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Sperm Cryopreservation for Male Cancer Patients: More than 10 Years of Experience, in Beijing China

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Background: Fertility preservation is very important for male cancer patients, especially adolescents. Unfortunately, the use of fertility preservation is very low among Chinese male cancer patients. Additionally, the cumulative rate of frozen sperm use is also low.

Material/Methods: We performed a retrospective study by collecting available information at the Human Sperm Bank, National Research Institute for Family Planning from July 2006 to December 2017 to examine the data in China.

Results: A total 145 male cancer patients underwent sperm cryopreservation. The patients were 29.3 ± 6.9 years old, and 6.2% (9 out of 145) of the patients were adolescents under the age of 18 years old. As of June 2018, only 9.7% (14 out of 145) of patients returned to use their cryopreserved sperm for assisted reproduction technology (ART). Of the 33 ART cycles, conceptions were achieved in 51.5% (17 out of 33), and the rate of patients who had a baby was 71.4% (10 out of 14). The data indicate men with testicular cancer or leukemia had lower total sperm counts and recovery rate of progressive sperm than did men with other types of cancer, while men with sarcoma had the lowest progressive sperm.

Conclusions: The physician should make an effort to promote fertility preservation for male cancer patients in China. And patients with testicular cancer and leukemia require additional attention.

MeSH Keywords: **Cryopreservation • Fertility Preservation • Infertility, Male • Reproductive Techniques, Assisted**

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Background

Human sperm cryopreservation is widely used for male fertility preservation, especially for cancer patients. Cancer is a major public health problem worldwide, and the newest data show that the probability of being diagnosed with a malignant tumor is 3.4% for men younger than 49 years of age [1]. Traditionally, cancer treatments have put emphasis on extending survival time. In recent years, the number of men who are long-term survivors of cancer, especially adolescents and young men, is growing due to progress in cancer treatments [2]. Therefore, the focus of treatment is increasingly shifting toward quality of life, and fertility is a critical aspect. The use of chemotherapeutic agents [3] and radiation [4] significantly increase the risk of reproduction dysfunction. Many patients suffer from infertility before or after their cancer treatments, and the incidence of azoospermia after chemotherapy ranges from 0% to 63% [5]. Therefore, preservation of reproductive potential is a crucial issue for cancer survivors [6].

In China, few articles have been published regarding fertility preservation, particularly with respect to male cancer patients [7]. Moreover, only a few studies have focused on the future fertility of these patients. Sperm cryopreservation was the only effective approach used for male fertility preservation in the clinic in China, by now [8]. The Human Sperm Bank of the National Research Institute for Family Planning in Beijing, China (CNHSB), has been offering this service for 10 years. So, in this study, we performed a retrospective review of sperm cryopreservation for male cancer patients in our unit by focusing on the main outcomes including the usage rate of cryopreserved semen and the reproductive outcomes after use of frozen spermatozoa. Additionally, we compared raw and post-cryopreservation semen quality among men with newly diagnosed cancer with cases, using sperm cryopreservation for procreation management.

Material and Methods

The study was approved by the Ethics Committee of the National Research Institute for Family Planning. Informed written consent was obtained from all patients prior to their enrollment in this study.

In accordance with the guidelines and processes established by our sperm bank, all relevant patients seeking fertility preservation were counseled by an andrology physician and fully informed about the procedure. The information provided included the cancer type and the treatment, the different methods for fertility preservation, and the costs for future use of Assisted Reproductive Technology (ART). All the patients received a physical examination (PE) and were screened for sexually transmitted diseases. A slow sperm freezing method was performed according to the standardized programmable freezers (Kryo 360-1.7,

Planner, United Kingdom) in our unit [9,10]. Briefly, 1 volume of GEYC cryoprotectant was added to 2 volumes of semen; and the program is cool the straws at 1.5°C per minute from 20°C to -6°C, at 6°C per minute to -100°C, at -100°C for 30 minutes, then the straws are transferred to liquid nitrogen. After being preserved in the liquid nitrogen for a minimum of 2 days, a small portion of the frozen sample was thawed for semen quality assessment. If there were no motile sperm before or after thaw, then the staff had a discussion with the patients or their parents, whether the sample was cryopreserved. The fresh semen and frozen-thawed specimens were analyzed for sperm number and motility according to the World Health Organization (WHO) standardized operating process (WHO, 2010). We then followed up with the patients annually by telephone or email to confirm that they wanted to continue the semen preservation. The last follow-up time was June 30, 2018.

Our retrospective study involved male cancer patients who had undergone sperm cryopreservation in our sperm bank from July 2006 to December 2017. The medical notes of these patients were reviewed, and the demographic and other data were logged. These data included the following: age at diagnosis and referral, type of cancer and treatment, pre- and post-cryopreservation semen analysis results, length of semen cryopreservation, usage of the frozen semen, and the reproductive outcome obtained with the use of spermatozoa frozen before cancer treatment.

In order to explore the correlation of the type of cancer and the semen quality, we characterized both the raw and post-cryopreservation semen quality among men with newly diagnosed cancer. And patients with other clear factors affecting semen quality were excluded from this part, such as a history of prior antineoplastic therapy, varicocele, and sexually transmitted diseases. Additionally, based on previous research [9], we also included control males who accepted sperm cryopreservation for procreation management as a comparison group. Only 1 ejaculate was recorded per visit.

The data are presented as the mean (standard deviation). The statistical methodology consisted of Student's *t*-tests and ANOVA after normal distribution and homogeneity of variance detected. All statistical analyses were performed using SPSS 22.0 (IBM, USA). All *P*-values were 2-sided and results with *P*<0.05 are considered statistically significant.

Results

Characteristics of patients

Our unit has been providing sperm cryopreservation for male patients since July 2006. There were a total of 167 patients

Table 1. Details of men using cryopreserved sperm and their reproductive outcomes.

Diagnosis	Age at cryopreservation (year)	Duration from cryopreservation (year)	Raw semen			Frozen-thawed semen		ART (cycle)	Reproductive outcome (clinical pregnancies/live birth)
			Volume (mL)	TMC (10 ⁶ /ejaculation)	PR (%)	RRPR (%)	SCPR (10 ⁶ /mL)		
Gastroenterological tumors	29	4	2.0	80	65	69	8.28	ICSI(2)	1/0
Brain tumor	29	5	4.5	306	73	58	12.04	IUI(2)+ ICSI(1)	1/1
Leukemia	33	5	2.6	202.8	60	53	10.88	PGD(1)	1/1
Leukemia	28	1	3.5	157.5	60	50	1.8	ICIS(2)	1/1
Testicular cancer	24	1	4.0	184	50	60	3.9	IUI(2)+ IVF(1)	1/1
Leukemia	25	4	2.0	22	50	4	0.04	IVF(3)	1/0
Leukemia	28	2	4.5	137.5	60	50	4.8	IVF(2)	1/1
Testicular cancer	23	2	5.3	20	55	2	0.005	IVF(4)	2/0
Testicular cancer	26	3	3.5	20	50	70	4.0	IVF(2)	2/1
Lymphoma	24	3	5.1	395	50	90	29.25	IUI(2)	2/1
Gastroenterological tumors	26	1	6.1	305	55	73	15.6	IUI(4)	2/1
Gastroenterological tumors	29	2	4.1	155	55	65	8.6	IUI(1)	1/1
Leukemia	28	1	3.5	87.5	60	50	5.8	IUI(3)	1/1
Lymphoma	31	2	2.2	110	55	53	8.12	IVF(1)	0/0

TMC – total sperm motility count; RRPR – recovery rate of progressive sperm; SCPR – sperm concentration of PR; ART – assisted reproduction technology; IUI – intrauterine insemination; ICSI – intracytoplasmic sperm injection; IVF – assisted reproductive technology; PGD – preimplantation genetic diagnosis.

referred for sperm cryopreservation due to malignancy before December 2017. However, 5.4% (9 out of 167) of patients refused sperm cryopreservation because either they or their parents considered it would impact the cancer treatment. An additional 7.8% (13 out of 167) of patients failed the sperm cryopreservation, including 7 patients who failed due to azoospermia, 4 patients for oligoasthenozoospermia, and 2 patients failed masturbation. Among the 7 patients with azoospermia, 3 patients underwent unilateral orchiectomy for testicular cancer, 3 patients received chemotherapy for leukemia or lymphoma and 1 patient was diagnosed with lymphoma without treatment.

There were 145 male cancer patients who underwent sperm cryopreservation from July 2006 to December 2017. A total of 482 semen samples were collected and 1627 tubes were cryopreserved in CNHSB. The age of patients was 29.3±6.9 years old, among whom 6.2% (9 out of 145) patients were

adolescents under the age of 18 years. The median semen volume was 3.2 (1.28) mL, sperm density 54.4 (36.1)×10⁶/mL and total sperm motility count (TMC) was 180.1 (142.6)×10⁶/ejaculation. The rate of progressive sperm (PR) in the raw semen was 44% (17%), while the PR of frozen and then thawed semen was 24% (15%).

The outcome of ART with cryopreserved semen

As of December 31, 2017, only 9.7% (14 out of 145) of patients returned to use their cryopreserved semen for ART. The mean time from cryopreservation to usage was 2.6 years. Of these 33 cycles, conceptions were achieved in 51.5% (17 out of 33), with 30.3% (10 out of 33) of the pregnancies resulting in delivery. Four pregnancies were chemical, 2 ended as spontaneous abortions. Of the 14 patients, the rate of patients who had a baby was 71.4% (10 out of 14).The details of these cases are listed in Table 1.

Table 2. The semen parameters of patients with newly diagnosed cancer (n=94).

	No.	Volume (ml)		TMC		PR (%)		RRPR (%)		SCPR (10 ⁶ /mL)	
		Mean (SD)	p	Mean (SD)	p	Mean (SD)	p	Mean (SD)	p	Mean (SD)	p
Leukemia	29	3.0 (0.9)	0.004	135 (121.4)	<0.001	41 (20.6)	<0.001	46 (23.2)	<0.001	6 (7.1)	<0.001
Testicular cancer	11	2.9 (0.8)	0.034	130 (77.5)	0.001	49 (11.0)	0.440	37 (22.8)	<0.001	5.5 (6.5)	0.001
Lymphoma	20	3.6 (1.5)	0.342	247 (142.9)	0.178	48 (12.5)	0.155	57 (21.1)	0.281	13.1 (10.6)	0.267
Brain tumor	13	3.4 (1.5)	0.223	255 (156.0)	0.342	52 (8.4)	0.977	62 (17.1)	0.986	15.0 (10.0)	0.814
Gastroentero-logical tumors	12	3.4 (1.6)	0.218	225 (149.4)	0.122	49 (14.3)	0.421	58 (29.1)	0.402	12.6 (7.5)	0.281
Sarcoma	3	2.7 (1.2)	0.149	96 (93.2)	0.027	27 (24.6)	0.001	48 (26.2)	0.720	7.3 (9.5)	0.130
Other or unknown	6	3.7 (1.7)	0.707	267 (213.3)	0.641	48 (12.5)	0.401	58 (22.6)	0.127	11.9 (10.4)	0.346
Comparison group	150	3.9 (1.6)	–	298 (164.6)	–	52 (9.9)	–	62 (21.6)	–	16 (9.9)	–

TMC – total sperm motility count; PR – progressive; RRPR – recovery rate of PR; SCPR – sperm count of PR. P value for two-tailed t test compared with comparison group: the person who accepted sperm cryopreservation for procreative management.

Semen analysis according to cancer group

At last, 94 patients were included in this part of the study. We compared the semen parameters among the 94 patients without treatment by cancer type to characterize the correlation between semen quality and tumor type. The details are shown in Table 2. For the pretreatment semen analysis, a statistically significant reduction in TMC was seen among men with sarcoma, testis cancer, and leukemia. The men with sarcoma had the lowest PR, followed by the leukemia patients. There were no statistical differences in PR between testis cancer and the control group (procreative management). Sperm freezing tolerance is very important for sperm cryopreservation. Therefore, we also examined the RP sperm survival of the different groups of oncologic diagnoses (Table 2). We found that testicular cancer and leukemia cases had a statistically significantly lower recovery rate of RP (RRPR) after cryopreservation.

Discussion

Fertility preservation is critical for male cancer patients. Cryopreservation of semen samples is a noninvasive procedure and is the main option for male fertility preservation [6]. The available methods of fertility preservation for men are evolving quickly. However, sperm cryopreservation is currently the only effective approach of male fertility preservation. Beginning in October 2013, cryopreservation of testicular tissue

or cells was provided to prepubertal boys lacking mature spermatozoon and at high risk for infertility. However, there were only a few units offering this service worldwide [11].

During the 10 years of our study, there were only 145 male cancer patients using the fertility preservation option in our unit. However, the estimated number of new male cases in China in 2013 exceeds 2 048 600. The practice of fertility preservation is far from widespread among oncologic patients [12]. In contrast to Europe and the USA, there are no practice guidelines advocating fertility preservation for men or physicians in China. Furthermore, only a limited number of patients know of this option and few physicians know how to perform the preservation. Our results indicate the understanding rate of male fertility is low and the number of participants is very limited in China. Another important issue is the practical difficulty of accessing this service in some institutions. In mainland China, there are only 26 officially approved sperm banks able to conduct sperm cryopreservation. In addition, the cost of the service is another key limitation. In France, the total cost is covered by national insurance and the patient does not have pay for sperm cryopreservation [13]. In China, all ART services are private, and this might be a heavy burden for the families that need to pay for cancer treatments. The average cost for sperm cryopreservation in China is 4000 CNY per year. The possibility of precancer treatment sperm banking is not yet common knowledge among patients and oncologists due to the lack of systematization. However, we need to be aware that the

number of these patients has been progressively increasing during the past few years [7,8].

In our sperm bank, only 9.7% (14 out of 145) men returned to use their cryopreserved sperm. The usage rate was similar to the rates that have been reported by other international oncology-infertility centers (2% to 60%) [14]. The usage rate of cryopreserved semen is low for many reasons, one of which is that many patients are still young or have no plans for offspring. The development of testicular sperm extraction (TESE) [15] and ART was important for patients with post-chemotherapy azoospermia and TESE is also an alternative for treatment [16]. A cohort study showed the following outcomes of micro-TESE: sperm retrieval rate of 47% (37 out of 66), clinical pregnancy rate of 35% (23 out of 66), and the live birth rate of 27% (18 out of 66) [17]. In China, many hospitals offer this service for cancer patients [18,19]. However, there are still no reports on the sperm retrieval rate or the reproductive outcomes. Moreover, the lack of a service mailing system for sperm transportation might also be another reason. In our bank, the semen must be transported by the staff in our unit or by the hospital corpsman.

In this study, we compared the raw and post-thaw semen parameters between newly diagnosed male cancer patients and those who accepted sperm cryopreservation for procreative management. We found that men with leukemia or testicular cancer had an inferior TMC and RRPR compared with men with other types of cancer. Regarding the freezing tolerance, patients with testicular cancer and leukemia had a significantly

lower recovery rate. Our results were similar to the findings of other research studies [11,20,21]. There are several causes of semen quality decline before cancer treatment including cytotoxic autoimmune response, lymphocytic infiltration, malnutrition, and reactive oxygen species [22,23]. In this study, we found statistically significant variation in both TMC and PR in patients with sarcoma. The possible reason is that sarcoma presents in a wide variety of different histological types and it can arise in any body location [24]. We should note that there were only 3 patients with sarcoma in our study. As a result, more investigations are needed to clarify this issue.

Conclusions

Fertility preservation is very important for male cancer patients, especially adolescents. Unfortunately, fertility preservation utilization is very low among Chinese male cancer patients, and the cumulative rate of frozen sperm use is also low. Therefore, reproductive physicians and oncologists are required to improve this situation. Especially for the initial clinician, it is necessary for them to provide a fertility preservation plan for these newly diagnosed patients: information about sperm cryopreservation before treatment should be provided. In our study, the patients with testicular cancer, leukemia, and sarcoma had poorer raw semen parameters compared with men without cancer. The freezing tolerance analysis showed patients with testicular cancer and leukemia had a significantly lower recovery rate. Thus, these patients require additional attention.

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