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EDITORIAL COMMENT

Agarwal *et al.* present a well-done study examining pretreatment subfertility and the efficacy of semen cryopreservation in men with testis carcinoma. Pretreatment subfertility was verified to be a significant phenomenon in such men, but the more important message is found in the cryopreservation results and interpreting those results in the setting of modern assisted reproductive technologies (ART). Despite differences in sperm quality between controls and testicular cancer patients, the percentage of sperm survival following cryopreservation and subsequent thawing was the same. However, the net effect is that the total motile sperm count yielded after cryopreservation and thawing in men with testicular cancer is still marginal.

In past years, the conclusion drawn from this information was that cryopreservation is not useful in men with testis cancer due to poor quality.¹ However, with recent advances in *in vitro* fertilization, particularly with the advent of intra-

cytoplasmic sperm injection (ICSI), pregnancies are now possible with extremely low numbers of spermatozoa (see authors' references Ng *et al.*²⁰ and Van Steirteghem *et al.*²¹). We truly are in an era where only a handful of viable sperm are necessary to induce a pregnancy. Certainly, as is evident from this article, most men with testis cancer would be candidates for ICSI using their cryopreserved specimens.

One wonders, as some have suggested (discussions at the 1995 American Society of Andrology and American Urological Association meetings), whether forcing fertilization in the setting of testicular dysfunction may bring about unwanted changes, such as genetic malformations, testis abnormalities, and infertility in the progeny. This concept remains theoretical, however, and there is some published evidence to the contrary. A recent study of children conceived with high-level ART procedures found no cytogenetic abnormalities, and no increased incidence of congenital malformations.²

With the information contained in the study by Agarwal *et al.*, and procedures currently available to allow pregnancy to occur with very low sperm counts, it seems prudent to offer semen cryopreservation to all men with testicular carcinoma, even those with extremely poor pretreatment semen quality.

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TABLE VII. Characteristics of sperm quality according to testicular cancer histology

	Embryonal (n = 7)			Mixed (n = 17)			Seminoma (n = 9)			P Value*
Age (yr, mean ± SD)	25.6 ± 4.5			25.1 ± 6.2			28.1 ± 5.8			0.43
Volume of ejaculates (mL)	3 ± 1.9			3.2 ± 2			2.9 ± 1			0.93
Sperm Characteristics	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	P Value*
Motile sperm count (×10 ⁶ /mL)										
Prefreeze	6.4	8.9	17.1	1.5	4	5.6	11.5	14	17	0.0096
Post-thaw	1.7	2.2	3.4	0.4	1.2	1.6	2.6	2.9	4.5	0.008
Change (%)	-81.5	-80	-69	-84.5	-72.6	-64.5	-80.4	-76.1	-72.9	0.85
Motility (%)										
Prefreeze	38	46	55	21	39.5	51.5	36	44	50	0.63
Post-thaw	12	16	22.0	9.5	14	24	16	20	21	0.42
Change (%)	-68.1	-62.8	-50	-69.5	-50.5	-31.5	-60.8	-52.3	-45.9	0.48
Velocity (μm/s)										
Prefreeze	26.3	52.2	63.1	32.1	39.4	46.6	41.4	47	51	0.14
Post-thaw	24	38.5	45.6	26.7	36.7	39.5	36.3	38.9	43	0.40
Change (%)	-48.2	-26.2	5.7	-28.8	-4.1	-0.25	-17.2	-15.7	-4.98	0.86
Motility index										
Prefreeze	9.8	2.40	33	6.2	14.4	20	15.3	17.5	25.5	0.17
Post-thaw	3.8	5.0	10.8	2.1	3.9	8.6	5.8	6.9	9.8	0.27
Change (%)	-81	-72.7	-60.60	-78.6	-60.9	-40.2	-69.5	-62.2	-54.5	0.72
Linearity (%)										
Prefreeze	5	5.4	6.8	4.8	5.2	5.8	4.7	5.3	5.5	0.44
Post-thaw	5.3	6.3	6.5	1.6	4.6	5.2	4.5	5	6.1	0.05
Change (%)	-16	6.6	23.3	-46	-3.9	7.2	-5.7	6.4	10.9	0.42
Amplitude of lateral head movement (μm)										
Prefreeze	1.6	3	3.3	1.7	2.2	2.7	2.3	2.6	3	0.08
Post-thaw	1.5	2.3	4.2	0	1.8	2.2	1.7	2.6	2.7	0.29
Change (%)	-45	-10.4	27.3	-43.8	-6.7	5.3	-18.4	-10	-2.7	0.97

*Comparison between patients and donors.

cryopreservation outcome; (3) in view of the introduction of new assisted reproductive techniques along with improvements in cryopreservation techniques and cryoprotectant media that further increase sperm quality after cryopreservation, semen cryopreservation should become routine for all men diagnosed with testicular cancer regardless of the extent of the disease.

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TABLE V. Percent change in sperm characteristics from before to after cryopreservation

Sperm Characteristic (% Change)	Patients				Donors				
	Q1	Median	Q3	P Value*	Q1	Median	Q3	P Value*	P Value†
Motile sperm count ($\times 10^6/\text{mL}$)	-81.5	-74.8	-65.8	0.0001	-78.8	-72.5	-65.1	0.0001	0.44
Motility	-64.9	-51.7	-36.8	0.0001	-58.1	-44.3	-28.1	0.0001	0.09
Velocity ($\mu\text{m/s}$)	-27.7	-16.1	-1.4	0.001	-33.3	-19.7	-4	0.015	0.66
Motility index	-75.1	-63.2	-44.6	0.0001	-65.6	-51.6	-41.4	0.0001	0.052
Linearity (%)	-19.6	2.04	12.5	0.66	-9.9	2	20.4	0.49	0.44
Amplitude of lateral head movement (μm)	-32.3	-11.5	0.0	0.008	-34.2	-21.7	-11.5	0.0001	0.31

*Comparing values before and after cryopreservation.

†Comparison between patients and donors.

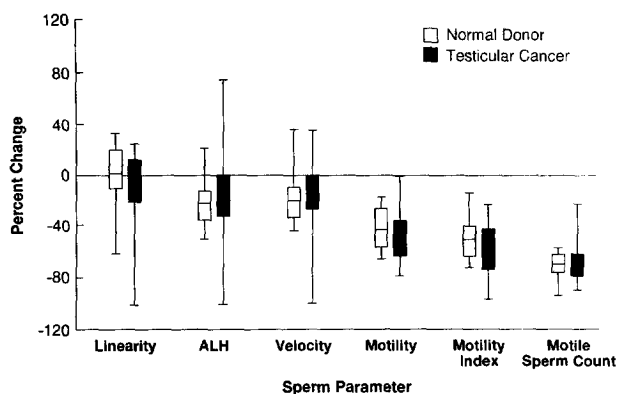


FIGURE 2. Influence of cryopreservation on semen parameters is displayed as box plots. There are no differences in the effect of cryopreservation between normal donors and testicular cancer patients. Except for linearity, all other semen parameters showed a large decline after cryopreservation in both study groups.

TABLE VI. Distribution of patients by histologic type of tumor

Tumor Type	Patients
Mixed germ cell tumor	17 (50%)
Pure seminoma	9 (26.5%)
Pure embryonal	7 (20.6%)
Pure choriocarcinoma	1 (2.9%)
Total	34

Cryopreservation of semen in this study significantly decreased MSC and motion characteristics in both patients and control subjects. Sperm motility, VCL, motility index, and MSC were significantly lower in post-thaw semen from patients than from normal donors, but the percentage decrease in these sperm motion characteristics in patients was not different from that in donors. This finding indicates that the effect of cryopreservation on semen quality in testicular cancer patients is similar to that in normal, healthy men. An intrinsic defect of sperm, which may magnify

deficiencies in motion characteristics, was not identified. It is, however, unclear if sperm from these patients have a lower fertilizing potential.¹⁵ With newer techniques in assisted reproduction, such as intracytoplasmic sperm injection,¹⁸ it remains to be seen whether these defects in sperm function even play a role in egg fertilization and pregnancy. Markert¹⁹ studied the fertilizing capacity of immotile and grossly defective mouse sperm by directly injecting them into the egg cytoplasm. This resulted in successful fertilization and delivery of mouse fetuses. He concluded that sperm phenotype was not related to the ability of the sperm to interact with the egg cytoplasm and form a pronucleus.

Approximately 115 live births have been reported using cryopreserved sperm from patients with various malignant diseases.⁷ Intrauterine insemination is the most common technique for artificial insemination using thawed semen. However, using this technique, the chances of a pregnancy are lower in patients than in normal donors, since the success rate depends on the MSC, which is lower among testicular cancer patients. Using in vitro fertilization, a much lower number of motile spermatozoa are necessary for a successful pregnancy.¹⁵ Furthermore, subzonal insertion of sperm²⁰ and intracytoplasmic sperm injection²¹ have increased the chances of fertility using cryopreserved sperm among patients with low sperm density.⁷

In conclusion, our results indicate that: (1) Although a majority of patients with testicular cancer are subfertile at the time of diagnosis, their sperm quality can be considered adequate for successful cryopreservation; (2) although semen cryopreservation in patients with abnormal sperm quality results in a poorer recovery rate than in normal men, the effect of the freezing and thawing processes on sperm quality does not appear to be different in this patient population. Therefore, a clinical diagnosis of testicular cancer is not an adequate predictor of

TABLE IV. Characteristics of sperm quality in patients with testicular cancer and normal donors, before and after cryopreservation

	Patients (n = 34)			Donors (n = 30)			P Value*
Age (yr, mean ± SD)	26.4 ± 5.7			31.4 ± 7.6			0.013
Volume of ejaculate (mL)	3 ± 1.7			2.9 ± 1.4			0.93
	Sperm Characteristics						
	Q1	Median	Q3	Q1	Median	Q3	P Value*
Motile sperm count (×10 ⁶ /mL)							
Prefreeze	3.4	6.7	14.4	24.6	50	72	0.0001
Post-thaw	1.2	2.0	3.2	7.8	11.6	20.8	0.0001
Motility (%)							
Prefreeze	24	42	51.0	49	60.5	73	0.0004
Post-thaw	12	16.5	23.3	29.0	32.5	40	0.0001
Velocity (μm/s)							
Prefreeze	38	42.9	51	43.4	51	59	0.03
Post-thaw	32	37.9	41.9	39.5	41	46	0.0083
Motility index							
Prefreeze	9.6	16.8	24	19.1	27.7	39.1	0.0022
Post-thaw	3.4	5.7	9.1	11	13.9	18.4	0.0001
Linearity (%)							
Prefreeze	4.7	5.3	5.6	4.3	4.7	6.1	0.30
Post-thaw	4.2	5.1	6.1	4.1	5.5	6.1	0.49
Amplitude of lateral head movement (μm)							
Prefreeze	1.92	2.5	3.0	2.4	2.7	3.4	0.027
Post-thaw	1.50	2.1	2.7	2	2.3	2.5	0.19

*Comparison between patients and donors.

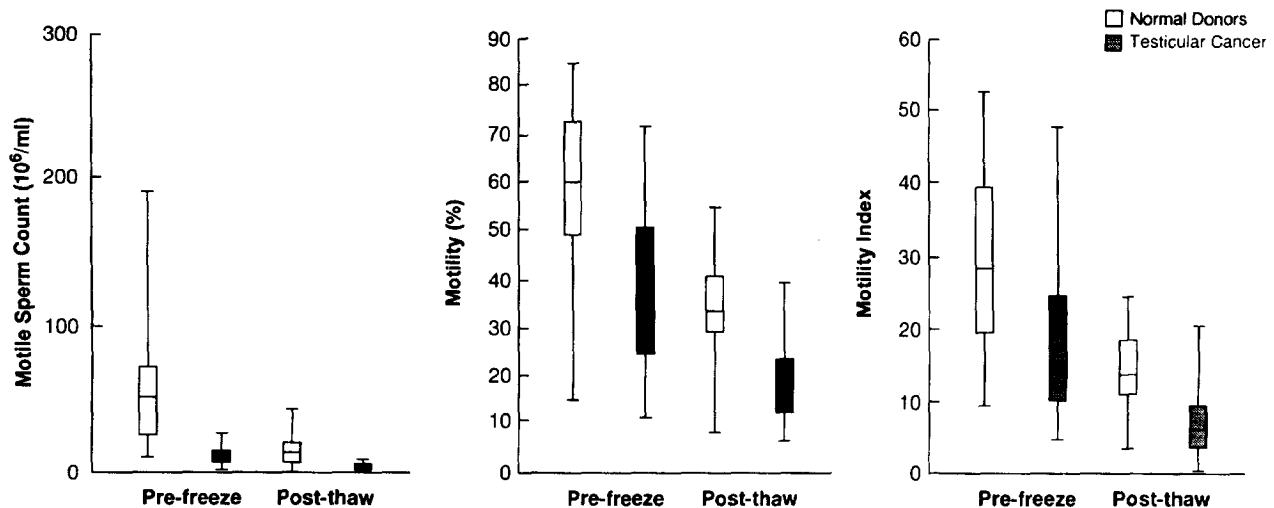


FIGURE 1. Box plots for three selected semen parameters before freezing and after thawing. Different shadings of the box plots indicate normal donors and testicular cancer groups. Testicular cancer patients had significantly lower semen values for the three parameters both before and after cryopreservation. The box covers the middle 50% of the data values, between the lower and upper quartile. The central line is the median and the whiskers extend out to 95% of the data.

our controls were a highly select group and may not truly be representative of all men in their reproductive years.

Although semen quality differed among histologic types of tumors, our findings show that this could also be attributed to the extent of the disease rather than the tumor cell type. This hypoth-

esis is also supported by the finding of decreased sperm counts in patients with extra gonadal germ cell tumors.¹⁷ Moreover, stress may adversely affect sperm production in humans.¹² It is possible that the stress of discovering that one has a potentially fatal disease requiring unpleasant treatment might be sufficient to reduce semen quality.

TABLE III. Characteristics of sperm quality in patients with Stage I testicular cancer and normal donors, before and after cryopreservation

	Stage I (n = 17)			Donors (n = 30)			P Value*
Age (yr, mean ± SD)	28.2 ± 6			31.4 ± 7.6			0.230
Volume of ejaculate (mL)	3 ± 1.6			2.9 ± 1.4			0.83
Sperm Characteristics							
	Q1	Median	Q3	Q1	Median	Q3	P Value*
Motile sperm count (× 10 ⁶ /mL)							
Prefreeze	4.8	11.5	16	24.6	50	72	0.0001
Post-thaw	1.6	2.6	3.2	7.8	11.6	20.8	0.0001
Change (%)	-80.4	-74.9	-69	-78.8	-72.5	-65.1	0.51
Motility (%)							
Prefreeze	26	44	50.0	49	60.5	73	0.0011
Post-thaw	15	18	21	29.0	32.5	40	0.0004
Change (%)	-60.8	-50	-37.9	-58.1	-44.3	-28.1	0.41
Velocity (μm/s)							
Prefreeze	40.2	47	51	43.4	51	59	0.12
Post-thaw	35	38.2	41.2	39.5	41	46	0.047
Change (%)	-20.1	-15.7	-2.63	-33.3	-19.7	-4	0.37
Motility index							
Prefreeze	10.2	17.5	24	19.1	27.7	39.1	0.0076
Post-thaw	4.5	6.5	8.4	11	13.9	18.4	0.0003
Change (%)	-69.5	-60.5	-41.3	-65.6	-51.6	-41.4	0.48
Linearity (%)							
Prefreeze	4.7	5.1	5.5	4.3	4.7	6.1	0.75
Post-thaw	4.5	5	5.4	4.1	5.5	6.1	0.68
Change (%)	-9.8	6.4	12.5	-9.9	2	20.4	0.82
Amplitude of lateral head movement (μm)							
Prefreeze	2.3	2.6	3.0	2.4	2.7	3.4	0.14
Post-thaw	1.7	2.1	2.6	2	2.3	2.5	0.22
Change (%)	-29.2	-12.5	-9.91	-34.2	-21.7	-11.5	0.49

* Comparison between Stage I patients and donors.

in those with mixed germ cell tumors ($P < 0.01$). Post-thaw linearity was also significantly better among patients with pure embryonal carcinomas than those with mixed germ cell tumors ($P < 0.01$). None of the other sperm motion parameters were significantly different among the three histologically defined groups. Patients with pure seminoma tended to have better quality than patients with pure embryonal tumors, who in turn had better quality than those with mixed germ cell tumors. Closer examination of this finding, however, reveals that 71.4% of patients with mixed germ cell tumors presented with extensive disease (Stage III), whereas all patients with pure seminomas presented at Stage I.

COMMENT

Our results support the previous findings that many patients with testicular cancer are oligospermic at the time of diagnosis.¹⁰⁻¹² The explanation for the poor semen quality in patients with testicular cancer is not immediately apparent. The impact of orchiectomy on the sperm count remains uncertain, because most patients are referred for sperm banking after unilateral orchiectomy.¹³ An

indirect effect of surgery and anesthesia could explain the decreased sperm production, assuming the removed testis showed spermatogenesis.^{14,15} Furthermore, the cancer itself might affect spermatogenesis, either because of a nonspecific effect or a disease-related mechanism. Abnormal histologic findings in the contralateral testis as well as a lower than expected sperm count after a unilateral orchiectomy in these patients suggest a germ cell defect.⁴

In previous studies, no association was found between disease stage and semen quality.¹⁶ Our study confirms this finding, although there was a trend toward a worsening of the sperm motion characteristics with increasing disease stage. In addition, the preefreeze and post-thaw MSC, percent motility, and motility index values were all significantly lower in patients with Stage I disease than in normal donors. This might indicate that dysfunctional germ cells may be responsible for both the development of cancer and defective spermatogenesis, or that precursors of malignancy present within testicular tissue may also affect fertility. One limitation of this study, however, is that

TABLE II. Characteristics of sperm quality among different testicular cancer stages

	Stage I (n = 17)			Stage II (n = 10)			Stage III (n = 7)			P Value*
Age (yr, mean ± SD)	28.2 ± 6			26.3 ± 4.6			22.1 ± 5.1			0.11
Volume of ejaculate (mL)	3 ± 1.6			3 ± 1.9			3 ± 2.1			0.87
Sperm Characteristics										
	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	P Value*
Motile sperm count (× 10 ⁶ /mL)										
Prefreeze	4.8	11.5	16	3.6	6.8	14.4	1.1	2.9	6.1	0.16
Post-thaw	1.6	2.6	3.2	0.4	1.8	4	0.4	1.2	2.1	0.17
Change (%)	-80.4	-74.9	-69	-86.8	-78	-69	-80	-65.7	-57.3	0.32
Motility (%)										
Prefreeze	26	44	50	38	42	66	11	39	54	0.57
Post-thaw	15	18	21.0	10	15.5	26	7	13	23	0.68
Change (%)	-60.8	-50	-37.9	-73.7	-64	-48.8	-60	-51.1	-31.5	0.19
Velocity (μm/s)										
Prefreeze	40.2	47	51	38	45.4	56.5	28.7	34	43	0.31
Post-thaw	35	38.2	41.2	35.9	39.3	43.2	0	28.3	38	0.12
Change (%)	-20.1	-15.7	-2.6	-28.3	-3.8	0.02	-100	-25.6	-1.4	0.43
Motility index										
Prefreeze	10.2	17.5	24	9.8	18.7	36.6	4.7	13.2	18.4	0.30
Post-thaw	4.5	6.5	8.4	3.8	4.8	9.8	0	2.2	8.7	0.12
Change (%)	-69.5	-60.5	-41.3	-81	-75.1	-49.9	-100	-67.3	-52.6	0.28
Linearity (%)										
Prefreeze	4.7	5.1	5.5	4.3	5.2	6.5	5.2	5.4	6.3	0.35
Post-thaw	4.5	5	5.4	2.9	5.4	6.3	0	5.1	5.4	0.70
Change (%)	-9.8	6.4	12.5	-35.3	4.6	23.3	-100	0	2	0.27
Amplitude of lateral head movement (μm)										
Prefreeze	2.3	2.6	3	1.6	2.6	3.3	1.6	1.9	3.1	0.42
Post-thaw	1.7	2.1	2.6	1.5	2.6	3	0	0	2.3	0.13
Change (%)	-29.2	-12.5	-0.9	-13.5	3.3	27.3	-100	-32.3	0	0.17

* Comparison among Stages I, II, and III.

motility index values than did control subjects, both before and after cryopreservation (Table III).

As a group, the cancer patients had significantly lower MSC, percent motility, and motility index values before cryopreservation than did controls (Table IV; Fig. 1). The preefreeze ALH, linearity, and VCL values were similar between the two groups. Based on the conventional normal range for each semen parameter, analysis of individual patient semen indicated that 23 (67.7%) of the cancer patients consistently had normal semen volume, but only 4 (11.8%) had both a normal MSC and motility percent values. Eleven patients (32.4%) had a normal MSC, five (14.7%) had a normal percent motility, 27 (79.4%) had normal velocity, and 29 (85.3%) had normal ALH values.

The MSC, percent motility, ALH, VCL, and motility index values declined significantly after thawing among patients with testicular cancer (Table V). Among controls, the pattern was similar except for a decrease in VCL, which was of marginal significance ($P = 0.015$). The MSC, VCL, percent motility, and motility index values ($P = 0.0001$) were all significantly lower after thawing among the cancer pa-

tients than among control subjects. The ALH and linearity values, however, were not significantly different between the two groups. Despite the significant changes in semen parameters, a comparison of the overall percentage changes after thawing shows no significant differences between patients and donors (Table IV; Fig. 2). These results indicate that the effect of cryopreservation on semen quality is the same for both testicular cancer patients and normal donors, even if the donor group had higher quality semen to begin with.

Finally, we divided the patients into groups according to type of tumor (Table VI). A single patient with pure choriocarcinoma was excluded from the analysis. The mean age and seminal volume were not significantly different among the histologic groups (Table VII). The median MSC value before freezing was higher in the patients with seminoma than in the group with mixed germ cell tumors ($P < 0.01$). Patients with pure embryonal carcinoma also had a higher median MSC value before freezing ($P < 0.01$). The MSC value was also significantly higher in patients with pure seminomas after thawing, and again the MSC was significantly better than

significantly decreases sperm quality. As a result, the usefulness of banking sperm from these patients has been questioned.⁶ Recent reports indicating the feasibility of pregnancies with cryopreserved sperm from this group of patients support the practice of sperm banking.^{7,8} In addition to the issue of poor pretreatment semen quality, whether sperm from these patients is more sensitive to cryopreservation and consequently more prone to lose quality after thawing is still unclear. The purpose of this study was twofold: first, to investigate the pretreatment semen quality and motion characteristics in a group of patients with testicular cancer, and second, to compare the effect of cryopreservation on sperm quality between a group of patients with testicular cancer and a group of normal volunteers.

MATERIAL AND METHODS

SUBJECTS

Records from 34 patients with newly diagnosed testicular cancer aged 16 to 40 years were available for review. Patients were referred to the Andrology Laboratory at The Cleveland Clinic Foundation for sperm cryobanking between 1987 and 1994 before undergoing cancer treatment. A total of 87 semen samples (1 to 3 per patient) were collected and frozen. For controls, semen from 30 healthy volunteers was analyzed. These men had requested semen analysis and cryobanking for various reasons: they were away from home a great deal but still wished to father a child; they wanted to donate sperm for laboratory research or for artificial insemination; or they wanted "insurance" before undergoing vasectomy. These men were highly selected from a large pool of candidates for inclusion into the control group on the basis of their semen quality; inclusion criteria included an ejaculate volume of at least 2.0 mL and a sperm count of at least $20 \times 10^6/\text{mL}$, of which at least 50% had to be motile and 30% had to be normal in morphology.⁹

SEMEN COLLECTION AND ASSESSMENT OF QUALITY

Semen specimens were collected by masturbation after at least 2 days of ejaculatory abstinence and liquefied at 37°C for 30 minutes. Five microliters of specimen was loaded on a 20- μL Microcell chamber (Conception Technologies, San Diego, Calif) and analyzed on a Cell-Track Semen Analyzer, CTS version 4.0 (Motion Analysis Corporation, Palo Alto, Calif). Semen analysis was performed before and after cryopreservation on each specimen. The motile sperm count (MSC), percent of sperm that were motile (percent motility), curvilinear velocity (VCL), linearity, amplitude of the lateral head movement (ALH), and motility index (motility \times velocity/100) were determined. The normal value for the MSC is more than $10 \times 10^6/\text{mL}$; VCL more than 30 μ/sec^9 ; motility more than 50%; and ALH more than 1.4 as determined by our laboratory.

CRYOPRESERVATION PROCEDURE

Test yolk buffer with glycerol (Irvine Scientific, Santa Ana, Calif) was used as a freezing agent for cryopreservation. A 5-mL vial of the medium was thawed by incubating the vial at 37°C. An aliquot of frozen medium equal to 25% of the original specimen volume was then added to the specimen. The specimen was gently mixed for 5 minutes using the Hema-Tek aliquot mixer (Miles, Elkhart, Ind). This was repeated until an equal volume of freezing medium had been added to the ejaculate. The specimen was then equally di-

TABLE I. Stage distribution among 34 patients with testicular cancer

Stage	Patients
I	17 (50%)
II	
a	2 (5.9%)
b	7 (20.6%)
c	1 (2.9%)
III	7 (20.6%)
Total	34

vided into vials for long-term cryopreservation. An additional vial was cryopreserved to assess the 24-hour survival. Cryovials were placed in the freezer at -20°C for 8 minutes and thereafter 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. One day after freezing, semen from the additional vial was removed and thawed by incubating the vial at 37°C for 20 minutes. The vial was mixed, and a 5- μL aliquot was analyzed as already described.

STATISTICAL ANALYSIS

A nonparametric Wilcoxon signed rank test was used to determine the effect of cryopreservation (percentage change) on both the normal group and the patient group. In addition, the Wilcoxon rank sum test was used to compare the semen analysis results between the two study groups. For the comparisons among three cancer stages, Kruskal-Wallis test was used. The same statistical method was also applied for the analysis of tumor histologic results. A *P* value of 0.01 or less was considered significant. All statistical analyses were performed using the SAS statistical software package (Cary, NC).

RESULTS

The mean age of the 34 testicular cancer patients (26.4 ± 5.7 years) was not statistically different (*P* = 0.013) from that of the controls (31.4 ± 7.6 years). There were also no statistically significant differences in ejaculate volume or abstinence time between the two groups.

Seventeen patients (50%) presented with Stage I disease, 10 (29.4%) with Stage II, and 7 (20.6%) with Stage III (Table I). The mean age did not differ significantly among the patients with different cancer stages, nor did the ejaculate volume (Table II). Similarly, there were no significant differences in any of the various sperm motion parameters among the three cancer stage groups, either before or after cryopreservation (Table II). However, there was a trend toward progressive worsening of the semen quality before freezing with increasing cancer stage. This was particularly true with respect to MSC, VCL, and motility. After thawing, a similar trend could be seen again with percent motility, MSC, and motility index. Stage III patients also had the worst ALH and motility index values before freezing and VCL and ALH values after thawing. Patients with Stage I disease had significantly lower MSC, percent motility, and

EFFECT OF CRYOPRESERVATION ON SEMEN QUALITY IN PATIENTS WITH TESTICULAR CANCER

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ABSTRACT—Objectives. Current techniques in cryopreservation of human semen substantially decrease sperm quality. In addition, the pregnancy rate using cryopreserved sperm obtained from testicular cancer patients is lower than when sperm from normal fertile men is used. However, it is still unclear whether cryopreserved sperm from these patients is inherently defective or if the sperm loses its motility after thawing. This study was undertaken to assess the effect of cryopreservation on the quality and motion characteristics of semen from patients with testicular cancer before definitive therapy compared with semen from normal volunteers.

Methods. We compared the sperm quality before and after cryopreservation in samples from 34 patients with testicular cancer and 30 normal volunteers who were referred for sperm banking over a 7-year period. The effects of cancer stage and histologic type on various semen parameters were also examined. A computer-assisted semen analysis was performed before and after cryopreservation on each specimen. The nitrogen vapor technique using Test yolk buffer with glycerol as a cryoprotective agent was used for cryopreservation. The motile sperm count and motion characteristics (motility, velocity, linearity, amplitude of the lateral head movement, motility index) were analyzed before and after cryopreservation and compared between the groups.

Results. Semen quality did not significantly differ among patients with Stage I, II, or III cancer. However, semen quality tended to be poorer at higher cancer stages. In general, semen quality was better among patients with pure seminomas than with pure embryonal tumors; quality was worst among patients with mixed germ cell tumors. However, 71.4% of patients with mixed tumors presented with Stage III disease, whereas all patients with seminomas presented with Stage I disease. Significant differences were also seen in prefreeze motility (median, 42% [interquartile range, 24 to 51] versus 60.5% [range, 49 to 73]; $P = 0.0004$) and motile sperm count ($6.7 \times 10^6/\text{mL}$ [range, 3.4 to 14.4] versus 50.0 [range, 24.6 to 72.0]; $P = 0.0001$) in patients compared with controls, respectively. The motile sperm count and percent motility significantly decreased in both patients and controls after cryopreservation ($P = 0.0001$). However, the percentage decline in motile sperm count and motion characteristics after cryopreservation did not differ significantly between patients and controls ($P > 0.01$).

Conclusions. We conclude that the effect of cryopreservation on sperm quality in patients with testicular cancer is identical to its effect on sperm from normal fertile men. Differences in values after preservation are explained by poor semen characteristics before freezing; semen quality declines with more extensive disease. Stage I patients also had poorer quality than control subjects. Thus, we recommend that routine sperm banking be encouraged among all patients with testicular cancer before the initiation of specific medical treatment. We also recommend that future efforts be focused on improving the technique of sperm banking. *UROLOGY*[®] **46**: 382–389, 1995.

Recent advances in treating patients with testicular cancer have greatly improved their long-term survival. However, infertility is a common long-term side effect of testicular cancer, surgery, chemotherapy, or radiotherapy among these young patients. Since the first report of a successful

pregnancy resulting from frozen spermatozoa,¹ there have been many improvements in sperm cryobanking techniques. The use of complex cryopreservatives has resulted in better post-thaw semen quality.² Moreover, programmed freezing techniques and ice-crystal induction (seeding) also improve the recovery rate of spermatozoa.³ With the advent of newer methods in assisted reproduction, research in improving cryopreservation is becoming of utmost importance, especially in malignant diseases affecting young men, such as testicular cancer and lymphoma.

Many patients with testicular cancer have low sperm density at the time of diagnosis.^{4,5} In addition, cryopreservation of human semen per se also

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