Journal of Andrology, Vol. 23, No. 1, January/February 2002

Sperm Cryopreservation for Men With Nonmalignant, Systemic Diseases: A Descriptive Study

PAVITHRA RANGANATHAN, AYMAN M. MAHRAN, JORGE HALLAK, AND ASHOK AGARWAL

From the Center for Advanced Research in Human Reproduction and Infertility, Urological Institute, The Cleveland Clinic Foundation, Cleveland, Ohio.

ABSTRACT: Cytotoxic drugs and immunosuppressive therapies are used to treat patients with nonmalignant, nontesticular systemic diseases. These therapies can permanently suppress spermatogenesis. Sperm cryopreservation before treatment theoretically could give these men the opportunity to achieve a pregnancy with a woman later in life when the couple decides to do so. However, it is not known whether pretreatment sperm quality in these men is good enough to be used for assisted reproductive techniques. The main objective of this study was to determine the usefulness of cryopreservation in this patient population by: 1) assessing their pretreatment semen quality (eg, count, motility, and motion kinetics) and comparing it with that of healthy donors before and after cryopreservation; 2) comparing patients' pretreatment semen characteristics with World Health Organization reference values for normal sperm; and 3) examining the differences in semen parameters among patient groups. Semen specimens were obtained from 25 healthy donors and from 23 patients with a variety of disorders (12 had autoimmune disorders, 4 had kidney disorders, 3 had diabetes, 2 had ulcerative colitis, and 2 had heart transplants). All patients, except those with diabetes, required immunosuppressive or cytotoxic therapy. Although the pretreatment quality of the semen of these patients was not as good as that of donors, semen samples were within the normal reference range of the World Health Organization. No statistically significant differences in sperm parameters were found within the 4 patient groups except for those with diabetes ($n = 3$), who showed poorer sperm counts ($P < .04$). However, no conclusive evidence can be reached due to the small sample size. Our results indicate that pretreatment semen quality in these patients is adequate for reproductive techniques. We believe that cryopreservation should be offered to patients of reproductive age with disease or treatment regimens that may cause infertility.

Key words: Assisted reproduction, autoimmune disorders, cytotoxic drugs, male infertility, semen quality, sperm banking.

J Androl 2002;23:71–75

Cytotoxic drugs such as alkylating agents are commonly used to treat people with malignant diseases. One of the main adverse effects of these drugs is impaired fertility in men. After treatment, recovery of spermatogenesis is often unpredictable (Thachil et al, 1981; Baker, 1998) and, in many cases, these drugs render men permanently infertile (Gams, 1980; Marmor, 1994). This can have particularly devastating consequences for young men who have not yet started their families (Muller at al, 2000).

Many studies have evaluated sperm cryopreservation for patients with cancer (Padron et al, 1997; Naysmith et al, 1998; Hallak et al, 1999). Even though cryopreservation can decrease sperm quality (Agarwal et al, 1995), recent advances in assisted reproductive techniques make it possible to achieve fertilization with a single sperm (Tournaye et al, 1991). Thus, in men with impaired fertility due to cytotoxic cancer treatments, pretreatment cryopreservation can give them an opportunity to initiate a pregnancy later in life.

Patients with nonmalignant systemic diseases may receive cytotoxic therapy or immunosuppressive therapy, which can also impair spermatogenesis. Offering these men the option of sperm cryopreservation before treatment would seem to be useful as well. However, the usefulness of cryopreservation in patients with nonmalignant systemic diseases has not yet been evaluated objectively. Specifically, no one has studied whether the semen quality in these men is adequate for reproductive techniques such as in vitro fertilization and intracytoplasmic sperm injection. It is possible that pretreatment semen quality in these men may be too poor for use in assisted reproduction techniques because systemic diseases such as diabetes or chronic renal failure may affect testicular function by causing decreased levels of testosterone and increased levels of gonadotropins.

To determine whether sperm cryopreservation would be useful in men with nonmalignant systemic diseases, we: 1) assessed the pretreatment semen quality in this patient population and compared it with that of healthy donors before and after cryopreservation, 2) compared the patients' pretreatment semen characteristics with World Health Organization (WHO) reference values for normal

Correspondence to: Ashok Agarwal, PhD, HCLD, Director, Andrology Clinical and Research Laboratories, Urological Institute, 9500 Euclid Ave, A19.1, Cleveland, OH 44195 (E-mail: agarwaa@ccf.org).

Received for publication April 2, 2001; accepted for publication July 25, 2001.

Table 1. *Prefreeze and postthaw semen quality in normal donors and patients with systemic disease*

	Donors (n = 25)			Patients (n = 23)			*P* value from Wilcox
		IQR*			IQR*		
Variable	Median	25%	75%	Median	25%	75%	Rank-Sum Test
Age	25.00	22.00	29.00	33.00	20.00	40.00	0.10
Volume	2.20	2.00	3.40	2.40*	1.60	3.60	0.90
Concentration	96.99	70.46	122.91	15.0*	7.00	77.52	0.002
Total count	213.42	165.27	323.27	37.6*	8.4	231.0	0.004
Total motile sperm							
Prefreeze	158.40	96.71	213.00	21.06	1.94	133.86	0.0009
Postfreeze	65.80	55.60	90.80	5.17	1.40	41.94	0.0001
Percentage change	−47.79	−60.80	−38.41	−60.83	−79.07	−51.57	0.03
Motility							
Prefreeze	66.0	51.00	79.00	47.00*	18.00	67.00	0.007
Postfreeze	37.0	33.00	45.00	15.00	10.00	33.00	<.0001
Percentage change	−40.91	−48.19	−27.91	−59.26	−76.60	−46.34	0.001
Curvilinear velocity							
Prefreeze	50.90	38.80	56.40	42.60	33.80	55.20	0.12
Postfreeze	40.60	35.00	46.00	31.30	16.20	35.70	0.0007
Percentage change	−22.09	−29.86	−10.68	−30.59	−49.29	−15.94	0.054
Linearity							
Prefreeze	6.30	5.60	40.00	4.80	4.10	33.00	0.04
Postfreeze	6.40	5.90	44.00	5.00	4.00	47.00	0.03
Percentage change	8.93	−2.13	14.04	2.63	−5.20	18.90	0.86
Lateral head displacement							
Prefreeze	2.50	2.40	3.30	3.40	2.41	4.30	0.18
Postfreeze	2.25	1.90	2.70	2.35	0.00	2.80	0.99
Percentage change	−22.18	−28.26	−4.47	−14.90	−48.89	4.65	0.89

* Volume ($P = .07$), concentration ($P = .16$), total sperm count ($P = .20$), and prefreeze motility ($P = .46$) not significantly different from World Health Organization guildelines for normal semen parameters, using Wilcoxon sign-rank test. IQR indicates interquartile range.

sperm, and 3) examined the differences in semen parameters among patient groups.

Materials and Methods

Patient Selection

The Institutional Review Board of the Cleveland Clinic Foundation approved this study, and all subjects granted their written consent. The patients included 23 men with nonmalignant, systemic, nontesticular diseases who had their sperm cryopreserved before treatment with immunosuppressive or cytotoxic drugs (this does not include patients with diabetes). The patients were broadly categorized into 5 groups: those with autoimmune disorders (n = 12), kidney diseases (n = 4), diabetes (n = 3), ulcerative colitis (n = 2), and heart transplants (n = 2).

Donor Selection

Semen specimens were collected from 25 normal healthy men who met WHO (1999) criteria for normal, healthy sperm. To be included in this study, donors had to have an ejaculate volume of at least 2 mL, a sperm concentration greater than 20×10^6/mL, sperm motility greater than 50%, and normal morphology greater than 30%.

Assessment of Semen Variables

All semen specimens were collected by masturbation after 48 to 72 hours of sexual abstinence, and were liquefied at 37°C for 30 minutes. Five μL of the specimen were loaded into a 20-μL Microcell counting chamber (Conception Technologies, San Diego, Calif). The specimens were analyzed by a computer-assisted semen analyzer (Cell Trak Semen Analyzer, CTS version 4.0, Motion Analysis Corporation, Palo Alto, Calif) for 7 characteristics: concentration, total count, total motile sperm count, motility, and motion kinetics (curvilinear velocity, linearity, and lateral head displacement). The age of the patient and volume of ejaculate was also noted. Semen analysis results were manually verified by microscopic examination. To prevent observer bias, laboratory personnel who analyzed the samples were blinded to the study purpose and patient characteristics.

Sperm Cryopreservation

Semen specimens were cryopreserved with a glycerol-based protectant, TEST-Yolk buffer (Irvine Scientific, Santa Ana, Calif). An aliquot of the freezing medium equal to 25% of the original specimen volume was added to the specimen and gently mixed for 5 minutes using an aliquot mixer (Hema-Tek, Miles Scientific, Elkhart, Ind). This procedure was repeated until the volume of the cryoprotectant added equaled the volume of the ejaculate. The mixture was then aliquoted and stored in cryovials. The volume and count of sperm in each vial depended on the initial

volume and count of the specimen. Cryovials were frozen at −20°C for 8 minutes, and then in liquid nitrogen vapor at −100°C for 2 hours. The vials were then transferred to liquid nitrogen at −196°C for long-term storage. Twenty-four hours after the semen was frozen, a vial was removed and thawed by incubation at 37°C for 20 minutes. A 5-μL aliquot was analyzed as described above (Hallak et al, 2000).

Statistical Analysis

All statistical analyses were performed with the Statistical Analysis System statistical software version 8.1 (SAS Institute Inc, Cary, NC). The Wilcoxon rank-sum test was used to compare the prefreeze and postthaw semen characteristics of donors with those of the patients, and also to compare the semen characteristics among the 4 patient groups. The Wilcoxon signed-rank test was used to compare patients' prefreeze semen parameters with WHO normal cutoffs. The data conformed to the assumptions of the statistical tests. A P value less than .05 was considered statistically significant, and all summary statistics are presented as medians and interquartile ranges (25th and 75th percentiles). Based on variability of several indices, the sample size has greater than 90% power to detect about 20% differences in percentage changes between patients and donors; 30% differences between autoimmune disorders, and other patients; and 40% differences between the other patient subgroups and the remaining patients.

Results

Semen Quality in Patients Before Cryopreservation

In general, the prefreeze semen characteristics of patients were lower than those of the healthy donors (Table 1), but they were within the WHO normal reference values for semen parameters (Table 2). Sperm motion kinetic parameters such as velocity, linearity, and amplitude of lateral head displacement were comparable in both groups.

Semen Quality in Patients After Cryopreservation

The postthaw median total motile sperm count in patients was much lower than that in donors, and the difference was statistically significant ($P < .001$). However, the percent change in motility (from prefreeze to postthaw) was comparable between the 2 groups. Postthaw sperm motility in the patients was similar to the WHO reference range. The percentage decline in sperm motion kinetics such as curvilinear velocity, linearity, and lateral head displacement were similar in the patient and donor groups (Table 1).

Of the 23 patients, 7 (30%) had more than 40 million total motile sperm in their postthaw specimens (mean ± SD 120.6 ± 155.0 × 10^6, range 41–448 × 10^6), which means that they had adequate semen quality for multiple intrauterine insemination procedures. The remaining patients had a total motile sperm count of 6.0 ± 0.38 × 10^6, range 0.5–25.3 × 10^6), which means that they had adequate semen for other assisted reproductive techniques such as in vitro fertilization and intracytoplasmic injection (WHO, 1999).

Intra-Group Analysis

No significant differences in semen quality were observed between patients with different clinical diagnoses. The patients with diabetes had lower sperm counts and lower velocity compared with patients in the other 3 groups, and these differences were statistically significant. However, this trend was inconclusive because of the small sample size (n = 3; Table 2).

Discussion

The literature lacks information on the importance of semen cryopreservation in men with systemic diseases as well as on the fertilizing capacity of their sperm. This study was designed to explore these issues. We found that: 1) the pretreatment semen quality in patients was poorer than in normal healthy donors but within normal WHO reference values and 2) intragroup analysis showed no valid statistically significant differences between patients with different diagnoses. We therefore concluded that semen quality in men with nonmalignant systemic diseases is adequate for reproductive techniques.

The fact that the semen quality in these patients was within the WHO reference range indicates that spermatozoa were adequate for assisted reproductive procedures. Thirty percent of the patients had semen quality sufficient for multiple intrauterine insemination procedures, and the remaining men had semen that was adequate for other assisted reproductive techniques such as in vitro fertilization and intracytoplasmic sperm injection.

Currently, information and data on the fertilizing capacity of sperm cryopreserved from men with systemic diseases is sparse. Reports on the fertilizing capacity of cryopreserved sperm from men with cancer appear promising (Hallak et al, 1998a).

Chapman et al (1981) studied the effect of Hodgkin disease and subsequent chemotherapy on gonadal function and reported that high proportions of these men (43%) had gonadal dysfunction prior to treatment with cytotoxic drugs. Patients with malignancies have a higher incidence of gonadal dysfunction than patients with systemic diseases. Although it is clear that chemotherapy induces infertility, the sterilizing effects of chemotherapy depend on the nature of the drug and the genetic makeup of patients more than it does on disease type. Hence, irrespective of disease state, treatment modality or semen quality, the option to cryopreserve sperm should be offered to all men before they start any form of suppressive therapy. This provision is critical for adolescents and men

Table 2. *Prefreeze and postthaw semen quality within various patient groups with systemic disease**

	Autoimmune Disorder (n = 12)			Kidney Disorder (n = 4)			Diabetes (n = 3)			Other (n = 4)			*P* Values			
		IQR†			IQR†			IQR†			IQR†		1 vs	2 vs	3 vs	4 vs
Variable	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Others	Others	Others	Others
Age	33.00	26.00	34.00	19.00	17.50	32.00	40.50	38.00	43.00	44.00	41.00	47.00	0.36	0.20	0.19	0.07
Volume	2.50	2.15	4.50	2.25	0.75	4.25	2.00	0.50	2.00	2.45	1.25	3.45	0.12	0.74	0.13	0.72
Concentration	37.80	7.50	123.26	13.94	10.64	97.00	3.68	0.45	8.80	55.41	20.51	77.78	0.45	0.71	0.07	0.84
Total count	91.55	31.25	223.65	26.75	7.95	470.05	**1.84**	**0.90**	**17.60**	148.15	32.74	269.50	0.22	0.90	**0.04**	0.72
Total motile sperm																
Prefreeze	55.75	13.22	139.43	5.14	2.29	84.16	0.86	0.58	1.94	67.15	22.53	122.15	0.13	0.65	0.052	0.84
Postfreeze	14.85	4.30	50.08	1.94	0.92	26.74	0.30	0.18	2.82	28.81	7.84	56.67	0.14	0.40	0.07	0.63
Percentage change	−58.78	−82.58	−51.03	−67.04	−69.60	−60.07	−48.28	−79.07	45.45	−59.56	−82.59	−53.49	1.00	0.49	0.26	0.75
Motility																
Prefreeze	56.50	45.00	68.50	18.00	17.00	29.50	47.00	11.00	64.00	48.00	27.00	68.00	0.15	0.07	0.62	0.69
Postfreeze	18.00	10.50	35.00	6.00	6.00	14.00	16.00	11.00	42.00	19.00	7.00	29.00	0.50	0.12	0.47	1.00
Percentage change	−56.80	−81.41	−46.03	−64.58	−66.67	−54.42	−34.38	−76.60	45.45	−58.47	−82.61	−50.49	0.90	0.77	0.23	0.58
Curvilinear velocity																
Prefreeze	45.40	39.85	56.10	42.50	34.45	57.00	33.80	0.00	59.20	34.85	17.35	41.00	0.24	0.84	0.55	0.22
Postfreeze	32.30	22.45	38.35	29.85	23.10	35.00	**0.00**	**0.00**	**16.20**	26.10	8.75	35.85	0.22	0.71	**0.04**	0.90
Percentage change	−17.72	−45.21	−13.14	−34.06	−45.08	−21.80	−76.04	−100.00	−52.07	−21.28	−50.00	0.00	0.35	0.81	0.07	0.60
Linearity																
Prefreeze	5.05	4.35	28.25	4.35	3.40	4.85	4.80	0.00	33.00	28.25	2.25	52.50	0.41	0.32	0.71	0.81
Postfreeze	5.35	4.05	30.35	5.00	4.80	5.15	0.00	0.00	50.00	25.75	2.25	49.00	0.52	0.96	0.34	0.97
Percentage change	3.64	−8.47	18.75	13.28	2.07	52.94	−24.24	−100.00	51.52	−1.92	−11.32	0.00	0.88	0.25	0.90	0.17
Lateral head displacement																
Prefreeze	3.15	2.60	3.95	3.25	1.20	4.45	4.50	0.00	5.10	3.25	1.25	4.00	0.81	0.80	0.58	0.57
Postfreeze	2.50	1.50	3.20	2.15	1.05	2.40	0.00	0.00	2.30	3.15	1.05	4.45	0.23	0.49	0.14	0.64
Percentage change	−11.18	−27.78	−3.85	−46.34	−56.25	8.33	−74.44	−100.00	−48.89	5.00	−16.00	17.50	0.86	0.81	0.11	0.17

* Bold indicates statistically significant difference.
† IQR indicates Interquartile Range.

who have not completed their families but have the potential to become infertile from a disease or its treatment.

We recommend that sperm banking be offered to all men who have systemic diseases at any stage of progression and before they are treated with cytotoxic drugs. This gives patients a chance to establish pregnancy with assisted reproduction, even when normal conception may not be possible. Cryopreservation is safe, convenient, and inexpensive. If patients die or choose not to have children, the sperm bank can be instructed to destroy their samples (Hallak et al, 1998b).

Acknowledgments

The authors thank Karen Seifarth, Cheryl Wellstead, and Lora Cordek, of the Clinical Andrology Laboratory for technical assistance, and Robin Verdi for secretarial support.

References

Agarwal A, Tolentino MV, Sidhu RS, Ayzman I, Lee JC, Thomas AJ Jr, Shekarriz M. Effect of cryopreservation on semen quality in patients with testicular cancer. *J Urol.* 1995;46:382–389.

Baker HWG. Reproductive effects of non-testicular illness. *Endocrinol Metab Clin.* 1998;27:832–850.

Chapman RM, Sutcliffe SB, Malpas JS. Male gonadal dysfunction in Hodgkin's disease. A prospective study. *JAMA.* 1981;245:1323–1328.

Gams RA. Complications of chemotherapy in the treatment of Hodgkin's disease. *Semin Oncol.* 1980;7:184–186.

Hallak J, Hendin BN, Thomas AJ Jr, Agarwal A. Investigation of fertilizing capacity of cryopreserved spermatozoa from patients with cancer. *J Urol.* 1998a;159:1217–1219.

Hallak J, Mahran A, Chae J. Why cancer patients request disposal of cryopreserved semen specimens post-therapy: a retrospective study. *Fertil Steril.* 1998b;69:889.

Hallak J, Mahran A, Chae J, Agarwal A. The effects of cryopreservation on semen from men with sarcoma or carcinoma. *J Assist Reprod Genet.* 1999;17:218–221.

Hallak J, Sharma R, Wellstead C, Agarwal A. Cryopreservation of human spermatozoa: comparison of TEST-Yolk buffer and glycerol. *Int J Fertil.* 2000;45:38–42.

Marmor D. Fertility after antimitiotic treatments. *Bull Cancer.* 1994;81: 764–769.

Muller J, Sonksen J, Sommer P, Schmiegelow M, Petersen PM, Heilman C, Shmiegelow K. Cryopreservation of semen from pubertal boys with cancer. *Med Pediatr Oncol.* 2000;34:191–194.

Naysmith TE, Blake DA, Harvey VJ, Johnson NP. Do men undergoing sterilizing cancer treatments have a fertile future? *Hum Reprod.* 1998; 13:3250–3255.

Padron OF, Sharma RK, Thomas AJ Jr, Agarwal A. Effects of cancer on spermatozoa quality after cryopreservation: a 12-year experience. *Fertil Steril.* 1997;63:326–331.

Thachil JV, Jewett MAS, Rider WD. The effects of cancer and cancer therapy on male fertility. *J Urol.* 1981;126:141–145.

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 disease. *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. Cambridge, United Kingdom: Cambridge University Press; 1999.