INVESTIGATION OF FERTILIZING CAPACITY OF CRYOPRESERVED SPERMATOZOA FROM PATIENTS WITH CANCER

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Abstract

Purpose: There are few published reports concerning fertilization and pregnancy outcomes achieved with cryopreserved spermatozoa from cancer patients. Controversy exists regarding the value of sperm banking for these patients before therapy, whether the spermatozoa are viable after long-term storage and whether they can fertilize the ovum. We assess fertilization and pregnancy outcomes achieved with cryopreserved spermatozoa from cancer patients using assisted reproductive techniques.

Materials and Methods: We studied 10 cancer patients who transferred cryopreserved semen specimens from our sperm bank to outside in vitro fertilization programs for assisted reproductive technique. Of these patients 5 had Hodgkin's disease, 2 testicular cancer, 1 leukemia and 2 prostate cancer. The length of specimen storage ranged from 14 to 135 months (median 49, interquartile range 24 and 82).

Results: The median pre-freeze motility was 44% (interquartile range 36 and 55%) and the median total sperm count was 31.1 x 10^6 (interquartile range 6.3 and 53.9 x 10^6). At 24 hours after banking the median post-thaw motility was 11% (interquartile range 6 and 35%) and the median total sperm count was 6.6 x 10^6 (1.2 and 17.1 x 10^6). A total of 18 cycles of assisted reproductive technique were performed among 10 couples with an overall pregnancy rate of 50% per couple, with 2 deliveries, 1 ongoing pregnancy and 2 miscarriages. The pregnancy rate per cycle of in vitro fertilization and intracytoplasmic sperm injection was 36.4% with an implantation rate of 13%.

Conclusions: These results indicate that poor quality cryopreserved spermatozoa from cancer patients, irrespective of the length of storage, may provide successful results with the latest micromanipulative techniques such as intracytoplasmic sperm injection.

Key Words: neoplasms, spermatozoa, cryopreservation, fertilization

An estimated 30,000 malignancies are diagnosed annually among men 18 to 35 years old in the United States. [1] Testicular cancer, Hodgkin's disease and leukemia are the most frequent malignancies in this population. [2] Therapeutic advances have substantially improved long-term survival, with more than 70% of treated patients now expected to survive more than 5 years after the initial diagnosis. [1] However, cytotoxic therapies used to treat malignancies also affect the testes and sterility is a potential consequence. [3,4] Cancer patients in this age group may not have completed families when they begin the treatment regimens that may cure the malignancy. Hence, in an effort to circumvent chemotherapy-induced sterility, sperm cryopreservation before treatment has been recommended for men with newly diagnosed malignancies who desire to have children. [5-8] However, the value of sperm banking in patients with cancer is controversial. [2].

Despite advances in technique, cryopreservation decreases sperm quality. [6,9] Furthermore, sperm quality is often poor in men with systemic malignancies even before treatment has begun. [1,10] Whether these spermatozoa will remain viable after long term storage and whether they can fertilize ova are not assured. No difference in pregnancy rates were seen when ejaculated, fresh and frozen-thawed epididymal and testicular sperm were used for intracytoplasmic sperm injection (ICSI). [11,12] However, the pregnancy rates achieved with cryopreserved sperm from cancer patients are lower than those achieved with fresh or cryopreserved sperm obtained from otherwise healthy men. [3] Few reports in the literature examine the fertilization and pregnancy outcome when cryopreserved spermatozoa from cancer patients are used. Successful fertilization and pregnancy using cryopreserved spermatozoa in in-vitro fertilization (IVF) programs in patients with cancer were reported before the advent of ICSI. [13,14] The introduction of ICSI in the last few years has revolutionized the treatment of patients with severe male factor infertility. ICSI has also become the treatment of choice.
for some patients with cancer with poor post-thaw semen quality. We assess the outcomes achieved with cryopreserved sperm from patients with cancer using assisted reproductive technique, including the latest micromanipulative technique, ICSI.

**MATERIAL AND METHODS**

Sperm bank records were used to identify individuals who withdrew semen specimens from our sperm bank between January 1, 1993 and December 1996. All patients had the specimens transferred to IVF programs outside our institution (closer to residence) to establish pregnancy using assisted reproductive techniques. Patient charts at our institution were reviewed for clinical data regarding the reason for which semen was cryopreserved. Individuals were identified who had a newly diagnosed malignancy at the time of sperm banking and who had not yet undergone therapy. We contacted the outside IVF programs and obtained data on treatment and outcome by a telephone survey of the treating physicians as well as the patients. The number of cycles of intrauterine insemination (IUI), standard IVF and ICSI were obtained, and the outcomes for each cycle were recorded with respect to the number of ova harvested and successfully fertilized, the number of ova or embryos stored, the number of embryos transferred and the outcomes of resultant pregnancies.

Semen samples were collected by masturbation after 2 to 3 days of sexual abstinence and liquefied at room temperature. Manual and computer assisted semen analysis was performed using a semen analyzer before the specimens were processed for cryopreservation. Test yolk buffer with 20% egg yolk and 12% glycerol was added in a 1:1 ratio to semen as a cryoprotectant. Specimens were vortexed and divided equally between vials for long-term storage. The cryovials were placed in a -20C freezer for 8 minutes and thereafter in liquid nitrogen vapor at -96C for 2 hours. The vials were then transferred to liquid nitrogen at -196C for long-term cryopreservation. One cryovial was used from each patient for an initial post-thaw analysis after 24 hours. The vial was placed at room temperature for 5 minutes and then incubated at 37C for 20 minutes and vortexed. A 5 micro l. aliquot of semen then was analyzed for count and motility and compared with the pre-freeze results to calculate the concentration of spermatozoa that survived the freeze-thaw process. Because of the small sample size and small numbers of patients in the cancer groups no statistical analysis was done.

**RESULTS**

**Patient characteristics**

We identified 10 patients who banked sperm and then withdrew it for use in assisted reproduction after successful treatment for malignancy. Patient age ranged from 26 to 72 years (mean 32) at initial diagnosis. Clinical diagnoses for these patients were Hodgkin’s disease in 5, testicular carcinoma in 2, leukemia in 1 and localized prostate carcinoma in 2. Female partner age ranged from 25 to 38 years (mean 31) at the time of the initial assisted reproductive technique cycle.

**Semen parameters**

A total of 94 cryopreserved sperm vials were withdrawn by the 10 patients with an average of 9 vials per patient (range 2 to 32). The duration of sperm storage ranged from 14 to 135 months (mean of 50.4 +/- 49.8). The median pre-freeze motility was 44% (interquartile range 36 and 55%) and the median total sperm count was 31.1 x 10^6 (interquartile range 6.3 and 53.9 x 10^6). At 24 hours after banking the median post-thaw motility was 11% (interquartile range 6 and 35%) and the median total sperm count was 6.6 x 10^6 (1.2 and 17.1 x 10^6). Results of post-thaw sperm quality at the time of assisted reproduction were not available.

**Assisted reproduction outcomes**

All assisted reproductive technique procedures were performed at 7 institutions other than our own, and none of the female partners was evaluated or treated at our institution. The assisted reproductive technique procedures and outcomes are detailed in Table 1. They consisted of 7 cycles of IUI in 4 couples, 2 cycles of standard IVF in 1 and 9 cycles of IVF/ICSI among 8. One pregnancy was established with IUI and has resulted in a healthy baby. In the couples undergoing IVF no pregnancy was achieved. In the couples having ICSI 4 pregnancies were established, with 1 resulting in a live delivery, 1 ongoing pregnancy in a patient with Hodgkin's disease with sperm cryopreserved for more than 12 years and the other 2 in spontaneous abortions. Examining the assisted reproductive technique treatments and outcomes by diagnosis revealed that for 1 patient with localized prostate cancer who underwent an ICSI cycle pregnancy resulted but spontaneous abortion subsequently occurred. The patient with leukemia did not establish a pregnancy regardless of assisted reproductive technique procedure used.
Table 1. Number of cycles, eggs fertilized and embryos transferred in assisted reproduction using cryopreserved spermatozoa

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<tr>
<td>Hodgkin's disease -5</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>19</td>
<td>21</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>4</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
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<td>Prostate Ca (2)</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>3</td>
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<td>14</td>
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<tr>
<td>Totals</td>
<td>7</td>
<td>2</td>
<td>6</td>
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<td>9</td>
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The overall success rate for couples withdrawing banked sperm for use in assisted reproductive technique after cancer treatment included pregnancy in 50% of couples, with live deliveries in 2 couples and 1 currently 6-week ongoing pregnancy. A total of 55 eggs were fertilized (55 of 89, 62%) in 18 cycles of assisted reproductive technique with 34 transferred embryos. For IVF/ICSI overall there was a 36% pregnancy rate per cycle (4 of 11) with an implantation rate of 13% (4 of 31). For all assisted reproductive technique procedures the overall pregnancy rate was 28% (5 pregnancies in 18 cycles). Pregnancies were achieved in all diagnostic groups except leukemia when ICSI was performed.

**DISCUSSION**

Poor quality sperm may be used to achieve fertilization and pregnancy when assisted reproductive technique procedures, particularly ICSI, are used. [15] ICSI may result in fertilization rates of up to 47.7% per oocyte-cumulus complex retrieved and 66.4% per successfully injected metaphase II oocyte. [16] Ongoing pregnancy rates of 31 to 37% per retrieval have been reported by many highly experienced infertility specialists, a greatly enhanced chance of achieving pregnancy when compared to standard IVF. [16,17] The high fertilization rate achieved with ICSI (62% per injected oocyte cycle, 55 of 89) among our cohort underscores the efficacy of this intervention. Our data indicate that ICSI may be successful even in cancer patients with extremely poor sperm quality who have been unable to achieve pregnancy with other types of assisted reproduction. Oocyte fertilization was achieved in all 7 patients who underwent ICSI. Overall, a total of 5 pregnancies were established among the 10 patients (50%), with 2 live deliveries and 1 ongoing pregnancy (28%).

Sexual abstinence time has a minimal impact on semen quality in men with cancer, and specimens have been found to be of similar quality when comparing abstinence times of 24 to 48 hours and 48 to 72 hours or longer. [18] Hence, sperm banking should not cause significant delay between the diagnosis and treatment of a malignancy.

Many theories have been proposed to explain poor semen quality in cancer patients. The explanations vary but it is likely that a disease related mechanism [19] or neoplastic cells in the testis [20] adversely affect spermatogenesis. The effect of testicular cancer and treatment on spermatogenesis has been studied but diagnostic laboratory tests are not available. [3] It seems that an increase in intrascrotal temperature that occurs with these tumors is important in the decreased quality observed in these patients. [21] Also, a change in blood flow results in functional and structural changes in the epididymis. [22] These alterations may be associated with vasomotion abnormalities with consequent loss in transfer of nutrients from the blood to interstitial fluid. [23] Within any particular treatment group it is not possible to predict which patients will be irrevocably sterilized and which may regain fertility. Some investigators have questioned whether the cryopreserved sperm from testicular cancer patients is inherently defective or whether the sperm loses its motility after thawing. [9] Others have found that poor quality semen after cryopreservation in testicular cancer is attributable to poor pre-freeze sperm quality as well as to progressive impairment in semen quality in more advanced disease. [6]

Previous reports have revealed that patients with Hodgkin's disease achieve greater success with IVF compared to those with other types of cancer, [14] particularly testicular cancer. Khalifa et al found a 60% fertilization rate with pre-ovulatory oocytes and a 40% pregnancy rate, with 3 live deliveries and 1 miscarriage. [14] In their study patients were selected who banked semen specimens and used assisted reproductive technique at 1 institution.

Our study is limited in several ways. The sample size was small with few patients in any of the 4 diagnostic categories (range 1 to 4 patients), which precluded statistical analysis. Limited data were available regarding the female factors. The
study is retrospective and all data regarding fertility treatments and outcomes were collected by telephone survey. Fertility treatments were performed at 7 different institutions, and the outcome data are still incomplete in that not all withdrawn semen vials have been used and some of the study patients are scheduled for further assisted reproductive technique. Despite the differences between institutions in equipment, personnel experience, and technique, a normal pregnancy rate of 36% per transferred embryo and a normal implantation rate of 13% were achieved. [24]

CONCLUSIONS

Our overall results indicate that successful pregnancy can be achieved when pre-freeze semen quality is poor in patients with cancer, even when stored for more than a decade. ICSI appears to be a good option in these patients if other assisted reproductive techniques fail. Therefore, semen cryopreservation should be recommended for all patients with cancer even when pre-freeze semen quality is poor.

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REFERENCES

EDITORIAL COMMENT

Although the study reported by the authors has several limitations, which are acknowledged in the article, the message about the fertility potential of sperm that are cryopreserved before oncological therapy nevertheless is powerful. Limitations of the study regard the small sample size of patients in various categories and uncertainty about the level of expertise with IVF and ICSI at the institutions where those procedures were performed.

The fact that 1 couple achieved a pregnancy with IUI suggests that a sufficient number of motile sperm were obtained even after thawing for IUI to be considered. However, it is unlikely that a sufficient number of motile sperm would be available after thawing to consider IUI in most such cases. As the authors indicate, the power of ICSI concerns the ability to achieve conceptions with IVF when unusually low numbers of motile sperm are available. Indeed, the pregnancy rates in this series may have been higher if ICSI had been used in all IVF procedures.

A factor that was not mentioned by the authors deserves consideration. When semen samples are cryopreserved the quality of sperm concentration and motility in each specimen, and the number of specimens that will be available before oncological therapy is initiated are important issues that must be considered by the urologist and by the sperm bank personnel before cryopreservation. If several semen specimens with relatively high sperm concentration and motility will be available, then it may be reasonable to cryopreserve the semen in aliquots that will provide a sufficient number of motile sperm for IUI to be performed on several occasions. However, if only a single semen specimen of good quality or several specimens of poor quality will be available, then the specimens probably should be preserved in aliquots that will be suitable for IVF/ICSI. Such aliquots useful for IVF/ICSI each would contain considerably smaller numbers of motile sperm than would be useful for IUI but would ensure that the couple would have a maximum number of opportunities to achieve conception(s) when using thawed sperm. The urologist also should counsel patients who contemplate cryopreservation of sperm for the possibility of IVF/ICSI in the future about its expense, which ranges from approximately $6,000 to $14,000 in this country and which often is not covered by health insurance.

The authors have provided information in this article to support their conclusion in the abstract that "poor-quality cryopreserved spermatozoa from cancer patients, irrespective of the length of storage, may provide successful results with the latest micromanipulative techniques such as ICSI." Urologists should be encouraged to make this information available to their oncological colleagues, not only by personal communications, but also hopefully by future publications of similar articles in the oncological literature.

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