, 273 (94%) were tested on factor-V Leiden and the prothrombin G20210A mutation. The concomitance of these mutations was demonstrated in 52 women (19%); 6% were double heterozygotes (see Table 3 for the separate and pooled cohorts). These thrombophilic defects were observed more frequently in both women who were deficient and women who were non-deficient than is expected in the normal

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics of 289 women with hereditary deficiencies of antithrombin, protein C or protein S, and their non-deficient female relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antithrombin</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Women, n</strong></td>
</tr>
<tr>
<td><strong>Age at first pregnancy, median (range)</strong></td>
</tr>
<tr>
<td>1 pregnancy, n (%)</td>
</tr>
<tr>
<td>2 pregnancies, n (%)</td>
</tr>
<tr>
<td>3 pregnancies, n (%)</td>
</tr>
<tr>
<td>4 or more pregnancies, n (%)</td>
</tr>
<tr>
<td>At least 1 fetal loss, n (%)</td>
</tr>
<tr>
<td>Recurrent fetal loss, n (%)</td>
</tr>
<tr>
<td>VTE at fertile age, n (%)</td>
</tr>
<tr>
<td>Concomitance, n/n (%)*</td>
</tr>
</tbody>
</table>
*Concomitance of factor-V Leiden and/or prothrombin G20210A; n affected/n tested (%).
Table 2. Total, early and late fetal loss rates in 289 women with 860 pregnancies with hereditary deficiencies of antithrombin, protein C or protein S, and their non-deficient relatives

<table>
<thead>
<tr>
<th>Deficiency (families, n)</th>
<th>Antithrombin (12)</th>
<th>Protein C (40)</th>
<th>Protein S type I (39)</th>
<th>Protein S type III (81)</th>
<th>Total (172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female probands</td>
<td>7</td>
<td>21</td>
<td>22</td>
<td>39</td>
<td>89</td>
</tr>
<tr>
<td>Female relatives</td>
<td>95</td>
<td>120</td>
<td>111</td>
<td>215</td>
<td>541</td>
</tr>
<tr>
<td>Total women</td>
<td>102</td>
<td>141</td>
<td>133</td>
<td>254</td>
<td>630</td>
</tr>
<tr>
<td>Exclusion</td>
<td>33</td>
<td>32</td>
<td>40</td>
<td>70</td>
<td>175</td>
</tr>
<tr>
<td>Eligible women</td>
<td>69</td>
<td>109</td>
<td>93</td>
<td>184</td>
<td>455</td>
</tr>
<tr>
<td>Never pregnant</td>
<td>27</td>
<td>37</td>
<td>27</td>
<td>47*</td>
<td>138*</td>
</tr>
<tr>
<td>Women with only pregnancies after VTE</td>
<td>4</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>Evaluable women</td>
<td>38</td>
<td>63</td>
<td>57</td>
<td>131</td>
<td>289</td>
</tr>
<tr>
<td>Deficient</td>
<td>19</td>
<td>31</td>
<td>29</td>
<td>83</td>
<td>162</td>
</tr>
<tr>
<td>Non-deficient</td>
<td>19</td>
<td>32</td>
<td>28</td>
<td>48</td>
<td>127</td>
</tr>
</tbody>
</table>

*Odds ratios (ORs) were adjusted for clustering of pregnancies in women, and compared with all non-deficient women using the generalised equation estimation methodology. In this, a logistic regression analysis with individual women as clustering variable was performed to estimate ORs with confidence limits based on the Wald statistic.

Figure 1. Recruitment of women from families with hereditary deficiencies of antithrombin, protein C or protein S.
population (i.e. factor-V Leiden, 5%, and the prothrombin G20210A mutation, 3%).

The risks of total, early and late fetal loss in women with deficiencies of either AT or PC, and the reference group, with or without the concomitance of thrombophilic defects, are presented in Table 4. In women deficient in AT or PC, the total fetal loss rates were 44% (four of nine) in women with a concomitance of thrombophilic defects, versus 44% (16 of 36) in women without a concomitance of thrombophilic defects (Table 4). In women from the reference group, consisting of women who were not deficient in AT or PC, but who were deficient in type-I or type-III PS, and non-deficient women, the fetal loss rates with and without a concomitance of thrombophilic defects were 23 and 29%, respectively. The observed differences in risk of early and late fetal loss within subgroups (women deficient in AT or PC and women from the reference group) with and without the concomitance of thrombophilic defects were not statistically significant. Again, the numbers were small.

### Discussion

This study showed a high absolute risk of fetal loss in women with hereditary deficiencies of AT or PC. In women with hereditary deficiencies of type-I and type-III PS, the risk was comparable with non-deficient women. Odds ratios of total fetal loss were not statistically significant in deficient women, although there was some suggestion that late as opposed to early fetal loss rates were different. The concomitance of factor-V Leiden and/or the prothrombin G20210A mutation apparently did not influence the risk of fetal loss.

The absolute risk of total fetal loss in our study was 23% in women deficient in AT and 26% in women deficient in...
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PC: 2.3- and 2.1-fold higher than in non-deficient women. Previous studies showed odds ratios of total fetal loss ranging from 1.5 to 2.5 in women deficient in AT, and ranging from 1.4 to 2.5 in women deficient in PC, compared with controls.\textsuperscript{11–13} Although the odds ratios in our study were in agreement with previous studies, the absolute risks in relatives not deficient in AT and PC (11 and 18%, respectively) were higher than in the risks in the controls reported by Preston et al.\textsuperscript{11} In that study, the controls were partners of male participants in the European Prospective Cohort on Thrombophilia (EPCOT) or were acquaintances of women in the study. As we compared deficient women with their non-deficient relatives, the comitance of other thrombophilic defects in these families might explain the higher risk of fetal loss in non-deficient as well as deficient women. The results suggest that the higher risk of fetal loss in women deficient in AT and PC mainly reflects an 11.3- and 4.7-fold increased rate of late fetal loss, although further research is needed as numbers are small.

Early fetal loss was comparable with non-deficient relatives. Preston et al.\textsuperscript{11} found an odds ratio for early fetal loss of 1.7 (95% CI 1.0–2.8) in women deficient in AT and 1.4 (95% CI 0.9–2.2) in women deficient in PC, whereas this was 5.2 (95% CI 1.5–18.1) for late fetal loss in women deficient in AT and 2.3 (95% CI 0.6–8.3) in women deficient in PC.

We observed the lowest excess risk for total fetal loss in women deficient in type-I and type-III PS. The total fetal loss risks, as well as early and late fetal loss risks, were comparable with non-deficient relatives for both types of PS deficiency. Preston et al.\textsuperscript{11} found a comparable odds ratio of 1.3 (95% CI 0.8–2.1) for total fetal loss in women deficient in PS, whereas it was 3.3 (95% CI 1.0–11.3) for late fetal loss. In a meta-analysis the risk for total fetal loss was supported by our data. It is remarkable, however, that type-I PS deficiency did not influence the risk of fetal loss, considering that it is comparable with AT deficiency and PC deficiency as a risk factor for VTE.\textsuperscript{5,6}

In our study, the comitance of factor-V Leiden and the prothrombin G20210A mutation apparently did not influence the risk of fetal loss in either deficient or non-deficient women. In fact we observed a comparable, rather than higher, risk of fetal loss in women deficient in AT and PC with the comitance of factor-V Leiden and the prothrombin G20210A mutation, although the numbers were small. The exclusion of pregnancies after prior VTE could be an explanation for this finding. The comitance of other thrombophilic defects results in a higher risk and earlier onset of VTE in women with deficiencies of AT, PC or PS.\textsuperscript{5} One would expect that the comitance of other thrombophilic defects also increased the risk of fetal loss.\textsuperscript{11} By excluding pregnancies after prior VTE from the analysis, we probably excluded women at the highest risk of VTE, i.e. deficient women with comitance, and consequently women with potentially the highest risk of fetal loss. Indeed, the comitance of factor-V Leiden and the prothrombin G20210A mutation was more frequently observed in excluded women than in analysed women (data not shown), whereas the former showed a higher total fetal loss rate. Our assumption that thromboprophylaxis during pregnancy in deficient women reduced the estimated risk of fetal loss was further supported by the previously reported results of a prospective observational study on the same family cohort.\textsuperscript{21} That study showed a fetal loss rate of 0% in deficient women, who received thromboprophylaxis during pregnancy, compared with 45% in deficient women who did not (P = 0.001).

A comparison of our results with previous reports on fetal loss related to thrombophilic deficiencies and other thrombophilic defects is hampered by differences in methodology. The majority of previous studies addressed the incidence of thrombophilic defects in women with adverse pregnancy outcomes.\textsuperscript{7,9,13,14,22–24} We assessed fetal loss rates in families with hereditary deficiencies, which were identified by testing consecutive patients with VTE. Furthermore, gestational ages ranged widely in previous studies, and some studies did not differentiate between early and late fetal loss. However, placental function depends on gestational age,\textsuperscript{25,26} and the mechanisms of early and late fetal loss differ. Although placental thrombosis is a plausible explanation for (late) fetal loss in deficient women, deficiencies of AT, PC and PS may also contribute to another pathophysiological mechanism. Experiments in mice provided evidence that fibrin degradation products induce apoptosis of throphoblasts, resulting in fetal loss.\textsuperscript{27} As it is likely that deficiencies of AT, PC and PS are associated with the increased generation of thrombin and, consequently, fibrin and fibrin degradation products, we speculate that deficient women will be more prone to fetal loss than non-deficient women. In deficient women, early fetal loss may be a result of apoptosis of throphoblasts, whereas late fetal loss may be a result of placental thrombosis. In accordance with this hypothesis, anticoagulant treatment during pregnancy might have a beneficial effect on both early and late fetal loss in deficient women, as suggested by the results of a prospective study mentioned previously.\textsuperscript{21}

This study has some limitations. The numbers of subanalyses were small, leading to wide imprecise confidence intervals. The absolute risk of fetal loss in deficient women may have been underestimated by excluding preg-
nancies after prior VTE, and consequently women at higher risk of fetal loss. It is also important to keep in mind that the group of deficient women we studied are from highly thrombophilic families, and these results can therefore not be applied to women proven to be thrombophilia deficient without positive family members. In addition, different genetic forms of hereditary deficiencies of AT, PC or PS, regarding mutations or polymorphisms, may carry a higher risk for pregnancy complications; this should be addressed in future studies. Although a systematic search for the causes of early and late fetal loss (e.g. chromosomal abnormalities and infections) was not performed because of the retrospective design of our study, it is not likely that these would strongly differ among deficient and non-deficient women. In our analysis we did control for clustering of pregnancies within women. Because of the small numbers, additional adjustment for confounding was not feasible. We can only speculate about the impact of other important risk factors of fetal loss among deficient and non-deficient women. We feel that this lack of further control, however, did not significantly influence our findings. Recall bias regarding fetal loss may have been introduced by its retrospective design, but its influence remained limited as clinical data was collected prior to the classification of women as deficient or non-deficient. In addition, patients were not selected because of their compromised obstetrical history. Referral bias cannot be excluded by the setting of a university hospital. Selection bias is not very likely, as consecutive patients with VTE were tested to identify probands and their relatives, rather than women with fetal loss.

Conclusion

There was some suggestion that hereditary deficiencies of AT and PC were associated with a higher absolute risk of late fetal loss, in contrast with PS deficiency. An additional effect of the concomitance of other thrombophilic defects, although plausible, was not demonstrated, maybe as a result of excluding women at the highest risk of VTE. Further research is needed.

Disclosure of interests

None of the authors declare any financial, personal, political, intellectual or religious conflicts of interest, or conflicts of any other nature.

Contribution to authorship

JvdM conceived the study idea and all authors contributed to the study design, data abstraction and interpretation. NJGMV performed the statistical analysis. FJK wrote the article and all authors took part in its revision and approved the final version.

Details of ethics approval

The procedures of the study received ethics approval from the institutional review board of our hospital: the ethics committee University Medical Centre Groningen in the Netherlands, responsible for human experimentation.

Funding

There was no external funding for the study.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Powerpoint slides summarising the study.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author.

References


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**Journal club**

**A retrospective family study investigating the risk of fetal loss in women with hereditary deficiencies of antithrombin and protein C, compared with protein S, and contribution of the concomitance of other thrombophilic defects**

**Background**

- Describe recommendations for thrombophilia testing in women with late fetal loss, with reference to whether such testing should be routine or selective. Debate whether the recommendations should be modified in light of the findings from this study.
- How common are thrombophilias, e.g. factor-V mutation?

**Methods**

- Critically appraise the method of selecting patients and controls for this study.
- Debate the authors’ decision to exclude pregnancies with prior venous thromboembolism, and the impact of this decision on the results.

**Results and implications**

- Absolute risks of total fetal loss were high and odds ratios of late fetal loss were significant for women with anti-thrombin and protein C deficiency, compared with their non-deficient relatives: discuss.
- Consider the possible influence of body mass index on the results.
- Can results from this study be used to counsel women with specific thrombophilia deficiencies pre-pregnancy and antenatally? (Data S1)
Disclosure of interests
None to declare.

D Siassakos
NIHR Clinical Lecturer in Obstetrics and Gynaecology,
School of Clinical Sciences, University of Bristol, Bristol, UK
Email jsiasakos@gmail.com

Katherine O’Brien
Southmead Hospital, Obstetrics and Gynaecology,
Westbury on Trym, Bristol, UK

Reference