Is the male involved in the aetiology of ectopic pregnancy?

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We have previously observed a low incidence of ectopic pregnancies in couples having gamete intra-Fallopian transfer (GIFT) with donated spermatozoa. Based on findings in both animal and human models, we proposed the hypothesis that sperm defects may be associated with the expression of paternal genes which cause abnormal early embryo development and predispose the embryos to interact inappropriately with the genital tract epithelium, and so increase the risk of an ectopic implantation. To both confirm and extend the initial observation, GIFT and in-vitro fertilization (IVF) pregnancies entered on the Australian and New Zealand national database between 1979 and 1993 were analysed with regard to the incidence of ectopic pregnancy. There was an increased risk of ectopic pregnancy for IVF relative to GIFT and when spermatozoa from the male partner were used rather than donor spermatozoa. However, when couples were categorized with respect to the aetiology of their infertility, we were unable to show a significant association between ectopic pregnancy and whether spermatozoa from the male partner or a donor were used. We have therefore been unable to confirm a direct association between the source of spermatozoa and ectopic pregnancy.

Key words: ectopic pregnancy/GIFT/IVF/sperm donors

Introduction

There is increasing interest in the clinical implications of the finding that parental genomes are not equivalent and are imprinted to express differentially during embryo development (Hochberg et al., 1993). Paternal genes are critical to the development of the placenta but the extent of their involvement in preimplantation events remains conjectural. It was demonstrated in the early 1980s that paternally and maternally derived genetic factors could affect cleavage and the rate of development of mouse embryos (Shire and Whitten, 1980). Furthermore, in the DDK strain of mice, the paternal genome has deleterious interactions with the maternal cytoplasm causing embryo destruction prior to blastocyst formation (Renard et al., 1988), and embryo development in mice is negatively influenced by antisperm antibodies (Ahmad and Naz, 1991; Naz, 1992) which are present on the surface of the zygote during the first few cleavage divisions (O’Rand, 1977). Paternal effects on preimplantation development and blastocyst formation have also been reported in the human (Janny and Ménézo, 1994). These observations strongly suggest a paternal effect on early embryonic development and viability and, by inference, that sperm quality may play a role in embryo development following fertilization either in-vitro (IVF) or by gamete placement in the Fallopian tubes.

In assisted reproduction programmes, there are a number of artificially contrived events. These include the transfer of embryos directly into the uterine cavity without prior passage through the Fallopian tube, the establishment of pregnancies without exposing the cervix and reproductive tract to the products of ejaculation, and using spermatozoa from donors for uterine or cervical insemination or to produce embryos by IVF for uterine transfer. Furthermore, while IVF was initially used to overcome physical barriers to fertilization and implantation, the methodology has subsequently been successfully applied to other indications for infertility. While pregnancy rates in these situations have been widely reported, there has been limited critical examination of the outcome of these pregnancies or basic research into the effects on the offspring of by-passing normal reproductive processes or barriers to fertility (Ménézo and Dale, 1995; Seamark and Robinson, 1995).

As part of a continuing study of the influence of the paternal genome on reproductive health, we examined pregnancy rates and outcomes in women having gamete intra-Fallopian transfer (GIFT) where spermatozoa from either the husband or an altruistic donor were used for the GIFT procedure. For the period studied (1985–1993), there were 993 GIFT cycles (1/3 donor spermatozoa) and not only was the pregnancy rate significantly higher with donor spermatozoa than when the husband’s spermatozoa were used (48.6% compared with 32.4%) but also there was only one ectopic pregnancy from donor spermatozoa and the incidence of ectopic pregnancy was significantly lower (1.4% donor versus 5.3% husband: Petrucco et al., 1994). To explain this observation we suggested that differences in paternal genes relating to sperm morphology or function, when expressed in preimplantation embryos, could influence the propensity for implantation at ectopic sites. To test this hypothesis the original study has been extended by examining pregnancies on the Australian Institute of Health and Welfare (AIWH), National Perinatal Statistical Unit database. All units in Australia and New Zealand are required annually to provide details on the outcomes of their pregnancies to this database. We have compared the ectopic pregnancy rates...
following both GIFT and IVF in the treatment programmes at the Reproductive Medicine Unit (RMU) with the AIWH National Perinatal Statistical Unit data. The incidence of ectopic pregnancy was analysed for couples with infertility of different causes and whether spermatozoa from the male partner or a donor were used.

Materials and methods

Throughout the study period patient selection and treatment in the RMU treatment programmes conformed with established clinical protocols. Ovarian stimulation protocols were modified following the introduction of a gonadotrophin-releasing hormone agonist in 1989 (leuprolide acetate; Abbot Pharmaceuticals Australasia, Kurnel, NSW, Australia), however, the laboratory methods were essentially unchanged. All embryo transfers following IVF were performed with a ‘tom cat’ catheter (Sherwood Medical, St. Louis, MO, USA) whilst a Cook transfer catheter (Cook IVF, Brisbane, NSW, Australia, cat # K-GIFT-1010) was used for GIFT transfers, which were performed laparoscopically under general anaesthesia. Policy regarding the number of oocytes or embryos for transfer was modified during the study period, with the number of oocytes transferred at GIFT being reduced from three to two for women who were less than 37 years of age and a progressive trend for patients to have only two embryos transferred following IVF. A maximum of three oocytes or embryos was transferred throughout the study period.

Pregnancy was confirmed by an elevated level of human chorionic gonadotrophin (HCG) 16 days after oocyte recovery and clinical pregnancy by the presence of a gestational sac on ultrasound scan at 6 to 7 weeks gestation. Ectopic pregnancies were diagnosed by a combination of clinical symptoms, β-HCG and progesterone levels and pelvic ultrasound or laparoscopy. So that the statistical power of the analyses could be increased, data on all clinical and ectopic IVF and GIFT pregnancies for the years 1979–1993 were extracted from the AIWH National Perinatal Statistics Unit database in the form of composite tables. Data from individual clinics remained confidential and each clinic was autonomous with respect to patient selection and clinical and laboratory protocols. All pregnancies resulting from assisted fertilization techniques (subzonal sperm injection, intracytoplasmic sperm injection) were excluded.

Pregnancy outcomes were analysed according to whether the fertilizing spermatozoa were obtained from the husband or a donor and the type of treatment, i.e. IVF or GIFT. Information regarding the infertility status of couples on the pregnancy database was also analysed. Three broad categories were defined: (i) male factor only; (ii) male factor in combination with one or more other factor; and (iii) the absence of infertility due to the male partner. The fertility status of couples was centrally recorded but classification was at the discretion of each unit and may have varied. In particular ‘male factor infertility’ depended on the criteria used to make the diagnosis. Couples with ‘normal semen’ included those with a diagnosis of female infertility, most frequently related to damage to one or more Fallopian tubes but also as a result of endometriosis or pelvic adhesions involving the reproductive tract, and women with cervical factors. Couples with ‘unexplained infertility’ were also included in this category.

Table I. The effect of source of spermatozoa on the incidence of ectopic pregnancy following either in-vitro fertilization (IVF) or gamete intra-Fallopian transfer (GIFT) in Australia and New Zealand (National Perinatal Statistics Unit (NPSU) and the Reproductive Medicine Unit (RMU: University of Adelaide, Australia))

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Database</th>
<th>No. of clinical pregnancies</th>
<th>No. of ectopic pregnancies</th>
<th>Ectopic pregnancy rate (%)</th>
<th>Intrauterine: ectopic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIFT + donor</td>
<td>RMU</td>
<td>167</td>
<td>3</td>
<td>1.80</td>
<td>55:1</td>
</tr>
<tr>
<td></td>
<td>NPSU</td>
<td>957</td>
<td>24</td>
<td>2.51</td>
<td>39:1</td>
</tr>
<tr>
<td>GIFT + husband</td>
<td>RMU</td>
<td>222</td>
<td>11</td>
<td>4.95</td>
<td>19:1</td>
</tr>
<tr>
<td></td>
<td>NPSU</td>
<td>4753</td>
<td>197</td>
<td>4.14</td>
<td>23:1</td>
</tr>
<tr>
<td>IVF + donor</td>
<td>RMU</td>
<td>73</td>
<td>1</td>
<td>1.37</td>
<td>72:1</td>
</tr>
<tr>
<td></td>
<td>NPSU</td>
<td>907</td>
<td>37</td>
<td>4.08</td>
<td>24:1</td>
</tr>
<tr>
<td>IVF + husband</td>
<td>RMU</td>
<td>1060</td>
<td>34</td>
<td>3.21</td>
<td>30:1</td>
</tr>
<tr>
<td></td>
<td>NPSU</td>
<td>9280</td>
<td>540</td>
<td>6.17</td>
<td>15:1</td>
</tr>
</tbody>
</table>

The effect of source of spermatozoa on the incidence of ectopic pregnancy following either in-vitro fertilization (IVF) or gamete intra-Fallopian transfer (GIFT) in Australia and New Zealand (National Perinatal Statistics Unit (NPSU) and the Reproductive Medicine Unit (RMU: University of Adelaide, Australia))

Results

Following GIFT the overall incidence of ectopic gestation in the RMU treatment programmes was similar to those of the combined Australian and New Zealand units (Table I). With GIFT the incidence of ectopic pregnancy in the combined data was significantly lower when spermatozoa were used from a donor rather than from the male partner ($\chi^2 = 7.36; P = 0.007$). Following IVF, however, in pregnancies resulting from the use of spermatozoa from the male partner, the ectopic pregnancy rate in the RMU treatment programmes was significantly lower than for the combined AIWH National Perinatal Statistics Unit data (3.2% compared with 6.2%: $\chi^2 = 11.9; P = 0.0006$). Where donor spermatozoa were used, the difference was not significant. In the AIWH National Perinatal Statistics Unit data, couples having IVF with spermatozoa from the male partner made up 59% of the total pregnancies.
and 64% of all ectopic pregnancies. In both the RMU and the combined data, the risk of ectopic pregnancy was significantly increased for couples having IVF when compared with GIFT \( \left( \chi^2 = 26.6; \ P < 0.001 \right) \) or when spermatozoa from the male partner were used rather than from a donor \( \left( \chi^2 = 18.2; \ P < 0.001 \right) \).

The combined data from the AIHW National Perinatal Statistics Unit database therefore confirmed our previous observation on RMU pregnancies that, following GIFT, there were significantly fewer ectopic pregnancies with donor spermatozoa than with spermatozoa from the husband. The pattern of ectopic pregnancies following IVF, however, suggested that other differences between the treatment groups may have contributed to this observation. To determine whether the use of spermatozoa from the male partner or a donor influenced the incidence of ectopic pregnancy, independently of the infertility diagnosis or type of treatment, further analyses were performed according to the infertility diagnosis of each couple. The frequencies of ectopic gestation in the different treatment and patient groups are shown in Table II. The lowest incidence of ectopic pregnancy (1.4%) was in couples where the only indication for treatment was a semen defect in the male partner necessitating the use of donor semen for the GIFT procedure, and the highest incidence (>6%) occurred following IVF in couples whose infertility was unrelated to the male partner.

Interactions between pregnancy outcome \( (p) \), cause of infertility \( (i) \), source of spermatozoa \( (s) \) and type of treatment \( (t) \) were determined by fitting a log linear model to the cell frequencies of the categorical variables. Good agreement between the observed and expected frequencies was obtained \( (\text{Pearson} \chi^2 = 19.52; \ P = 0.034) \). The strongest associations found were between infertility status and the type of treatment \( (i \times t) \), the infertility status and sperm source \( (i \times s) \) and between the type of treatment and sperm source \( (t \times s) \). The categorical variable of greatest interest to this study, i.e. pregnancy outcome (intrauterine or ectopic), was significantly associated with the type of treatment \( (p \times t) \) and infertility status \( (p \times i) \), but a relationship between the source of spermatozoa used and ectopic pregnancy was not apparent from the model which best fitted the observed data. This suggests that the type of treatment and infertility category were more important determinants of ectopic pregnancy than whether the spermatozoa were obtained from the male partner or a donor.

Among the 170 donor spermatozoa/GIFT pregnancies in the RMU treatment programmes, there were three ectopic pregnancies, two being heterotopic. We therefore analysed the incidence and outcome of all heterotopic pregnancies in the AIHW National Perinatal Statistics Unit database for 1979–1995. Details are included in Table III. The overall frequency of heterotopic pregnancy was 0.6% and was similar following IVF (0.62%) and GIFT (0.55%) and where spermatozoa from the male partner (0.62%) or a donor (0.48%) were used. With regard to the outcome of the heterotopic pregnancies, the proportion of pregnancies resulting in a live birth was higher for donor spermatozoa (7/11, 64%) than when spermatozoa were provided by the male partner (46/130, 35%) but this finding was not statistically significant (odds ratio 3.2, 95% confidence interval = 0.9–11.5; \( P = 0.10 \)).

### Discussion
Ectopic pregnancy remains a major complication of pregnancy and is associated with significant patient morbidity and mortality. The reasons for an increase in the incidence of ectopic pregnancy in recent decades (Chamoun et al., 1996) has been the subject of considerable speculation but is generally attributed to an increase in established risk factors (James, 1996). Pelvic inflammatory disease and the spread of *Chlamydia trachomatis* may explain much of the increase (Ankum, 1996). However, maternal smoking at the time of conception (Campbell and Gray, 1987), difficulty in conceiving (Chow et al., 1987), delaying the age of conception, increasing maternal age and environmental exposure to mutagenic drugs (reviewed by Job-Spira et al., 1996) are risk factors. Modulation of tubal motility secondary to an imbalance of oestradiol and progesterone (Fernandez et al., 1996; James, 1996; Parazzini,
1996) and chromosomal abnormalities resulting from abnormal embryogenesis (Cohen et al., 1993; Toikkanen et al., 1993) or of maternal origin (Karikoski et al., 1993; Job-Spira et al., 1996) have also been proposed as alternative mechanisms to explain ectopic pregnancy. However, others have reported a low incidence of chromosomal abnormality (Goddijn et al., 1996) and their significance remains conjectural. Furthermore, Mol et al. (1995) in a meta-analysis of pregnancies following discontinuing contraception found that intrauterine contraceptive devices, but not oral contraceptives or tubal sterilization, were associated with an increased risk of ectopic pregnancy. While Speirs (1996) has rightly highlighted the need to control for tubal disease because of its strong association with ectopic pregnancy (Ankum et al., 1996), others have suggested that 1/4 to 1/3 of ectopic pregnancies cannot be attributed to tubal risk factors (see James, 1996).

While information on the relationship, if any, between sperm phenotype and genotype in the human is limited to a small number of specific conditions, such as globozoospermia, IVF using spermatozoa with poor morphology or motility may result in delayed fertilization and poor embryo morphology (Ron-El, 1991), impaired embryo viability (Enginsu et al., 1992) and reduced blastocyst formation in vitro (Janny and Ménézo, 1994). In BALB/c mice, injection of spermatozoa with grossly abnormal morphology directly into the oocyte results in normal pups (Burruel et al., 1996), suggesting that abnormal sperm morphology acts primarily as a barrier to normal fertilization in this model, but the human reports suggest that embryo viability may be affected by expression of paternal genes during early embryo development. We therefore proposed that the increased incidence in ectopic pregnancy in couples having GIFT with spermatozoa from the male partner rather than a donor may result from abnormal embryo development resulting from the expression of paternal genes.

To test this hypothesis we used data on IVF and GIFT pregnancies from 1979 to 1993, a period when the main focus of treatment was on IVF for couples where there was pathology associated with the reproductive tract of the female partner (“tubal factor”) and on GIFT in couples with unexplained infertility. The contribution of GIFT to the total pregnancies was particularly significant from 1985 to 1993. The widespread use of intracytoplasmic sperm injection (ICSI) since 1993 has resulted in a marked decrease in the contribution of both donor spermatozoa and GIFT cycles to the total activity (NPSU, 1997). More than 16,000 IVF and GIFT pregnancies in Australia and New Zealand IVF were analysed. The overall incidence of ectopic gestation was 5.0%, which is similar to that reported for assisted reproduction in a survey of 27 countries where the incidence varied from 3 to 12% (de Mouzon and Lancaster, 1995). Our analyses have shown that there are significant interactions between the incidence of ectopic pregnancy and treatment (IVF or GIFT: male partner or donor spermatozoa) and also with the infertility category of couples having treatment. Because treatment modality depends on the infertility diagnosis (i.e. GIFT is offered where the female partner has at least one normal Fallopian tube and the male has good semen; donor spermatozoa are offered if the semen analysis indicates a severe defect or the couple had a history of poor fertilization following IVF; IVF is used where there is damage to the Fallopian tubes or poor fertilization is anticipated), strong statistical associations between infertility category and the modality of treatment were also confirmed.

When patients were separated with respect to the cause of their infertility, however, we were unable to demonstrate an association between incidence of ectopic pregnancy and whether the spermatozoa came from the male partner or a donor. We therefore conclude that expression of paternal genes is probably not a significant factor in the overall aetiology of ectopic pregnancy, at least in comparison with other known risk factors. There are, however, possible reasons why we may not have been able to show an effect. While statistical power was increased by the use of a large database (i.e. the AIHW National Perinatal Statistics Unit database is among the most comprehensive multicentre registers available for IVF and GIFT pregnancies), there were limitations with respect to defining specific subgroups and controlling important variables. For these analyses, clinical practices or procedures were assumed not to differ significantly between units or to influence the rate of ectopic pregnancy. Because the choice of treatment for a particular couple can vary according to clinical practices, unit preference for particular treatments or even preference by the individual couple, this may not be a valid assumption. For example, in the RMU treatment programmes, donor semen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total no. of pregnancies</th>
<th>No. of heterotopic pregnancies</th>
<th>Abortion</th>
<th>Birth</th>
<th>Heterotopic pregnancies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIFT-D</td>
<td>1312</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>IVF-D</td>
<td>1255</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>2567</td>
<td>11</td>
<td>4</td>
<td>7</td>
<td>0.43b</td>
</tr>
<tr>
<td>GIFT-H</td>
<td>6315</td>
<td>37</td>
<td>24</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>IVF-H</td>
<td>14,496</td>
<td>93</td>
<td>60</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>20,811</td>
<td>130</td>
<td>84</td>
<td>46</td>
<td>0.62</td>
</tr>
</tbody>
</table>

*Either donated spermatozoa (D) or from the male partner (H) used for IVF or GIFT.

The differences in both the incidence and outcome of heterotopic pregnancies were not statistically significant (see text for details).
was only offered where the male partners were azoospermic or the semen failed to meet minimum requirement (i.e. <200,000 motile spermatozoa after washing) but in the combined database there were pregnancies reported with donor semen in couples classified as having ‘normal semen’. Similarly the RMU only offers GIFT to couples with a normal semen analysis and where there is laparoscopic evidence of normal Fallopian tubes and a pelvis free of active endometriosis (i.e. for couples with unexplained infertility) but the AIHW National Perinatal Statistics Unit results included GIFT pregnancies for couples with ‘female’ infertility. Given that more than 30 individual clinics contribute to the AIHW National Perinatal Statistics Unit register and there is no uniform consensus on how to define specific categories of infertile couples or treatment options for couples, interpreting analyses based on pooled data from a diversity of treatment programmes can be questioned. This problem may well increase if the emphasis on the accuracy of the infertility diagnosis decreases as the tendency to offer standardized treatment increases. Many units now choose not to offer GIFT and use IVF or ICSI to treat unexplained infertility. Methodological differences between reporting units could also be significant. In this study, we observed a statistically lower incidence of ectopic pregnancy following IVF in the RMU than for the AIHW National Perinatal Statistics Unit data. While there was no obvious reason for this difference, the method of embryo transfer has been shown to affect the rate of ectopic pregnancy (Nazari et al., 1993; Marcus and Brinsden, 1995).

To improve the test of our hypothesis, we would require a cohort of patients with a defined abnormality in sperm morphology as the sole cause of their infertility and randomized treatment with regard to site of transfer (uterus for IVF; tube for GIFT) and the source of the spermatozoa (male partner or donor). Such a trial is clearly neither logistically nor ethically feasible. An alternative model would be to examine the incidence of ectopic pregnancy following ICSI using spermatozoa with a sufficiently stringent classification of ‘male factor’ to enable any relationships between sperm morphology and embryo development (or ectopic pregnancy) to be tested. However, this model would also suffer from the need for multicentre results for statistical power and from a trend towards a reduced emphasis on detailed investigation of the male and female partner as ICSI increasingly becomes the treatment of choice in many units.

If the embryonic genome plays a role in ectopic pregnancy because embryos with poor viability lack the potential to interact appropriately with the uterine endometrium, a high incidence of miscarriage in heterotopic pregnancies might be predicted. Tal et al. (1996) recently reviewed the literature on heterotopic pregnancies following ovulation induction and assisted reproduction. The incidence was approximately 1% and was consistent with the estimated incidence based on the relative incidence of ectopic pregnancy and multiple gestation. They reviewed 139 cases of heterotopic pregnancy following assisted reproduction and in this cohort the live birth rate was 68%. The AIHW National Perinatal Statistics Unit database between 1979 and 1995 includes more than 23,000 IVF and GIFT pregnancies. The incidence of heterotopic pregnancies was 0.6% but, even with this large database, statistical power is limited. Statistical significance was not achieved (P = 0.1) despite the viability of the non-ectopic pregnancy with donor spermatozoa being almost twice that when spermatozoa from the male partner was used, a result which might be expected if the use of donor spermatozoa were beneficial to embryo development in this cohort of couples.

References


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