Semen quality: is there a paternal effect on pregnancy outcome in in-vitro fertilization/intracytoplasmic sperm injection?

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The objective of this study was to investigate the role of the spermatozoon (paternal effects) on implantation and pregnancy outcome in in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI). Male individuals of three types were analysed: infertile men with oligoasthenoteratozoospermia (OAT), fertile men with normozoospermia and fertile men (donors). Female counterparts were judged to have comparable egg quality within two groups studied, i.e. infertile women with pure mechanical (tubal) infertility and recipients of donor eggs. There were significantly higher differences in implantation and pregnancy rates in groups using donor spermatozoa and donor egg recipients. Analyses of key set groups revealed a trend toward a poorer implantation and pregnancy outcome when comparing OAT versus normozoospermic patients within IVF, but not within ICSI treatments, in couples with tubal infertility. In couples who were recipients of donor eggs, no differences were observed between OAT patients treated by ICSI and normozoospermic patients treated with IVF. No significant differences were observed in miscarriage rates within any groups studied. In conclusion, the poorer results observed in OAT patients undergoing IVF may be secondary to spermatozoal effects due to a high insemination concentration. Overall, there does not seem to be a significant effect of severe male infertility (OAT) on implantation and pregnancy outcome. However, this does not preclude that specific sperm aberrations may exert a negative effect on embryogenesis and therefore on implantation potential following assisted or in-vivo reproduction.

Key words: oligoasthenoteratozoospermia/implantation/pregnancy

Introduction

There is an ongoing debate regarding the effects of sperm quality on the implantation potential of human embryos. Janny and Ménézo (1994) provided evidence for a strong paternal effect on human preimplantation embryo development in blastocyst studies in vitro. Ron-El et al. (1991) reported delayed fertilization and poor embryonic development associated with impaired semen quality following in-vitro fertilization (IVF) therapy. Parinaud et al. (1993) suggested that the spermatozoon is involved in the embryonic quality, even in the early stages of development, and showed an association between abnormal sperm morphology and poor embryo morphology. We have also observed reduced implantation and pregnancy rates in men with severe teratozoospermia, associated with oligoasthenozoospermia (OAT), undergoing IVF (Oehninger et al., 1988; Grow et al., 1994). A comparative analysis of embryo implantation in patients with severe teratozoospermia treated with either IVF using a high insemination concentration or intracytoplasmic sperm injection (ICSI) revealed that ICSI produced a significantly higher proportion of morphologically superior embryos with a tendency towards a higher implantation potential (Oehninger et al., 1996). This suggested a negative effect of putative spermatozoal factors released in the vicinity of the eggs on early embryogenesis (Hall et al., 1995; Oehninger et al., 1996). It is a known fact that the first two cell cycles of the embryo are controlled by maternal genes (Braude et al., 1988). The paternal effect on the embryo does not begin until the 4-cell stage; therefore, potential detrimental effects on embryo competence could be observed in vitro after that stage of development and up to blastocyst formation, or in vivo, looking at implantation, pregnancy viability and loss following embryo transfer.

Thus, the goal of this study was to test whether or not men with poor sperm parameters contribute to poor embryo quality. In order to achieve this objective, a comparison was made of implantation, pregnancy and miscarriage rates for different groups of individuals undergoing IVF and ICSI therapies. This was a retrospective, controlled analysis of three groups of men including infertile men with OAT, infertile men with normozoospermia (NZ) and fertile men (donor spermatozoa, DS). Female counterparts were considered to have comparable egg quality within two groups of patients, i.e. infertile women with pure mechanical (tubal) infertility and recipients of donor eggs (DE).

Materials and methods

In our programme, clinical and laboratory data were stored for individual patients in a Pentium computer (Zios International Ltd., Minneapolis, MN, USA) using a customized software program written in APL Plus (originally developed by K.Iverson of Harvard University, Boston, MA, USA). Eight groups of patients were categorized in the following manner: IVF-NZ; IVF-OAT; ICSI-NZ; ICSI-OAT; IVF-NZ/OAT; ICSI-NZ/OAT; IVF-NZ/DS; ICSI-NZ/DS.

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NZ-DE; ICSI-OAT-DE; IVF-DS; and IVF-DS-DE. The total number of patients (cycles) studied was 1322. All attempts included cycles with an embryo transfer.

OAT was defined following World Health Organization criteria for sperm concentration (<20 million spermatozoa/ml) and motility (<50%) (World Health Organization, 1992), and strict criteria for sperm morphology (<4% normal forms, ‘poor prognosis pattern’) (Oehninger et al., 1988; Grow et al., 1994). Infertile men not pertaining to this group were categorized as NZ. Candidates for donor spermatozoa and donor egg selection followed routine guidelines established in our programmes at Norfolk. Except for the DE groups, all other female counterparts were selected based upon a diagnosis of pure mechanical, tubal infertility. This was done in order to have a controlled, homogeneous group of women with a similar aetiological factor. The recipients of DE constituted a group where the egg factor could be maximally controlled (donors were selected based upon age <35 years with normal serum day 3 follicle stimulating hormone concentrations) and therefore contributed the best quality eggs to the study.

All patients except those belonging to the DE, DS and IVF-OAT groups underwent therapy between April, 1994 and September, 1996. All DE and DS groups were treated between April, 1994 and March, 1997 (the study period for these groups was enlarged in order to be able to include more cycles of treatment). Patients from the IVF-OAT group underwent therapy from May, 1988 through December, 1991. Our programme started offering ICSI to all patients with diagnosis of OAT or previous poor or failed fertilization in April, 1994. Consequently, male infertility patients undergoing IVF therapy had to be gathered from a previous time-frame. Information from this group of IVF-OAT patients was published in a previous communication (Grow et al., 1994).

Ovarian stimulation protocols, sperm preparation, IVF and ICSI techniques, embryo transfer procedures and luteal support used in our assisted reproduction programmes have been published extensively. For the IVF-OAT group, eggs were inseminated using a high insemination concentration (Grow et al., 1994). Implantation rate was defined as the number of gestational sacs visualized by ultrasonography divided by the total number of embryos transferred. The clinical pregnancy rate was calculated as the number of pregnancies per cycle divided by the total number of transfers. Miscarriages were defined as pregnancies that had aborted by the first trimester. Data were analysed by contingency tables using $\chi^2$ (with Yates’ correction) and Fisher’s exact tests as appropriate, in order to determine whether significant differences existed in implantation, pregnancy and miscarriage rates between specific groups.

**Results**

The mean age of the women and number of embryos transferred per transfer (± SD) for all groups were as follows: IVF-NZ: 35.3 ± 0.2 and 4.0 ± 0.1; IVF-OAT: 35.3 ± 0.3 and 3.6 ± 0.1; ICSI-NZ: 35.3 ± 0.3 and 3.9 ± 0.1; ICSI-OAT: 34.0 ± 0.6 and 4.0 ± 0.2; IVF-NZ-DE: 40.0 ± 0.3 and 3.5 ± 0.2; ICSI-OAT-DE: 40.3 ± 0.3 and 3.6 ± 0.2; IVF-DS: 37.3 ± 0.2 and 3.6 ± 0.1; and IVF-DS-DE: 40.4 ± 0.2 and 3.8 ± 0.1. There were no significant differences in the age or in the number of embryos transferred for the different groups.

Table I summarizes implantation, pregnancy and miscarriage rates for each of the eight groups used in the study. Table II presents statistical results of key comparisons. An overall comparison of the eight groups of patients revealed significant differences in implantation ($P = 0.001$) and pregnancy ($P = 0.001$) rates but not in pregnancy loss. Analysis of key groups of patients was then performed in order to test whether infertile men with poor sperm parameters (OAT) show an impairment in embryo quality. For IVF, there was a clear trend for higher implantation and pregnancy rates in normozoospermic patients (IVF-NZ) than in patients with OAT (IVF-OAT), but the differences were marginally significant ($P = 0.08$ and $P = 0.05$, respectively). Comparison of patients with IVF-OAT and IVF-DS showed marginal differences in implantation ($P = 0.05$) and pregnancy ($P = 0.07$) rates in favour of donor sperm cases. There were no differences in implantation and pregnancy rates within ICSI groups with NZ and OAT (ICSI-NZ versus ICSI-OAT). Also, there were no differences when comparing IVF-NZ with ICSI-NZ or IVF-OAT with ICSI-OAT groups.

Groups of patients receiving DE had the best results in terms of implantation and pregnancy rates. Importantly, there

<table>
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<tr>
<th>Groups</th>
<th>Implantation</th>
<th>Pregnancy</th>
<th>Miscarriage</th>
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<tbody>
<tr>
<td>IVF-NZ</td>
<td>175/1737 (10)</td>
<td>130/455 (28)</td>
<td>36/130 (27)</td>
</tr>
<tr>
<td>IVF-OAT</td>
<td>40/538 (7)</td>
<td>30/150 (20)</td>
<td>9/30 (30)</td>
</tr>
<tr>
<td>IVF-DS</td>
<td>43/377 (11)</td>
<td>32/105 (30)</td>
<td>6/32 (19)</td>
</tr>
<tr>
<td>ICSI-NZ</td>
<td>101/1066 (10)</td>
<td>81/255 (32)</td>
<td>13/81 (16)</td>
</tr>
<tr>
<td>ICSI-OAT</td>
<td>32/362 (9)</td>
<td>28/92 (30)</td>
<td>10/28 (36)</td>
</tr>
<tr>
<td>IVF-NZ-DE</td>
<td>141/712 (20)</td>
<td>76/201 (38)</td>
<td>14/76 (18)</td>
</tr>
<tr>
<td>ICSI-OAT-DE</td>
<td>28/181 (15)</td>
<td>20/50 (40)</td>
<td>4/20 (20)</td>
</tr>
<tr>
<td>IVF-DS-DE</td>
<td>17/52 (33)</td>
<td>10/14 (71)</td>
<td>1/10 (10)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

a Defined as number of gestational sacs/number of embryos transferred.
b Defined as clinical pregnancies/number of cycles.
c Defined as pregnancies miscarried/total number of pregnancies.

OAT = oligoasthenoteratozoospermia; NZ = normozoospermia; DS = donor spermatozoa; DE = donor eggs; IVF = in-vitro fertilization; ICSI = intracytoplasmic sperm injection.

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<th>Table II. Statistical analysis of key selected groups (P-values)</th>
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<td>Groups</td>
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<tr>
<td>IVF-NZ</td>
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<td>IVF-OAT</td>
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For abbreviations, see Table I.
were no significant differences between recipients having undergone IVF-NZ-DE compared with those with ICSI-OAT-DE. There were significant differences in implantation and pregnancy rates, however, when recipients of both DS and DE were included in the analysis: IVF-NZ-DE versus ICSI-OAT-DE versus IVF-DS-DE; \( P = 0.02 \) and \( P = 0.04 \), respectively. Comparison of all severe male infertility groups showed significantly higher implantation and pregnancy rates for DE recipients (IVF-OAT versus ICSI-OAT versus ICSI-OAT-DE \( (P = 0.001 \) and \( P = 0.01 \), respectively).

There were no significant differences in miscarriage rates between any of the groups studied, except a marginally significant difference between IVF-NZ and ICSI-NZ. However, the power for detection of differences in pregnancy loss was only 41% (a total of 830 pregnancies would be needed in order to detect differences with at least an 80% power). On the other hand, the statistical power of the observed differences in implantation and pregnancy rates exceeded 90%.

Discussion

In this study we assessed whether the presence of severe male infertility might be associated with an effect on embryo quality as judged by implantation, pregnancy and miscarriage rates following uterine embryo transfer. It could be speculated that abnormalities of the male gamete might be associated with abnormal embryo development resulting in either impaired implantation or increased pregnancy loss. Spermatozoal deficiencies including nuclear or cytoplasmic aberrations of those organelles/molecules involved in oocyte activation and embryogenesis might be responsible for such a negative effect.

Here, we studied infertile men with a variety of sperm disorders, judged to be severe as classified by World Health Organization (oligoasthenozoospermia) and strict criteria (teratozoospermia). Careful data analysis revealed that male infertility is not associated with an impaired implantation or pregnancy potential within the assisted reproduction setting. This was demonstrated for several of the different groups studied, but particularly by the lack of impact of OAT within the DE setting (IVF-NZ-DE versus ICSI-OAT-DE). Oocyte donation provides the best model to answer our question by allowing for maximal control of the egg factor. Additionally, the results of couples with tubal infertility and OAT did not differ between IVF or ICSI therapies. Furthermore, the results of ICSI-OAT and ICSI-NZ were similar, again pointing out the absence of impact of male infertility on implantation or pregnancy outcome.

The only exception to this overall conclusion was the observation of a trend toward decreased implantation and pregnancy rates in the IVF-OAT group (compared with IVF-NZ or IVF-DS). We and others have speculated that the use of a high insemination concentration in such cases (with the techniques of multiple oocytes per dish or insemination in a smaller volume of medium) may be associated with the release of toxic factors by abnormal spermatozoa (reactive oxygen species and/or others) resulting in oocyte/embryo damage. This technique usually results in an improved fertilization rate but pregnancy results, although improved, have been suboptimal. Therefore, we now recommend using ICSI in these situations (Hall et al., 1995; Oehninger et al., 1996).

As expected, superior conception results were observed within the DE groups (in spite of the presence of severe OAT). However, the use of DS in the group of infertile couples with a tubal factor did not improve results significantly. This probably points to the presence of subtle defects of egg quality in the infertility population. On the other hand, the combination of DE and DS in IVF produced outstanding implantation and pregnancy rates (33% and 71%, respectively), demonstrating the true potential of the assisted reproductive technologies.

We must acknowledge that this study had limitations that arise mainly from its retrospective nature. Retrospective studies may have different types of bias in terms of patient selection, time-frame and others. Here, we studied patients whose IVF/ICSI results were stored in our computerized database with no exclusions (inclusion criteria were the presence of females with tubal infertility or recipients of egg donation) and who were allocated into the study groups based upon an accepted definition of male infertility. The IVF-OAT group had to be selected from a previous time-frame since our programme has performed ICSI in male infertility cases since April, 1994. However, we judged this to be a reasonable approach since a prospective study comparing IVF versus ICSI results in OAT patients seems not to be logical at this time. Furthermore, although other discrepancies existed among the study periods for some of the remaining groups, any changes implemented in our programme in terms of ovarian stimulation protocols, gamete manipulation or embryo transfer techniques would have affected all groups similarly.

Finally, although the conclusions reached in terms of implantation and pregnancy outcomes are validated by an adequate statistical power, unfortunately we could not collect sufficient numbers of pregnancies to validate the miscarriage results. Although initial reports of the normalcy of children born after ICSI are reassuring (Bondouelle et al., 1996; Wennerholm et al., 1996), more studies are needed in order to answer unequivocally these critical questions. This is particularly relevant in light of the reports of increased aneuploidy in spermatozoa of OAT patients and the presence of microdeletions of the Y chromosomes in the infertile population (In’t Veld et al., 1995; Moosani et al., 1995; Reijo et al., 1995). Furthermore, alterations in sperm chromatin might result in defective decondensation and DNA activation during fertilization, leading to a delay in the formation of the male pronucleus and/or the first division events. One consequence of this might be early embryonic wastage or poor embryonic development (Hamamah et al., 1997). Consequently, prospectively designed studies of the chromosomal/genetic characteristics of spermatozoa and lost products of conception in cases of severe male infertility are warranted.

In conclusion, it would seem reasonable to state that, overall, men with poor sperm parameters do not contribute to poor embryonic development. Infertile patients with OAT (except when a high insemination concentration is used) seem to yield embryos with similar implantation and developmental potential to those of infertile and fertile men with normozoospermia. To the best of our knowledge, this is the first attempt to
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examine the role of the spermatozoon on pregnancy outcome within assisted reproduction using a model that controls for egg quality. These data give support to and extend the benefits of ICSI as the technique of choice for OAT within the assisted reproduction setting. These results, on the other hand, do not preclude the possibility that specific (known or not yet identified) sperm abnormalities may be responsible for defective embryogenesis. Sperm morphology and zona binding, among others, are functional bio-markers of fertilization disorders (Liu et al., 1988; Oehninger et al., 1992). It is our challenge to try to identify other sperm deficiencies (genetic, cellular–molecular) that may be associated with a negative impact in post-fertilization development.

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References


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