

- frozen-thawed sperm: a method for preserving the progenerative potential of Hodgkin patients. *Fertil Steril* 1991;55:443-5.
17. Vigersky RA, Chapman RM, Berenberg J, Glass AR. Testicular dysfunction in untreated Hodgkin's disease. *Am J Med* 1982;73:482-6.
 18. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of a single spermatozoon into an oocyte. *Lancet* 1992;340:17-8.
 19. Palermo GD, Cohen J, Alikami M, Adler A, Rosenwaks Z. Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. *Fertil Steril* 1995;63:1231-40.
 20. Nagy Z, Liu J, Cecile J, Silber S, Devroey P, Van Steirteghem A. Using ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa gives rise to comparable results after intracytoplasmic sperm injection. *Fertil Steril* 1995;63:808-15.
 21. Sanger WG, Olson JH, Sherman JK. Semen cryobanking for men with cancer—criteria change. *Fertil Steril* 1992;58:1024-7.
 22. Richie JP. Neoplasms of the testis. In: Walsh PC, Retik AB, Stamey TA, Vaughan ED, editors. *Campbell's urology*. 6th ed. Philadelphia: Saunders, 1992:1222-63.
 23. Ng SC, Bongso A, Ratnam SS, Sathananthan H, Chan CL, Wong PC, et al. Pregnancy after transfer of sperm under zona [letter]. *Lancet* 1988;2:790.
 24. Van Steirteghem AC, Liu J, Joris H, Nagy Z, Janssenwillen C, Tournay H, et al. Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum Reprod* 1993;8:1055-60.
 25. Bonduelle M, Desmyttere S, Buysse A, Van Assche E, Schietecatte J, Devroey P, et al. Prospective follow-up study of 55 children born after subzonal insemination and intracytoplasmic sperm injection. *Hum Reprod* 1994;9:1765-9.

respective of the type of cancer. Within the testicular cancer group, tumor type appeared to affect semen quality, a finding that is in agreement with our previous report (10).

There was no significant difference in prefreeze motility among patients that died of the disease as compared with those who survived. Patients in the leukemia or advanced soft tissue cancer represented one third of the patients that died of disease and this may explain the trend to higher prefreeze motility.

A significant observation of this study is the post-thaw predictability of the motile sperm. This has an important bearing on the type of assisted reproductive strategy to be recommended. A prefreeze sperm motility of $>15\%$ could assure a post-thaw motility of $\geq 10\%$. We feel that suboptimal semen analysis should not be used as a criteria to preclude sperm banking (23) as sperm can fertilize in vitro after thawing even in cases of grossly impaired semen parameters. A high cumulative pregnancy rate with ICSI can be achieved in severe male factor cases (22, 24) and in cancer patients (Batzofin J, Tran C, Tan T, Nelson J, Serafini P, abstract). Post-thaw semen quality will dictate the type of assisted reproductive technique to be used in establishing a pregnancy.

Physicians should discuss sperm cryopreservation with all young men who are about to begin cancer treatments. The possibility of genetic malformations and infertility in the progeny due to the use of cryopreserved semen specimens for artificial reproduction remains a theoretical concept. Recent studies have not found cytogenetic abnormalities or increased incidence of congenital malformations in children conceived by assisted reproductive technique procedures (25). Cryopreservation counseling also should include ethical issues of what is to be done with the semen specimens, should the patient succumb to his illness.

In conclusion, we found that type of cancer is not related to the prefreeze or post-thaw quality of semen specimen, and there is no relationship between the semen quality and the treatment outcome. Significantly higher motile sperm counts seen in patients with leukemia and advanced soft tissue cancer is interesting. It appears that advanced disease stage does not affect adversely the semen quality in these men with poor prognosis. As cryopreserved semen specimens from cancer patients are better than fresh or frozen epididymal aspirates or sperm obtained from testicular biopsy in infertile men, it is important that they be frozen when there is a minimum prefreeze motility of $\geq 15\%$. These samples can provide a post-thaw specimen with adequate motility for several attempts with IVF and ICSI. Given these findings and the latest advances in the treatment of male factor infertility, cryopres-

ervation of semen should be offered to cancer patients regardless of the type or the extent of disease.

Acknowledgment. The authors thank Jar-Chi Lee, M.S., for her help in the statistical analysis of the results, and Karen Seifarth, M.T. (A.S.C.P.), for technical assistance.

REFERENCES

1. Drasga RE, Einhorn LH, Williams SD, Patel DN, Stevens EE. Fertility after chemotherapy for testicular cancer. *J Clin Oncol* 1983;1:179-83.
2. Glick JH. Hodgkin's disease. In: Wyngaarden JB, Smith LH, Bennett JC, editors. Cecil textbook of Medicine. 19th ed. Philadelphia: Saunders, 1992:955-63.
3. Meirou D, Schenker JG. Cancer and male infertility. *Hum Reprod* 1995;10:2017-22.
4. Buchanan JD, Fairley KF, Barrie JU. Return of spermatogenesis after stopping cyclophosphamide therapy. *Lancet* 1975;2:156-7.
5. Nijman JM, Schraffordt Koops H, Kremer J, Sleijfer DT. Gonadal function after surgery and chemotherapy in men with stage II and III non-seminomatous testicular tumors. *J Clin Oncol* 1987;5:651-6.
6. Kreuser ED, Harsch U, Hetzel WD, Schreml W. Chronic gonadal toxicity in patients with testicular cancer after chemotherapy. *Eur J Cancer Clin Oncol* 1986;232:289-94.
7. Rothmann SA, Schroeder-Jenkins M, Henrich L, Thomas AJ Jr. Rationale for sperm banking in men with cancer. *Cleve Clin Q* 1986;53:89-94.
8. Fossa SD, Ous DS, Aybyholm T, Norman N, Loeb M, Jetne V. Post-treatment fertility in patients with testicular cancer. II. Influence of cisplatin-based combination chemotherapy and of retroperitoneal surgery on hormone and sperm cell production. *Br J Urol* 1985;57:210-5.
9. Hendry WF, Stedronska J, Jones CR, Blackmore CA, Baret A, Peckham MJ. Semen analysis in testicular cancer and Hodgkin's disease: pre-freeze and post-treatment findings and implications for cryopreservation. *Br J Urol* 1983;55:769-73.
10. Berthelsen JG. Sperm counts and serum follicle-stimulating hormone levels before and after radiotherapy and chemotherapy in men with testicular germ cell cancer. *Fertil Steril* 1984;41:281-6.
11. Richter MA, Haning RV Jr, Shapiro SS. Artificial donor insemination: fresh versus frozen semen; the patient as her own control. *Fertil Steril* 1984;41:277-80.
12. Agarwal A, Tolentino MV, Sidhu RS, Ayzman A, Lee J-C, Thomas AJ Jr, et al. Effect of cryopreservation on semen quality in patients with testicular cancer. *Urology* 1995;46:382-9.
13. Shekarriz M, Tolentino MV, Ayzman I, Lee JC, Thomas AJ Jr, Agarwal A. Cryopreservation and semen quality in patients with Hodgkin's disease. *Cancer* 1995;75:2732-6.
14. Agarwal A, Sidhu RK, Lee J-C, Shekarriz M, Thomas AJ Jr. Value of clinical diagnosis in predicting cryopreservation outcome in patients with malignant diseases. *J Urol* 1996;155:934-9.
15. Khalifa E, Oehninger S, Acosta AA, Morshedi M, Veeck L, Bryzski RG, et al. Successful fertilization and pregnancy outcome using cryopreserved/thawed spermatozoa from patients with malignant diseases. *Hum Reprod* 1992;7:105-8.
16. Tournaye H, Camus M, Bollen N, Wisanto A, Van Steirteghem AC, Devroey P. In vitro fertilization techniques with

Table 3 Predicting the Post-Thaw Sperm Motility ($\leq 10\%$ to $>10\%$) From Prefreeze Sperm Motility Cutoff Values (Range from ≤ 10 to >40) (n = 105)*

Prefreeze sperm motility	Post-thaw sperm motility ($\leq 10\%$)	Post-thaw sperm motility ($>10\%$)	Total predictive accuracy†
%			%
≤ 10	2 (1.9)	1 (0.95)	73.3
>10	27 (25.7)	75 (71.4)	
≤ 15	6 (5.7)	1 (0.95)	77.1
>15	23 (21.9)	75 (71.4)	
≤ 20	9 (8.6)	5 (4.8)	76.2
>20	20 (19)	71 (67.6)	
≤ 25	16 (15.2)	11 (10.5)	77.1
>25	13 (12.4)	65 (61.9)	
≤ 30	19 (18.1)	20 (19.1)	71.4
>30	10 (9.5)	56 (53.3)	
≤ 35	20 (19.1)	22 (21)	70.5
>35	9 (8.6)	54 (51.4)	
≤ 40	23 (21.9)	27 (25.7)	68.6
>40	6 (5.7)	49 (46.7)	

* Values in parentheses are percentages.

† Percent accuracy of predicting post-thaw sperm motility $\leq 10\%$ or $>10\%$ for a given preefreeze cutoff motility.

Predictive Power of Prefreeze Sperm Motility

Prefreeze motility results were analyzed to find a cutoff value that could be used to predict the post-thaw sperm motility. In the analysis, a number of preefreeze sperm motility cutoff points ranging from $<10\%$ to $>40\%$ were used to see how accurately preefreeze sperm motility could predict a post-thaw sperm motility less than or greater than 10% . For any given preefreeze cutoff value, the accuracy of predicting the post-thaw motility was calculated (Table 3). A post-thaw motility of $>10\%$ was seen in 75 specimens (71.4%) when the preefreeze motility was $>15\%$. This post-thaw motility could be predicted from the preefreeze motility values with an accuracy of 77.1%. (Table 3). Prefreeze motility with cutoff values between 15% and 25% were able to predict a post-thaw motility $>10\%$ with high accuracy (77%).

DISCUSSION

Abnormal spermatogenesis is seen in 40% to 70% of patients with Hodgkin's disease (3, 11, 15, 16) or testicular cancer (3, 10, 15–17) before treatment with radiation, chemotherapy, or surgery. The causes of poor semen quality in cancer patients are not well understood. Many mechanisms contribute to the impairment of semen; these include the direct effect of the tumor in testicular cancer to constitutional symptoms such as fever (3). As curative chemotherapy results in permanent azoospermia in up to 50% of the patients (1–4), use of cryopreserved semen to father children is the only viable option in young men with cancer. Recent advances in the

treatment of male factor infertility during the last 3 years have made pregnancy potentially possible in most patients except in azoospermic men (18, 19). Similar fertilization and pregnancy rates have been reported with IVF-ET and intracytoplasmic sperm injection (ICSI) when sperm are obtained from either the ejaculate, epididymis, or directly from the testis (20).

Poor semen quality before freezing has been associated with poor post-thaw outcome (15, 17, 21). Lack of a relationship between disease type and post-thaw sperm quality in our study does not support the view that the outcome of successful artificial insemination is poor with cryopreserved spermatozoa from young men with malignant disease (21). Although the results of the current study were not compared with a normal control group, similar results were seen when normal volunteers were used as controls as reported previously by us (10, 12). Recent reports of 115 live births with cryopreserved semen from men with Hodgkin's disease, testicular, and other types of cancer (22) encourages the use of these spermatozoa for assisted reproduction. In our study, approximately 40% of cancer patients who banked their semen were oligospermic. This is similar to earlier reports (1, 3, 7, 10, 13, 16, 17). High cumulative pregnancy rates can be achieved by assisted reproductive techniques with cryopreserved specimens, and fertilization rates of up to 80% and pregnancy rates of 100% were reported in Hodgkin's patients (14).

Absence of the differences in semen quality (preefreeze and post-thaw) between the patients with testicular cancer and those with Hodgkin's disease in our study suggest that spermatozoa from patients with these two types of cancer respond similarly to the freezing process. These results support the earlier observations in which normal controls also were used as reference and the response of semen specimen to freezing was similar in cancer patients (12–14).

The finding of a significantly higher motile sperm counts seen in patients with the leukemia may be due to the lack of a direct effect of the disease on the testis. In Hodgkin's and testicular cancer groups, spermatogenesis is impaired due to the direct effect of the disease on the testicles (3).

Patients with nonseminomatous germ cell tumors and worse prognosis present at a more advanced stage than patients with seminoma (23). In our study, these patients had lower preefreeze and post-thaw motile sperm count than the seminoma group. However, the percentage change in semen quality was not different between these two groups, suggesting that semen from men with testicular cancer responds to cryopreservation in a similar fashion ir-

Table 1 Comparison of Sperm Motility and Total Sperm Count in 106 Patients According to Cancer Type*

Sperm characteristics	Hodgkin's disease	Testicular cancer	Leukemia and advanced soft tissue cancer	P†
Total sperm count ($\times 10^6$)	24.9 (14.0, 60.9)	29.2 (15.1, 62.0)	65.7 (35.8, 106.1)	0.13
Motility (%)				
Prefreeze	33.0 (25.0, 50.0)	44.0 (26.0, 51.0)	48.5 (27.0, 57.0)	0.23
Post-freeze	15.0 (9.0, 23.0)	17.0 (12.0, 23.0)	18.0 (12.0, 22.0)	0.57
Percent change	-55.2 (-68.5, -44.4)	-53.9 (-64.9, -40.0)	-52.5 (-74.5, -21.7)	0.83
Motile sperm count ($\times 10^6$)				
Prefreeze	7.6 (3.9, 31.1)	7.3 (4.3, 7.1)	31.8 (13.7, 44.8)	0.03†
Post-thaw	1.7 (0.9, 4.4)	2.1 (1.2, 3.4)	5.1 (1.3, 14.2)	0.16
Percent change	-75.0 (-80.4, -68.3)	-74.5 (-81.5, -65.8)	-68.5 (-87.2, -60.9)	0.83

* Values are medians with interquartile ranges in parentheses.
† $P < 0.05$ was considered significant by Kruskal-Wallis test.

nificantly between the three groups (Table 1). Prefreeze and post-thaw motion variables such as VCL, LIN, and ALH did not differ significantly between the three groups; the percentage change (from prefreeze to post-thaw) between the three groups also was not significant (Fig. 1). Semen quality was affected inversely in patients with leukemia and advanced soft tissue. Compared with the other two cancer groups, patients in leukemia and advanced soft tissue cancer group had higher prefreeze motile sperm count ($P = 0.03$, Table 1).

When comparisons were made within the testicular cancer group, patients with seminoma had higher prefreeze motile sperm count (median 17.0×10^6 sperm, interquartile ranges 7.5, 26.5) than patients with nonseminomatous germ cell (median

5.7×10^6 sperm, interquartile ranges 3.4, 16.0) ($P = 0.04$). Similar results were seen in the post-thaw motile sperm count in patients with seminoma (median 2.9×10^6 sperm, interquartile ranges 2.6, 4.5) and in nonseminomatous patients (median 1.6×10^6 sperm, interquartile ranges 0.9, 3.3) ($P = 0.06$). Percentage change in motile sperm count was also similar in both patients with seminoma and nonseminomatous germ cell tumor. Prefreeze and post-thaw motion parameters (VCL, LIN, and ALH) did not differ significantly between the testicular cancer groups.

Patient Status After Treatment

Sperm characteristics were poor predictors of treatment outcome. Patient status after treatment (alive or dead) was not related to prefreeze or post-thaw semen parameters. However, patients who died after treatment were older than those who survived ($P = 0.05$). Cancer patients that died after the treatment ($n = 24$) had the following disease type (Hodgkin's, $n = 12$; testicular cancer, $n = 4$; leukemia or advanced soft tissue cancer, $n = 8$) (Table 2).

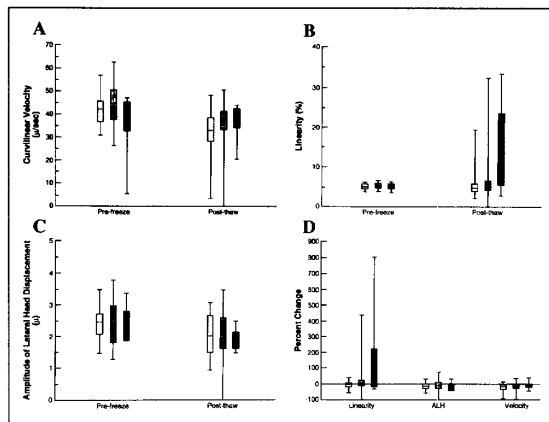


Figure 1 Prefreeze and post-thaw curvilinear velocity (A), linearity (B), amplitude of lateral head displacement (C), and percent change in sperm motion characteristics (post-thaw minus prefreeze) (D), for Hodgkin's disease, testicular cancer, and patients with leukemia or advanced soft tissue cancer were not significantly different. 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 of 95% of the data.

Table 2 Comparison of Semen Quality With the Patient Status (Alive Versus Dead) After Completion of Chemotherapy

Characteristics	Alive (n = 82)	Dead (n = 24)	P†
Age (y)	25.0 (21, 29)	28.0 (26.0, 30.5)	0.05
Total sperm count ($\times 10^6$)	29.0 (14.5, 85.1)	30.4 (25.0, 66.8)	0.85
Motility (%)			
Prefreeze	39.0 (24.0, 50.0)	49.0 (32.0, 54.5)	0.07
Post-thaw	16.0 (10.0, 2.0)	17.5 (11.5, 25)	0.35
Percent change	-52.6 (-66.7, -40.3)	-68.2 (-58.1, -4.4)	0.43
Motile sperm count ($\times 10^6$)			
Prefreeze	7.6 (4.0, 25.6)	13.8 (5.6, 31.9)	0.17
Post-thaw	2.0 (0.9, 4.0)	2.1 (1.2, 8.7)	0.39
Percent change	-74.7 (-81.3, -68.3)	-74.5 (-81.8, -3.3)	0.89

* Values are medians with interquartile ranges in parentheses.
† $P < 0.05$ was considered significant by the Wilcoxon Rank sum test.

quality, cryopreserved specimens from cancer patients are capable of producing pregnancies using assisted reproductive techniques (13, 14).

This study determined whether prefreeze and post-thaw semen quality in men diagnosed with Hodgkin's disease, testicular cancer, and leukemia or advanced soft tissue cancer is related to type of disease and the response to treatment. Additionally, we investigated if the post-thaw sperm motility could be predicted from the prefreeze motility.

MATERIALS AND METHODS

This study was approved by our Institutional Review Board. The medical records of 106 cancer patients referred to the andrology laboratory of a tertiary care institution for cryobanking were reviewed retrospectively before beginning cancer treatment (between 1983 when sperm banking became available to 1995). The criteria for inclusion of the patients in this study was that all of them had their sperm specimens banked before initiating cancer treatment. Patients were placed in three groups according to the type of cancer. These groups were those with Hodgkin's disease ($n = 49$, median age 24 years, interquartile ranges 21, 28), testicular cancer ($n = 47$, median age 28 years, interquartile ranges 22, 31), or leukemia and advanced soft tissue cancer ($n = 10$, median age 24 years, interquartile ranges 27, 31). Patients with leukemia ($n = 7$) had three cases of acute lymphocytic leukemia and four cases of acute myelogenous leukemia. Because of the poor prognosis, patients with leukemia were grouped for comparison with advanced soft tissue cancer other than testicular cancer or Hodgkin's disease (sarcoma, $n = 2$; lung cancer, $n = 1$). Compared with the testicular cancer group, patients with Hodgkin's disease had a lower mean age. Each patient banked multiple specimens before initiating treatment.

Semen Collection and Analysis

Semen specimens were collected by masturbation after 2 days of sexual abstinence. Because of the urgency of treatment, 10% of patients had an abstinence of <2 days. After liquefaction at 37°C for 30 minutes, 5 μL of the fresh semen specimens were loaded on a 20- μL sperm counting chamber and analyzed on a computer-assisted semen analyzer (Motion Analysis Corp., Palo Alto, CA). Semen analysis was performed before and after cryopreservation on each specimen. Additionally, 8 to 10 fields with a minimum of 200 sperm were examined manually using a microscope to validate the semen analyzer results. The motile sperm count, percentage motility, curvilinear velocity (VCL), linearity (LIN), and am-

plitude of the lateral head displacement (ALH) were determined.

Cryopreservation of Semen

A glycerol-based freezing medium was used as the cryoprotectant. An aliquot of 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, IN). This procedure was repeated until an equal volume of cryoprotectant (1:1 vol:vol) was added to the specimen. Cryogenic vials (size: 1.2 mL; Fisher Scientific, Pittsburgh, PA) were placed in the freezer at -20°C for 8 minutes, followed by immersion in liquid nitrogen vapors for 2 hours, and finally submersion in liquid nitrogen at -196°C for long-term storage. An additional vial containing 0.1 mL of aliquot was prepared for post-thaw evaluation after 24 hours. In brief, the post-thaw vial was removed from liquid nitrogen and thawed at room temperature for 5 minutes followed by incubation at 37°C for 20 minutes. A 5- μL aliquot then was analyzed as described above. Throughout the study period, the same protocol was followed for processing semen specimen for cryopreservation.

Statistical Analysis

Semen analysis results of the first banked specimen from each patient were used for prefreeze and post-thaw analysis. The Kruskal-Wallis test was used to determine the differences in semen quality between the three cancer groups as well as for comparisons between nonseminomatous germ cell tumors to those with seminoma. The Wilcoxon Rank-sum test was used to determine if the patient status (alive or dead after treatment) was related to prefreeze or post-thaw semen characteristics. A P value ≤ 0.05 was considered significant. All statistical analyses were performed using the SAS statistical package (Cary, NC).

RESULTS

Semen Quality at Presentation

Of the patients in all three disease groups, 40% were oligospermic ($<20 \times 10^6/\text{mL}$). Oligospermia occurred in 37% of the patients in Hodgkin's group ($n = 18$), 50% in the testicular group ($n = 23$), and 20% in leukemia and advanced soft tissue cancer group ($n = 2$).

Disease Type and Cryopreservation

Total sperm count, prefreeze and post-thaw sperm motility, and motile sperm count did not differ sig-

Effects of cancer on spermatozoa quality after cryopreservation: a 12-year experience*†

Osvaldo F. Padron, M.D.
Rakesh K. Sharma, Ph.D.
Anthony J. Thomas, Jr., M.D.
Ashok Agarwal, Ph.D.‡

Andrology Research and Clinical Laboratories, Department of Urology, The Cleveland Clinic Foundation, Cleveland, Ohio

Objective: To determine whether type of cancer and response to treatment was related to prefreeze or post-thaw semen quality and to predict post-thaw sperm motility from prefreeze motility.

Design: Retrospective study.

Setting: Tertiary care institution.

Patient(s): One hundred six cancer patients cryopreserving their semen specimens.

Intervention(s): Computer-assisted semen analysis was performed before and after cryopreservation on each patient specimen.

Main Outcome Measure(s): The relationship of sperm motility and motion characteristics to type of cancer and patient's response to treatment.

Result(s): Prefreeze and post-thaw semen quality did not differ between patients presenting with testicular cancer and Hodgkin's disease. Patients with leukemia or advanced soft tissue cancer had a higher prefreeze and post-thaw motility and higher total and motile sperm count than testicular and Hodgkin's disease patients. A prefreeze sperm motility of $\geq 15\%$ could predict a post-thaw motility of $>10\%$.

Conclusion(s): Prefreeze or post-thaw semen quality in cancer patients is not affected (except the prefreeze motile sperm count within the testicular cancer patients) by the type of disease. Prefreeze motility can predict post-thaw motility. Cryopreservation of semen should be offered to cancer patients irrespective of the type of disease. *Fertil Steril*® 1997;67:326-31

Key Words: Spermatozoa, cryopreservation, Hodgkin's disease, testicular cancer, leukemia

Testicular germ cell malignancies are rare; however, they are the most common solid organ tumors in individuals aged 15 to 35 years (1). Hodgkin's disease is the second most common tumor that affects this age group and occurs twice as commonly in men than women (2, 3). The cure rate has changed

dramatically in these groups of young patients and is mainly the result of better chemotherapeutic agents and regimens (1-3). Alkylating and other chemotherapeutic agents cause azoospermia in 90% to 100% of treated adult men, with only 20% to 50% eventually recovering spermatogenesis (1-4). Maximal spermatogenic function may not be realized until 2 to 3 years after the completion of treatment for testicular cancer (5-7). Currently, it is not possible to predict accurately which of these men will regain spermatogenic function (8).

Unfortunately, cancer patients have a disease-intrinsic suppression of spermatogenesis (3, 5). Cryopreservation of human semen is known to result in a 25% to 75% decrease in sperm motility (9). A similar decrease is seen in patients with testicular cancer and Hodgkin's disease (10-12). Despite poor semen

Received April 16, 1996; revised and accepted September 27, 1996.

* Presented at the 21st Annual Meeting of the American Society of Andrology, Minneapolis, Minnesota, April 25 to 29, 1996.

† Supported by a research grant (RPC 4925) from The Cleveland Clinic Foundation, Cleveland, Ohio.

‡ Reprint requests: Ashok Agarwal, Ph.D., HCLD, Andrology Research and Clinical Laboratories, Department of Urology, A100, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland Ohio 44195 (FAX: 216-445-6049; e-mail: agarwaa@cesmtp.ccf.org).