Early pregnancy loss following assisted reproductive technology treatment

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BACKGROUND: In women treated by assisted reproductive technology (ART), early pregnancy loss (EPL) reduces the initial success. Risk factors for EPL, however, have not been comprehensively studied. This study assesses some potential risk factors in ART pregnancies. METHODS: Altogether 1196 pregnancies, defined as serum hCG ≥ 10 IU/l on day 16 ± 1 after oocyte retrieval, were included in this study. EPL was defined as pregnancy loss that occurred before 6–7 weeks gestation. Risk factors investigated were maternal age, body mass index (BMI), smoking and polycystic ovary syndrome (PCOS) status, infertility aetiology, response to stimulation, quality and number of embryos replaced and treatment type. RESULTS: Overall EPL was 16%. The risk of EPL was not linearly related to either age or BMI. Though women >40 years old had an increased risk, this was not significant after adjusting for other factors. The risk in both lean (BMI < 18.5 kg/m²) and very obese (BMI > 35 kg/m²) women was also not significantly higher in multivariate analysis. There was no effect of PCOS. Smoking or transfer of ‘poor quality’ embryo(s) was associated with a significant increased risk of EPL after adjusting for other factors. CONCLUSION: Smoking and transferring poor quality embryos increased EPL, while the effects of age, obesity and other risk factors were not significant in a multivariate analysis.

Key words: ART/early pregnancy loss/risk factors

Introduction

A rise in hCG concentration in either urine or serum has been used to detect the establishment of pregnancy within the first few weeks of conception. Failure to confirm the presence of an embryonic sac(s) or fetal heart by subsequent ultrasound scan at 6–7 weeks gestation occurs in 18–22% of all pregnancies (Alfredsson, 1988; Wilcox et al., 1988; Kolstad et al., 1999) and is sometimes defined as early pregnancy loss (EPL). In assisted reproductive technology (ART), pregnancy loss before clinical detection by ultrasound scan is also commonly referred to as biochemical pregnancy. While studies carried out in the general population can give some estimates of the risk of EPL, the sample size of these studies has usually limited the statistical power for a detailed investigation of the potential risk factors for EPL (Wilcox et al., 1990). Women treated by ART are routinely monitored for early detection of pregnancy by measuring serum hCG concentration on a specific day, usually 14–17 days following oocyte retrieval, equivalent to ovulation in the general population, and again by ultrasound scan at about 6–7 weeks gestation. Therefore, they represent an ideal population to study the potential risk factors for EPL, with the results possibly applicable to the general population.

High rates of EPL, ranging from 12 to 48%, have been reported in ART (Barlow et al., 1988; Acosta et al., 1990; Levy et al., 1991; Schmidt et al., 1994; Simon et al., 1999; Fedorcsak et al., 2000; Sugantha et al., 2000). EPL significantly reduces the initial success of ART treatment, decreases the efficiency of treatment and increases the psychological burden on patients. Risk factors for EPL in pregnancies after ART, however, have not been comprehensively studied, despite its apparent significance. With a view to altering practice, an important conceptual issue relates to the mutability of risk factors for EPL, as potentially modifiable factors can form the basis for clinical or preventive interventions. For example, maternal age is not ‘reversible’; the age at which women have children is, however, amenable to modification within populations, as indicated by secular trend of increasing maternal age at the birth of the first child. A recent report has linked obesity with greater risk of EPL (Fedorcsak et al., 2000), and obesity has been shown to be a detrimental factor for pregnancy rate (Wang et al., 2000) and spontaneous abortion (Hamilton-Fairley et al., 1992) in ART programmes. Obesity is also potentially modifiable, possibly amenable to low cost, non-invasive, self-management by patients. The objective of this retrospective study is to investigate the effect of some potential risk factors on EPL in a large cohort of pregnant women treated by ART.

Materials and methods

During the period from 1994 to 1999, a total of 6045 embryo transfer cycles were carried out in the Reproductive Medicine Unit at its three
sites located at the Queen Elizabeth Hospital and the Wakefield Clinic in Adelaide, and Darwin Private Hospital in Darwin, Australia. In the whole population of 6045 embryo transfer cycles, 2270 had a period on or before the designated day of hCG measurement (day 16 after ovulation). In the remaining 3775 cycles, 2354 (62% of 3775 cycles) had at least one hCG measurement done on day 16 (±1 day). Amongst them, 1196 were pregnancy cycles based on the definition as stated below and included in the final analysis of this retrospective cohort study. In the 1421 cycles (38%) with no hCG measurement, the risk of EPL could not be determined. Amongst them, 489 were clinical pregnancy cycles and 728 non-pregnancy cycles and 204 had no records of period day (though none of them was a pregnancy cycle).

Treatment addressed various infertility aetiologies, including tubal blockage (23%), endometriosis (9%), male factor (50%), unexplained infertility (12%) and other factors (15%). A total of 27% of all patients had multiple infertility factors. The main stimulation protocol used during the study period has been described previously (Norman et al., 1991), including both down-regulation (>90%) with leuprolide and gonadotrophin and <10% of the cycles under a flare regime. The treatments were IVF, ICSI, and gamete intra-Fallopian transfer (GIFT).

While hCG measurement was routinely obtained in the morning of day 16 after oocyte retrieval (85% of the study population), a small proportion of patients had their hCG measured on day 15 or day 17. In 1994, hCG was measured with an automated fluorometric enzyme immunoassay using two monoclonal antibodies (Stratus II, Baxter, Miami, FL, USA). Sensitivity of the assay was 2 IU/l with coefficients of variation consistently <5% across a wide dynamic range (2–500 IU/l). Since 1995, the assay has been performed with an automated system, using a two-site chemiluminometric sandwich immunoassay [automated chemiluminescence (ACS) 180; Chiron Diagnostics Corporation, East Walpole, MA, USA]. Sensitivity was 0.01 nmol/l (2.5 IU/l) and 8% over the dynamic range (2–100 IU/l). Pregnancy was defined as a serum hCG concentration of ≥20 IU/l on day 16 after ovulation (equivalent to 30 days after last menstrual period in the general population).

Response

<table>
<thead>
<tr>
<th>BMI category</th>
<th>EPL (%)</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5</td>
<td>12%</td>
<td>1.00</td>
</tr>
<tr>
<td>18.5–25</td>
<td>16%</td>
<td>1.27</td>
</tr>
<tr>
<td>25.1–30</td>
<td>18%</td>
<td>1.34</td>
</tr>
<tr>
<td>&gt;30</td>
<td>18%</td>
<td>1.46</td>
</tr>
</tbody>
</table>

*Seventy-three cases with BMI values missing.

**Embryo quality is defined as: 1 for transferring one or two embryos electively; 2 for transferring three embryos electively; 3 for transferring two or three embryos non-electively; 4 for transferring one embryo non-electively.

The risk factors for EPL investigated in this study were: maternal age, body mass index (BMI, kg/m2), smoking and polycystic ovary syndrome (PCOS) status, infertility aetiology, response to stimulation (both maximum estradiol concentration and the number of oocytes recovered), a quasi measurement of embryo quality (refer to Table I for details), and treatment type. Patient age and BMI were categorized for the clarity of data analysis and presentation of results. Four age subgroups were formed: age ≤30 years, 30.1–35 years, 35.1–40 years and >40 years. BMI subgroups were: <18.5 kg/m2 (lean), 18.5–25 kg/m2 (normal), 25.1–30 kg/m2 (overweight), 30.1–35 kg/m2 (obese) and ≥35.1 kg/m2 (very obese). In 73 cases (6%), BMI was not recorded and was treated as missing in the analysis. PCOS was determined by both the ultrasound scan result and hormonal measurement as defined previously (Norman et al., 1995).

Analysis of variance (ANOVA) and χ²-test or Fisher’s exact test were used employing SPSS (version 10.0 for windows, Chicago, USA). Multivariable logistic regression analysis was used to assess all potential risk factors simultaneously (SAS version 10, Cary, USA). The results were shown as odds ratios (OR) and its 95% confidence interval (CI) calculated from the logistic regression model.

Results

The mean age of the study cohort at the time of treatment was 32.7 years (SD = 4.7, range 19.2–47.1). The mean BMI was 24.7 kg/m² (SD = 5.0, range 14.8–50.9). Sixty-eight percent of the women were aged ≤35 years. Fifty-nine percent had a normal BMI (18.5–25 kg/m²), while the obese (BMI >30 kg/m²) and very obese women (BMI >35 kg/m²) constituted 13% of the study population.

Univariate analysis

A total of 195 (16%) pregnancies ended as EPL in the study population. No clear linear trend in the risk of EPL in relation to either age or BMI was observed (Table I). In women up to 40 years old, the risk of EPL was very similar at around 15–17%, while women >40 years old had a significantly increased risk (23%) compared with the rest (P < 0.05). There was some variation between the BMI groups in their risk of EPL, with the lowest risk of EPL (12%) occurring in the
overweight women (25–30 kg/m²), while lean women (<18.5 kg/m²) had a much greater risk, 35% (P < 0.01) and very obese women (>35 kg/m²) had a non-significant increased risk (20%, P = 0.16). The normal weight (18.5–25 kg/m²) women also had a significantly higher risk of EPL than the overweight group (18 versus 12%, P < 0.05). There was a significant increase of risk (P < 0.05) in women who received IVF treatment compared with those who received ICSI or GIFT treatment (combined). Smoking was linked with a significant increase of risk (P < 0.001) in EPL. There seemed to be little effect of the level of response to ovarian stimulation defined by either the maximum estradiol concentration or the number of oocytes recovered. Women who received embryo(s) of poorest ‘quality’ as characterized by non-elective transfer of a single embryo had the greatest risk (P < 0.01) in contrast to that following multiple embryo transfer, particularly elective transfer of two or three embryos, where the women probably received the best quality embryos. The PCOS status of patients was marginally (P = 0.05) associated with an increased risk, while aetiology of infertility was not associated with any significantly increased risk of EPL.

**Multivariate analysis**

After adjusting for possible confounding effects from these risk factors with multivariate logistic regression analysis, only smoking and poorest embryo quality remained as significant risk factors for EPL (Table I). Smoking doubled the risk of EPL, while transferring the poorest quality embryos more than tripled the risk of EPL compared with the group receiving the best quality embryos.

**Discussion**

There was 16% EPL in this population. While both smoking and transferring the poorest quality embryo were significant risk factors for EPL, there was no evidence that other factors considered in the study had a significant effect on the risk of EPL.

There is a high wastage of pregnancy as EPL in both general population and ART population. The risk of EPL, 16%, reported in this study is lower than the estimates given in the general population. However, direct comparison of the two populations can be problematic since generally speaking, more sensitive assays, earlier hCG measurement and lower criterion of hCG for pregnancy detection have been utilized in general population studies (Wilcox et al., 1988, for example). Another possible factor that may complicate the early detection of pregnancy in ART is the common use of large doses of hCG for inducing ovulation or luteal support. Early work, however, has suggested that the residual hCG from either ovulation induction or luteal support would have been reduced to an undetectable level at the time of early pregnancy detection (Lenton et al., 1988). The practice of multiple embryo transfer in ART also means that some EPL may be ‘hidden’ by the continuing growth of companion embryo(s). It is likely that the real risk of EPL would be higher in the ART population than in the general population, although direct evidence to support this assertion is yet to be obtained. In fact, many methodological differences also exist between the reports of EPL in an ART population, which may account for the varying risks of EPL reported. The risk of EPL of 16% reported in this study, by and large, is within the range of a few other reports of ART population using similar criteria (Simon et al., 1999; Fedorcsak et al., 2000). The level of hCG used here for pregnancy detection was well within the sensitivity of hCG assay, although it was lower than the common clinical criterion for positive pregnancy (>30–50 IU/l), which is usually for informing patients and is considered conservative. On the other hand, since the criterion used in this study, hCG >10 IU/l at day 16, was much higher than that used in general population studies (Wilcox et al., 1988), it can be anticipated that some very early pregnancy loss would not have been counted.

Couples who are planning their pregnancies want to know the potential hazards, while those receiving infertility treatment will try to avoid any potential risk factors causing pregnancy loss. Since EPL causes a big reduction of the initial success of ART, it reduces its efficiency and increases the burden of psychological stress to infertile couples. Therefore, establishing the risk factors for EPL has important clinical implications in improving the efficiency of ART treatment, in addition to increasing the understanding of possible mechanisms of early pregnancy failure. The lack of effect of maternal age on EPL until women reached 40 years of age appeared to be consistent with that reported in two previous studies (Wilcox et al., 1990; Dickey et al., 1993). Alternatively, Balmaceda et al suggested that there is an increased incidence of EPL and chromosomal abnormalities of oocytes in older women (Balmaceda et al., 1994). It is possible that embryos in older women may have lost their ability to develop even before implantation, although the uterus may have the capacity to conceive with donated embryos from younger women (Sauer, 1997). In this study, there appeared to be a U-shaped effect of body mass. Both lean and very obese women had increased risk, while the optimal group was the slightly overweight women with a BMI between 25 and 30 kg/m². This is different from the results reported recently (Fedorcsak et al., 2000). It is possible that other obesity related factors, such as fat distribution or fat type, have confounded the effect of BMI and resulted in inconsistency between different studies. Further investigation is needed to clarify the matter. The association of smoking with a higher risk of EPL is an interesting observation. Detrimental effects of smoking on spontaneous abortion have been reported recently (Ness et al., 1999; Mishra et al., 2000). The association between embryo quality and the risk of EPL is another interesting finding which needs to be confirmed. Though the embryo quality defined here is not a direct measurement, it is known that the selection from large number of embryos available leads to a better clinical pregnancy rate. So the high risk of EPL suffered by embryos transferred with little or no selection does suggest that current selection criteria seem to be able to discriminate the embryos’ ability to grow after the initial implanting stage. The lack of relationship between the stimulation level and the risk of EPL was supported by one earlier study (Dickey et al., 1993).
There may be other possible risk factors that should be considered such as the endometrium thickness (Dickey et al., 1993) or bacterial vaginosus, which is associated with adverse pregnancy outcomes across all gestational ages (Ugwumadu, 2002). Lack of data, due to the retrospective nature of the study, prevented us adjusting for their possible effect and this may have reduced the sensitivity of the study. Another limitation of this study is the relative large proportion of patients (38%) who did not come back for their early pregnancy test, even though they had a luteal period possibly longer than 16 days. Reasons for not coming back for early pregnancy detection were usually personal. Comparisons showed that this group were 0.8 years older and had lower BMI, 0.5 kg/m² less, both statistically significant. Given the finding that age and BMI were not risk factors for EPL, however, small differences may not be meaningful. On the other hand, there was no difference in their likelihood of being a smoker or of having had poor quality embryos transferred. Finally, the smoking status was based on self-reporting, which can be a source of error, in particular under-reporting bias. However, in the present study, doctors obtained the data during consultation and the population can be characterized as one seeking specialist care over a long period of time. Both are elements known to be compatible with relatively accurate self-reporting of smoking status (Schofield and Hill, 1999; Donnelly et al., 2000). Even if under-reporting was a likely scenario in this study, then it would have made the observed smoking effect a conservative estimate instead of changing the direction of the effect.

Although we could not investigate the mechanism of EPL in this retrospective study, it has been suggested that EPL may be due to failure of the maternal support system or through impairment of the fetus itself. Due to the extreme difficulty in obtaining pregnancy material in EPL, the determination of either a pathological or a chromosomal cause is hard. A recent review (Norwitz et al., 2001) has discussed in detail early implantation, implantation failure and the possible mechanism involved.

In summary, this study has investigated many factors for their potential association with the risk of EPL and found that smoking doubled the risk of EPL and poor quality of embryos was also associated with an increased risk of EPL. An effect of age, obesity and several other factors on the risk of EPL was not evident.

Acknowledgements
We thank Ms Barbara Godfrey and Mr Allan Gilmore for helping to access the original data.

References
Submitted on February 5, 2002; resubmitted on June 5, 2002; accepted on August 7, 2002.