

# OPTIMUM ABSTINENCE TIME FOR CRYOPRESERVATION OF SEMEN IN CANCER PATIENTS

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## ABSTRACT

Although an abstinence period of 48 to 72 hours is the most commonly prescribed interval for diagnostic semen analysis, to our knowledge the association between the abstinence period and sperm quality after cryopreservation has not been identified. Patients with a malignant disease who are consulting for semen banking most often require urgent therapy. Consequently, defining the shortest abstinence period that allows for frequent semen collection within a limited interval and the best post-thaw sperm quality is necessary. We investigated the relationship between abstinence period, and the pre-freeze and post-thaw motility variables in semen specimens obtained from cancer patients for sperm banking. Samples collected from 95 patients were divided according to abstinence period: group 1—15 patients at 24 to less than 48 hours, group 2—53 at 48 to less than 72 hours and group 3—27 at more than 72 hours. Pre-freeze and post-thaw motile sperm count and motion variables (motility, velocity, linearity, amplitude of lateral head movement and motility index), and percentage decrease in sperm variables after cryopreservation were analyzed. Semen volume, the pre-freeze and post-thaw motile sperm count, motion parameters and the percentage decrease in semen variables did not differ significantly among the groups. We conclude that semen collection for cryopreservation after 24 to less than 48 hours of abstinence results in post-thaw quality comparable to that after an abstinence of 48 to less than 72 hours or longer. Thus, an abstinence period of 24 to less than 48 hours can be recommended for sperm banking in cancer patients.

KEY WORDS: spermatozoa, infertility, cryopreservation, sperm banks

Sperm cryopreservation has become a complementary part of the therapeutic management in young men with malignant diseases, such as testicular cancer or Hodgkin's disease.<sup>1</sup> A substantial number of these patients, however, present with poor semen quality at semen banking. Moreover, aside from direct cryopreservation damage, sperm preparation procedures may further decrease the number of sperm available for subsequent intrauterine insemination. In general, one would ideally place at least  $5 \times 10^6$  motile sperm in the uterine cavity. Fewer numbers can certainly be used successfully with the more sophisticated assisted reproductive techniques (micromanipulation of gametes) but these methods may be too expensive or morally unacceptable to some couples.

A minimum of 3 and preferably 6 ejaculates, each obtained after an abstinence of 48 to 96 hours, has been commonly recommended for therapeutic semen banking.<sup>1,2</sup> Consequently, for patients who bank sperm to preserve future fertility, the current semen collection recommendations may significantly delay planned treatment, such as retroperitoneal lymphadenectomy, radiation therapy or chemotherapy.<sup>3</sup>

The World Health Organization recommended abstinence period for semen collection (48 to 96 hours) is based on the generally accepted protocol for routine semen analysis and is explained by studies revealing that ejaculatory abstinence of 48 to 96 hours may improve some characteristics of the semen, such as semen volume and sperm concentration, in normal fertile men.<sup>4,5</sup> A recent study suggests that, in contrast to normospermic men, a second successive ejaculate from oligospermic men may contain equal or even greater numbers of motile sperm than the initial ejaculate.<sup>6</sup>

Assuming that the pattern of changes in semen quality

differs between normal and oligospermic men after various abstinence periods, the question arises as to whether the standard abstinence period of 48 to 96 hours in patients with malignant diseases is justified or even desirable. In view of the urgency of treatment in many of these patients, determining the optimum abstinence period that allows for frequent semen sampling within a limited period and adequate post-thaw semen quality seems to be important. We investigate the relationship between abstinence period, and the pre-freeze and post-thaw semen qualities in ejaculates obtained from patients with malignant disease.

## PATIENTS AND METHODS

*Selection of subjects.* Pre-freeze semen analysis results of specimens obtained from 95 patients with malignant disease (testicular cancer in 36, Hodgkin's disease in 39 and other cancers in 20) who were referred for sperm banking between 1989 and 1993 were analyzed. Patients were selected regardless of the status of the disease. Only those with a negative history of chemotherapy or radiation therapy at the time of semen banking were included. Based on the abstinence period, patients were divided into 3 groups: group 1—15 at 24 to less than 48 hours, group 2—53 at 48 to less than 72 hours and group 3—27 at longer than 72 hours. Of the 15 patients in group 1, 13 had an abstinence duration of 24 hours or less.

*Semen collection and assessment of semen variables.* Semen specimens were collected by masturbation and liquified at 37°C for 30 minutes. Then, 5  $\mu$ l. of the specimen were loaded on a 20  $\mu$ l. Microcell\* chamber and analyzed on a sperm motility analyzer. Each semen specimen was analyzed before and after cryopreservation.

*Cryopreservation procedure.* Test-yolk buffer with glycerol was used as a freezing agent for cryopreservation. A 5 ml.

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vial of the medium was thawed by incubating the vial at 37C. A volume of freezing medium equal to 25% of the original specimen volume was then added to the sample. The specimen was vortexed and divided into equal aliquots for long-term cryopreservation. An additional aliquot was cryopreserved to assess 24-hour survival. Cryopreservation vials were frozen at -20C for 8 minutes and in liquid nitrogen vapor at -100C for 2 hours. The vials were then transferred to liquid nitrogen at -196C for long-term storage. At 24 hours after freezing, semen from the additional vial was removed and thawed by incubating the vial at 37C for 20 minutes. The semen was mixed gently with a sterile pipette and a 5  $\mu$ l. aliquot was used for semen analysis as described.

**Statistical analysis.** Kruskal-Wallis and Wilcoxon signed rank tests were performed to compare the semen analysis results. Semen volume, pre-freeze and post-thaw motile sperm count, and motion variables (motility, velocity, linearity, amplitude of lateral head movement and motility index) were analyzed and compared among the groups. The motility index was calculated as percent motility times velocity divided by 100. A p value of  $\leq 0.01$  was considered significant. All statistical analyses were performed using a statistical software package.

### RESULTS

There were no statistically significant differences among the groups for any of the pre-freeze and post-thaw semen variables (table 1). After cryopreservation, post-thaw motile sperm count, motility and motility index decreased significantly in all 3 groups ( $p < 0.0001$ ). The sperm velocity decreased significantly only in group 2 ( $p < 0.0001$ ) and marginally in groups 1 ( $p = 0.03$ ) and 3. Similarly, the amplitude of lateral head movement decreased significantly only in group 2 ( $p < 0.002$ ). The percent change from pre-freeze to post-thaw values for sperm linearity did not differ significantly in any of the groups. The percentage decrease in semen variables from pre-freeze (100%) to the post-thaw values did not differ significantly among the 3 groups (table 2).

### DISCUSSION

Our results suggest that the routine abstinence interval of 48 to 96 hours for semen banking in patients with malignant disease does not improve post-thaw semen quality compared to an abstinence of 24 to less than 48 hours. It is generally believed that substantial decreases in sperm concentration

and semen volume are associated with a shortened abstinence period in normal men.<sup>4,6</sup> The pattern of these changes in normal men is still inconclusive. Recently, Schwartz et al showed no linear relationships between the duration of abstinence, and sperm concentration, semen volume and total sperm count for 1 to 7-day intervals.<sup>7</sup> However, in another study decreases in semen volume, total sperm count and concentration were noted during the initial periods of semen collection.<sup>8</sup> Values for semen variables remained relatively constant after the exhaustion of extra gonadal reserves. It is possible that with initially poor quality sperm and decreased extra gonadal reserves, subfertile patients lack the capability to recover by simply increasing the abstinence period between ejaculations. Thus, the commonly prescribed abstinence period may not be superior compared to a shorter abstinence period between the successive ejaculations. Our findings support this hypothesis.

The correlation of sperm motility and morphology with abstinence interval is controversial.<sup>9</sup> Recent studies suggest that these variables do not change greatly with increasing abstinence interval.<sup>9,10</sup> Even though the effects of abstinence on semen quality have been extensively explored in normal men, few studies have been done on infertile men. The relationship between abstinence and semen variables in oligospermic men has been reported to be different from that in normal fertile men.<sup>6,11</sup> Also, the comparison of a group of oligospermic men with a mean sperm concentration of less than  $20 \times 10^6$ /ml. to a group of asthenospermic men (sperm concentration less than  $20 \times 10^6$ /ml., sperm motility less than 50%) showed that semen volume and sperm concentration increased with increasing abstinence duration. However, the effect of abstinence on sperm concentration was significantly greater in the asthenospermic group than in the oligospermic group.<sup>12</sup>

The relationship between abstinence interval and post-thaw semen quality also has not been fully studied. The abstinence interval has been reported not to affect post-thaw motility in normal subjects.<sup>13</sup> Our results indicate that the pattern and magnitude of decreases in post-thaw sperm motion variables are similar in specimens obtained after varying abstinence periods. The effect of cryopreservation on semen quality does not appear to depend on the duration of abstinence before collection of the semen specimen.

In conclusion, an abstinence interval of 24 to less than 48 hours can be recommended for therapeutic semen banking in

TABLE 1. Effect of abstinence interval on sperm quality in 95 patients with malignant disease before and after cryopreservation (values are reported as median and interquartile range)

Sperm Parameters	Group 1 (15 pts.)*			Group 2 (53 pts.)			Group 3 (27 pts.)			p Value†
	25%	Median	75%	25%	Median	75%	25%	Median	75%	
Motile sperm count:										
Pre-freeze	3.1	6.2	19.8	5.2	12	27.3	6.6	13	20.1	0.22
Post-thaw	6	1.7	3.1	1.1	2.8	6.6	1.9	3.2	5	0.13
Motility (%):										
Pre-freeze	27	41	56	32	44	57	33	43	54	0.65
Post-thaw	9	17	36	15	22	28	15	18	27	0.48
Velocity ( $\mu$ /sec.):										
Pre-freeze	37	43.7	59.5	38	43	51	34	42	48	0.53
Post-thaw	27.4	36	40	29.6	36.6	43	29	33.6	41	0.60
Motility index:										
Pre-freeze	10.1	19.8	28.2	12.5	19.6	25.5	9.6	17.2	23.9	0.54
Post-thaw	3.8	7.8	12.8	3.4	7.3	11.7	2.4	5.6	8.7	0.40
Linearity (%):										
Pre-freeze	4.1	5.2	23.4	4.7	5.2	5.7	4.6	4.9	5.5	0.66
Post-thaw	4.4	5.1	7.3	4	4.8	5.5	4.7	5.2	5.8	0.31
Amplitude of lateral head movement ( $\mu$ ):										
Pre-freeze	2.1	2.7	2.8	2	2.4	2.9	2.2	2.8	3.2	0.21
Post-thaw	1.6	2.2	2.6	1.7	2.1	2.6	2.1	2.6	2.7	0.07

\* The number of patients studied was 15 except for velocity, motility index and linearity (pre-freeze 14, post-thaw 13) and for amplitude of lateral head movement (pre-freeze 14, post-thaw 12).

† The differences among the 3 groups were analyzed by the Kruskal-Wallis test.

TABLE 2. Percent change in sperm parameters from before to after cryopreservation (values are reported as median and interquartile range)

Sperm Parameters (% change)	Group 1 (15 pts.)*				Group 2 (53 pts.)				Group 3 (27 pts.)				p Value†
	25%	Median	75%	p Value	25%	Median	75%	p Value	25%	Median	75%	p Value	
Motile sperm count	-84.9	-67.9	-59	<0.0001	-80.6	-75	-65.8	<0.0001	-83	-75	-68.2	<0.0001	0.57
Motility (%)	-69.6	-36.6	-18	<0.0002	-61.1	-50	-31.8	<0.0001	-66	-50	-36.4	<0.0001	0.49
Velocity ( $\mu$ /sec.)	-38.5	-21.6	-11.6	0.03	-32.3	-15.3	-2.7	<0.0001	-26.3	-19.2	0	0.02	0.62
Motility index	-79.9	-61	-26.6	0.001	-77.6	-61.1	-45.3	<0.0001	-72.8	-60.8	-51.9	<0.0001	0.90
Linearity (%)	0	8.2	22.2	0.27	-19.6	-1.8	-7	0.072	-5.6	2.1	10.2	0.17	0.04
Amplitude of lateral head movement ( $\mu$ .)	-34.7	-11.6	6	0.20	-28	-10.3	1.02	0.002	-22.9	-6.7	8.3	0.12	0.70

\* The number of patients studied was 15 except for velocity (13), motility index (13), linearity (13) and amplitude of lateral head movement (12).

† The differences among the 3 groups were analyzed by the Kruskal-Wallis test.

cancer patients. Shortening the abstinence period would minimize the delay in receiving specific medical therapy and would encourage semen cryopreservation for patients in whom postponing therapeutic intervention for malignancy cannot be justified.

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