No evidence for a decreased fertilizing potential after in-vitro fertilization using spermatozoa from polyzoospermic men

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Polyzoospermia is generally recognized as a male factor contributing to infertility and/or recurrent abortion. Although a reduced spermatozoal fertilizing capacity is assumed to be involved, so far there is no conclusive explanation for the assumed reduced reproductive performance in these patients, and data on the fertilizing capacity of spermatozoa from polyzoospermic men are lacking. The present study therefore aimed at analysing the outcome after in-vitro fertilization (IVF)–embryo transfer in polyzoospermic patients. Retrospective analysis showed that only 0.5% out of 7863 IVF cycles were performed with spermatozoa from polyzoospermic men. The outcome of these IVF cycles shows neither a reduction in spermatozoal fertilizing capacity nor an increase in pregnancy wastage in cycles in which a pregnancy was obtained. These results may suggest a normal reproductive potential in polyzoospermic patients and therefore the question may be raised whether polyzoospermia represents a real pathological entity leading to infertility.

Key words: in-vitro fertilization/male infertility/polyzoospermia/semen

Introduction

Polyzoospermia is currently defined as a condition in which ejaculates with a volume of at least 1.5 ml repeatedly contain >250×10⁶ spermatozoa per ml (Glezerman et al., 1982). The incidence of polyzoospermia in the infertile male population ranges from 0.2% (Amelar et al., 1979) to 4.2% (Glezerman et al., 1982). According to many authors and textbooks, polyzoospermia is invariably associated with a reduced reproductive performance, i.e. impaired fertility and/or a higher risk for spontaneous abortion (Joel and Gibor, 1966; Amelar et al., 1979; Glezerman et al., 1982; Schirren, 1995). Many causative factors have been proposed for this impairment in reproductive performance: a reduction in seminal fructose concentration (Singer et al., 1979; Barnea et al., 1980; Schirren, 1995), a reduced seminal DNA content (Joel and Gibor, 1966), a decrease in spermatozoal ATP (Calamera et al., 1987) and defective acrosomal function (Schill and Feifel, 1984; Calamera et al., 1987; Topfer-Petersen et al., 1987; Schill et al., 1988; Schill, 1990). However, data on the fertilizing capacity of spermatozoa from polyzoospermic men are lacking. The present study therefore aims at analysing the results of in-vitro fertilization (IVF) in a subpopulation of men with polyzoospermia.

Materials and methods

A retrospective analysis of 7863 in-vitro fertilization (IVF) cycles performed in the period from January 1987 till December 1993 showed that spermatozoa from polyzoospermic men (≥250×10⁶/ml spermatozoa in at least two consecutive semen analyses with semen volume ≥1.5 ml) was used for IVF in 40 cycles.

In the male partners all semen samples were assessed according to the WHO criteria (World Health Organization, 1987, 1992) for normality. Sperm morphology was either assessed using the WHO criteria or the strict criteria according to Kruger et al. (1988). All patients were instructed to deliver a semen sample after an abstinence period of 3 days. Immunological testing for immunoglobulin (Ig) G and IgA was performed on all ejaculates using the mixed antiglobulin reaction test (MAR test; FertiPro, Ghent, Belgium) and was found to be negative on all samples tested. Medical history and physical examination did not reveal any specific problems and all men had a normal endocrine profile, including serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone and a normal 46, XY karyotype.

All female patients had a basic infertility work-up, including an endocrine and immunological investigation, hysterosalpingography and/or laparoscopy.

The female partners underwent ovulation induction using a gonadotrophin-releasing hormone analogue (GnRH) suppression protocol combining busrelin (Suprefact nasal spray, Hoechst, Frankfurt, Germany) with human menopausal gonadotrophins (HMG, Humegon; Organon, Oss, The Netherlands; Pergonal, Serono, Brussels, Belgium). Human chorionic gonadotrophin (HCG) was administered if at least three follicles measured at least 17 mm in diameter and serum oestradiol concentrations were at least 1000 ng/l. Oocytes were recovered by transvaginal ultrasound-guided retrieval 36 h after HCG. Oocytes were inseminated within 3–4 h after retrieval by adding ~3000 motile spermatozoa per oocyte in a 25 µl microdrop under quality-controlled paraffin oil (British Drug House, London, UK) (insemination concentration 120 000 motile spermatozoa/ml).

A modified Earle’s medium was used for sperm preparation and for insemination (Staessen et al., 1990). Inseminated oocytes were individually cultured in 25 µl droplets of B2 medium (Bio-Mérieux, Brussels, Belgium) under oil. Eighteen to 20 h after insemination, oocytes were examined under an inverted microscope after removing cells of the cumulus oophorus and corona radiata with fine glass micropipettes. The presence of two distinct pronuclei was considered evidence of normal fertilization. If more than two pronuclei (PN) were observed, the embryo was referred to as polyplid. After an additional 24–30 h of in-vitro culture, embryos were examined under
The total sperm count was 500,000, and the concentration was 250,106/ml (median, 95% confidence interval (CI) 150,000–350,000). The median age for the male partner was 42.4 years, and the median age for the female partner was 35.4 years. In 40 cycles (27.5%) of 37 couples, polyzoospermia was the sole indication for IVF. In 25 cycles an additional female factor was present as an indication for IVF treatment (tubal infertility: n = 20, endometriosis: n = 3 and antisperm antibodies: n = 2).

The volume of semen used in the 40 IVF cycles was 2.0 ml [median, 95% confidence interval (CI) 1.5–2.3] with a concentration of 250,106/ml (median, 95% CI 250–252). The total sperm count was 500,000,000,000 (median, 95% CI 431,400,000–575,000,000), progressive motility was 55% (median with a 95% CI of 50.0–60.0) and sperm morphology was within normal limits in all samples used.

After insemination of 344 oocyte–cumulus complexes, 230 (66.9%) showed 2 PN and 16 (6.9%) showed 3 PN (Table I). The mean fertilization rate per cycle was 62.6%. In three cycles no fertilization was obtained, two in which only one oocyte was recovered after ovum retrieval. In the third cycle, none of the eight oocytes was fertilized. Of the 230 normally-fertilized oocytes, 153 (66.5%) developed into embryos with ≥50% of their volume filled with fragments. In three cycles no further development was obtained of the normally fertilized oocytes (n = 1, 1 and 3). After replacement of 90 embryos in 34 intratransfer transfers (2.6 ± 0.6 embryo per transfer), 13 patients became pregnant (32.5% per cycle or 38.2% per embryo transfer). The ongoing pregnancy rate was 25.0% per cycle and the implantation rate was 15.6% per embryo. Of the 13 pregnancies observed two were biochemical and one was an ectopic pregnancy occurring in a patient with tubal infertility. No patient miscarried. Ten patients delivered 14 babies. Because of the neonatal death of a triplet, the take-home baby rate (at least one baby taken home) was 22.5% per cycle or 24.3% per couple. There was no difference in fertilization, in further embryonic development or in implantation between cycles in which polyzoospermia was the sole indication or cycles in which other causes for subfertility were found (see Table I).

### Results

Spermatozoa from polyzoospermic men were used for IVF in only 40 cycles (0.5%) of 37 couples. The average duration of infertility of these couples was 6.0 ± 4.2 years and the female age 34.4 ± 4.3 years. In only 15 cycles was polyzoospermia the sole indication for IVF. In 25 cycles an additional female factor was present as an indication for IVF treatment (tubal infertility: n = 20, endometriosis: n = 3 and antisperm antibodies: n = 2).

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After insemination of 344 oocyte–cumulus complexes, 230

### Table I. Outcome of IVF cycles using sperm from polyzoospermic men

<table>
<thead>
<tr>
<th></th>
<th>All cycles</th>
<th>Polyzoospermia-only cycles</th>
<th>Cycles with co-existent female factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles (A)</td>
<td>40</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Oocytes inseminated</td>
<td>344</td>
<td>164</td>
<td>180</td>
</tr>
<tr>
<td>2-PN fertilization</td>
<td>230</td>
<td>98</td>
<td>132</td>
</tr>
<tr>
<td>2-PN fertilization rate</td>
<td>62.6 ± 4.3</td>
<td>55.8 ± 5.2</td>
<td>66.6 ± 8.4</td>
</tr>
<tr>
<td>3-PN fertilization</td>
<td>16</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Cycles without any fertilization</td>
<td>3 (7.5)</td>
<td>1 (6.6)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Cleaving embryos</td>
<td>153</td>
<td>63</td>
<td>90</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>69.2 ± 5.1</td>
<td>66.6 ± 8.4</td>
<td>70.8 ± 6.6</td>
</tr>
<tr>
<td>Embryos replaced</td>
<td>90</td>
<td>33</td>
<td>57</td>
</tr>
<tr>
<td>Pregnancies</td>
<td>13 (32.5)%</td>
<td>6 (40.0)%</td>
<td>7 (28.0)%</td>
</tr>
<tr>
<td>Implantations</td>
<td>14 (15.6)%</td>
<td>5 (15.1)%</td>
<td>9 (15.8)%</td>
</tr>
</tbody>
</table>

PN = pronuclei.

aDifferences between polyzoospermia-only cycles and cycles with co-existent female factor were not statistically different.

bValues are mean of fertilization rate per cycle ± SEM.

Percentage of A.

cValues are mean of cleavage rate per cycle ± SEM.

dImplantation rate.

the microscope to assess cleavage, and embryos with <50% of their volume filled with anucleate fragments were considered for transfer. A maximum of three cleaving embryos was transferred into the uterine cavity.

The luteal phase was supplemented by vaginal administration of 200 mg micronized progesterone three times daily (Utrogestan; Laboratoires Piette, Brussels, Belgium).

Pregnancy was diagnosed at least 10 days after transfer by rising HCG concentrations of at least 20 IU/ml in serum on two occasions. Preclinical abortion was designated in pregnancies where no gestational sac was detected and/or where hormone concentrations were falling. Clinical pregnancies were confirmed by the presence of a gestational sac detected by transvaginal ultrasonography 4–6 weeks after transfer. Implantation rate was defined as the ratio of the number of gestational sacs containing a fetus with a positive heartbeat to the number of replaced concepti. Clinical pregnancies reaching 20 weeks of gestation were considered ongoing. Differences between fertilization and cleavage rates of cycles in which IVF was performed for polyzoospermia and cycles in which a co-existent female factor existed were compared using the Mann–Whitney test. Differences in pregnancy and implantation rates were compared using the χ² test.

### Discussion

Although many textbooks dealing with male infertility will list polyzoospermia as a definite cause of subfertility, the WHO manual on semen analysis neither defines nor addresses polyzoospermia as such (WHO, 1987, 1992). The existence of polyzoospermia as a pathological entity is still under debate. Part of this debate may be the result of a deficiency in formulating a consistent and persuasive pathophysiological explanation for the postulated decrease in fertility. However, the lack of a uniform definition of polyzoospermia too will certainly contribute to this debate. Most authors now refer to polyzoospermia when the ejaculate contains >375×10⁶ spermatozoa (Eliasson et al., 1970).
In our retrospective series, only 0.5% of 7863 IVF cycles were performed with spermatozoa from men with polyzoospermia according to this definition. In only 0.2% of 7863 cycles was IVF performed with polyzoospermia as the sole indication. This percentage is in agreement with that reported by Dubin and Amelar (1971) but is lower than the figure of ~3–4% reported in the rest of the literature (Glezerman et al., 1982; Schirren, 1995). This may be explained by the retrospective character of this study in which only polyzoospermic individuals undergoing IVF were included.

The treatment for infertility due to polyzoospermia is extremely varied. Some authors advocate decreasing the numbers of spermatozoa in the ejaculate by increasing the frequency of intercourse (Schirren, 1995) or by administering androgens (Schirren, 1995). Others have reported casuistic successes from diluting the semen by means of a precoital vaginal douche using a 5% dextrose in Ringer’s lactate solution or by artificial insemination with semen diluted by the same solution (Amelar et al., 1979). Finally, IVF and related techniques such as gamete intra-Fallopian transfer (GIFT) have been suggested (Wolk, 1990). However, even after IVF, a reduction in the fertilization rate is assumed and overnight chilling of the sperm samples has been suggested to improve IVF rates (Edwards and Brody, 1995).

The outcome of IVF cycles in this series shows neither a reduction in spermatozoal fertilizing capacity nor an increase in pregnancy wastage when compared retrospectively to a population of normozoospermic men undergoing IVF because of tubal disorders in their partners (Tournaye et al., 1992). In a series of 169 polyzoospermic patients, spontaneous conception was reported to decrease drastically in subjects with $>350 \times 10^6$/ml spermatozoa (Schirren, 1995). The average sperm concentration in this study was $270 \times 10^6$/ml. It may be that we have analysed a borderline subgroup of patients with moderate polyzoospermia. Furthermore, more than half of our polyzoospermic subjects were associated with a coexistent ‘female factor’.

Yet our results are in agreement with the results of hamster ovum penetration tests (HOPT) using spermatozoa from polyzoospermic men where no decrease in penetration was observed (Chan et al., 1986). Testart et al. (1983) also found no affect on IVF in a small retrospective series, although they used a less strict definition of polyzoospermia ($>130 \times 10^6$/ml). The above findings, together with the present results, may suggest a normal reproductive profile in-vitro for polyzoospermic patients. It may therefore also be questioned whether polyzoospermia represents a real pathological entity which may lead to a decrease in natural fecundity or increased pregnancy wastage in vivo.

References


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IVF in polyzoospermic men