In-vitro fertilization in cases with severe sperm defect: use of a swim-across technique and medium supplemented with follicular fluid

C. Giorgetti¹, E. Hans, J-L. Spach, P. Auquier² and R. Roulier

¹Institut de Médecine de la Reproduction, 6 rue Rocca, 13008 Marseille and ²Laboratoire de Santé Publique, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille, France

Introduction

Various techniques have been developed for preparing semen for in-vitro fertilization (IVF). Most groups utilize one- or two-step centrifugation of the semen sample followed by swim-up (Purdy, 1982; Wong et al., 1986) or Percoll gradient (Hyne et al., 1986; Geller-Mortimer et al., 1988; Guerin et al., 1989). The Percoll gradient technique appears to be better than swim-up in terms of the quantity of recovered spermatozoa (Berger et al., 1984); however, in cases of severe oligoasthenozoospermia (motile sperm count < 1 × 10⁶/ml of semen), conventional methods of sperm preparation are ineffective (Yovich and Stanger, 1984). This failure is due to the low recovery yield and poor motility in vitro.

We propose a new swim-across insemination technique in which spermatozoa in the centrifugation pellet are placed directly in a well with oocytes, thus eliminating the recovery step. The incubation medium is supplemented with follicular fluid from the female partner. Two studies were conducted. The first (protocol I) was a randomized study aimed at comparing swim-across with swim-up. After 15 IVF attempts, a significant difference was noted in favour of swim-across with regard to fertilization rate. The second study (protocol II) was undertaken to evaluate the efficacy of our new technique for men with very poor semen characteristics.

Subjects

Protocol I. A total of 15 couples entered protocol I. In all male partners, motile sperm counts were < 1 × 10⁶/ml on the day of retrieval. Oocytes were randomly divided into two batches according to the order of collection and inseminated with either the swim-up or swim-across technique.

Protocol II. A total of 124 couples entered protocol II. In 79 of these couples, the only cause of infertility was sperm defects. In 45 couples, tubal damage was also involved. All male partners had motile sperm counts < 1 × 10⁶/ml on the day of retrieval.

The mean age of the female partners was 34 years (range 22–41 years) and the mean duration of infertility was 5.3 years (range 1–20 years). Oocytes were collected during 168 cycles in 1989 and 1990. We compared couples who did (group A) and did not (group B) achieve fertilization. Using logistic regression analysis, we showed a significant correlation between fertilization and progressive motility, percentage normal spermatozoa, number of recovered oocytes and tubal damage. The efficacy of this method may be due to two factors: more efficient utilization of motile spermatozoa, and follicular fluid supplementation. These results demonstrate that fertilization and full-term pregnancy can be achieved with extremely low motile sperm counts.

Key words: follicular fluid/severe sperm defect/swim-across

Materials and methods

Follicular fluid and oocyte preparation

Follicular recruitment and cycle monitoring have been described previously (Giorgetti et al., 1990). Oocytes were collected under ultrasonic control. Follicular fluid was aspirated into a 30 ml bottle (Ovucap 1, Cometh France). The aspiration system was flushed with an additional 1 ml of IMV UA 023 medium (BICEF, l’Aigle, France) to evacuate any oocytes that may have been...
trapped in the needle. All oocytes collected were pre-incubated for 1–2 h in Petri dishes (Falcon 1007, Becton Dickinson) containing 10 ml of IMV UA 023 medium at 37°C in a humidified 5% CO₂ atmosphere (CO₂ Incubator, Model Cytoferm 8088, Heraeus). In protocol I, oocytes for swim-up and swim-across were pre-incubated in separate dishes whereas in protocol II, all oocytes were pre-incubated in the same dish.

Immediately after oocyte collection, follicular fluid from the various follicles of each patient was pooled and an aliquot (4 ml) was centrifuged at 1500 g for 15 min in a sterile tube. The supernatant was aspirated and filtered through a Millex filter with a 0.22 μm pore size (Millipore, France) and mixed with B2 medium (API Bio-Mérieux, France) to a final concentration of 20% in follicular fluid. The resulting medium (B2 + 20% follicular fluid) was used in the swim-across technique during protocol I and II. Follicular fluid was not used during swim-up.

**Semen preparation and insemination**

Semen samples were collected by masturbation after at least 2 days of sexual abstinence. After complete liquefaction, a standard semen analysis (sperm count, progressive motility and morphology) was performed by light microscopy according to WHO guidelines (WHO, 1987). Morphological classification was based on the method described by David et al. (1975). If the semen was of very poor quality, a second specimen was collected 3–4 h after the first.

In protocol I, IVF was attempted using both the swim-up and swim-across techniques, whereas in Protocol II only the swim-across technique was used in all cases.

The swim-up procedure was performed as follows: 2 ml of semen was diluted in 4 ml of IMV UA 023 and the resulting mixture was centrifuged at 400 g for 10 min. IMA UA 023 is a ready-to-use medium containing 4 g albumin/l. After repeating this washing step, the supernatant was discarded and the pellet was resuspended in 50 μl of B2, placed at the bottom of a tube containing 1 ml of B2 medium, and placed in a CO₂ incubator. After 30–60 min, the upper fraction of supernatant (0.5 ml) was recovered. Insemination was conducted using 80 000–120 000 motile spermatozoa in a multi-dish well containing 350 μl of B2 medium without supplementation. In many cases, a large volume of supernatant (100–200 μl) was needed.

The swim-across technique was performed as follows: the semen sample was diluted with four parts of IMA UA 023 medium in sterile 5 ml round-bottom polystyrene tubes (Falcon 2058, Becton Dickinson) and the suspension was centrifuged once at 400 g for 10 min. After carefully aspirating all of the supernatant with a Pasteur pipette, a precision pipette (Pipetman P20, Gilson, France) mounted with a sterile yellow tip, was used carefully to transfer 1–5 μl of the pellet to one side of a multi-dish well containing 350 μl of B2 medium without supplementation. In many cases, a large volume of supernatant (100–200 μl) was needed.

The swim-across technique was performed as follows: the semen sample was diluted with four parts of IMA UA 023 medium in sterile 5 ml round-bottom polystyrene tubes (Falcon 2058, Becton Dickinson) and the suspension was centrifuged once at 400 g for 10 min. After carefully aspirating all of the supernatant with a Pasteur pipette, a precision pipette (Pipetman P20, Gilson, France) mounted with a sterile yellow tip, was used carefully to transfer 1–5 μl of the pellet to one side of a multi-dish well containing 350 μl of B2 medium without supplementation. In many cases, a large volume of supernatant (100–200 μl) was needed.

The fertilization and cleavage rates for all oocytes retrieved (n = 1703) were 24.4% and 88.7% respectively. This should be compared with 66.0% and 91.3% for patients in our laboratory with normal spermatozoa (Giorgetti et al., 1990). In all, 415 embryos were transferred. Of these, 18 cleaved embryos were observed after swim-across compared to only five after swim-up. Fertilization failed with both techniques in five cases and with swim-up alone in seven cases. In no case was the number of embryos obtained with swim-up greater than or equal to the number obtained with swim-across. Based on these observations, we switched exclusively to the new technique.

**Protocol II**

Mean sperm characteristics of the male partners are presented in Table II. The overall results of IVF are depicted in Table III. The fertilization and cleavage rates for all oocytes retrieved (n = 1703) were 24.4% and 88.7% respectively. This should be compared with 66.0% and 91.3% for patients in our laboratory with normal spermatozoa (Giorgetti et al., 1990). In all, 415 embryos were transferred. Of these, 18 cleaved embryos were observed after swim-across compared to only five after swim-up. Fertilization failed with both techniques in five cases and with swim-up alone in seven cases. In no case was the number of embryos obtained with swim-up greater than or equal to the number obtained with swim-across. Based on these observations, we switched exclusively to the new technique.

**Statistical analysis**

Data were analysed using the Statistical Analysis System (SAS). To determine the effect of sperm treatment on fertilization, the non-parametric Wilcoxon test was performed. The relationship between semen characteristics and female parameters, on the one hand, and successful fertilization, on the other hand, was examined using logistic regression analysis. The comparisons group A versus group B and patients with tubal infertility versus patients with other types of infertility were performed using the ANOVA procedure. Results were expressed as means ± SD.

**Results**

**Protocol I**

As shown in Table I, swim-across insemination achieved a significantly higher fertilization rate than swim-up. Interestingly, 18 cleaved embryos were observed after swim-across compared to only five after swim-up. Fertilization failed with both techniques in five cases and with swim-up alone in seven cases. In no case was the number of embryos obtained with swim-up greater than or equal to the number obtained with swim-across. Based on these observations, we switched exclusively to the new technique.

**Protocol II**

Mean sperm characteristics of the male partners are presented in Table II. The overall results of IVF are depicted in Table III. The fertilization and cleavage rates for all oocytes retrieved (n = 1703) were 24.4% and 88.7% respectively. This should be compared with 66.0% and 91.3% for patients in our laboratory with normal spermatozoa (Giorgetti et al., 1990). In all, 415 embryos were transferred. Of these, 18 cleaved embryos were observed after swim-across compared to only five after swim-up. Fertilization failed with both techniques in five cases and with swim-up alone in seven cases. In no case was the number of embryos obtained with swim-up greater than or equal to the number obtained with swim-across. Based on these observations, we switched exclusively to the new technique.

**Table 1. Influence of sperm treatment on fertilization rate during a randomized study of oocyte insemination (protocol I)**

<table>
<thead>
<tr>
<th></th>
<th>Swim-up</th>
<th>Swim-across</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Oocytes inseminated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74 (4.9 ± 2.1)</td>
<td>71 (4.7 ± 2.0)</td>
<td>145 (9.7 ± 4.1)</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>5/74 (6.7%)</td>
<td>19/71 (26.7%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24/145 (16.5%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values in parentheses are means ± SD per cycle.

<sup>b</sup>P < 0.01.
fertilized eggs and 368 cleaved embryos were obtained. The mean number of embryos obtained in cycles with fertilization was 3.9 ± 3.4; an average of 2.7 ± 1.2 were transferred (range 1–4). The total number of full-term pregnancies was 12, with 16 live-births (nine singletons, two twins, one triplet).

Comparison of couples who did (group A) and did not (group B) achieve fertilization showed no statistically significant differences with regard to female age (33.7 ± 4.7 versus 33.8 ± 5.0 years respectively) and duration of infertility (4.9 ± 2.9 versus 5.9 ± 3.5 years respectively). Conversely, the number of oocytes obtained (12.0 versus 7.7 respectively) and the incidence of tubal damage (50% versus 24.3% respectively) were significantly higher (P < 0.001) in the group that achieved fertilization (Table III). Progressive motility in groups A and B (6.8 ± 5.4 versus 3.8 ± 2.7 respectively) and the percentage of normal spermatozoa in groups A and B (44.9 ± 14.2 versus 39.6 ± 17.4 respectively) were higher in group A (P < 0.001). Interestingly, fertilization was not achieved in eight cases with >80% abnormal spermatozoa. The lowest motile sperm counts at which fertilization and full-term pregnancy were achieved were 0.03 × 10^6/ml and 0.15 × 10^6/ml respectively. Table IV compares patients with confirmed tubal infertility (n = 65) and patients with other types of infertility (n = 103). There was no significant difference between these groups with regard to female age, duration of infertility, number of oocytes received, sperm counts, progressive motility and the percentage of normal spermatozoa. Only the fertilization rate was significantly higher in patients with tubal damage as opposed to other causes of infertility.

A logistic regression analysis was done to determine the predictive value of male and female parameters for successful fertilization (Table V). Variables were selected according to the parameter estimate. On this basis, female age, duration of infertility, sperm counts and motile sperm counts were excluded. In Table V, results are presented in descending order of importance of variables, which was determined by standardized estimate. Progressive motility was the variable most significantly related to fertility. In the present study, predicted probability was associated with successful fertilization in 84.6% of cases.

### Discussion

Cohen et al. (1984) was the first to propose IVF as a treatment for male factor infertility. Recently Ord et al. (1990) reported

---

### Table II. Semen parameters for all attempts with Protocol II between couples who did (group A) and did not (group B) achieve fertilization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>94</td>
<td>74</td>
<td>168</td>
</tr>
<tr>
<td>Sperm concentration (×10^6/ml)</td>
<td>7.5 ± 4.6 (0.1–20.0)</td>
<td>8.4 ± 5.5 (0.1–20.0)</td>
<td>7.8 ± 5.0 (0.1–20.0)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>6.8 ± 5.4 (2–30)</td>
<td>3.8 ± 2.7 (1–10)</td>
<td>5.5 ± 4.6 (1–30)</td>
</tr>
<tr>
<td>Total motile count (×10^6/ml)</td>
<td>0.41 ± 0.24 (0.01–0.95)</td>
<td>0.32 ± 0.20 (0.01–0.95)</td>
<td>0.37 ± 0.26 (0.01–0.95)</td>
</tr>
<tr>
<td>Normal forms (%)</td>
<td>44.9 ± 14.2 (18–84)</td>
<td>39.6 ± 17.4 (3–80)</td>
<td>44.4 ± 16.1 (3–84)</td>
</tr>
</tbody>
</table>

*Values are means ± SD with range in parentheses.

### Table III. Overall results of in-vitro fertilization by Protocol II and comparison of clinical parameters between couples who did (group A) and did not (group B) achieve fertilization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>94</td>
<td>74</td>
<td>168</td>
</tr>
<tr>
<td>No. with tubal damage</td>
<td>47 (50.0)</td>
<td>18 (24.3)</td>
<td>65 (38.7)</td>
</tr>
<tr>
<td>No. collected oocytes</td>
<td>12.0 ± 6.5</td>
<td>7.7 ± 4.8</td>
<td>10.1 ± 6.2</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>36.7</td>
<td>0</td>
<td>24.4</td>
</tr>
<tr>
<td>No. of clinical pregnancies</td>
<td>21</td>
<td>–</td>
<td>21</td>
</tr>
<tr>
<td>No. of full-term pregnancies</td>
<td>12</td>
<td>–</td>
<td>12</td>
</tr>
</tbody>
</table>

*Values in parentheses are percentages.

### Table IV. Comparison of characteristics in patients with tubal infertility and patients with other types of infertility in Protocol II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tubal infertility</th>
<th>Other infertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>65</td>
<td>103</td>
</tr>
<tr>
<td>Female age (years)</td>
<td>32.7 ± 4.9</td>
<td>31.0 ± 4.3</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>5.7 ± 3.6</td>
<td>5.1 ± 2.3</td>
</tr>
<tr>
<td>Mean no. of collected oocytes</td>
<td>10.2 ± 5.6</td>
<td>10.1 ± 6.5</td>
</tr>
<tr>
<td>Sperm concentration (×10^6/ml)</td>
<td>8.4 ± 4.7</td>
<td>7.5 ± 5.1</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>5.2 ± 3.3</td>
<td>5.6 ± 5.3</td>
</tr>
<tr>
<td>Normal forms (%)</td>
<td>46.4 ± 15.6</td>
<td>42.9 ± 16.4</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>31.8</td>
<td>19.5</td>
</tr>
</tbody>
</table>

*There were no significant differences between the two groups.

### Table V. Significant variables correlated with fertilization by stepwise logistic regression analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter estimate</th>
<th>P-value</th>
<th>Standardized estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive motility</td>
<td>+ 0.28</td>
<td>0.0004</td>
<td>+ 0.72</td>
</tr>
<tr>
<td>Recovered oocytes</td>
<td>+ 0.15</td>
<td>0.0002</td>
<td>+ 0.52</td>
</tr>
<tr>
<td>Tubal infertility</td>
<td>+ 1.25</td>
<td>0.0044</td>
<td>+ 0.34</td>
</tr>
<tr>
<td>Normal forms</td>
<td>+ 0.29</td>
<td>0.02</td>
<td>+ 0.26</td>
</tr>
</tbody>
</table>
a fertilization rate of 40% for subjects with total motile sperm counts $<5 \times 10^6$/ml. The first sperm recovery procedure used for human IVF was a simple one-step washing (Edwards et al., 1969). More recently, various techniques have been proposed to improve the yield (Lessing et al., 1987); swim-up and Percoll gradient are the most widespread. However, in severe oligoasthenozoospermia, recovery of motile spermatozoa is often poor and leads to failure of IVF.

Protocol I demonstrated that swim-across was significantly more effective than swim-up; in no case did swim-up achieve fertilization when swim-across failed. Conversely in 46% of cases (7/15), swim-across achieved fertilization when swim-up failed. Based on these observations, we discontinued the randomized study for ethical reasons and began protocol II using only the swim-across technique. With the swim-across technique, the presence of debris and dead spermatozoa did not seem to hinder fertilization. This finding contrasts with that of Aitken et al. (1988), who reported that centrifugal pelleting of unslected spermatozoa can induce irreversible damage to spermatozoa by production of superoxide radicals.

Another factor that may account for the greater effectiveness of swim-across in cases of severe oligoasthenozoospermia is that horizontal swimming may increase the chance of contact between spermatozoa and oocytes; indeed failure of the swim-up technique in this condition could be due to the fact that numerous motile spermatozoa remain at the bottom of the migration tube and thus are not recovered in the supernatant.

The positive effects of follicular fluid on acrosome reaction rates have been confirmed in various animals including the hamster (Yanagimachi, 1969), mouse (Iwamatsu and Chang, 1969) and rat (Murkherjee and Lippes, 1972), as well as in man (Tesarak, 1985). More recently follicular fluid was shown to prolong the motility of human spermatozoa (Mendoza and Tesarak, 1990) and enhance sperm penetration (Yee and Cummings, 1988). In a previous study, we showed an improvement in sperm motility after a 24 h incubation in B2 supplemented with 20% follicular fluid (Mattei et al., 1987). We also showed that follicular fluid increases the fertilization and pregnancy rates in cases involving sperm defects (Giorgetti et al., 1988).

The importance of sperm quality for the outcome of IVF has been reported in numerous studies (Cohen et al., 1984; Mahadevan and Trounson, 1984; Kruger et al., 1986; Giorgetti et al., 1990). Comparison of the group which achieved fertilization (group A) and the group which did not (group B) in this study showed that progressive motility and sperm morphology were the only determinant traditional semen parameters for IVF. This study confirmed the absence of correlation between fertilization and sperm concentration in patients with severe oligoasthenozoospermia as shown by Mahadevan and Trounson (1984).

Traditional semen parameters are unable to predict the fertilizing potential of spermatozoa and other tests are required. In this regard, Jeulin et al. (1986) demonstrated that lateral head displacement was predictive of successful fertilization and Barratt et al. (1989) showed that the sperm mucus penetration test was the only independent test related to fertilization.

Female parameters must also be taken into account. It appeared that fertilization was more likely to be successful in cases when the female partner presented tubal damage as opposed to other types of infertility. This is reasonable since in such cases infertility cannot be conclusively attributed to sperm defects as the gametes were never actually in contact with each other. Conversely, when the genital tract is normal, spermatozoa have had every opportunity to fertilize eggs. A corollary observation was proposed in the donor artificial insemination model of Emperaire et al. (1982) who stated that women whose husbands were azoospermic had a better chance of achieving pregnancy than women whose husbands were oligozoospermic.

In conclusion, the swim-across technique described in this report allowed fertilization and full-term pregnancy in patients with extremely low motile sperm counts. Logistic regression analysis showed that progressive motility, normal forms, number of oocytes retrieved and tubal damage have a significant positive association with fertilization. Further study is needed to compare this simple, inexpensive, laboratory technique with microsurgical fertilization techniques (Sathananthan et al., 1989; Cohen et al., 1991).

Acknowledgements

We thank Drs Jean Marie Barbeault and Jean Pierre Franquebalme who performed oocyte retrievals, Mrs M.H. Stura for secretarial assistance in the preparation of the manuscript and Andy Corsini for revising the English manuscript. We are also grateful to Dr Jacques Testart for helpful advice.

References


1124


Received on January 27, 1992; accepted on June 5, 1992