Long-term effects of cryopreservation on human spermatozoa

We evaluated the long-term effects of cryopreservation on spermatozoa of men with testicular cancer, lymphoma, and benign conditions. No statistically significant decrease in semen parameters was noted. Duration of follow-up was up to 5 years and mean follow-up was 2 years. (Fertil Steril® 2005;84:536–7. © 2005 by American Society for Reproductive Medicine.)

Semen quality is often impaired by the process of cryopreservation. In a recent study, sperm viability and normal morphological sperms were reduced by 31% and 37% (P =.001) after thawing, respectively (1). Up to half of men presenting with testicular cancer or lymphoma have impaired semen quality (2). Some investigators have shown that damage to sperm cryopreserved from cancer patients is more severe than that from normal donors (3), whereas other researchers have demonstrated a similar decline in quality after cryopreservation in control and cancer groups (4, 5). Unlike the described effects of short-term freezing, the results of long-term cryopreservation on sperm quality remain largely unknown. This study was conducted to evaluate the post-thaw motility and viability of the human sperm after long periods of freezing.

The statistical database of a single, licensed, and accredited sperm bank was reviewed from 1997 to 2001 (North Shore-Long Island Jewish Institutional Review Board 04-03-115). The initial (24 hours after initial cryopreservation) and delayed (at the time of authorized destruction) post-thaw sperm motility and viability were evaluated. All specimens were collected before the start of any treatment (surgery, chemotherapy, or radiation) in cancer patients. Our cryopreservation protocol for all specimens was as follows: ejaculated specimens were collected by masturbation into a sterile polypropylene container and processed within 1 hour. The ejaculated specimens were slowly mixed in a 1:1 ratio with freezing medium TEST Yolk Buffer (Irvine Scientific, Santa Ana, CA) and glycerol and placed in a 1-mL Nunc Cryotube (Nunc A/S, Roskilde, Denmark). Cryotubes were then placed in a planer Kryo 10 Series III programmed freezer (Planer Products LTD, Middlesex, England). A three-step programmed freeze was used. First, the specimens were brought to −10°C at −1°C/min and then to −80°C at −10°C/min. The specimens were then plunged into liquid nitrogen. All specimens were cryopreserved using the same technique. Sperm motility was measured using a Makler chamber (Haifa, Israel) and viability was assessed using eosin Y-nigrosin staining. The t test and linear regression analysis were used for data analysis.

Sixty patients with cryopreserved ejaculates were identified. The mean age was 29.5 years (17–48 years). Eighteen men had testicular cancer (30.0%), 19 had lymphoma (31.7%), and the rest were diagnosed with other conditions. The mean ages of men with and without cancer were 27.4 and 36.1 years, respectively (P<.0001). The mean cryopreservation time was 711 days (143–1,817 days). Delayed motility and viability (at the time of disposal) were not significantly different from the immediate post-thaw values for the entire group of cancer patients (Table 1). Linear regression analysis did not show a significant correlation between the length of cryopreservation and change in motility or viability over that time period (correlation coefficients = 0.16 and 0.01, respectively).

Cryopreservation of the human spermatozoa was first reported in 1954 (6). Free radicals, superoxides, osmotic changes, and intracellular and extracellular ice formation have all been shown to affect sperm integrity after short periods of freezing. The initial semen quality (7) and the method of freezing and thawing also contribute to the preservation of semen viability. Previously we demonstrated that motility and viability could be maintained up to seven repetitive thaw–refreeze cycles even in specimens from cancer patients with severe semen impairments (8). Fast freezing preserved motility and viability for 2.75 and 2.0 cycles longer than slow freezing, respectively (8). In addition, sperm preserved in seminal fluid has been shown to suffer less cryodamage than the frozen-prepared sperm (9).

This study demonstrates that sperm integrity remains unchanged after the initial cellular damage induced by the freezing process. Semen from men with lymphoma and testicular cancer also tolerated the cryopreservation well up to 5 years (mean 2 years). This contrasts with a 1990 study by Kolodziej et al. (10). In their study, after a mean storage time of 44 months, only 28% of the specimens could be recovered and the concentration of motile sperm was re-

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TABLE 1

Immediate and delayed motility and viability.

n	Immediate motility[a] (%)	Delayed motility[a] (%)	*P* value	Immediate viability[a] (%)	Delayed viability[a] (%)	*P* value
All patients (60)	27.9	24.8	.23	37.0	34.5	.43
Testicular cancer (18)	31.8	28.5	.37	35.9	36.6	.86
Lymphoma (19)	29.6	26.9	.59	37.4	34.2	.57

[a] Mean post-thaw values are shown.

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duced so severely in men with testicular cancer that chances for fertility were extremely poor.

Advances in cryopreservation techniques and better initial semen quality in cancer patients, perhaps due to early referral and semen collection before the start of gonadotoxic therapy (11), may explain our better outcome compared to the Kolodziej et al. (10) study conducted more than a decade ago. In addition, with advanced assisted reproductive techniques (ART) only few spermatozoa are needed to achieve fertilization. As Hallak et al. (12) demonstrated, after a median storage time of 49 months for cancer patients, the pregnancy rate per cycle of ART was 36.4%. This finding is significant for cancer patients where infertility can last up to 5 years after radiotherapy or chemotherapy (13). This study demonstrates that sperm motility and viability are well preserved up to 5 years after cryopreservation (mean follow-up of 2 years) in patients with testicular cancer, lymphoma, as well as benign conditions.

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