ANDROLOGY

The Effects of Cryopreservation on Semen from Men with Sarcoma or Carcinoma

J. HALLAK,¹ A. MAHRAN,¹ J. CHAE,¹ and A. AGARWAL^{1,2}

Submitted: October 1, 1999

Accepted: December 27, 1999

Purpose: This study evaluated prefreeze and postthaw semen quality before treatment in patients with different types of sarcomas and carcinomas to determine whether cryopreservation would be of value for these patients.

Methods: Semen specimens were obtained from 50 normal donors and from 21 patients with carcinoma and from 14 patients with sarcoma. The specimens were cryopreserved by a standard freezing procedure using TEST-Yolk buffer. Prefreeze and postthaw sperm motion characteristics were measured.

Results: Prefreeze total motile sperm count was significantly higher in donors (median: $129.6 \times 10^6/\text{ml}$) than in men with carcinoma ($46.9 \times 10^6/\text{ml}$, $P < .001$) or sarcoma ($66.3 \times 10^6/\text{ml}$, $P = .04$). The percent motility and percent linearity were significantly lower in patients with carcinoma. In postthaw specimens, total motile sperm count, curvilinear velocity, and linearity were significantly lower in patients with carcinoma.

Conclusions: The two patient groups in this study had poor semen quality when compared with healthy donors both before and after cryopreservation. Sarcoma patients had better semen quality than carcinoma patients. As cancer therapy in these men could significantly impair their reproductive potential, these men should be advised to preserve their semen before starting treatment.

KEY WORDS: assisted reproduction; cancer; cryopreservation; male infertility.

INTRODUCTION

During the past two decades, major strides have been made in the curability of malignant tumors, so that survival after the treatment of cancer in general and adolescent or childhood cancer in particular has improved (1,2). However, the price that many young men pay for the cure of their malignant disease is temporary or permanent infertility caused by cytotoxic therapies (3,4). In addition, several prospective studies have reported that semen quality is poor in patients with cancer, suggesting that some patients with cancer have decreased potential fertility even before starting treatment (5–8). Cryopreserving spermatozoa before starting treatment is proven to be the only effective method to circumvent the sterilizing effect of cytotoxic therapy in patients with malignant diseases (9). Recent advances in assisted reproductive techniques, namely intracytoplasmic sperm injection (ICSI), have made it possible to use a single motile sperm for fertilization (10,11).

The purpose of our study was to (a) examine semen quality in a group of patients with different types of sarcoma and a group of patients with different types of carcinoma who were referred for sperm banking before beginning cancer therapy over a 15-year period, and (b) compare prefreeze and postthaw semen analyses in patients and a group of normal healthy donors to establish the value of cryopreservation in these patients before they start therapy.

PATIENTS AND METHODS

Patient Selection

This study was approved by the Institutional Review Board of the Cleveland Clinic Foundation and written

¹ Center For Advanced Research in Human Reproduction and Infertility, The Cleveland Clinic Foundation, Cleveland, Ohio 44195.

² To whom correspondence should be addressed at Andrology Research and Clinical Laboratories, Department of Urology, A 19.1, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195.

consent was obtained from all subjects. Records of 14 patients with different types of sarcoma aged 22 to 31 years and 21 patients with different types of carcinoma referred to the sperm bank at our institution for sperm cryopreservation between 1982, when the sperm bank was established, and 1997 were reviewed. The results were compared to those of 50 normal healthy donors aged 23 to 35 years. Patients were included in this study only if they banked specimens before starting treatment. Patients with sarcoma had the following diagnoses: prostate sarcoma ($n = 1$), malignant schwannoma ($n = 1$), osteogenic sarcoma ($n = 2$), neurofibrosarcoma ($n = 1$), primitive neuroectodermal tumor ($n = 1$), Ewing's sarcoma ($n = 5$), synovial sarcoma ($n = 1$), and rhabdomyosarcoma ($n = 2$). Patients with carcinoma had the following diagnoses: thyroid carcinoma ($n = 1$), squamous cell carcinoma of the larynx ($n = 1$), adenocarcinoma of the tongue ($n = 1$), small cell carcinoma of the lung ($n = 2$), gastric adenocarcinoma ($n = 1$), colon adenocarcinoma ($n = 2$), prostate adenocarcinoma ($n = 5$), and undifferentiated carcinoma ($n = 8$).

Patient information (age, type of disease) was obtained from medical records and when necessary by phone calls to the patients or to the referring physician.

Donor Selection

For the donor population, inclusion criteria were an ejaculate volume of at least 2 ml and a sperm concentration of at least $20 \times 10^6/\text{ml}$ of which at least 50% were motile and 30% had normal sperm morphology according to the World Health Organization (WHO) classification (12).

Assessment of Semen Variables

Semen specimens were collected by masturbation after 48–72 h of sexual abstinence and liquefied at 37°C for 30 min. Five microliters of the specimen were loaded on a 20- μl MicroCell counting chamber (Conception Technologies, San Diego, CA) and analyzed by a computer-assisted semen analyzer (Cell Trak Semen Analyzer, CTS Version 4.0, Motion Analysis Corporation, Palo Alto, CA). Manual verification of the semen analyzer results was performed by microscopic examination. (TMS), percent motility, curvilinear velocity (VCL), linearity (LIN), and amplitude of lateral displacement (ALH) were measured.

Semen Cryopreservation

Sperm were cryopreserved with a glycerol-based cryoprotectant, TEST-Yolk buffer (Irvine Scientific,

Santa Ana, CA). An aliquot of freezing medium equal to 25% of the original specimen volume was added to the specimen and gently mixed for 5 min using an aliquot mixer (Hema-tek; Miles Scientific, Elkhart, IN). This procedure was repeated until the volume of cryoprotectant added equaled the volume of the ejaculate. Cryovials were frozen at -20°C for 8 min and then in liquid nitrogen vapor at -100°C for 2 h. The vials were then transferred to liquid nitrogen at -196°C for long-term storage. On the day after the semen was frozen, a vial was removed and thawed by incubation at 37°C for 20 min. A 5- μl aliquot was analyzed as described above.

Statistical Analysis

All statistical analyses were performed with the SAS statistical software package (SAS Institute Inc., Cary, NC). The pairwise Wilcoxon rank-sum test was performed to compare the prefreeze and postthaw semen analysis results of the healthy donors and the patients. In addition, age and ejaculate volumes were compared using the pairwise Wilcoxon rank-sum test. A P value of $<.05$ was considered significant, and all summary statistics are presented as median and interquartile ranges.

RESULTS

Sperm characteristics before and after cryopreservation in healthy donors, men with sarcoma, and men with carcinoma are compared in Table I. Patients with carcinoma did not differ from the healthy donors with respect to age (median 31, range 26–53 years vs. median 29, range 23–35 years; $P = .18$); or ejaculate volume [1.95 (1.35–3.15 ml), vs. 2.45 (1.8–3.5 ml); $P = .19$]. Similarly, patients with sarcoma showed no difference in age or ejaculate volume from normal donors [age 26 (22–31 years) vs. 29 (23–35 years), $P = .1$; ejaculate volume 3.9 (2–5 ml) vs. 2.45 (1.8–3.5 ml), $P = .05$]. However, patients with carcinoma were significantly older than patients with sarcoma ($P = .04$). Also, the ejaculate volume of patients with sarcoma was significantly higher than that of patients with carcinoma ($P = .007$).

Semen Quality Before Cryopreservation

Median TMS, percent motility, and LIN were significantly lower in patients with carcinoma than in healthy donors (Table I). Median TMS was signifi-

Table I. Sperm Characteristics Before and After Cryopreservation in Healthy Donors, Men with Sarcoma, and Men with Carcinoma^a

Sperm characteristics	Healthy donors (n = 50)	Men with sarcoma (n = 14)	Men with carcinoma (n = 21)	P1 ^b	P2 ^c	P3 ^d
Motile sperm count ($\times 10^6$ /ml)						
Prefreeze	129.6 (61.5–240.0)	66.3 (26.7–139.0)	46.9 (12.7–84.6)	.04	<.001	.029
Postthaw	59.1 (23.0–90.8)	32.4 (17.7–76.0)	17.3 (5.3–33.9)	.27	.003	.11
Motility (%)						
Prefreeze	63.5 (50–74)	57.0 (31–76)	48.0 (39–59)	.43	.02	0.41
Postthaw	33.0 (26–40)	21.0 (12–39)	24.5 (13–38)	.08	.05	0.71
VCL ^e (μ m/sec)						
Prefreeze	49.9 (37.1–56.4)	40.4 (35.9–46.3)	46.5 (37.5–50.3)	.12	.14	.48
Postthaw	40.6 (32.0–46.0)	35.5 (29.7–38.6)	33.0 (20.6–40.9)	.09	.03	.43
LIN (%)						
Prefreeze	6.2 (4.4–39.0)	5.1 (4.7–6.2)	5.4 (4.6–5.6)	.44	.03	.78
Postthaw	6.2 (5.0–37.0)	5.3 (5.2–6.0)	5.5 (4.1–5.8)	.22	.006	.39
ALH (μ m)						
Prefreeze	2.7 (2.4–3.2)	3.2 (2.2–4.5)	3.2 (2.5–4.3)	.34	.07	.76
Postthaw	2.2 (1.8–2.5)	2.7 (2.3–3.8)	2.5 (1.8–3.3)	.03	.13	.29

^a Data compared with Wilcoxon rank-sum test. *P* considered significant at <.05.

^b Difference between donors and sarcoma patients.

^c Difference between donors and carcinoma patients.

^d Difference between sarcoma patients and carcinoma patients.

^e Abbreviations: VCL, curvilinear velocity; LIN, linearity; ALH, amplitude of lateral head displacement.

cantly lower in patients with carcinoma than in patients with sarcoma.

Semen Quality After Cryopreservation

Median postthaw TMS was significantly lower in patients with carcinoma than in healthy donors (Table I). Median VCL and LIN were lower in carcinoma patients than in healthy donors. Only ALH was significantly lower in the sarcoma patients than in healthy donors.

DISCUSSION

Sarcomas are rare tumors accounting for approximately 8000 cases per year in the United States, whereas the different types of carcinomas combined are responsible for more than 500,000 new cases per year (13). Surprisingly, there are few reports on sperm cryopreservation in patients with any type of carcinoma or sarcoma (13–15).

In testicular cancer, the cancer may be directly related to poor semen quality (8,16). Also, several brain tumors can cause hyperprolactinemia, which in turn causes gonadal dysfunction, decreased libido, and impotence (17). However, the explanation of the poor semen quality in many patients with other types of malignant diseases, such as sarcoma and carcinoma,

is poorly understood (17, 18). Some authors (19) have suggested that the stress of discovering that one has a potentially fatal disease requiring unpleasant treatment might be sufficient to lower semen quality. It also may result from nutritional deficiency or from the systemic consequences of cancer itself (17).

Therapeutic advances have made curing many malignant diseases a realistic goal (19). Unfortunately, treatment regimens such as chemotherapy, radiotherapy, surgery, or a combination of these treatments frequently result in permanent infertility (1). Gonadal damage induced by chemotherapeutic agents usually occurs within 90 to 120 days of chemotherapy induction, resulting in various degrees of oligozoospermia, necrozoospermia, or azoospermia (20). The duration and severity of spermatogenic suppression are dependent on the agents involved, the time course of treatment, and the total dose of agents. Alkylating agents and procarbazine are the most damaging to spermatogenesis (20,21).

However, surviving malignancy patients, especially those of reproductive age, may wish to have children after an interval free of the disease (22). Therefore, sperm banking is considered “fertility insurance” in patients with malignancy who require potential spermatotoxic treatment for cure (23), especially with the recent advances in assisted reproductive techniques. With ICSI, men with even the most severe forms of

infertility can establish pregnancy, because an oocyte can be fertilized with a single motile sperm (24,25).

Our data show that patients with carcinoma and patients with sarcoma have poorer prefreeze semen quality than healthy donors, and those patients with sarcoma have a better prefreeze semen quality than carcinoma patients. Also, postthaw semen quality is better in patients with sarcoma than in patients with carcinoma, but both are poorer than that of healthy donors.

We recommend that sperm cryopreservation be offered to all men of reproductive age who have carcinoma or sarcoma before starting treatment. Our data suggest that patients with sarcoma may have semen quality adequate for use in a simple assisted reproductive technique such as IUI. Minimum semen quality criteria for successful IUI was found to be a postwash sperm motility of >40% (unpublished observation). Semen quality in carcinoma patients is so poor that they may require advanced assisted reproductive techniques, such as ICSI. Cryopreservation is safe and inexpensive, and if cancer survivors regain fertility, choose not to have children, or die, they can instruct the sperm bank to destroy the samples. There appears to be much to gain and little to lose by offering sperm cryopreservation to patients with sarcoma or carcinoma before they start therapy for cancer.

REFERENCES

- Levron J, Lightman A, Stein DW, Brandes JW, Itskovitz-Eldor J: Pregnancy after subzonal insertion of cryopreserved spermatozoa from a patient with testicular seminoma. *Fertil Steril* 1992;58:839–840
- Myers SE, Schlisky RL: Prospects for fertility after cancer chemotherapy. *Sem Onc* 1992;19:597–604
- Milligan DW, Hughes R, Lindsay KS: Semen cryopreservation in men undergoing cancer chemotherapy—A UK survey. *Br J Cancer* 1989;60:966–967
- Meistrich ML: Effects of chemotherapy and radiotherapy on spermatogenesis. *Eur Urol* 1993;23:136–142
- Chapman RM, Sutcliffe SB, Rees LH, Edwards CR, Malpas JS: Cyclical combination chemotherapy and gonadal function: Retrospective study in males. *Lancet* 1979;1:285–289
- Slanina J, Musschoff K, Rahner T, Tiansky R: Long-term side effects in irradiated patients with Hodgkin's disease. *Int J Rad Oncol Biol Phys* 1977;2:1–19.
- Bracken RB, Smith KD: Is semen cryopreservation helpful in testicular cancer? *Urol* 1980;15:581–583
- Sanger WG, Armitage JO, Schmidt MA: Feasibility of semen cryopreservation in patients with malignant disease. *J Am Med Assoc* 1980;244:789–790
- Khalifa E, Oehninger S, Acosta AA, Morshedi M, Veeck L, Bryzyski RG, Muasher SJ: Successful fertilization and pregnancy outcome in in-vitro fertilization using cryopreserved/thawed spermatozoa from patients with malignant diseases. *Hum Reprod* 1992;7:105–108
- Padron O, Sharma RK, Thomas AJ, Agarwal A: Effects of cancer on spermatozoa quality after cryopreservation: A 12-year experience. *Fertil Steril* 1997;67:326–331
- Tournaye H, Camus M, Bollen N, Wisanto A, Van Steirteghem AC, Devroey P: In vitro fertilization techniques with frozen-thawed sperm: A method for preserving the progenitive potential of Hodgkin's patients. *Fertil Steril* 1991;55:443–445
- World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th ed. New York, Cambridge University Press, 1999
- Fraumeni JF Jr, Hoover RN, Devesa SS, Kinlen LJ: Epidemiology of cancer. In DeVita V, Hellman S, Rosenberg S, ed, *Principles and Practice of Oncology*, 4th ed. New York, Lippincott-Raven, 1993, pp 150–165
- Barth V, Schonfelder M: Fertility studies in malignancy (tumors of the testicle, malignant melanomas, cancer of the thyroid gland). *Andrologia* 1988;20:75–82
- Handelsman DJ, Conway AJ, Donnelly PE, Turtle JR: Azoospermia after iodine-131 treatment for thyroid carcinoma. *Br Med J* 1980;281:1527–XXXX
- Agarwal A, Tolentino MV Jr, Sidhu RS, Ayzman I, Lee JC, Thomas AJ Jr, Shekariz M: Effect of cryopreservation on semen quality in patients with testicular cancer. *Urol* 1995;46:382–389
- Shekariz M, Tolentino MV Jr, Ayzman I, Lee JC, Thomas AJ Jr, Agarwal A: Cryopreservation and semen quality in patients with Hodgkin's disease. *Cancer* 1995;75:2732–2736
- Meirow D, Schenker JG: Cancer and male infertility. *Hum Reprod* 1995;10:2017–2022
- Sanger WG, Olson JH, Sherman JK: Semen cryobanking for men with cancer - criteria change. *Fertil Steril* 1992;58:1024–1027
- Amelar RD, Dubin L: Other factors affecting male fertility. In *Male infertility*, Amelar RD, Dubin L, Walsh PC, eds. Philadelphia, WB Saunders, 1977, pp 73–75
- Parvinen M, Lahdetie J, Parvinen LM: Toxic and mutagenic influences on spermatogenesis. *Arch Toxicol* 1984;7:128–139
- Neumann F: Effects of drugs and chemicals on spermatogenesis. *Arch Toxicol* 1984;7:109–117
- Thachil JV, Jewett MAS, Rider WD: The effects of cancer and cancer therapy on male fertility. *J Urol* 1981;126:141–145
- Kaempfer S, Hoffman D, McKinney F: Sperm banking: A reproductive option in cancer therapy. *Cancer Nurs* 1983;6:31–38
- Sherins RJ, Thorsell LP, Dorfmann A, Dennison-Lagos L, Calvo JP, Krysa L, et al.: Intracytoplasmic sperm injection facilitates fertilization even in the most severe forms of male infertility: Pregnancy outcome correlates with maternal age and number of eggs available. *Fertil Steril* 1995;64:369–375