The contribution of subtle oocyte or sperm dysfunction affecting fertilization in endometriosis-associated or unexplained infertility: a controlled comparison with tubal infertility and use of donor spermatozoa

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This study aims to determine the relative contribution of oocyte and/or sperm dysfunction to the reduction of fertilization rates in vitro in cases of minor endometriosis and prolonged unexplained infertility. The results of in-vitro fertilization (IVF) treatment with ovarian stimulation have been compared between couples with the above conditions and women with tubal infertility (as control for oocyte function) and the use of donor spermatozoa (as control for sperm function). Fertilization and cleavage rates using husband’s spermatozoa were significantly reduced in endometriosis couples (56%, n = 194, P < 0.001) and further significantly reduced in couples with unexplained infertility (52%, n = 327, P < 0.001) compared with tubal infertility (60%, n = 509). Using donor spermatozoa the rates were the same as using husband’s spermatozoa in tubal infertility (61%, n = 27) or endometriosis (55%, n = 21) but significantly though only partly improved with unexplained infertility (57%, n = 60, P < 0.02). In unexplained infertility, a significantly increased proportion of couples experienced complete failure of fertilization and cleavage in a cycle (5–6% versus 2–3%). However, complete failure was not usually repetitive, and the affected couples did not account for the overall reduction in fertilization and cleavage rates, which remained significantly lower in the rest of the unexplained and endometriosis groups. Implantation and pregnancy rates appeared similar in all groups. The benefit of IVF treatment in cases of minor endometriosis and prolonged unexplained infertility is due to superabundance of oocytes obtained by stimulation. The reduction in natural fertility associated with endometriosis appears to be at least partly due to a reduced fertilizing ability of the oocyte. In unexplained infertility, there is distinct impairment due to otherwise unsuspected sperm dysfunction but probably also oocyte dysfunction.

Key words: endometriosis/fertilization/oocyte/spermatozoa/unexplained infertility

Introduction

Undefined mechanisms cause subfertility associated with minor endometriosis or prolonged unexplained infertility in apparently normal couples. Our own studies employing controlled comparison with tubal infertility have suggested that endometriosis is associated with oocyte and follicular dysfunction, as indicated by reduced fertilization rates observed in in-vitro fertilization (IVF) treatment using various stimulation regimens (Wardle et al., 1985; Mills et al., 1992; Fleming et al., 1994) or without stimulation (Cahill et al., 1997), and by reduced steroidogenic capacity of the granulosa cells (Harlow et al., 1996). Some authors have found no significant reduction in fertilization rates, but two of the biggest studies apart from our own have found a highly significant reduction in fertilization rates and an overall analysis combining all available reports indicates a proportional reduction by about one-third (Table I).

By contrast, various reports suggest that unexplained infertility may be due, despite normal semen microscopy, to unsuspected sperm dysfunction, as indicated by the failure of various fertilizing steps (Albert et al., 1992; Mackenna et al., 1993) and reduced fertilization rates of human oocytes (Audibert et al., 1989), as well as failure to penetrate zona-free hamster oocytes in vitro (Aitken et al., 1982), which in turn can be linked to impaired ability of the spermatozoa to penetrate the cervical mucus (Schaats et al., 1984). These possibilities are unfortunately confused by varied definition of unexplained infertility, but even when sperm–mucus penetration is a required diagnostic criterion, as in our own practice, there remains the possibility of subtle degrees of sperm dysfunction contributing to the observed subfertility.

The same possibility might apply to endometriosis, particularly in minor cases without any structural damage. Endometriosis as a disease entity and direct cause of infertility has been questioned (Wardle and Hull, 1993). It may be functionally linked to unexplained infertility, since many women with unexplained infertility are later found to have developed endometriosis (Pepperell and McBain, 1985).

The present study therefore aims to determine the possibility of subtle oocyte and/or sperm dysfunction, which may not be indicated by standard fertility tests, contributing to the reduced fertilization rates, and therefore to the natural subfertility, associated with minor endometriosis or prolonged unexplained infertility. We have undertaken a controlled comparison of the results of standard IVF treatment, not using intracytoplasmic sperm injection (ICSI), using women with tubal infertility as the female controls and donor spermatozoa as the male controls.

Materials and methods

We studied couples who had IVF treatment employing standardized methods from July 1990 to December 1996 and who met the following
Fertilization, or strictly fertilization and cleavage leading to the development of a normal embryo suitable for transfer, was defined by the formation of two pronuclei and progressive cleavage. The fertilization rate was based on the total number of oocytes collected, irrespective of apparent maturity. Pregnancy was defined by ultrasound evidence of a gestation sac, including ectopic gestation. Implantation rate was defined by the number of gestation sacs as a proportion of the number of embryos transferred.

Results were first analysed by comparison between groups of pooled oocyte or embryo data in all cycles of oocyte collection. Further analysis was undertaken on individual fertilization rates but was limited to the first three cycles for each couple and to cycles producing at least five mature oocytes, to minimize potential bias due to unbalanced cycle numbers and minimize distortion by extreme fertilization rates due to the small number of oocytes. Rates based on pooled embryos and eggs within each group were compared using the χ² test employing Yates’ correction where appropriate. Means of individual fertilization rates in the groups were tested by one-way analysis of variance for significant differences (P < 0.05) and, if found, differences between individual groups were tested by Student’s t-test. Means of continuous variables (age, duration of infertility) were compared by two-way analysis of variance, using logarithmic transformation to normalize data as appropriate (duration of infertility).

### Table I. Fertilization rates in previously reported studies of IVF treatment for infertility due to endometriosis compared with tubal disease. (The results of the present study include in part those reported in abstract by Fleming et al., 1994)

<table>
<thead>
<tr>
<th>Authors and hormonal stimulation</th>
<th>Tubal</th>
<th>Endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oocytes Embryos (%)</td>
<td>Oocytes Embryos (%)</td>
</tr>
<tr>
<td>Mahadevan et al. (1983)</td>
<td>594</td>
<td>476 (80)</td>
</tr>
<tr>
<td>Clomiphene/HCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wardle et al. (1985)</td>
<td>77</td>
<td>54 (70)</td>
</tr>
<tr>
<td>Clomiphene/HMG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dlugi et al. (1989)</td>
<td>81</td>
<td>69 (85)</td>
</tr>
<tr>
<td>Clomiphene/HMG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matson and Yovich, (1989)</td>
<td>174</td>
<td>132 (76)</td>
</tr>
<tr>
<td>Clomiphene/HMG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahmood et al. (1991)</td>
<td>8</td>
<td>5 (63)</td>
</tr>
<tr>
<td>Unstimulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melbourne et al. (1992)</td>
<td>937</td>
<td>642 (69)</td>
</tr>
<tr>
<td>Lelaider et al. (1993)</td>
<td>7181</td>
<td>5726 (80)</td>
</tr>
<tr>
<td>Stimulation not stated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fleming et al. (1994)</td>
<td>2745</td>
<td>1949 (71)</td>
</tr>
<tr>
<td>GnRH/HMG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simon et al. (1994)</td>
<td>1162</td>
<td>670 (58)</td>
</tr>
<tr>
<td>Cahill et al. (1997)</td>
<td>51</td>
<td>39 (76)</td>
</tr>
<tr>
<td>Unstimulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>12 929</td>
<td>9762 (76)</td>
</tr>
</tbody>
</table>

*Numbers calculated from rates given.

HMG = human menopausal gonadotrophin; FSH = follicle stimulating hormone; HCG = human chorionic gonadotrophin; GnRH = gonadotrophin-releasing hormone analogue.

### Results

The numbers of couples, their background information, and cycles of oocyte collection studied in each group, followed by the outcomes of treatment based on pooled numbers of oocytes and transferred embryos are given in Table II. The woman’s age and duration of infertility were significantly greater (in both cases by 1–2 years) in couples treated with donor spermatozoa, but did not differ significantly between diagnostic groups. Significantly fewer oocytes were collected in the donor sperm groups, which may be explained by the differences in age. There were no significant differences in oocyte numbers between diagnostic groups. The numbers of embryos were too dependent on the number of oocytes to be usefully compared.

Fertilization rates using husband’s spermatozoa were highly significantly reduced in both the endometriosis and unexplained infertility groups compared with the tubal group, and the rate was further reduced significantly in the unexplained compared with the endometriosis group. Using donor spermatozoa, however, the fertilization rate in the unexplained group was significantly improved compared with husband’s spermatozoa, but the rate in the endometriosis group appeared to remain as low as with husband’s spermatozoa.

Embryos were available for transfer in nearly all cycles of oocyte collection in all groups, but the lack of embryos due to complete failure of fertilization and/or cleavage was significantly more frequent in the unexplained subgroups. Of the couples who failed to achieve embryos in a cycle, the
numbers who had additional cycles of treatment were too few for useful statistical comparison. However, 12 couples with tubal infertility all achieved fertilization and cleavage in another cycle with husband’s (n = 11) or donor spermatozoa (n = 1), at a median rate of 55%. In two patients with endometriosis, both using their husband’s spermatozoa, one failed and one produced embryos from all three oocytes. In 12 patients with unexplained infertility, nine achieved embryos, five out of eight with husband’s spermatozoa and four out of four with donor spermatozoa, at an overall median rate of 33%. Even after excluding the repeated failures, the rate was 36%. Those findings suggest a persistent impairment, though more likely to be due to dysfunction of the husband’s spermatozoa than the woman’s oocytes.

To determine whether the reduced fertilization rates in the endometriosis and unexplained infertility groups could be explained by failure affecting only a small minority of individuals, fertilization rates were compared after excluding those couples who experienced complete failure. However the same differences were found as between the full groups (whose results are given in Table II). Using the husbands’ spermatozoa, the fertilization and cleavage rate in the tubal group was 61%, but by comparison was significantly reduced in the endometriosis group (57%, \( P < 0.001 \)) and unexplained group (55%, \( P < 0.001 \)); and the rate in the unexplained group was significantly lower than in the endometriosis group (\( P < 0.01 \)). Using donor spermatozoa the rates in the three groups were 62, 56 and 58% respectively; the apparent increase in the unexplained group using donor compared with husband’s spermatozoa was not now significant.

Similar numbers of embryos were transferred each time when available. The resulting pregnancy and implantation rates were not significantly different between groups although there appeared to be a greater variation in the donor sperm groups associated with relatively small numbers.

Table III compares the individual fertilization and cleavage rates expressed as the mean and standard error of the mean (rather than pooled rates) between the study groups after limitation to the first three cycles and production of at least five oocytes. In the groups using husband’s spermatozoa, one-way analysis of variance indicated highly significant effect of diagnosis on fertilization and cleavage rates (\( P < 0.0001 \)) and \( t \)-tests confirmed the differences between individual groups (probabilities given at foot of Table III) which had been found in the analysis of pooled data in Table II.

In the groups using donor spermatozoa (Table III), one-way analysis of variance failed to detect a significant effect of diagnosis although the observed results were very similar to those in Table II. The numbers in the donor sperm groups were again much lower than in the husband’s sperm groups.

The results in Table III are almost identical to those based on the larger set of pooled data in Table II and eliminate the possibility of distortion caused by the pooled analysis, which therefore provide valid, representative findings.

**Discussion**

The results of the present study first of all confirm the reduction in fertilization and cleavage rates in women with endometriosis, compared with tubal infertility, as generally found in other reports as reviewed in Table I. We found the rate was reduced from 60 to 56%. Furthermore there was an even greater reduction in fertilization and cleavage rates in couples with unexplained infertility (52%), despite a requirement for preliminary diagnostic evidence of favourable sperm function in this and all the study groups using the husband’s spermatozoa.

**Table II. Background information on couples studied, and results of IVF treatment based on all oocyte collections and pooled oocytes in each group, comparing women with endometriosis, unexplained and tubal infertility, and using husband’s or donor spermatozoa**

<table>
<thead>
<tr>
<th>No. of couples</th>
<th>Husband’s spermatozoa (H)</th>
<th>Donor spermatozoa (D)</th>
<th>Statistical probability of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubal</td>
<td>Endometriosis</td>
<td>Unexplained</td>
<td>Tubal</td>
</tr>
<tr>
<td>No. of couples</td>
<td>509</td>
<td>194</td>
<td>327</td>
</tr>
<tr>
<td>Woman’s age (years) (mean ± SEM)</td>
<td>33.1 (0.16)</td>
<td>33.3 (0.27)</td>
<td>33.3 (0.21)</td>
</tr>
<tr>
<td>Duration infertility (years) median (quartiles)</td>
<td>4 (2–6)</td>
<td>4.5 (3–6)</td>
<td>4 (3–7)</td>
</tr>
<tr>
<td>Cycles of oocyte collection</td>
<td>813</td>
<td>252</td>
<td>402</td>
</tr>
<tr>
<td>Embryos</td>
<td>5118</td>
<td>1421</td>
<td>2141</td>
</tr>
<tr>
<td>Fertilization and cleavage rate (%)</td>
<td>60a</td>
<td>56b</td>
<td>52c</td>
</tr>
<tr>
<td>Embryo transfers</td>
<td>790</td>
<td>241</td>
<td>377</td>
</tr>
<tr>
<td>Per cycle (%)</td>
<td>97a</td>
<td>96</td>
<td>94b</td>
</tr>
<tr>
<td>Embryos transferred (per transfer)</td>
<td>2219 (2.8)</td>
<td>664 (2.8)</td>
<td>1036 (2.7)</td>
</tr>
<tr>
<td>Pregnancies</td>
<td>223</td>
<td>75</td>
<td>112</td>
</tr>
<tr>
<td>Per cycle (%)</td>
<td>27</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Gestation sacs</td>
<td>292</td>
<td>97</td>
<td>153</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>13</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

Only statistically significant differences given (NA = not applicable).
Normal sperm function had been predicted by favourable penetration of cervical mucus and motility characteristics in culture medium.

The possibility that subtle impairment of sperm function might have accounted for reduced fertilization rates was examined by comparison of fertilization results using normal donor spermatozoa. The women treated using donor spermatozoa differed slightly in that they produced fewer oocytes, probably due to being 1–2 years older (Table II). The difference in age was matched by greater duration of infertility before proceeding to treatment, probably because of the more difficult decisions regarding donor spermatozoa. However, these differences were consistent in all the female diagnostic groups and did not affect the key outcome measures.

When donor spermatozoa were used, the fertilization and cleavage rate in the tubal infertility group (61%) was virtually the same as with husband’s spermatozoa (60%) (Table II), confirming the normal functional ability of the husband’s spermatozoa.

In the endometriosis group, use of donor spermatozoa resulted in no improvement in the fertilization and cleavage rates (55%) compared with husband’s spermatozoa (56%) (Table II), which would imply that the cause of impaired fertilization is primarily due to impaired ability of the oocyte. Although that conclusion could not be confirmed by statistically significant difference from the tubal group using donor spermatozoa, there is an unavoidable problem of a small number of couples undergoing IVF treatment with donor spermatozoa. Because of increasing use of ICSI to treat associated sperm defects, the problem may be difficult to resolve. However, publication of other similar studies may help to reach a definite conclusion by meta-analysis.

In the unexplained infertility group, use of donor spermatozoa led to a significant improvement in the fertilization and cleavage rate (57%) compared with the use of husband’s spermatozoa (52%). However, the rate appeared to remain lower than in the tubal control group (61%) and the frequency of complete failure in a couple appeared to remain higher (5% versus 2%; Table II). These findings together indicate that in unexplained infertility subtle impairment of sperm function is partly responsible for the impairment of fertilization, and it is likely that there is also a contribution due to impaired function of the oocyte.

Oocyte dysfunction was suggested by Gabrielson et al. (1996) to account for previous unexplained fertilization failure by standard IVF treatment, though the study model was unreliable in some respects. When they turned to ICSI treatment they found a fertilization and cleavage rate of 58%, compared with 63% using ICSI as first-time treatment in cases of distinct sperm defects.

In our study, oocyte or sperm dysfunction could not be accounted for as a specific defect affecting only a minority of individuals. Complete failure of fertilization and cleavage in a cycle did not usually occur repeatedly in the same couples, and after excluding them from analysis, fertilization and cleavage rates remained significantly impaired in the endometriosis and unexplained groups. Thus, in the absence of any obvious defects, gamete dysfunction associated with endometriosis and unexplained infertility appeared to be a slight and generalized disorder, very unlikely to affect any individual in a severe, repetitive or predictable way.

The impaired fertilizing ability of the oocyte in both endometriosis-associated and unexplained infertility was not associated with any reduction in ovarian follicular capacity, in terms of oocyte number obtained in response to stimulation. Given usually plentiful oocytes, nearly all couples achieved embryos. Also, because of the maximum limit on embryos transferred, the average numbers transferred per cycle were similar in all groups. Implanting ability of the embryos appeared similarly favourable and consequently pregnancy rates were similar.

Thus the favourable clinical success rates with IVF and other assisted conception methods, as generally reported, are due to the benefits of ovulation stimulation to produce a superabundance of oocytes, usually sufficient to overcome the slight reduction in fertilizing ability which remains evident even after maximal follicular stimulation. The reduction in fertilization and cleavage rates which we found with endometriosis, was much smaller than that generally observed: proportionately by about one-third, as shown in the results reviewed in Table I. It is possible that the observed variations are due to artefactual differences in stimulation regimens or culture conditions. On the other hand, if such conditions could partly overcome impairment of oocyte quality it would imply a greater reduction of fertilizing ability in natural cycles (as we and others have previously found: see Table I), therefore substantial reduction in the chance of natural conception.

Inherent ovarian/oocyte dysfunction could not entirely explain the subfertility associated with endometriosis. Peri-
tional factors of the inflammatory type, including macrophage activity and toxic agents, could affect the reproductive process at various stages, e.g. oocyte retrieval by the tubal fimbria, and oocyte and sperm function affecting fertilization and post-fertilization events, but the evidence is conflicting (see review by Oral et al., 1996).

Whether the endometriosis is itself a cause or consequence of the evident pro-inflammatory conditions is also unknown (Oral et al., 1996). However, recent evidence that surgical destruction of peritoneal endometriosis leads to doubling of pregnancy rates (Marcoux et al., 1997) suggests a direct effect of the ectopic endometrium. On the other hand, the improved pregnancy rates were still markedly subnormal, therefore independent factors must play an important role in the associated subfertility and possibly in the pathogenesis of endometriosis. Follicular fluid could be an important source, as suggested by McLaren et al. (1997).

In our study, any adverse factors in peritoneal fluid affecting sperm function (e.g. Tasdemir et al., 1995) or oocyte–sperm interaction (Oral et al., 1996) were avoided by the IVF process. It is possible, however, that fertilization was affected by humoral factors in the woman’s serum used to supplement the culture medium. We tested and excluded that possibility by comparing fertilization rates in the women with endometriosis using either the woman’s serum or human serum albumin as substitute. The latter was used when serology on the woman showed the presence of antisperm antibodies, which occurred in a minority of cases in all diagnostic groups. The fertilization rate using albumin was not greater than with own serum in any of the study groups (unpublished data).

The reduced fertilizing ability of the oocyte in endometriosis and unexplained infertility may be due indirectly to disordered function of the granulosa cells on which the oocyte depends for support and control of maturation. We have shown that the steroidogenic capacity of granulosa cells in vitro is reduced, not only in unstimulated cycles but also after maximal stimulation in vivo with exogenous gonadotrophins (Harlow et al., 1996). We have also found reduced sensitivity of granulosa cells to stimulation with LH in vitro in unstimulated cycles (Harlow et al., unpublished data). Others have previously demonstrated reduced LH receptors (Ronnberg et al., 1984), and the concentration of endothelin-1 in follicular fluid has been found to be increased (Tedeschi et al., 1994); endothelin-1 is an inhibitor of granulosa cell steroidogenesis.

It is tempting to speculate that the oocytes are inherently normal in endometriosis and unexplained infertility and that the impairment in fertilizing ability is due rather to maturational impairment dependent on granulosa cell function. Once the oocytes are fertilized, the resulting embryos appear to implant well. Quite different effects are expressed due to ageing of the woman. There is no impairment of fertilization and cleavage with advancing age, but implanting ability of the resulting embryos is progressively reduced (Abdelmassih et al., 1996; Hull et al., 1996). However, the age relationship appears to be due to chromosomal defects of the oocyte (Munné et al., 1995), which would not affect fertilization but would affect embryo development. By contrast, the favourable implantation rates in endometriosis suggest that the oocytes are chromosomally normal. They may, however, lack important factors controlling granulosa (cumulus) cell function (Eppig et al., 1993) on which full oocyte maturation and fertilization could depend.

In summary, in a study of IVF treatment following ovarian stimulation, fertilization and cleavage rates in women with tubal infertility (the female control group) were the same with either the husband’s spermatozoa or with cryopreserved donor spermatozoa (the male control group). By contrast, in endometriosis fertilization rates were reduced with husband’s spermatozoa but equally with donor spermatozoa. In unexplained infertility, the reduction was even greater with husband’s spermatozoa but only partly improved with donor spermatozoa. Implanting ability of resulting embryos appeared to be similar in all groups.

In conclusion, the reduction in natural fertility associated with endometriosis appears to be at least partly due to reduced fertilizing ability of the oocyte. In unexplained infertility there is distinct impairment due to otherwise unsuspected sperm dysfunction, despite prior diagnostic testing of function, but probably also due to oocyte dysfunction. These effects on natural fertility are likely to be even greater because in our study the follicles were maximally stimulated by exogenous gonadotrophins. The findings depend on use of donor spermatozoa as one of the controls and need to be supported by more extensive studies, which will have to come from multiple centres due to the diminishing use of donor spermatozoa in IVF treatment.

Pregnancy rates through IVF treatment are favourable despite reduced fertilizing ability with endometriosis and unexplained infertility, because oocytes are superabundant due to stimulation and implanting ability of the resulting embryos appears favourable.

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References


