CLINICAL ARTICLE

Survival and infertility treatment in male cancer patients after sperm banking

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Objective: To evaluate the relationship between sperm pathology and cancer diagnosis, determine the mortality rate, and evaluate the outcomes of the use of frozen sperm from the sperm bank.
Design: Prospective study.
Setting: University fertility center.
Patient(s): A total of 619 male patients were referred for sperm freezing before gonadotoxic therapy from 1995 to 2006.
Intervention(s): Semen analysis, data verification in the National Oncologic Register, assisted reproduction technologies, and statistical evaluation.
Main Outcome Measure(s): Cancer diagnosis and sperm pathology analysis, survival of patients, and infertility treatment success.
Result(s): Malignant testicular cancer was diagnosed in 43.6% of patients, and malignant neoplasms of the lymphatic and hematopoietic tissues were found in 31.7% of patients. Azoospermia or severe oligospermia ($\leq$1 million/mL) was detected in 9.7% and 22.6% of patients, respectively. To date, 32 patients (5.2%) sought infertility treatment. Cryopreserved semen was used in 28 couples (87.5%), and 44 intracytoplasmic sperm injection (ICSI) cycles resulted in 13 pregnancies. In total, 74 deaths (11.9%) were reported, 61 of them (82.4%) within 30 months of the cryopreservation of their sperm.
Conclusion(s): A significant number of patients survived. Intrauterine insemination and ICSI with cryopreserved sperm resulted in deliveries. (Fertil Steril® 2008;■:■–■. ©2008 by American Society for Reproductive Medicine.)

Key Words: Cryopreservation, semen, cancer survivors, male infertility

Damage to reproductive function is a very frequent and well documented side effect associated with the treatment of malignant tumors. The first work describing chemotherapy-induced azoospermia was published in 1948 (1). Variation in sperm quality in relation to the type of malignant tumor was also investigated (2). The increasing success of cancer treatment and determined efforts to improve the quality of life after successful treatment has turned attention to the preservation of reproductive function in young men (3, 4). The development of assisted reproduction technologies has brought about effective qualitative changes in this field (5, 6). The collection, freezing, and long-term storage of sperm is currently considered to be the most effective method.

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The Assisted Reproduction Center of the Department of Gynecology and Obstetrics, Faculty of Medicine, Masaryk University, and the Faculty Hospital in Brno launched a program of freezing sperm for long-term storage in 1995. The main aim of the present paper was to analyze the sperm counts of cancer patients, examine possible correlation between sperm pathology and cancer diagnosis, determine the mortality rate, and provide an overview of the use of the frozen sperm during the twelve years of sperm banking.

MATERIALS AND METHODS

Between October 1995 and the end of December 2006, a total of 619 male adolescents and adults aged 13 to 64 years (mean 26.2 $\pm$ 6.8 years, median 26 years) were referred to the Assisted Reproduction Center for sperm cryopreservation before treatment for malignant tumours using chemotherapy, actinotherapy, or orchidectomy. Sperm counts were evaluated according to the World Health Organization laboratory manual using the Neubauer counting chamber (7).

Commercial media, including Medi-Cult (Jyllinge, Denmark) and Vitrolife (Kungsbacka, Sweden), were used. Semen was mixed with a cryopreservation medium and placed in 2-mL Nunclon Cryotubes (Roskilde, Denmark) and followed by freezing. Cryopreservation technology and the procedures used in the storage of frozen sperm samples were aimed at minimizing the potential risks, including mistaken identity and transmission of infection. Sperm samples were frozen in the programmable Planer Kryo F10 (Sunbury-On-Thames, U.K.) instrument using a standard cooling curve or in nitrogen vapor (used only in the absence of the instrument). Samples from 1–3 collections before starting cancer treatment were frozen. The cryotubes were stored in liquid nitrogen at a temperature of −196°C in an LS 4800 container (Tailor-Wharton Harsco, Husum, Germany) with an indicator of the surface level and an alarm.

The assisted reproduction methods used comply with the respective standards of the department. Diagnosis and the time of death were verified with the database of the National Oncologic Register of the serving area, in compliance with personal data protection.

The study group was described using basic descriptive statistics, where categoric variables were characterized using the percentage representations of individual categories and continuous variables (age, sperm concentration and motility) were described using the mean, the median, standard deviation, and the range of values.

Statistical testing was used to confirm the hypothesis of whether or not the results of sperm counts correlate with the patient's diagnosis. The differences among a group of patients were tested using the Kruskal-Wallis test. When the influence of the diagnosis on the sperm count was significant, partial hypotheses were tested to see which particular diagnoses differ by their values (i.e., multiple comparisons of mean ranks). The critical limit for the level of significance was set to $P=.05$.

The project of fertility protection in male cancer patients was approved by the Brno Faculty Hospital scientific council and ethics commission.

RESULTS

Malignant testicular tumor (a total of 270 patients, 43.6%) was the most common diagnosis in patients who were referred for sperm cryopreservation, followed by patients with Hodgkin lymphoma (103 patients, 16.6%), leukemia (50 patients, 8.1%), or non-Hodgkin lymphoma (44 patients,7.1%). Forty-one men were treated for malignant tumors of bone and cartilage (6.6%). Other malignant diseases occurred only sporadically.

A concentration of spermatozoa <20 million/mL was found in 53.1% of patients, and 22.6% showed a concentration ≤1 million/mL. The lowest mean values of sperm count were found in men with malignant testicular tumors (17.2 ± 21.4 million/mL, median 8.0 million/mL), as shown in Table 1. Azoospermia was found in 60 men (9.7%), with the highest incidence in leukemia patients (24.0%). Progressive sperm motility ≥40% was found in only 4.4%, asthenospermia ≤10% in 64.6%, and sperm motility <1% in 6.8% of cases. The lowest mean percentage of progressive motility was also seen in patients with malignant testicular tumours, namely 9.8 ± 11.3%, median 5.0%.

A statistically significant correlation was found between the concentration of spermatozoa and the diagnosis (Kruskal-Wallis test: $P<.001$). Detailed analysis revealed a difference between testicular tumors and malignant tumors of the digestive tract ($P=.012$) and Hodgkin disease (p=0,003). No statistically significant correlation was confirmed between the diagnosis and progressive sperm motility (Kruskal-Wallis analysis of variance: $P=.149$).

The Department of Pediatric Oncology was established in the Brno Faculty Hospital in 2000 and adolescent boys began

TABLE 1

Sperm counts (×10^6/mL) with type of malignancies.

Diagnosis	Mean	Median	Min.	Max.	SD
Testicular cancer	17.2	8.0	0	122	21.4
Hodgkin disease	29.9	25.8	0	100	26.3
Leukemia	32.6	23.5	0	130	35.4
Non-Hodgkin lymphoma	29.4	23.0	0	172	31.1
Bone and cartilage MT	29.5	32.0	0	86	27.2
Digestive system MT	44.1	37.5	0	110	35.4
CNS MT	44.7	33.0	0	130	46.4
Urinary system MT	25.9	14.0	1.5	82	26.0
Respiratory system MT	48.0	36.0	2	93	33.9
Unspecified cancer	28.4	22.0	0	125	26.9
Total	25.3	16.0	0.0	172	27.7

Note: CNS = central nervous system; MT = malignant tumor.

Crha. Sperm banking for cancer patients. Fertil Steril 2008.

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to be referred for sperm cryopreservation to our center. In the years 2000–2006, 36 young men aged 13–16 years were referred. The most frequent diagnosis was malignant tumor of bone and cartilage (25.0%), followed by leukemia (16.7%), Hodgkin lymphoma (13.9%), and testicular tumor (11.1%). Azoospermia was detected in 8 cases (22.2%). The mean concentration of spermatozoa was 14 million/mL (median 1.8 million/mL), with mean sperm motility of 5.2% (median 2%) —significantly lower compared with the mean values for the whole group.

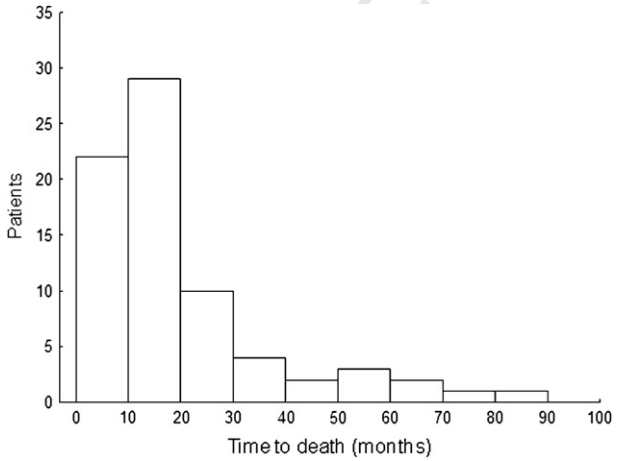
Of all the 619 patients referred for sperm cryopreservation, 74 (11.9%) died. The average time interval between the referral and death was 20.5 ± 17.3 months, median 16 months (Fig. 1). The lowest mortality rate was found in patients with malignant testicular tumor (3.0%) and Hodgkin lymphoma (4.9%) (Table 2).

Out of the 32 men treated, 56.3% were successful in their treatment for testicular cancer, 28.1% were successful in treating Hodgkin lymphoma, and 15.6% successful in treating leukemia. The interval between cryopreservation and infertility treatment was in the range of 7–70 months (mean 22.2 ± 14.7 months, median 18 months). Cryopreserved samples were used in 28 couples (nine cycles of intrauterine insemination, 38 intracytoplasmic sperm injection [ICSI] cycles), and fresh sperm was used in four cases (six ICSI cycles). Intrauterine insemination was performed for four couples (12.5%) and ICSI for 28 couples (87.5%). ICSI (44 cycles) resulted in 13 pregnancies and nine deliveries. Intrauterine insemination (nine cycles) resulted in two clinical pregnancies and two deliveries.

After the failure of two ICSI cycles, four couples (12.5% of men seeking infertility treatment after sperm cryopreservation) decided to use intrauterine insemination with donor sperm, from which seven cycles resulted in two pregnancies and deliveries.

FIGURE 1

[Q1] ■■■.



Crha. Sperm banking for cancer patients. Fertil Steril 2008.

TABLE 2

Incidence of malignancies and number of deceased patients (n = 619).

Diagnosis	n	Deceased	%
Testicular cancer	270	8	3.0
Hodgkin disease	103	5	4.9
Leukemia	50	18	36
Non-Hodgkin lymphoma	44	7	15.9
Bone and cartilage MT	41	11	26.8
Digestive system MT	20	5	25.0
CNS MT	13	3	23.1
Urinary system MT	9	2	22.2
Respiratory system MT	7	3	42.9
Unspecified cancer	62	12	19.4
Total	619	74	12.0

Note: 100% = patients with the same diagnosis. Abbreviations as in Table 1.

Crha. Sperm banking for cancer patients. Fertil Steril 2008.

DISCUSSION

The frequency of certain diagnoses in this group of patients, the prevalent ones being malignant testicular tumors and tumors of lymphatic and hematopoietic tissue, corresponds to the high incidence rate of such diagnoses in men of a fertile age. Similar findings are reported by other clinics (6, 8, 9). Tumors of bone and cartilage, Hodgkin lymphoma, and leukemia were the most frequently diagnosed malignancies in young men under 16 years.

Azoospermia was found in 9.7% of males referred for sperm cryopreservation, compared with Lass et al., who reported 17.3% (10). Severe abnormalities in sperm concentration (≤5 million/mL in 36.4%) and progressive motility (≤10% in 64.6%) were frequently detected. Similarly to other studies (11, 12), the lowest concentration of spermatozoa and the lowest progressive sperm motility were found in men with malignant testicular tumors. The etiology of impaired spermatogenesis in cancer patients is not fully understood and is thought to be associated with involvement of the immune system (13). Furthermore, damage to the DNA of sperm due to the malignancy has been confirmed (14). The correlation between sperm pathology and testicular tumors is also known. The impaired quality of sperm production is most likely associated with disturbed differentiation of the testicle during the embryonic development of the gonad (15). Testicular dysgenesis syndrome is manifested by the increased incidence of developmental defects of the genitals (cryptorchism, hypospadias), spermatogenesis disorders, and testicular carcinomas. Testicular dysgenesis is caused by alteration in the development of the testicle, determined by the factors affecting endocrine regulation ("endocrine disruptors"). Because spermatogenesis disorders correlate well with testicular carcinoma, close urologic examination of men with severe sperm abnormalities is of particular importance (16).

When analyzing impaired spermatogenesis in relation to the type of malignancy, we found a significant difference only between testicular tumors and malignant tumors of the digestive tract. Some studies have shown that the concentration of spermatozoa and sperm motility in men with Hodgkin lymphoma is significantly lower compared with patients with non-Hodgkin lymphoma (17). However, like Agarwal et al., we failed to confirm such a difference (18).

We succeeded in obtaining and freezing sperm samples from young men aged 13–16 years (77.8%), which is similar to other studies (19). Sperm samples were collected by masturbation. We did not perform electroejaculation or surgical collection. Although sperm count and sperm motility were very low, sperm cryopreservation may also be used in this age group. One of the major tasks of assisted reproduction is to preserve reproduction in patients who undergo childhood treatment for malignant tumors.

After the completion of gonadotoxic therapy, the quality of sperm was significantly impaired (20, 12). The resulting function of the gonad is affected by a number of factors, such as the diagnosis of the malignant disease, the chemotherapy regimens used, and the sperm count as determined before the start of therapy. In the case of azoospermia, the methods of assisted reproduction based on the surgical collection of sperm provide inferior results; however, recovery of spermatogenesis has also been described (21, 22). Sperm cryopreservation performed before cancer therapy is therefore a prerequisite for the successful treatment of subsequent infertility.

In the present group, only 5.2% of the men had come for infertility treatment as of the time of writing. This finding corresponds with data from other published studies (2). The reasons are not only in the area of patient health, but also in the social area, i.e., patients usually plan to start a family long after they have successfully completed therapy. Another important aspect is that patients are afraid of the increased risk of congenital defects and malignant tumors in their offspring. Many detailed studies that have investigated this risk have failed, however, to prove its increase (23, 24). Most men from the present group who came for infertility treatment were aaround 29 years old, had undergone successful treatment for testicular cancer or lymphoma, and usually presented 18 months after sperm cryopreservation. Intrauterine insemination was performed in our clinic much less frequently (17.0%) compared with Agarwal et al. (6); ICSI was used in 83.0% of treatment cycles.

Intrauterine insemination with donor sperm was used after failure of ICSI cycles. Couples electing to use donor sperm preferred its improved pregnancy rate, easier procedure, and decreased risk of malignant disease in the offspring.

The mortality rate of the present group of patients was analyzed also. According to the data obtained from the Oncologic Register, 11.9% of the men referred for sperm cryopreservation died; 82.4% of these died within 30 months of referral. The lowest mortality rate was found in patients with malignant testicular tumors and Hodgkin lymphomas, which corresponds to a total survival rate in patients with the early stage of testicular seminoma surpassing 95% (25).

Sperm cryopreservation before gonadotoxic therapy is the basic method used to preserve reproductive potential for the survivors of cancer treatment. It can also be used in the period of adolescence. The lowest sperm counts were found in men with malignant testicular tumors. Cancer patient sperm banking programs require close cooperation between the respective assisted reproduction centers and the cancer clinics. Sperm cryopreservation should be offered to every patient before therapy that causes the destruction of spermatogenesis.

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1 **Survival and infertility treatment in male cancer patients after sperm banking**

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The results of infertility treatment, sperm pathology, and survival of 619 cancer patients after sperm banking over 12 years are described and analyzed.

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