Permanent impairment of embryo development by hydrosalpinges*

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Recent reports suggest a deleterious effect of hydrosalpinges on pregnancy outcome for in-vitro fertilization (IVF) and improvement following surgical treatment. We compared the effect of hydrosalpinges on pregnancy outcome in 286 patients having 348 IVF cycles and followed the development of untransferred embryos for 7 days to determine if hydrosalpinges affect oocyte quality or embryo development. The delivery rate per retrieval was significantly lower for patients with hydrosalpinx, but was restored by surgical treatment to that of patients without hydrosalpinx. However, the implantation rate per embryo transferred and normal blastulation of untransferred embryos, which were significantly decreased in patients with hydrosalpinx, and growth arrest and degeneration of untransferred embryos, which were significantly increased compared to patients without hydrosalpinx, were not restored by surgical treatment of hydrosalpinges. We conclude that surgical treatment of hydrosalpinges decreases early pregnancy loss and improves pregnancy outcome, possibly by diminishing reversible deleterious effects exerted on the endometrium. As we have seen in our laboratory, hydrosalpinges may have a permanent negative influence on ovarian function, follicular development and oocyte quality since implantation of transferred embryos and normal blastulation of untransferred embryos remain low, and in-vitro growth arrest and degeneration remain high despite surgical treatment of hydrosalpinges.

Key words: blastocyst/hydrosalpinx/in-vitro fertilization/salpingectomy/tubal disease

Introduction

In vitro fertilization (IVF) was initially developed as a treatment for patients with tubal factor infertility (Edwards et al., 1984) and remains one of the major therapies for patients with tubal disease. However, the IVF success rate for patients with tubal infertility remains disappointingly low despite adequate ovarian response to gonadotrophins and normal oocyte retrieval conditions yielding an average number of oocytes, normal fertilization rates and transfer of good quality embryos. Recent reports have suggested that patients with tubal disease do not comprise a homogeneous group. Variable factors include extent of tubal patency or blockage, adhesions and the presence of unilateral or bilateral hydrosalpinges (Strandell et al., 1994; Katz et al., 1996).

A common factor in failed IVF cycles for many patients with tubal factor infertility has been shown to be the presence of a hydrosalpinx (Anderson et al., 1994; Strandell et al., 1994; Vandromme et al., 1995; Katz et al., 1996). Indeed, several reports have indicated that the presence of a unilateral or bilateral hydrosalpinx adversely affects both implantation and pregnancy rates (Anderson et al., 1994; Strandell et al., 1994; Vandromme et al., 1995; Katz et al., 1996).

To determine if there is a correlation between the presence of hydrosalpinx and pregnancy rate, implantation rate, and in-vitro embryo development, we conducted a retrospective analysis of the IVF results of patients with tubal and non-tubal factor infertility in our centre. In addition, we sought to determine whether these parameters would be improved after surgical treatment of hydrosalpinges.

Materials and methods

The records of 286 patients, aged 24–39 years, undergoing 348 consecutive IVF–embryo transfer cycles between January 1993 and June 1996 were analysed. The only patient exclusions were patients aged >39 years, donor oocyte cycles and frozen-embryo transfer cycles.

For the purpose of this study, patients were classified into two major groups: tubal factor infertility and non-tubal factor infertility. Tubal factor infertility was divided into three subgroups: without hydrosalpinx (group A), with surgical treatment of hydrosalpinx (group B), and with unilateral or bilateral hydrosalpinx (group C). Non-tubal factor infertility is group D. Patients with hydrosalpinx who entered our IVF programme between June 1994 and June 1996 were informed of the possible negative influence of hydrosalpinx on IVF outcome that was being reported in the current literature (Kassabji et al., 1994; Strandell et al., 1994), and were given the opportunity to have surgical treatment or not prior to their upcoming IVF cycle. This information was not available to patients entering our IVF programme prior to the publication of this information. Therefore, these patients would have proceeded with IVF with hydrosalpinx or may have had surgical treatment of hydrosalpinx for other indications such as ectopic pregnancy. Eight patients in group B also appear in group C for IVF cycles completed before surgical treatment. The diagnosis of hydrosalpinx was documented by hysterosalpingography within 2 years of the IVF cycle, surgery (laparoscopy or laparotomy), or ultrasonography prior to initiation of an IVF cycle. Hydrosalpinx was defined as a dilated distal tube with minimal or no patency. Surgical treatment of hydrosalpinges was accomplished by salpingectomy or neosalpingostomy with post-

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operative documentation of tubal patency and absence of hydrosalpinges by hysterosalpingography.

Methods of ovulation induction, follicular monitoring, oocyte retrieval, autologous granulosa–lutein cell/embryo co-culture, embryo transfer and blastocyst cryopreservation have been described previously (Freeman et al., 1995). Embryos were graded on the day of embryo transfer by the protocol of Hill et al. (1989). Briefly, the embryos were graded according to the symmetry of the blastomeres and the extent of fragmentation and were assigned a score: 4: symmetrical, no fragmentation; 3: <10% fragmentation and/or asymmetrical; 2: 10–25% fragmentation; 1: >25% fragmentation. After embryo transfer on day 3, untransferred embryos were monitored daily until day 7 and were cryopreserved if they developed into normal expanded blastocysts with a single blastocoele cavity and a distinct inner cell mass. Embryos with more than one blastocoele or absent inner cell mass were considered abnormal. Cleaved embryos that failed to compact and blastulate within the 7 day period were considered arrested. Degenerate embryos had many atretic cells and/or vacuoles within the trophoblast and are considered abnormal. Cleaved embryos that failed to compact and blastulate within the 7 day period were considered arrested. Degenerate embryos had many atretic cells and/or vacuoles within the trophoblast and were assigned a score: 4: symmetrical, no fragmentation; 3: <10% fragmentation and/or asymmetrical; 2: 10–25% fragmentation; 1: >25% fragmentation. After embryo transfer on day 3, untransferred embryos were monitored daily until day 7 and were cryopreserved if they developed into normal expanded blastocysts with a single blastocoele cavity and a distinct inner cell mass. Embryos with more than one blastocoele or absent inner cell mass were considered abnormal. Cleaved embryos that failed to compact and blastulate within the 7 day period were considered arrested. Degenerate embryos had many atretic cells and/or vacuoles within the trophoblast and inner cell mass. Clinical pregnancies were confirmed by ultrasound visualization of an intrauterine gestational sac. The implantation rate was defined as the total number of gestational sacs visible on ultrasound divided by the total number of embryos transferred ×100. Data were analysed by χ² analysis, Fisher’s exact test or analysis of variance, where appropriate. P < 0.05 was considered significant.

Results
Included in this study were 136 non-tubal factor infertility patients having 175 IVF cycles and 150 tubal factor infertility patients having 173 IVF cycles. The tubal factor infertility patients were distributed into the three subgroups as follows: 83 patients in group A having 88 IVF cycles, 32 patients in group B having 40 IVF cycles, and 35 patients in group C having 45 IVF cycles. Eight patients appear in group B (11 IVF cycles) and group C (14 IVF cycles).

Details of IVF cycles are presented in Table I. There were no significant differences between the four groups for average number of retrievals per patient, average number of oocytes retrieved, average number of embryos transferred and average embryo quality score of transferred embryos. However, the average patient age in group C (31.7 years of age) was significantly lower than the other three groups (33.6–34.5 years of age). In addition, embryo degeneration and growth arrest was significantly higher for untransferred embryos in groups B and C and significantly fewer embryos developed to the blastocyst stage and were cryopreserved than for the other two groups.

Pregnancy outcome is summarized in Table II. Despite no significant differences in the number or quality of embryos transferred and a significantly lower mean age, patients with hydrosalpinges (group C) had a significantly lower clinical pregnancy rate and implantation rate compared to groups A and D. In addition, early pregnancy loss (spontaneous abortion, ectopic pregnancy or biochemical pregnancy) was significantly higher for group C compared to groups B and D. Despite an improvement in pregnancy outcome for group B with surgical treatment of hydrosalpinges, implantation rate remained low compared to groups A and D.

Discussion
In this study, we evaluated the impact of hydrosalpinges on implantation, pregnancy loss and in-vitro embryo development during IVF and analysed IVF outcome following surgical treatment of hydrosalpinges. We have shown that the presence of hydrosalpinges during IVF is associated with decreased implantation and increased early pregnancy loss and that surgical treatment of hydrosalpinges restores the live birth rate to that of patients without hydrosalpinges. Most importantly, this is the first report to demonstrate that hydrosalpinges have a lasting deleterious effect on oocytes with impairment of embryo development in subsequent IVF cycles. This is shown by the lower implantation rate per embryo transferred and the high growth arrest and degeneration rate and depressed blastulation of supernumerary embryos that persists following surgical treatment of hydrosalpinges.

Our results support the hypothesis that hydrosalpinges impair delivery rates and suggest that surgical treatment of hydrosalpinges is beneficial. Other groups have observed similar effects of hydrosalpinges on pregnancy rate and implantation. Strandell et al. (1994) found a decreased pregnancy rate per embryo transfer (13.2 versus 26.0%) and increased pregnancy

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<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Average age (range; years)</th>
<th>No. of retrievals</th>
<th>No. of oocytes (average/retrieval)</th>
<th>No. of embryos transferred (average/retrieval)</th>
<th>Embryo quality score</th>
<th>No. of untransferred embryos</th>
<th>No. of embryos cryopreserved (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>83</td>
<td>34.5 (26–39)</td>
<td>36</td>
<td>1361 (15.5)</td>
<td>297 (3.4)</td>
<td>3.4</td>
<td>424</td>
<td>223 (52.6)</td>
</tr>
<tr>
<td>B</td>
<td>32</td>
<td>30.6 (27–39)</td>
<td>40</td>
<td>714 (17.9)</td>
<td>157 (3.9)</td>
<td>3.4</td>
<td>157</td>
<td>114 (40.4)</td>
</tr>
<tr>
<td>C</td>
<td>35</td>
<td>35</td>
<td>45</td>
<td>637 (14.2)</td>
<td>153 (3.4)</td>
<td>3.3</td>
<td>198</td>
<td>87 (43.9)</td>
</tr>
<tr>
<td>D</td>
<td>136</td>
<td>34.5 (24–39)</td>
<td>175</td>
<td>2391 (13.7)</td>
<td>565 (3.2)</td>
<td>3.2</td>
<td>535</td>
<td>324 (60.6)</td>
</tr>
</tbody>
</table>

Values represent the average embryo quality score of transferred embryos.

p < 0.005 versus groups A, B and D.

p < 0.05 versus groups A and D.

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Table I. Details of in-vitro fertilization cycles for tubal-factor infertility patients without hydrosalpinx (group A), with surgical treatment of hydrosalpinx (group B), with hydrosalpinx (group C) and non-tubal factor infertility patients (group D)
loss (50 versus 30%) in patients with hydrosalpinges compared to patients with tubal disease without hydrosalpinges. Their results from frozen embryo replacements were similar. Lower pregnancy rates (7.1 versus 24.5%) and implantation rates (5.0 versus 10.4%) following frozen embryo replacement were also seen by Akman et al. (1996) for patients with tubal factor infertility compared to patients without hydrosalpinx. Similarly, Anderson et al. (1994) found that the presence of a hydrosalpinx was associated with a significant reduction in pregnancy rate per retrieval (19.2 versus 32.6%), implantation rate (2.9 versus 10.3%) and delivery rate per retrieval (5.8 versus 20.9%) and increased pregnancy loss (70 versus 36%) compared to patients without hydrosalpinx. Likewise, Katz et al. (1996) demonstrated a decreased pregnancy rate per retrieval (13.6 versus 28.3%) and decreased implantation rate (3.9 versus 11.5%) for patients with hydrosalpinx compared to tubal patients without hydrosalpinx.

Several groups have observed similar improvement in pregnancy and implantation following surgical treatment of hydrosalpinges. Shelton et al. (1996) compared clinical pregnancy rates per transfer for eight patients who had 23 cycles pre-salpingectomy and 12 cycles post-salpingectomy and found a significant improvement in clinical pregnancy rate for post-salpingectomy cycles (42 versus 0%). Similarly, Vandromme et al. (1995) observed significant improvement in clinical pregnancy rates per retrieval (38.1 versus 10.1%) and implantation rates (17.4 versus 4.2%) for patients following surgical treatment of hydrosalpinges compared to patients with hydrosalpinges present.

In contrast, Sharara et al. (1996) found slightly decreased pregnancy and implantation rates in patients with hydrosalpinx compared to tubal factor patients without hydrosalpinx (24.8 versus 33.7% and 9.8 versus 12.6% respectively), however, the differences were not statistically significant.

There are several possible explanations for the negative effect exerted by hydrosalpinges on the outcome of IVF. It has been suggested that irreversible endometrial damage and acute tubal damage may arise concurrently resulting in permanent diminished potential for implantation (Strandell et al., 1994). Our results do not support this hypothesis. We suggest that the diminished capacity of the endometrium for implantation is transient, but the effects of the hydrosalpinx on the ovary and developing follicles is not transient, but remains after surgical treatment of hydrosalpinges. This suggestion may be supported by our observation that the implantation rate in group B is diminished, as is the in-vitro embryo development to the blastocyst stage, indicating decreased developmental potential of both transferred and untransferred embryos. This effect is mirrored in the lower clinical pregnancy rate in group B. However, due to a decrease in clinical pregnancy loss in this group, the live birth rate is comparable to groups A and D.

It is possible that endometrial receptivity may be altered by the leakage of fluid into the uterine cavity from hydrosalpinges (Strandell et al., 1994) which have been shown to enlarge during ovarian hyperstimulation (Hill et al., 1986). This premise is supported by a case report of Bloechle et al. (1997) who have shown that transvaginal aspiration of the hydrocele during the IVF cycle did not alleviate the problem since the tubes refilled with fluid within several days and a sonometra was detectable in the uterine cavity on the day of embryo transfer. The authors concluded that aspiration of hydrosalpinx was not beneficial since the underlying pathology cannot be cured with this method. The deleterious effect of hydrosalpinges may be purely mechanical or may involve an inflammatory response (Sharara et al., 1996) which in turn impedes implantation. A recent study (Meyer et al., 1997) has demonstrated that inflammatory hydrocele fluid is a transudate that has a similar concentration of electrolytes to that of serum (David et al., 1969), it may contain microorganisms and cellular debris that are toxic to embryos (Strandell et al., 1994). In a recent study, hydrosalpinx fluid was shown to have physiological osmolarity (280–282 mOsmol/L) but an alkaline pH (8.45–8.65) and had a significant embryotoxic effect on mouse embryo cavitation in vitro at concentrations as low as 1% (Mukherjee et al., 1996).

### Table II. Pregnancy outcome for tubal factor infertility patients without hydrosalpinx (group A), with surgical treatment of hydrosalpinx (group B), with hydrosalpinx (group C) and non-tubal factor infertility patients (group D)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte retrievals</td>
<td>88</td>
<td>40</td>
<td>45</td>
<td>175</td>
</tr>
<tr>
<td>Positive βHCG (%)</td>
<td>62 (70.5)</td>
<td>23 (57.5)</td>
<td>18 (40.0)</td>
<td>104 (59.4)</td>
</tr>
<tr>
<td>Biochemical pregnancy (%)</td>
<td>6 (9.7)</td>
<td>5 (21.7)</td>
<td>4 (22.2)</td>
<td>11 (10.6)</td>
</tr>
<tr>
<td>Ectopic pregnancy (%)</td>
<td>5 (8.1)</td>
<td>0</td>
<td>1 (5.6)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Spontaneous abortion (%)</td>
<td>12 (19.4)</td>
<td>0</td>
<td>5 (27.8)</td>
<td>13 (12.5)</td>
</tr>
<tr>
<td>Total pregnancy loss (%)</td>
<td>23 (37.1)</td>
<td>5 (21.7)</td>
<td>10 (55.6)</td>
<td>26 (25.0)</td>
</tr>
<tr>
<td>Clinical pregnancy (%)</td>
<td>51 (88.0)</td>
<td>18 (45.0)</td>
<td>13 (28.9)</td>
<td>91 (52.0)</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>39 (44.3)</td>
<td>18 (45.0)</td>
<td>8 (17.8)</td>
<td>78 (44.6)</td>
</tr>
<tr>
<td>No. of gestational sacs (implantation rate %)</td>
<td>80 (26.9)</td>
<td>28 (17.8)</td>
<td>17 (11.1)</td>
<td>155 (27.4)</td>
</tr>
</tbody>
</table>

Values represent percentage of oocyte retrievals.

Values represent percentage of patients with positive β-human chorionic gonadotrophin (βHCG).

Values represent percentage of embryos transferred (Table I).

Values represent percentage of patients with positive β-human chorionic gonadotrophin (βHCG).

Values represent percentage of patients with positive β-human chorionic gonadotrophin (βHCG).

P < 0.05 versus group C.

P < 0.05 versus groups B and C.
deleterious effect on ovarian function, follicular development and oocyte quality (Strandell et al., 1994). Our results support this premise. In our embryo culture system, embryos are co-cultured with autologous granulosa lutein cells for 2 days and transferred on day 3. The untransferred embryos are co-cultured for an additional 2–4 days and are cryopreserved if they develop into normal expanded blastocysts with a well-defined inner cell mass, single blastocoele cavity and little or no fragmentation. We observed an increase in embryo degeneration, growth arrest and abnormal development of untransferred embryos with a concurrent decrease in normal blastulation and cryopreservation of blastocysts for patients with hydrosalpinx compared to patients without hydrosalpinx. These factors were not improved by surgical treatment of hydrosalpinges. Dokras et al. (1993) have demonstrated a correlation between in-vitro development of embryos into normal blastocysts and their developmental potential that may also be a reflection of the overall quality of the oocytes.

We have demonstrated that the presence of hydrosalpinx during IVF is associated with decreased pregnancy and implantation rates and increased pregnancy loss resulting in decreased live birth rates. Possible mechanisms may include the alteration of endometrial receptivity by mechanical factors, inflammatory response or embryotoxicity. This effect appears to be reversible since surgical treatment of hydrosalpinges decreases early pregnancy loss and improves pregnancy outcome, possibly by diminishing deleterious effects exerted on the endometrium. Furthermore, hydrosalpinges may adversely affect ovarian function, follicular development and oocyte quality. Our findings suggest a permanent negative influence of hydrosalpinges on oocyte quality and embryo development in IVF cycles subsequent to surgical treatment of hydrosalpinges. This is demonstrated by the low implantation rate, increased growth arrest and degeneration and depressed normal blastulation of untransferred embryos despite surgical treatment of hydrosalpinges. These data are important in counselling patients with hydrosalpinges, since the presence of hydrosalpinx during IVF is associated with poor outcomes. However, this information is also important for patients who have been treated for hydrosalpinges since they are unlikely to have as many blastocysts available for cryopreservation. Further studies are needed to determine what factor(s) associated with the hydrosalpinx might be responsible for this permanent effect on oocyte and embryo development.

References

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