Cumulative pregnancy rate following IVF and intracytoplasmatic sperm injection with ejaculated and testicular spermatozoa

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Abstract

The purpose of this study was to calculate the cumulative pregnancy rates of IVF cycles with ICSI using ejaculated or testicular spermatozoa. A computerized database for the IVF cycles with ICSI performed between January 1996 and December 1998 was utilized. Cycles with spermatozoa obtained after electro-ejaculation were excluded. A multifactorial analysis was performed to define the impact of different factors on the success rate of IVF and ICSI. During a 36-month period, 229 pregnancies were achieved by 643 couples using ejaculated spermatozoa, and 83 pregnancies by 167 couples who required testicular spermatozoa. The pregnancy rates (PR) per cycle, including all treatment cycles with ejaculated spermatozoa, remained similar during the first five consecutive cycles achieving a cumulative PR of 80.44%. The cumulative pregnancy rates for cycles with testicular spermatozoa showed a consistent rise during four consecutive treatments and reached 61.84%. The regression analysis of pregnancy rate showed that it was significantly positively correlated with oocyte fertilization rate ($P = 0.02$), and negatively correlated with maternal age ($P = 0.03$). Thus, according to the present results, couples with infertility who require IVF with ICSI should be offered at least five consecutive attempts if ejaculated spermatozoa are used, and at least four cycles whenever testicular spermatozoa are used.

Keywords: ICSI, infertility, IVF, male factor

Introduction

IVF with intracytoplasmatic sperm injection (ICSI) has become the standard treatment for couples who suffer from infertility due to severe male factor, and recently even for couples in whom the male partner is azoospermic. The complexity of this method is inevitably associated with high physical, emotional and financial costs. It is therefore of prime importance constantly to assess treatment outcome and, specifically, the influence of various factors on this outcome, in order to provide the most efficient treatment to these defined groups of patients (Wilcox et al., 1993). Several recent studies assessed the success rates of single treatment cycles (Madgar et al., 1996, 1998; Hourvitz et al., 1998), rather than the pregnancy rates (PR) of the entire treatment course in the individual couples involved. In this context, cumulative pregnancy rates (CPR) were often used to assess fertility treatment efficacy (Dor et al., 1996). The current study is a retrospective-prospective life-table analysis of an entire computerized database of the ICSI procedure in an IVF unit between January 1996 and 31 December 1998. IVF cycles with spermatozoa received after electro-ejaculation were deliberately omitted. The CPR for IVF cycles with ICSI from ejaculated spermatozoa and with spermatozoa retrieved by testicular sperm extraction (TESE) were independently calculated.

Recent policy in this clinic is to transfer two embryos in patients up to 35 years, and three for older patients, and those failing to conceive. Four are transferred, if available, for patients >42 years.
Materials and methods

The computerized database contained information on patient’s age, hormonal status and previous obstetric history as well as type and duration of infertility. For the male partner, the database contained information about age, hormonal status, previous fertility performances and sperm parameters. If the male partner had azoospermia, the type, whether obstructive or non-obstructive, as well as the histopathological diagnosis, was recorded.

TESE

TESE was always performed on the day of ovum retrieval under general anaesthesia, anticipating the female procedure. TESE was performed as described by Silber \textit{et al.} (1995). A horizontal incision measuring 2 cm was made in the scrotal skin and carried through the peritoneal tunica vaginalis. The testis was withdrawn and a small incision of the albuginea was made. A small piece of extruding testicular tissue was excised under general anaesthesia, anticipating the female procedure. The specimen obtained by TESE was gently crushed under direct microscopy. The number of retrieved spermatozoa and their motility status at the end of the tissue screening was recorded, as well as the motility status at the time of injection into the oocyte. The ICSI procedure was performed according to the method described by Palermo \textit{et al.} (1992), including the immobilization step. The state of oocyte fertilization was assessed 16–18 h after the injection. Fertilization was considered normal when two pronuclei and two extruded polar bodies were present. Embryos that cleaved and continued to develop were transferred to the uterus 44–48 h after the microinjection procedure. Up to five embryos were placed in the uterine cavity. Any additional embryos of good morphological quality were cryopreserved for possible later use. The detection of rising $\beta$-HCG concentrations at least twice and at least 12 days after embryo replacement confirmed pregnancy. Clinical pregnancies were diagnosed only when a gestational sac and fetal pole with a beating heart were noted at ultrasonography.

Follow-up

Each of the pregnant patients in the programme was followed up by a physician (as part of the IVF treatment) until the fetal heart activity was seen, and at least for 5 weeks after the embryo transfer. For the rest of the follow-up, a nurse coordinator was in continuous contact (face to face or telephone) with the pregnant patient. The computerized database and the patient personal file were filed with ‘on line information’ around 22–23 weeks of gestation (the time of level II ultrasonography), again around 32–33 weeks (ultrasound growth assessment), and finally for 1 week after the delivery. For the purpose of this study, cycles that follow a delivery. For the purpose of this study, cycles that follow a pregnancy (in the same patient) were excluded.

Statistical analysis

Patient age, gravidity and parity, baseline FSH and LH concentrations, number of retrieved oocytes, as well as fertilization rate and number of replaced embryos were the main variables tested against CPR in the regression analysis. The cumulative pregnancy rate was calculated by using the 

### Table 1. Life-table analysis of cumulative pregnancy rates (CPR) resulting in clinical pregnancies after ICSI fertilization from ejaculated spermatozoa.

<table>
<thead>
<tr>
<th>Treatment cycle</th>
<th>Patients (n)</th>
<th>Pregnancies (n)</th>
<th>Pregnancies/ cycle (%)</th>
<th>CPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>167</td>
<td>37</td>
<td>22.2</td>
<td>22.16</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>16</td>
<td>24.2</td>
<td>40.75</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>8</td>
<td>25.0</td>
<td>55.11</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>4</td>
<td>21.1</td>
<td>61.84</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>2</td>
<td>16.6</td>
<td>67.71</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>1</td>
<td>25.0</td>
<td>74.17</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1</td>
<td>50.0</td>
<td>87.00</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
<td>87.00</td>
</tr>
</tbody>
</table>

### Table 2. Life-table analysis of cumulative pregnancy rates (CPR) resulting in clinical pregnancies after ICSI fertilization from testicular spermatozoa.

<table>
<thead>
<tr>
<th>Treatment cycle</th>
<th>Patients (n)</th>
<th>Pregnancies (n)</th>
<th>Pregnancies/ cycle (%)</th>
<th>CPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>643</td>
<td>153</td>
<td>23.8</td>
<td>23.79</td>
</tr>
<tr>
<td>2</td>
<td>213</td>
<td>53</td>
<td>24.9</td>
<td>42.67</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>13</td>
<td>18.3</td>
<td>53.17</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>7</td>
<td>28.0</td>
<td>65.77</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>3</td>
<td>60.0</td>
<td>80.44</td>
</tr>
</tbody>
</table>
‘Proc lifetest’ (SAS Institute Inc., 1991). To estimate the expected CPR, the Kaplan–Meier method (Kaplan and Meier, 1958) was used, which is not based on a specific assumption with respect to the distribution of the data. Between-group differences were tested by the log rank test (Mantel and Haenszel, 1959). In order to assess homogeneity within various groups in this study, the chi-squared significance was noted when $P < 0.05$. Cut-off values for age, fertilization rates, etc. were determined by the regression statistics.

**Results**

Six hundred and forty-three couples underwent 1001 cycles of IVF with ICSI micromanipulation from ejaculated spermatozoa. In 44 cases, more than one pregnancy was obtained; thus for the purpose of the CPR only 957 cycles were analysed. In all, 229 pregnancies were obtained (23.92% pregnancies per cycle).

Clinical PR per cycle, including all treatment cycles, remained similar during the first five successive cycles (Table 1) reaching a CPR of 80.44% (Figure 1). Fifty percent of pregnancies were achieved during the first three cycles [mean $3.14 \pm 0.09$ (SE); 95% confidence interval, lower two cycles, upper four cycles]. Furthermore, 75% of the pregnancies were achieved during the first four cycles, even though many patients did not return for a second or later cycle.

Significant statistical differences in pregnancy rates were observed during the first four cycles between patients 30 years or younger (CPR = 79.6%) compared with patients older than 30 years (CPR = 61.3%), $P = 0.01$. The mean patient age was 33.78 (SD = 5.99), with the youngest treated patient being 22 years old and the oldest 46 years (median 34 years). Patients older than 30 years took part in one more cycle; thus the final CPR of this group after five cycles was 77.9% (Figure 2). Patients up to 30 years of age conceived after $2.61 \pm 0.1$ cycles, but only after $3.25 \pm 0.11$ ($P = 0.01$) cycles when older than 30 years (95% confidence interval, lower two cycles, upper four cycles for the younger group and lower three cycles, upper four cycles for the older group). Furthermore, 75% of the pregnancies were achieved during the first four cycles for the young group and after five cycles for the rest.

The pregnancy rates were significantly increased ($P = 0.04$) whenever the fertilization rates were higher than 45% of the retrieved eggs. Thus, 32.5% of couples with fertilization rates equal or lower than 45% became pregnant (CPR = 72.6%) compared with 40.8% (CPR = 85.8%) for those with higher fertilization rates (Figure 3). Furthermore, there was a statistically significant difference between the numbers of replaced embryos in pregnant versus non-pregnant cycles ($4.03 \pm 1.48$ and $3.42 \pm 1.64$, $P = 0.006$). Neither the day 3 FSH and LH concentrations nor the number of retrieved eggs had any impact on the pregnancy rates.

![Figure 1](image1.png)  
Figure 1. Cumulative pregnancy rate (%) in ICSI with ejaculated versus testicular spermatozoa.

![Figure 2](image2.png)  
Figure 2. Cumulative pregnancy rate (%) in ICSI with ejaculated spermatozoa according to patient age: ≤30 years versus >30 years.

![Figure 3](image3.png)  
Figure 3. Cumulative pregnancy rate (%) in ICSI with ejaculated spermatozoa according to oocyte fertilization rates: ≤45% versus >45%.

![Figure 4](image4.png)  
Figure 4. Cumulative pregnancy rate (%) for ICSI with testicular spermatozoa according to maternal age: ≤31 years versus >31 years.
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Furthermore, the CPR was calculated for patients who underwent ICSI with testicular spermatozoa. One hundred and five patients were diagnosed with non-obstructive azoospermia and 62 with obstructive type. Eighty-three clinical pregnancies were achieved in 167 couples who underwent 345 treatment cycles. Patients underwent between one (167) and eight (1) cycles. The patient who underwent eight cycles was exceptional for this clinic. In 12 patients, more than one pregnancy was achieved (14 pregnancies); thus for the purpose of the CPR, only 303 treatment cycles, resulting in 69 pregnancies, were included. The pregnancy rate per cycle did not change significantly for four consecutive cycles (Table 2). The CPR showed a consistent rise during four consecutive treatments and achieved a maximum of 61.8%. These figures did not differ significantly from the first four cycles for ICSI with ejaculated spermatozoa (Figure 1). Fifty percent of pregnancies resulting after ICSI with testicular spermatozoa were achieved after three cycles with a mean of 3.9 ± 0.3 cycles to achieve a pregnancy; 95% confidence interval, lower two cycles, upper four cycles).

The regression analysis of pregnancy rate showed that it was significantly positively correlated with oocyte fertilization rate (P = 0.02), and negatively correlated with maternal age (P = 0.03). Patients 31 years or younger conceived during the first 2.52 ± 0.15 cycles with 50% of pregnancies during the first two cycles; 95% confidence interval, lower two cycles, upper three cycles). Patients older than 31 years achieved a pregnancy after 4.42 ± 0.41 cycles, with 50% of total pregnancies at this age group only after five attempts; 95% confidence interval, lower three cycles, upper seven cycles) (Figure 4). The difference in pregnancy rates between the two age groups was not significant (P = 0.07). As for ICSI with ejaculated spermatozoa, a fertilization rate higher than 45% of the retrieved eggs increased the pregnancy rate significantly (CPR = 74.1%), with a mean of 3.18 (±0.39) cycles to conceive (P = 0.02) compared with 4.17 (±0.38) cycles to become pregnant when the fertilization rate was lower than 45% (CPR of 56.4%) (Figure 5).

In patients who achieved pregnancy, 4.36 ± 1.51 embryos were replaced, compared with 3.76 ± 1.66 embryos replaced in couples who failed to conceive (P = 0.006). The mean number of embryos replaced in successful cycles either with testicular spermatozoa or with ejaculatory spermatozoa did not differ significantly.

Of the 229 pregnancies obtained from ejaculated spermatozoa, full information on the pregnancy outcome was obtained for 228 couples; 33 were biochemical pregnancies. Out of 195 clinical pregnancies, 147 deliveries were noted, 101 singletons and 46 multiple births (41 pairs of twins and five sets of triplets). During the first trimester, 32 (16.4%) clinical pregnancies were lost spontaneously, and nine (4.6%) (of which three were twins) during the second trimester. Another four pregnancies were diagnosed as ectopic, and three other pregnancies were terminated due to the patient’s request and/or premature rupture of membranes after genetic amniocentesis. Seventeen percent of the patients who initially aborted become pregnant and delivered following a maximum of three successive cycles after the abortion. Patients with preclinical abortions (biochemical pregnancies) were statistically older than those who delivered (36.15 ± 2.76 versus 31.70 ± 5.07 years; P = 0.05). In the TESE group, 51 women delivered 28 singletons, 19 pairs of twins and four sets of triplets. There were 18 pregnancy losses (13 of them first trimester spontaneous abortions), two second trimester spontaneous abortions, two ectopic pregnancies, and one pregnancy lost after fetal reduction at 12 weeks. Five (21.7%) patients who initially aborted after TESE became pregnant and delivered during the two consecutive cycles.

Discussion

The need to provide couples seeking assisted conception with reliable prognostic information is of importance in the light of growing economic constraints (Wilcox et al., 1993). It is important for the candidate couples and for the counselling physicians to know the probability of conceiving, and furthermore delivering a baby following ICSI treatment with ejaculated or with testicular spermatozoa within a defined number of cycles.

Calculating CPR has become a widely used method for presenting couples with relevant estimates of therapy success rates (Dor et al., 1996; Tan et al., 1992). It has been suggested that cumulative studies should be calculated only when IVF treatment becomes freely available on demand (Walters et al., 1994). In this regard, the current data may have special merit, as the National Health Insurance Policy in Israel provides unlimited financial support for the first two children.

The present analysis demonstrated that the CPR following IVF with ICSI treatment increases constantly during the initial five cycles possessing equal chances to result in a pregnancy for each individual attempt. The data described here show that the chances to become pregnant increase in equal proportion for the first four cycles of ICSI with testicular spermatozoa. Although the data described here show equal chances after the sixth and fifth attempt (of ICSI with ejaculated spermatozoa and testicular spermatozoa), the present report was limited due to the relatively small sample available for further attempts. These results correspond with previous reports from the IVF cycles before the ICSI era (Dor et al., 1996) and also with the results reported recently by De Vries et al. (1999) for patients...
entering an IVF/ICSI programme. Although De Vries et al. reported in detail the CPR for the first three cycles only, they achieved a constant pregnancy rate also for cycle 4 and the success rate declined only from cycles 5 and 6 on, with 88% CPR after six cycles. In their study, they refer to either regular IVF or IVF with ICSI fertilization; thus it is not possible to distinguish for ICSI alone.

As previously described by Templeton et al. (1996), a number of patient characteristics, chiefly the woman's age, diminish the likelihood that a pregnancy will occur after IVF. Patients older than 30 years have a significantly lower pregnancy rate for cycles with ejaculated spermatozoa and ICSI, and due to the relatively small sample described in this study show only a tendency for statistical significance in cycles with TESE. This finding corresponds with the recent results of Templeton and Morris (1998) in regular IVF cycles. Stolwijk et al. (2000) found recently that the cut-off age for significant difference in pregnancy rates is 36 years. The difference between the present study and this reported by Stolwijk et al. (2000) consist in the mode the variables were calculated. They ‘forced into the model the variables were calculated. Multiple regression analysis was performed in the present study for all the variables and all the data simultaneously. Furthermore, like De Vries et al. (1999), they calculated the statistics for both regular IVF and ICSI treated cycles. As in an earlier study by Templeton et al. (1996) in regular IVF cycles, the present study demonstrated that in addition to these patient characteristics, the number of eggs fertilized, and hence the number of embryos available for transfer was an important factor in determining outcome. Patients with a fertilization rate lower than 45% needed statistically significantly more cycles to achieve a pregnancy. This may be explained partially due to very poor sperm quality and/or lower quality oocytes. Several recent studies have dealt with failure of fertilization after ICSI cycles (Ludwig et al., 1999) and there is increasing evidence that spermatozoa may be involved in this failure (Lopes et al., 1998; Ubaldi et al., 1999) the oocyte quality (Goud et al., 1999) or both of them (Flaherty et al., 1998). The fact that pregnancy rates after ICSI are much lower in the group with lower fertilization rate is explained partially by the lower number of embryos available for transfer and eventually by a sperm or oocyte defect that is expressed later, during or after the implantation phase. It has been suggested that embryonic gene expression start between the 4- and 8-cell stage of preimplantation development. Therefore it is possible that embryos from patients with lower fertilization rate may have genetic defects that prevent normal embryonic development.

The first trimester abortion rate in this study group (16.4%) is similar that found in other studies from IVF patients (Jones et al., 1983; Govaerts et al., 1996). Furthermore, the rate of the biochemical pregnancies (14.41%) is similar to that in other reports (Coulam et al., 1998), and as expected (Coste et al., 1991), increased among older patients. The mean age of patients who aborted a preclinical pregnancy in this study study was significantly higher (36.2) than of the patients who delivered (31.7). In a recent paper by Osmanagaoglu et al. (1999), a statistically significant drop in delivery rates of patients after 36 years of age was also found.

In conclusion, the data presented here suggest that at least five consecutive attempts of IVF with ICSI micromanipulation in patients with severe azoospermia, and at least four in patients with azoospermia ensures that they have equal chances to conceive. Couples with a young female partner and high fertilization rates can be assured that they will have a good prognosis, and realistically appropriate figures can be supplied for patients with low fertilization rates and/or older women.

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