Preliminary results using pentoxifylline in a pronuclear stage tubal transfer (PROST) program for severe male factor infertility

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The prognosis for severe male factor infertility (oligospermia and asthenospermia) with current in vitro fertilization and embryo transfer (IVF-ET) techniques is poor. One approach may be to use phosphodiesterase inhibitors to elevate intracellular levels of cyclic adenosine 3′5′-monophosphate (cAMP) and thus enhance the numbers of progressively motile sperm available for insemination. In this preliminary study, we evaluated pentoxifylline (PF), a methyl xanthine agent, in a program with pronuclear stage IVF followed by transfer to the fallopian tube (PROST).

MATERIALS AND METHODS

In Vitro Trial

Semen samples from 19 male volunteers were subjected to routine semen analysis and classified according to World Health Organization criteria as either oligospermic (<12 × 10^6 motile sperm/ml: N = 8) or normospermic (>12 × 10^6 motile sperm/ml: N = 11). Both nonprogressively motile and progressively motile spermatozoa were counted. The samples were divided equally into control and experimental aliquots: controls were washed with culture medium containing 10% heat-inactivated human serum; experimentals were washed with the same medium containing 1 mg/ml pentoxifylline (Hoechst, Melbourne, Australia). Motile spermatozoa were isolated from the semen by an overlay technique for normospermic samples and by sedimentation for the oligospermic samples. After 45 minutes in either control or PF-containing medium, the sperm suspension was centrifuged and the pellet layered with PF-free medium. After 1 hour more, samples of the supernatant were removed for analysis.

Clinical Study

After encouraging results in the in vitro trial, the technique was applied to nine patients with male factor problems in our PROST program. All of these had previous failed attempts (IVF-ET or PROST) (Table 1). In six treatment cycles, two consecutive ejaculates were collected 1 hour apart to maximize the number of spermatozoa available: the first sample was used as the control and the second treated with PF (prepared as above). In these cases, half of the oocytes were inseminated with PF-treated sperm, whereas the rest were inseminated with untreated sperm. In two cases, only one ejaculate could be produced, and in one, the numbers were so poor that the samples were pooled; in these cases, only PF-treated samples were used for insemination. Oocytes recovered by ultrasound-guided transvaginal aspiration were inseminated with 50,000 to 240,000 motile washed
Table 1 Effect of Pentoxifylline on Fertilization Rates and Pregnancy Outcome in Patients with Previous Fertilization Failure

<table>
<thead>
<tr>
<th>No. of previous failed fertilizations</th>
<th>Cases treated</th>
<th>Fertilization rate (%)</th>
<th>Pregnancy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pentoxifylline</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0/4</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>9</td>
<td>10/44 (22.7%)</td>
<td>3/17 (17.6%)</td>
</tr>
</tbody>
</table>

* Only one ejaculate could be collected.

** Two ejaculates pooled due to low sperm numbers.

spermatozoa 4 to 6 hours after collection. Fertilization was assessed by the presence of two pronuclei; up to four such pronuclear embryos were transferred to the fallopian tube by laparoscopy in the procedure known as PROST.2 Pregnancy was diagnosed 16 to 19 days after oocyte collection by rising serum levels of β-human chorionic gonadotropin (>25 IU/l standardized against 2nd International Standard 61/6) and confirmed approximately 5 weeks later by ultrasound. Results were evaluated with the Wilcoxon paired sample test.

RESULTS

The in vitro trial showed that the oligospermic samples were significantly improved in both the concentration of motile spermatozoa (0.5 ± 0.11 million/ml versus 1.02 ± 0.37: P < 0.05) and progressively motile sperm (0.18 ± 0.06 million/ml versus 0.44 ± 0.14 P < 0.02) after PF treatment. By contrast, no significant differences were noted for the normospermic samples in motile concentration (1.85 ± 0.56 to 2.00 ± 1.31 million/ml) or in progressively motile concentration (1.15 ± 0.87 to 1.36 ± 0.92 million/ml).

Clinical Study

The results are presented in Table 1, where couples are grouped according to their history of failed IVF attempts. Because two ejaculates were used in all cases, the results were analyzed to see if selection for control or PF treatment could have affected the outcome. Except for two cases where only one sample could be produced and one in which insufficient numbers were available, the first ejaculate was used as the control and the second was treated. As expected, the second ejaculate was lower in volume than the first (1.83 ± 0.55 ml versus 4.0 ± 0.89), had fewer total spermatozoa (9.85 ± 3.7 million versus 17.6 ± 8.0), and had fewer total progressively motile spermatozoa (5.92 ± 2.4 million versus 15.01 ± 6.9: P < 0.05). Thus the experimental design, if anything, was biased against the PF-treated samples in that fewer progressively motile spermatozoa were available.

Of the nine cases treated, six achieved fertilization and five pregnancies ensued after PROST, giving a pregnancy rate of 56% per treatment cycle and 83% per transfer. All the pregnancies involved the transfer of at least one embryo fertilized with PF-treated spermatozoa, and the triplet pregnancy resulted from one control and two experimental embryos. Pregnancies were also achieved in two cases where there were insufficient spermatozoa for control inseminations. Thus there is no doubt that some of the conceptuses resulted from PF-treated spermatozoa. As shown in Table 1, four infants have now been delivered, at least two of whom were derived from embryos generated with PF-treated spermatozoa. No congenital abnormalities were detected in these infants, and the ongoing pregnancies are clinically normal.

DISCUSSION

The results presented here, although preliminary, are encouraging for the treatment of severe male factor infertility with agents such as PF to enhance sperm motility. In the initial trials, we showed that treatment significantly improved the
numbers of motile spermatozoa that can be recovered from oligospermic samples. This encouraged us to progress to clinical evaluation, and as shown above, we achieved pregnancy in five of nine PROST treatment cycles. This success rate (56% per oocyte recovery or 83% per transfer) compares well with the overall pregnancy rate of 27% in our program for this procedure. The ongoing and completed pregnancy figures indicate that PF treatment of spermatozoa has no subsequent detrimental effect on human embryos, and we now have mice born after IVF-ET to pseudopregnant recipients with fertilization from PF-treated spermatozoa (results in preparation).

We emphasize, however, that this is a small preliminary trial and that further study is necessary before this treatment mode could be used routinely.

SUMMARY

In vitro trials with washed spermatozoa incubated in medium containing 1 mg/ml of the methyl xanthine phosphodiesterase inhibitor PF showed improved counts of total motile and total progressively motile spermatozoa in cases of oligospermia/asthenospermia. Application of this agent in a PROST program for a series of nine couples presenting for treatment with histories of failed fertilization in vitro resulted in five pregnancies (four singleton, one triplet) and the subsequent delivery of normal infants. The results warrant further evaluation of this sperm treatment for cases of severe male factor infertility.

REFERENCES