ARTIFICIAL STIMULANTS ON CRYOPRESERVED SPERMATOZOA FROM CANCER PATIENTS

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ABSTRACT

Purpose: We determined whether cryopreserved sperm samples obtained from cancer patients before treatment respond to artificial motility stimulants and if this response is related to the extent of disease.

Materials and Methods: Pre-freeze distribution of disease stage in the different types of cancer and the percentage of the population with or without oligosperma before cryopreservation were examined. Cryopreserved semen samples from 17 cancer patients (10 with testicular cancer, 5 with Hodgkin's disease and 2 with other metastatic disease) were examined for a relationship between post-thaw sperm motion characteristics and patient age or status (survived versus died) and type of disease. Motion characteristics (curvilinear velocity, straight line velocity, average path velocity, linearity and amplitude of lateral head displacement) were analyzed on a computer assisted semen analyzer before (time 0), and 30 and 60 minutes after addition of a 2.5 mM concentration of pentoxifylline and 2-deoxyadenosine.

Results: Post-thaw sperm motion characteristics were not correlated with patient age or status, whether they did or did not have oligosperma, or type of cancer. Compared to baseline values, sperm motion characteristics increased significantly after stimulation at time 0 (p <0.02) and at 60 minutes (p <0.05). Oligospermic or nonoligospermic specimens responded to the same extent with pentoxifylline and 2-deoxyadenosine. A negative correlation was noted between overall stage, and type of disease and motion characteristics.

Conclusions: Sperm banking should be encouraged at cancer diagnosis regardless of semen quality. Artificial stimulation of sperm motility results in significant improvement in sperm motion characteristics.

Key Words: spermatozoa, cryopreservation, carcinoma, pentoxifylline, deoxyadenosine

Although testicular malignancies are rare, they are the most common solid organ tumors in men 15 to 35 years old. Hodgkin's disease is the second most common cancer affecting young men.1 Chemotherapy with alkylating or other agents causes azoospermia in 90 to 100% of men with testicular carcinoma, and only 20 to 50% eventually recover spermogenesis.2,3 The success of artificial insemination by sperm from the husband has not been well studied.4,5

To preserve reproductive potential cryopreservation of spermatozoa before cancer treatment is the only effective method available. This procedure has become a complementary part of the treatment of young men with malignancy.4,7 Unfortunately, cryopreserved spermatozoa achieve lower fertilization rates than fresh spermatozoa because recovery of motile sperm is poor.5 Methylxanthine derivatives, such as pentoxifylline, increase the motility of ejaculated sperm by inhibiting cyclic adenosine 3',5' monophosphate diesterase.8-11 Motility is also enhanced by direct exposure to 2-deoxyadenosine, an adenosine analogue.12

The severity of disease at cryopreservation and type of cancer may influence the effectiveness of motility stimulants. We compared patient age and status (alive or dead) at analysis to post-thaw sperm motion characteristics and disease type. The effect of pentoxifylline and 2-deoxyadenosine on sperm motility and other motion characteristics was also examined for an association with the stage or type of the disease.

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MATERIALS AND METHODS

Subjects. Semen samples were obtained from cancer patients enrolled in the andrology laboratory sperm bank program at The Cleveland Clinic Foundation before undergoing cancer treatment (chemotherapy, radiation therapy or surgery). Patient information (age, type and stage of disease, and whether the patient survived treatment) was obtained from the clinical records and when necessary from the referring physician.

Cryopreservation of semen. TES-tris-yolk buffer with glycerol was used to freeze the semen samples via the liquid nitrogen vapor freezing technique. A 5 ml. vial of the freezing medium was thawed at 37°C. An aliquot of the freezing medium equal to 25% of the original specimen volume was then added to the specimen, which was gently mixed for 5 minutes using the Hema-Tek† aliquot mixer. This step was repeated to give a final 1:1 volume-in-volume concentration of the ejaculate and freezing medium. The specimen was divided into aliquots for long-term storage. An additional vial was cryopreserved to assess 24-hour survival. Cryopreservation vials were placed in a freezer at -20°C for 8 minutes. Then, the vials were submerged in liquid nitrogen vapor for 2 hours and finally in liquid nitrogen (-196°C) for long-term storage. All semen specimens were collected and stored under identical conditions for 4 to 8 years.

Motility stimulation. A written legal consent form was obtained from the patient for the release of the specimens. Specimens were randomly chosen from cancer patients who had died after treatment or from those who were alive but were no longer interested in storing the specimens. Cryopre-† Miles, Elkhart, Indiana.
served semen samples were grouped according to the type of disease (testicular cancer in 10 cases, Hodgkin’s disease in 5 and other metastatic type, that is acute myelogenous leukemia in 1 and synovial sarcoma in 1). Based on the pre-freeze sperm concentrations the specimens were classified into nonoligospermic and oligospermic groups. Cryopreserved samples were thawed at 37°C, post-thaw motility was assessed, the cryopreservation medium was removed and the sample was washed with modified human tubal fluid. The samples were centrifuged at 300 × gravity for 7 minutes and resuspended at a concentration of 5 to 10 × 10^6 sperm per ml. Pentoxifylline and 2-deoxyadenosine were dissolved in human tubal fluid to provide a final concentration of 2.5 mM, which is the optimum concentration for stimulating sperm motility in frozen specimens. Controls consisted of the human tubal fluid buffer alone. All samples were incubated for 5, 30 and 60 minutes.

**Assessment of semen variables.** Sperm samples were analyzed on a computer assisted motion analyzer before freezing, after thawing and after stimulation. For each measurement a 5 μl. sample aliquot from a control or treated sample was loaded on a slide. Percent motility, curvilinear velocity (total distance traveled by a given sperm divided by the total time elapsed), straight line velocity (straight line distance from the beginning of the sperm track divided by total time elapsed), average path velocity, linearity (ratio of curvilinear to straight line velocity) and amplitude of lateral head displacement (mean width of sperm head oscillation) were analyzed. The semen analyzer results (concentration and motility) were also verified manually.

**Statistical analysis.** Repeated measures analysis of variance was used to test the effect of stimulants. A paired Student t test was done to evaluate percentage changes in the motion characteristics. The Pearson test was used to determine whether disease severity correlated with the motion characteristics after stimulation. A value of p < 0.05 was considered significant. Semen samples in each group were studied individually to avoid any possible interaction from pooling the samples. The results were reported as mean plus or minus standard deviation. Statistical software was used to analyze the data.

## RESULTS

The mean pre-freeze sperm concentration plus or minus standard deviation was 71.9 ± 71.1 × 10^6/ml and sperm motility was 49.8% ± 17.4%. Of the patients 25% had oligospermia (less than 20 × 10^6 sperm per ml) at the time of sperm banking and the specimens were not specific to any particular stage or disease category. Patient age did not significantly differ in those who survived compared to those who died of the disease (28.5 ± 3.5 versus 31.5 ± 4.0 years). No significant differences were noted in post-thaw and post-wash motility, viability and other motion characteristics in patients who survived compared to those who died of the disease. When the type of disease was compared to post-thaw and post-wash sperm motility, viability and other motion characteristics, all of the groups responded similarly and did not show any significant association.

**Time of incubation did not significantly influence the stimulation outcome** (table 1). Compared to the baseline results, pentoxifylline and 2-deoxyadenosine significantly increased all motion variables (p < 0.0001) except linearity. When the 2 stimulants were compared to assess effectiveness, greater increase in motility (p < 0.013) was noted with 2-deoxyadenosine. Oligospermic specimens responded similarly to both stimulants compared to nonoligospermic specimens.

For statistical analysis, because the number of patients with different disease groups was small, the overall stage effect regardless of the disease type was examined for an association between cancer stage and motion variables. Pentoxifylline stimulation was negatively (but not significantly) correlated with all motion characteristics at both incubation times. Sperm incubation with pentoxifylline for 60 minutes showed a significant negative correlation for amplitude of lateral head displacement. Incubation with 2-deoxyadenosine for 30 minutes showed negative correlation for curvilinear velocity and average path velocity (table 2).

## DISCUSSION

Infertility is an important major sequela of cancer treatment. Urologists and oncologists frequently provide referrals to a sperm bank for cancer patients before initiating chemotherapy. Good sperm motility is crucial for zona pellucida penetration, and motility characteristics are highly correlated with fertilization outcome. However, sperm motility decreases 25 to 75% after cryopreservation, and pregnancy outcome after insemination, even in select patients, is disappointing. Our results do not show any correlation between the severity of disease, that is patient alive or dead, and the post-thaw semen characteristics. The semen characteristics of patients who survived did not significantly differ from those who died of disease, suggesting that sperm quality cannot be used to predict patient survival. Also, our study did not show any differences in post-thaw semen results among the 3 cancer types.

As many as 17 to 77% of testicular cancer patients have oligospermia before starting therapy and only approximately 1 in 4 with Hodgkin’s disease has semen suitable for

### Table 1. Effect of incubation period and stimulants (pentoxifylline and 2-deoxyadenosine) on post-thaw sperm motion characteristics

<table>
<thead>
<tr>
<th>Mean incubation ± SD (min):</th>
<th>% Motility</th>
<th>Curvilinear Velocity (μ/sec.)</th>
<th>Straight Line Velocity (μ/sec.)</th>
<th>Av. Path Velocity (μ/sec.)</th>
<th>% Linearity</th>
<th>Amplitude of Lateral Head Displacement (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.28 ± 10.26</td>
<td>57.07 ± 11.7</td>
<td>17.86 ± 6.8</td>
<td>31.95 ± 8.9</td>
<td>30.4 ± 6.6</td>
<td>2.45 ± 0.2</td>
</tr>
<tr>
<td>30</td>
<td>19.69 ± 9.56</td>
<td>60.56 ± 11.2</td>
<td>19.2 ± 5.9</td>
<td>34.0 ± 7.5</td>
<td>30.4 ± 6.0</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>60</td>
<td>20.18 ± 10.8</td>
<td>61.2 ± 13.7</td>
<td>19.4 ± 5.6</td>
<td>34.5 ± 8.9</td>
<td>30.9 ± 4.8</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Overall p value*</td>
<td>0.40</td>
<td>0.11</td>
<td>0.20</td>
<td>0.14</td>
<td>0.83</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Mean stimulant ± SD:

| None                        | 15.36 ± 8.2 | 51.0 ± 10.3 | 15.7 ± 5.3 | 28.8 ± 6.9 | 29.2 ± 5.4 | 2.2 ± 0.5 |
| Pentoxifylline             | 22.8 ± 10.4 | 63.6 ± 10.3 | 19.2 ± 5.3 | 35.3 ± 7.7 | 28.9 ± 5.8 | 2.7 ± 0.5 |
| 2-Deoxyadenosine           | 21.0 ± 10.4 | 64.3 ± 11.7 | 21.5 ± 6.4 | 36.3 ± 8.8 | 32.7 ± 5.9 | 2.7 ± 0.4 |
| Overall p value*           | 0.0001     | 0.0001       | 0.0001     | 0.0001     | 0.014     | 0.0001      |
| No stimulant vs. 2-deoxyadenosine† | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.015 | 0.0001 |
| No stimulant vs. pentoxifylline† | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.53 | 0.0001 |

2-Deoxyadenosine vs. pentoxifylline† | 0.013 | 0.67 | 0.045 | 0.34 | 0.028 | 0.76

* p ≤ 0.05 is considered significant.
† p ≤ 0.017 is considered significant.
cryopreservation. In our study, although the sample size was small (4), we found no significant differences in post-thaw semen characteristics in oligospermic and nonoligo- spermic specimens after stimulation with pentoxifylline and 2-deoxyadenosine regardless of the stage or type of disease.

Previously, we established the optimum concentrations and incubation time for pentoxifylline and 2-deoxyadenosine to maintain improved motion characteristics in cryopreserved semen samples in normospermic patients. In the present study pentoxifylline and 2-deoxyadenosine enhanced sperm motility as well as other motion characteristics in cryopreserved semen samples compared to respective controls (no stimulants). This increase in motility suggested that these stimulants may have acted on a subpopulation of cryopreserved sperm that was poorly mobile or metabolically quiescent, which became active as the cyclic adenosine mono-phosphate levels increased. A limitation of our study was the insufficient number of patient specimens among the 3 cancer groups, particularly with higher stage, to analyze adequately the extent of stimulation with pentoxifylline and 2-deoxyadenosine. Therefore, the specimens were grouped for overall stimulation effect regardless of the type or stage of the disease. However, we found a trend towards a negative correlation between improvement in sperm motion characteristics after stimulation with pentoxifylline and 2-deoxyadenosine and stage of the disease.

Pregnancies have been reported using pentoxifylline in patients with asthenospermia, oligoasthenospermia and oligospermia, neurologically impaired men undergoing electroejaculation, and patients undergoing intrauterine insemination-pentoxifylline treatment or pronuclear stage tubal transfer. Similarly, increased fertilization rates and pregnancies have resulted from using 2-deoxyadenosine. In vitro fertilization using cryopreserved spermatozoa after artificial stimulation seems to be a promising technique to maintain reproductive potential in patients with cancer. Because cancer treatment is urgent, frequent semen sampling in a short period and adequate post-thaw semen quality are critical, and the abstinence period is reported not to affect post-thaw motility. These motility stimulants, if used in conjunction with the assisted reproductive techniques, may further improve pregnancy outcome. With the availability of micromanipulative techniques, such as intracytoplasmic sperm injection, gross impairments in sperm characteristics can be overcome. For some patients cryopreservation will be their only chance to father a child. Therefore, the obvious benefit of pre-therapy storage of semen from cancer patients should be exploited even with conventional artificial insemination.

CONCLUSIONS

Artificial stimulation improves sperm motion characteristics in cancer patients regardless of the pre-freeze sperm quality. Pentoxifylline and 2-deoxyadenosine are promising motility stimulants, and are equally effective in improving sperm quality.

Ms. Jar-Chi Lee, Department of Biostatistics and Epidemiology, Cleveland Clinic Foundation, helped with the statistical analysis of the results.

REFERENCES


