

The effect of cancer on semen quality after cryopreservation of sperm

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Key words. Cancer - semen quality - cryopreservation - motility - velocity.

Summary. The results of cryopreservation of sperm from cancer patients were retrospectively reviewed in an effort to better understand the relationship between semen quality and the presence of different kinds of cancer. The semen analysis results for 146 patients referred to our infertility center for sperm banking over a 2-year period were examined. These patients were divided into three groups according to their diagnosis: group I, prevasectomy controls; group II, patients with lymphoma and Hodgkin's disease; and group III, patients with testicular cancer, e.g., seminoma, embryonal cell carcinoma, or teratocarcinoma. The seminal parameters assessed included sperm count and prefreeze and postthaw motility and velocity. For these parameters, significant decrease from control values ($P < 0.05$ to $P < 0.01$) was seen in groups II and III. The specimens from group I patients retained good motility and velocity after thawing. Our results indicate that semen quality is adversely affected by the presence of cancer in the body.

Introduction

The most common neoplasms in cancer patients between 20 and 35 years of age who desire cryopreservation of their semen to maintain fertility are testicular cancer, Hodgkin's disease, lymphoma, and leukemia. Testicular cancer was the third leading cause of death from cancer among men between the ages of 15 and 34 years in 1977, but by 1981 this disease was no longer among the top five causes of cancer death in this age group. Because of the increasingly successful treatment of testicular cancer as well as Hodgkin's disease, lymphomas, and leu-

kemia, there is now an increased focus on patients fertility. Studies over the past decade have revealed that as many as 50% of cancer patients show significant oligoasthenospermia (sperm count $< 20 \times 10^6/\text{ml}$; motility $< 40\%$) prior to treatment with potentially sterilizing drugs or irradiation. Perhaps this is one reason why these patients have shown a lack of success with attempted cryopreservation and an increased sensitivity to chemotherapy and irradiation (Thachil *et al.*, 1981; Berthelsen, 1987). However, a study by Scammell *et al.* (1985) showed a 45% cumulative probability of impregnation by men with cancer 6 months after cryopreservation compared with a 71% cumulative probability of impregnation by healthy men after 6 months (Berthelsen & Skakkeback, 1983); these results lent encouragement to efforts to cryopreserve the sperm of cancer patients prior to sterility-related chemotherapy or irradiation regimen. In this study we examined cryopreservation of sperm from cancer patients in an effort to better understand the effect of cancer on semen quality.

Materials and methods

Patients ($n = 146$) coming to our Infertility Center for evaluation were divided into three groups according to diagnosis. The first group of 20 patients included those seeking cryopreservation as prevasectomy insurance (group I, controls). The other 126 participants were cancer patients and were divided in two groups by diagnosis: lymphoma and Hodgkin's disease (group II, $n = 66$) and testicular cancer (group III, $n = 60$).

Semen analysis. Semen specimens were collected by masturbation after 48 hours of sexual abstinence and were kept for liquefaction at 37 °C for about 30 minutes. Sperm concentration and percentage motility were measured manually with a Makler counting chamber, velocity was measured with a laser Doppler velocimeter (Spermokinimetres, SKM 2000, Soro, Paris, France) [Serres *et al.*, 1980].

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Cryopreservation procedure. The specimen was considered unfreezable and rejected if the sperm count was below $1 \times 10^6/\text{ml}$ or the motility rate was $< 10\%$ (Czyglik & David, 1980). Semen specimens that met the minimal criteria of freezability were processed for cryopreservation as described by Barkay & Zuckerman (1980). Glycerol was added to a 0.75 ml vial of semen at a rate of one drop every 5 minutes until a 7% concentration of glycerol in semen was achieved. All vials were placed horizontally in a freezer for 5 minutes at -20°C and then transferred to the top of a storage tank containing liquid nitrogen vapors at -80°C . The specimens were stored in vapors for 6 to 24 hours and then transferred to liquid nitrogen at -196°C for long-term storage.

One day after freezing, spermatozoa from a single vial were removed, thawed at room temperature for 20 to 30 minutes, and examined for postthaw motility and velocity. The total number of motile sperm showing good velocity ($> 30 \mu/\text{sec}$) in each vial was termed as 'N'-value and was calculated as follows: count \times ejaculate volume in each vial \times dilution factor (1.08) \times postthaw motility.

Statistical analysis. Results were analyzed statistically by paired Student's *t*-test. For statistical evaluations, the Statview II program (Abacus Concepts, Inc., Berkeley, CA, USA) was run on a Macintosh SE-30 personal computer.

Results

Before freezing sperm counts were significantly lower in semen from group III patients than in that from controls ($P < 0.05$; Fig. 1). However, no difference was observed in the sperm concentration values for group I and group II patients (mean \pm SD, 73.2 ± 6.9 and mean \pm SD, 67.9 ± 7.0 , respectively; Table 1). Comparison of prefreeze sperm motility for controls (69%) and that for cancer patients 53% and 47% for groups II and III, respectively, showed a significant difference ($P < 0.01$; Fig. 2). Similarly, the percentage of spermatozoa showing good velocity before freezing was 60% for group I, 50% for group II, and 44% for group III ($P < 0.05$; Fig. 3).

After freezing and thawing of semen, the motility and velocity of sperm were significantly reduced from prefreeze values in all three groups. However, this decrease was more pronounced in cancer patients than in the control group. Postthaw motility was 37% in group I, 25% in group II and 26% in group III ($P < 0.01$ for controls vs. each group of cancer patients); 39%, 31%, and 30% spermatozoa in these three groups ($P < 0.05$ for controls vs. each group of cancer patients).

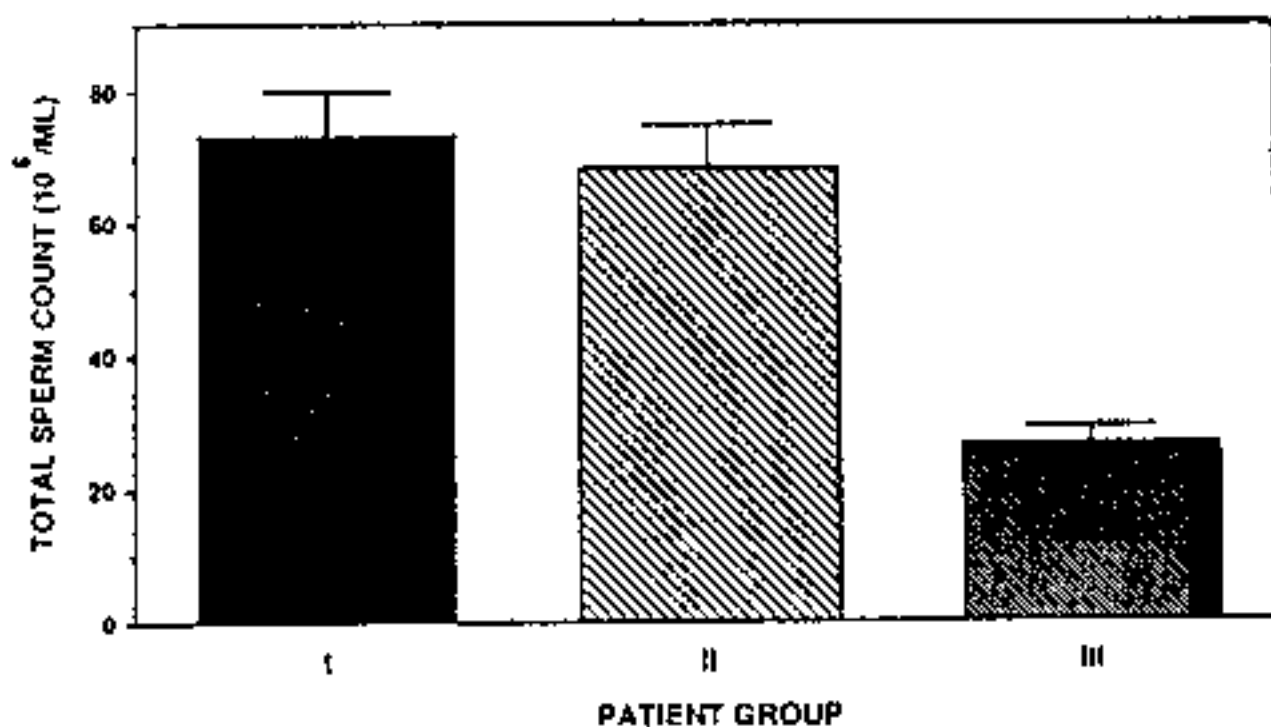


Figure 1. Comparison of total sperm count in controls (prevasectomy patients) and cancer patients (groups II and III).

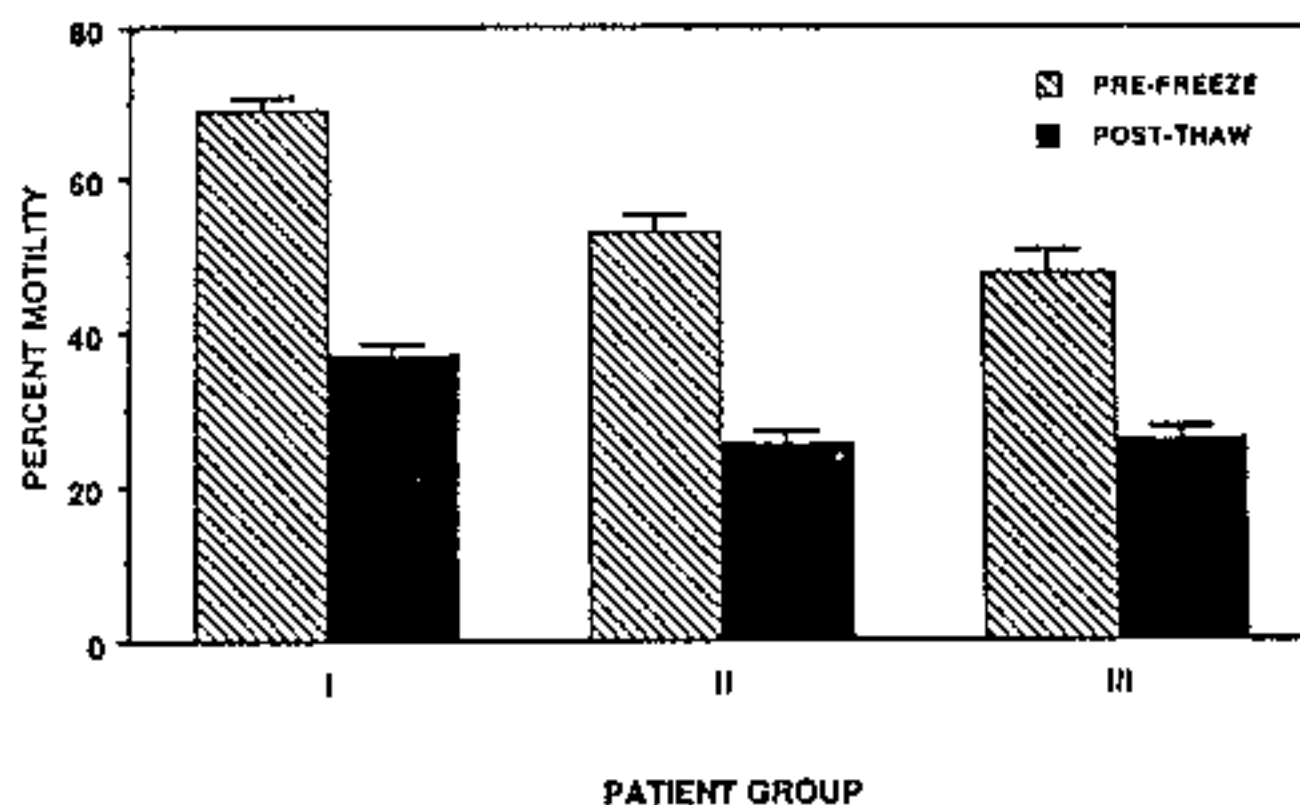


Figure 2. Comparison of prefreeze and postthaw sperm motility in controls (prevasectomy patients) and cancer patients (groups II and III).

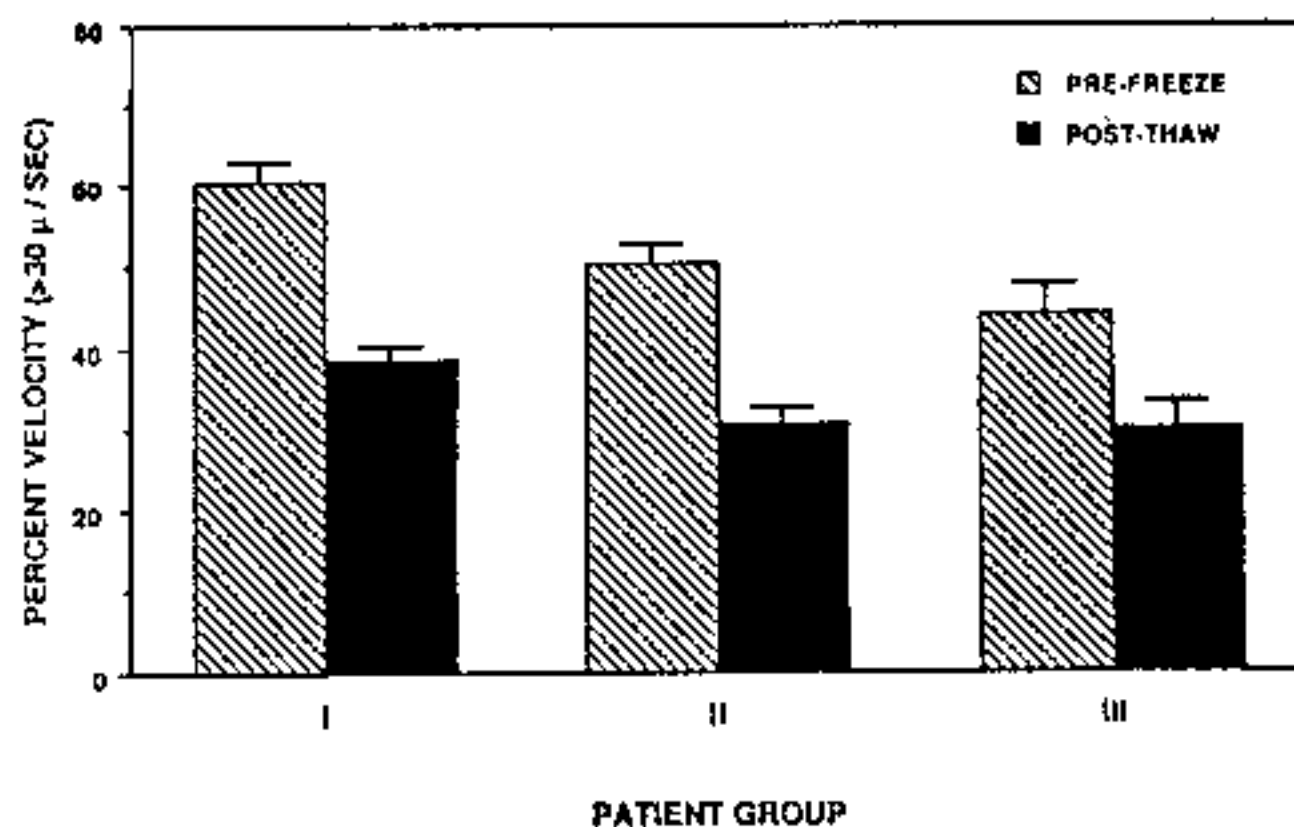


Figure 3. Percentage of controls (prevasectomy patients) and cancer patients (groups II and III) sperm with prefreeze and postthaw sperm velocity $> 30 \mu/\text{sec}$.

Comparison of prefreeze and postthaw motility revealed that even in the prevasectomy patients there was a significant reduction in the percentage of sperm motile after thawing. The same was true for patients with lymphoma and testicular cancer. However, prefreeze motility in the cancer patients was also significantly lower than in prevasectomy patients; thus we believe that, on average, the percentage of sperm found to be motile was below the

minimum of 30% considered necessary for impregnation.

The comparison of prefreeze and postthaw velocity also showed a reduction in the percentage of sperm with a velocity of greater than 30 μ /sec in all three groups. The prefreeze velocity was lower in the cancer groups than in the control group. However, the decrease in the postthaw percentage of sperm with a velocity of greater than 30 μ /sec was less pronounced in the cancer groups than in the prevasectomy group. This observation suggests that in the cancer groups those sperm that did survive the freezing process were of good quality.

The *N*-values for the three groups differed significantly from one another. The mean *N*-value was 25.2 for group I, 17.5 for group II ($P < 0.05$), and

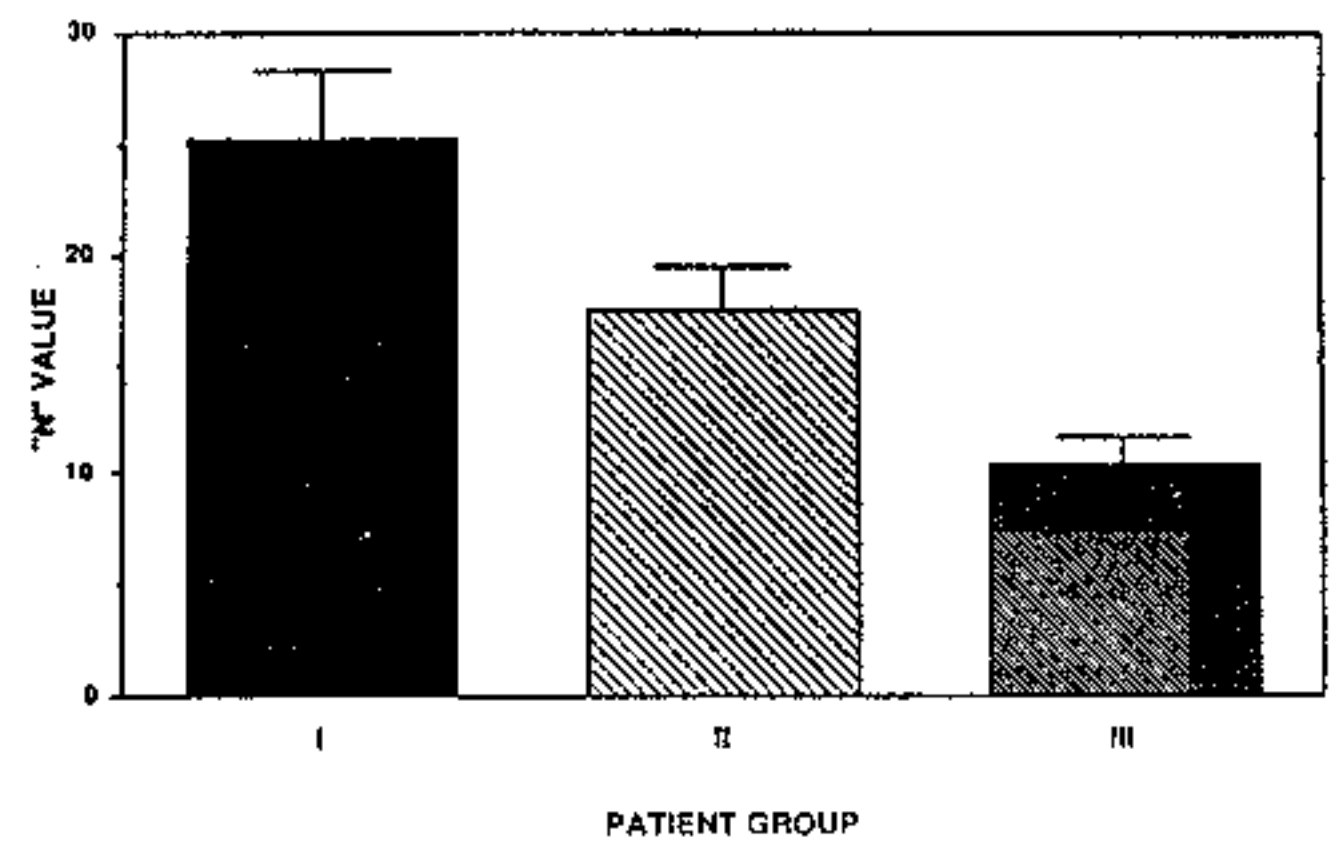


Figure 4. Comparison of *N*-values (postthaw motile sperm in each vial) in controls (prevasectomy patients) and cancer patients (group II and III).

Table 1. Semen characteristics of patients seeking cryopreservation of sperm. Data from control and cancer patients both before and after freezing is expressed as mean \pm SE. Comparison of results between control (group I) and cancer patients (groups II and III) if found statistically significant are reported as $P < 0.05$ or 0.01

Group	Volume (ml)	Sperm count (10^6 /ml)	Prefreeze		Postthaw		<i>N</i> ^b
			Motility %	Velocity ^a	Motility %	Velocity ^a	
I Prevasectomy (<i>n</i> = 20)	3.0 \pm 0.2	73.2 \pm 6.9	68.8 \pm 1.8	60.5 \pm 2.4	37.1 \pm 1.3	38.7 \pm 1.6	25.2 \pm 3.0
II Lymphoma & Hodgkin's (<i>n</i> = 66)	2.5 \pm 0.2	67.9 \pm 7.0	52.9 \pm 2.4**	50.2 \pm 2.6*	25.3 \pm 1.5**	30.6 \pm 2.1*	17.5 \pm 2.0*
III Testicular cancer (<i>n</i> = 60)	3.0 \pm 0.2	26.7 \pm 2.7*	47.5 \pm 3.1**	43.9 \pm 3.8**	25.6 \pm 1.8**	30.1 \pm 3.0*	10.4 \pm 1.3*

^a Percentage of spermatozoa showing velocity > 30 μ /sec.
^b Count \times ejaculate volume in each vial \times dilution factor (1.08) \times postthaw motility.
* $P < 0.05$, ** $P < 0.01$; comparison of results between control group I patients and the cancer patients (groups II and III).
n = Number of patients in each group.

Table 2. Relationship of semen quality to the stage of cancer. Patients with seminomatous and nonseminomatous tumors are divided into two groups based on sperm counts, which are expressed as mean values with a range

Diagnosis and stage	Number of patients ^a	Count ($> 20 \times 10^6$ /ml) ^b			Count ($< 20 \times 10^6$ /ml) ^c		
		Mean	(range)	<i>n</i> ^d	Mean	(range)	<i>n</i> ^d
Seminoma							
I	5	40	(31-56)	3	9	(2-17)	2
II & III	3	54		1	0		2
Nonseminoma							
I	19	35	(21-65)	8	8	(0-15)	11
II & III	10	31	(25-40)	5	9	(6-14)	5

^a Results of 37 patients are used in this table.
^b 17 patients have a sperm count $> 20 \times 10^6$ /ml.
^c 20 patients have a sperm count $< 20 \times 10^6$ /ml.
^d Number of patients in each category.

10.4 for group III ($P < 0.01$; Fig. 4). Patients with testicular cancer had the greatest decrease in the number of good quality sperm recovered after freezing.

Of the 37 cancer patients staged according to the method described by Garnick (1985), 8 had seminoma and 3 had stage II or stage III tumors. No significant difference in sperm concentration was seen, however, between patients with stage I lesions and those with stage II lesions (Table 2). Two patients with stage II lesions showed azoospermia. Among the 29 patients with nonseminomatous tumors, there was no significant difference in sperm concentration (less than or greater than 20×10^6 /ml) between those with stage I tumors and those with stage II or stage III tumors. In fact, essentially the same number of patients with stage I nonseminomatous tumors had sperm counts below 20×10^6 /ml as had counts above normal. The same was true for patients with stage II and stage III tumors. The range of counts above 20×10^6 /ml in all stages of tumors and tumor types was not significantly different.

Discussion

It is evident from this study that there is a significant reduction in the quality of semen from patients suffering from testicular cancer and lymphoma as compared with that from normal fertile patients. However, some cancer patients have good quality sperm both before and after the freezing of semen. Scammell *et al.* (1985) reported that of 22 men requesting artificial insemination of their partners with their banked semen, 11 had Hodgkin's disease and 3 had testicular tumor; of the 8 pregnancies that resulted 5 involved partners of patients with Hodgkin's disease and 3 involved partners of patients with testicular tumor. However, impregnation was achieved only when the sperm count was equal to or above 20×10^6 /ml before freezing and the rate of sperm motility was at least 30% before freezing.

Since little can be done to improve the fertility potential of cancer patients showing severe irreversible changes such as spermatogenic arrest, Sertoli-cell-only syndrome, hyalinized tubules, or carcinoma *in situ*, more emphasis should be given to the improved recovery in other cancer patients of motile sperm with good velocity. Recent studies have shown that the use of complex cryopreservatives

yields better thawed specimens (Weidel & Prins, 1987). The use of a programmed freezing technique, with semen maintained at -5°C for 10 minutes and the use of ice crystal induction (seeding) may also enhance the recovery of viable spermatozoa (Crister *et al.*, 1987).

In summary, although many cancer patients desiring cryopreservation of sperm for fertility purposes prior to chemotherapy or irradiation have severe oligoasthenospermia before treatment, some of these patients have pretreatment semen specimens of adequate quality for successful cryopreservation. Sanger *et al.* (1980) reported that 23% of men with testicular cancer or lymphoma had posttherapy specimens that were considered fertile; the corresponding figure was 60% for age-matched healthy men. Efforts should therefore be made to improve sperm banking capabilities by the use of better cryopreservatives and freezing techniques.

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