

# Sperm cryopreservation of male cancer patients for fertility preservation : 10-year monocentric experience

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## Research Article

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## Abstract

**Background:** Semen cryopreservation is an effective method to preserve fertility, which is very important for male cancer patients. Unfortunately, due to unaware of the opportunities for sperm cryopreservation for both physicians and cancer patients, not a lot of data on evaluating the semen parameters and dispositions of the cryopreserved samples of Chinese cancer population are available in the literature.

**Methods:** We retrospectively reviewed semen parameters as well as the clinical outcomes of assisted reproductive treatments (ART) of 339 male cancer patients of Chinese population who were referred to our center from 2010 to 2019 for fertility preservation.

**Results:** We first classify the male tumors into six major types according to body regions. The most prevalent cancer patients who came from our cohort for sperm banking were hematological neoplasms patients, and the second cancers were germ cell tumors. Patients with germ cell tumors had the lowest pre-thaw and post-thaw concentration among the six major cancer types. However, we separately compared among testicular tumors, lymphoma and leukemia, it turned out that leukemia had the lowest pre-thaw concentration. Most cancer patients (58%) chose to go on keeping their specimens in storage. The second proportion selected to discard their specimens electively (31%). Over the years, there were only 13 patients (4%) returned to use their sperm by ART. In the storage samples, germ cell tumors were the most proportion ones (29.3%). Moreover, in the unfrozen samples, the percentage of hematological neoplasms were the most (45.5%).

**Conclusions:** To our knowledge, we had owned the most numbers of male cancers who came to sperm bank for fertility conservation in the southwest of China. In our study we suggested that sperm quality could decrease even before antineoplastic treatment and sperm banking prior to treatment should be strongly recommended for male cancer patients.

## Introduction

According to World Health Organization (WHO) report, infertility will become the third largest disease after tumors and cardiovascular diseases affecting human life and health in the 21st century [1]. Studies have reported that distress about infertility was more prevalent among male survivors of cancer than unaffected men [2–4]. With the increasing number of patients surviving cancer, there is interest in long-term quality of life for cancer patients and reproductive physicians, especially with respect to cancer-related infertility [5, 6]. Therefore, Attaches great importance has been placed on studying the potential negative effects of cancer therapies: chemotherapy and radiotherapy, even malignancy itself on fertility [7]. At present, sperm banking before cancer treatment for male cancer patients is considered the most effective method to preserve fertility [8, 9].

In China, a few articles have been published regarding fertility preservation about male cancer patients [10]. There was one report performed a retrospective review of sperm cryopreservation for 143 male cancer patients about the Human Sperm Bank of the National Research Institute for Family Planning in Beijing [11]. Due to its late starting this program in China, man fertility preservation awareness is weak and we need to develop a systemic and patient-centered program for offering cryopreservation to all male cancer patients.

Human Sperm Bank, West China Second University Hospital, Sichuan University, has been offered sperm banking to patients for 10 years. Compare to other sperm banks in China, our unit own relatively more populations in which received 1039 men to cryopreserve sperm for birth demand and cancer patients was 339. In this study, the primary aim of our study was to evaluate semen parameters and the dispositions of the cryopreserved samples among the various types of cancer in our 10-year experience of sperm banking. We simultaneously focused on the reproductive outcomes used cryopreserved semen by ART.

## Materials And Methods

### Patients

The study was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University. 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 cancer patients seeking fertility preservation were counseled by an andrology physician and fully informed about the procedure. The information provided included the different methods for fertility preservation, and the procedures for future use of the specimens. All the patients received a physical examination. Medical records of all cancer patients who attempted sperm cryopreservation at Human Sperm Bank, West China Second University Hospital, Sichuan University from January 2010 to December 2019 were reviewed. These data included the following: age at diagnosis, cancer types, pre-and post-cryopreservation semen analysis results, the number of number of straws stored, current banking status, usage of the frozen semen, and the reproductive outcome of using of spermatozoa frozen. The disposition categories were ongoing storage within our unit, discarded, failure to bank, used, and death. The type of cancer was determined by oncologist's letter or histological diagnosis. We followed up cancer patients by telephone.

### Semen samples

According to semen quality, we will counsel patients on the number of samples to bank and potential usage of banked samples. The fresh semen and frozen-thawed specimens were analyzed for sperm concentration and motility according to WHO standardized operating process (WHO, 2010). All samples were collected by masturbation. Cryopreservation of the spermatozoa was performed as follows. On the grounds of the semen quality, the cryoprotectant medium was added to semen at a 1:1 or 1:3 medium: sample ratio. Then straws were stored at 4 °C for 15 minutes and then suspended above of liquid nitrogen 5 cm for 10 minutes before being stored in liquid nitrogen. For semen thawing, straws were placed at room temperature for 1 minute and then were transferred to a beaker containing 37°C water until all ice crystals had disappeared. A diagnosis of azoospermia was given by classical analysis of the semen and confirmed by centrifugation of the entire semen sample.

## Statistical analysis

The statistical methodology consisted of One-way analysis and Kruskal-Wallis tests. In all cases,  $p < 0.05$  was considered statistically significant.

## Results

### Population studied

There were 339 male cancer patients who attempted sperm cryopreservation from January 2010 to December 2019 (Fig. 1A). The cancer diagnoses of the 339 included patients were germ cell tumors (26%), hematological neoplasms (28%), head and neck cancers (19%), thoracic tumors (4%), abdominal tumors (10%), and other tumors (13%) (Fig. 1B). Germ cell tumors included testicular tumors and extragonadal germ cell tumors. Hematological neoplasms included lymphoma and leukemia. The "other" group was composed of skin, sarcoma and peripheral schwannoma. In our unit, we found not only testicular tumors but also extragonadal germ cell tumors had need for fertility preservation. This classification that cancers were devised into six major tumors by body parts was the first showed. The age of patients was  $26.7 \pm 6.8$  years old, among whom 31 patients (9.9%) were adolescents under the age of 18 years.

### Semen parameters

Median sexual abstinence was in the range of a minimum of 2 days and a maximum of 7 days [12]. While a few of the patients had a longer period of sexual abstinence than one week, due to emergency treatment (data not shown). The mean semen volume was  $3.4 \pm 1.5$  mL and a median number of straws stored was 4 per patient among different cancer groups. Before cryopreservation, the overall median sperm concentration was  $47 \times 10^6$ /mL and progressive motility (a + b %) of 49%. Post-thaw analyses after cryopreservation revealed a median concentration of  $30 \times 10^6$ /mL and motility (a + b %) of 28%. There was no difference in change from pre- to post-thaw motility and recovery rate between the various cancer groups. A statistically significant lower sperm pre- and post-thaw concentration was observed in germ cell tumors compared to the other five cancers (Table 1). When comparing the data of leukemia, lymphoma and testicular cancer group, patients with leukemia had the poorest samples, with lower pre-thaw concentration (Table 2).

Table 1  
Patient demographics and semen parameters by cancer diagnosis.

Parameter	Germ cell tumors (n = 81)	Hematological neoplasms (n = 83)	Head and neck cancers (n = 61)	Thoracic tumors (n = 14)	Abdominal tumors (n = 32)	Other Tumors (n = 42)	p-Value <sup>c</sup>
volume at banking (mL) <sup>a</sup>	3.7 ± 1.5	3.2 ± 1.3	3.3 ± 1.6	3.8 ± 1.3	2.7 ± 1.3	3.5 ± 1.6	< 0.0001 <sup>d</sup>
numbers of samples banked <sup>b</sup>	4(1–17)	4(1–10)	4(1–20)	4(2–5)	4(1–12)	4(1–8)	0.5431 <sup>e</sup>
pre-concentration (10 <sup>6</sup> /mL) <sup>b</sup>	26(0.9–159)	41(0.7–222)	64(0.4–620)	47.5(0.4–108)	64(3.3–229)	54.5(1.2–226)	0.0015 <sup>e</sup>
pre-motility a + b (%) <sup>b</sup>	48.5(3.1–83)	48(1.04–75)	51.5(4–73)	53(18–79)	45(8–70)	50(9–81)	0.2334 <sup>d</sup>
post-concentration (10 <sup>6</sup> /mL) <sup>b</sup>	16.5(0.6–89)	28(0.5–120)	42(3.7–193)	23(0.3–52)	40(2.2–120)	37(0.6–148)	0.0041 <sup>e</sup>
post-motility a + b (%) <sup>b</sup>	29(0.66–61)	29(2–60)	30.5(8–64)	23(6–55)	23(3–45)	30(0.7–66)	0.4099 <sup>d</sup>
Recovery rates (%) <sup>b</sup>	58.8(2.2–89.6)	60(9.1–89.6)	62.4(12.1–89.3)	52.5(31.9–73.3)	59(15.2–80.4)	59.3(2.8–89.2)	0.8639 <sup>e</sup>
a: mean ± standard deviation							
b: median (range)							
c: $p < 0.05$ significant							
d: Comparisons between groups were conducted using One-way analysis.							
e: Comparisons between groups were conducted using Kruskal-Wallis tests.							

Table 2  
Characteristics of testicular tumor, leukemia and lymphoma patients.

Parameter	Testicular tumors (n = 68)	Leukemia (n = 18)	Lymphoma (n = 65)	p-Value <sup>c</sup>
age at banking <sup>a</sup>	27 ± 7.5	24.7 ± 7.2	25.1 ± 5.5	0.0115 <sup>d</sup>
volume at banking (mL) <sup>a</sup>	3.8 ± 7.8	2.8 ± 1.3	3.4 ± 1.3	0.0388 <sup>d</sup>
pre-concentration (10 <sup>6</sup> /mL) <sup>b</sup>	30 (0.9–159)	18.5 (0.7–134)	48 (1.5–222)	0.0020 <sup>e</sup>
pre-motility a + b (%) <sup>b</sup>	48.5 (3.1–83)	41.5 (6.69-48)	50 (1.04-73)	0.1595 <sup>d</sup>
post-concentration (10 <sup>6</sup> /mL) <sup>b</sup>	16.4 (0.6–89)	10.9 (0.5–92)	32 (1-120)	0.1436 <sup>e</sup>
post-motility a + b (%) <sup>b</sup>	28 (2.6–61)	18.5 (2–37)	30 (5–60)	0.0563 <sup>d</sup>
Recovery rates (%) <sup>b</sup>	59.5 (6.8–89.6)	53.7 (9.1–77)	60.8 (25–89)	0.5558 <sup>e</sup>
a: mean ± standard deviation				
b: median (range)				
c: p < 0.05 significant				
d: Comparisons between groups were conducted using One-way analysis.				
e: Comparisons between groups were conducted using Kruskal-Wallis tests.				

## Disposition of cryopreserved sperm

We elaborated the cryopreserved sperm states to analyze the further study of improving the utilization. Disposition of cryopreserved sperm categories included ongoing storage (58%), discarded (31%), failure to bank (6%), death (1%) and used (4%) (Fig. 2). Twenty-two patients (6%) failed the sperm cryopreservation due to poor quality (azoospermia, oligoasthenozoospermia, and received chemotherapy). Moreover, we furtherly analyzed the details of men using cryopreserved sperm and their reproductive outcomes. The results showed that leukemia was the most cancers failed cryopreservation (45.5%). We concluded the urgent treatment time for further treatment might be the most reason for this result. The phenomenon needed we communicate with clinicians in a timely manner to ensure leukemia cancers bank sperm before cancer treatment. In the ongoing storage semen, germ cell tumors (29.3%) and hematological neoplasms (26.8%) were the higher proportion (Table 3).

Table 3  
Sperm banking outcomes of six types of cancers.

Parameter	Germ cell tumors	Hematological neoplasms	Head and neck cancers	Thoracic tumors	Abdominal tumors	Other tumors	Total
Ongoing storage	60(29.3%)	55(26.8%)	37(18.0%)	8(3.9%)	21(10.2%)	24(11.7%)	205
Destroyed	22(20.4%)	28(25.9%)	25(23.1%)	6(5.6%)	10(9.3%)	17(15.7%)	108
Used	2(15.4%)	4(30.8%)	4(30.8%)	0(0%)	2(15.4%)	1(7.7%)	13
Death	0(0%)	2(50%)	0(0%)	0(0%)	1(25%)	1(25%)	4
Unfrozen	5(22.7%)	10(45.5%)	4(18.2%)	0(0%)	1(4.5%)	2(9.1%)	22

## The outcome of ART with cryopreserved semen

As of December 31, 2019, only 3.8% (13 out of 339) of patients returned to use their cryopreserved semen for ART. Conceptions were achieved in 66.7% (10 out of 15), with 50% (5 out of 10) of pregnancies resulting in delivery, 20% of pregnancies spontaneous abortions (2 out of 10) and 30% (3 out of 10) clinical pregnancies. There were two couples failed concept, and one embryo cryopreservation. The details of these cases are listed in Table 4.

Table 4  
Details of men using cryopreserved sperm and their reproductive outcomes.

Diagnosis	Age at banking (year)	Numbers of samples	Raw semen			Frozen-thawed semen			ART	Reproductive outcome	
			Volume (mL)	Count (10 <sup>6</sup> /mL)	Motility (%)	Count (10 <sup>6</sup> /mL)	Motility (%)	Recovery rates (%)		Clinical pregnancies	Nur of birt
Testicular tumor	27	6	2.6	3.4	48	2.9	31	64.6	ICSI	live birth	1
Testicular tumor	27	6	6	14	49	10	22	45	ICSI	live birth	1
Leukemia	34	10	2.3	134	48	92	37	77	ICSI	live birth	1
Leukemia	28	4	3.9	9	47	3	30	63.8	ICSI	live birth	1
Lymphoma	37	8	4.3	42	68	28	33	48.5	ICSI/IVF/IUI	no pregnancy	0
Lymphoma	26	3	3.0	31	54	20	35	64.8	ICSI	clinical pregnancies	0
Nasopharyngeal Carcinoma	36	3	2.9	80	37	64	23	62.2	IVF	abortion	0
Nasopharyngeal Carcinoma	35	5	1.8	79	58	NS	NS	NS	IVF	live birth	1
Nasopharyngeal Carcinoma	41	4	3.1	108	52	NS	NS	NS	IVF	no pregnancy	0
Thyroid tumor	32	4	3.6	78	42	66	23	54.8	IVF	abortion	0
Colorectal cancer	46	4	1.6	56	39	43	23	59	ICSI	embryo cryopreservation	0
Gastric cancer	41	3	1.8	93	46	40	25	54.3	ICSI	clinical pregnancies	0
Hemangiosarcoma	28	6	2.5	26	28	16	11	39.3	ICSI	clinical pregnancies	0

Abbreviations: ART, assisted reproduction technology; ICSI, intracytoplasmic sperm injection; IVF, In vitro fertilization; IUI, intrauterine insemination.

Reproductive outcomes of 13 patients who used their banked samples for 15 assisted reproductive cycles.

## Discussion

As cancer survival rates improve, more attention is being directed from issues of cancer treatment toward enhancing quality-of-life issues related to long-term survivorship for cancer patients. Cryopreservation of semen samples is a noninvasive procedure and is the main option for male cancer patients [13].

In our study, we demonstrating that male cancer patients in the Sichuan, China, use sperm banking for fertility preservation. During the 10 years of our study, there were 339 male cancer patients attempted to conduct fertility preservation, the most population at present reported in China. To our knowledge, this is the first report demonstrating that sperm parameters of different cancers types according to body regions. We divide into six portions: germ cell tumors, hematological neoplasms, head and neck cancers, thoracic tumors, abdominal tumors and other tumors. We found that men with germ cell tumors (including testicular cancer and extragonadal germ cell tumor) had an inferior pre-and post-concentration compared with men with other types of cancer. However, when we separately compared testicular cancer, leukemia, and lymphoma we found that leukemia showed the lowest sperm concentration among them. Although there were reported that sperm count and motility was significantly lower in testicular tumors in some prior studies [14–17]. There are several causes of semen quality decline before cancer treatment, such as disruption of the blood-testis barrier and endocrine derangements [18, 19]. Some studies show that cancer patients especially leukemia patients have an innate suppression of spermatogenesis, but the mechanism for suppression is under studied [15, 20, 21]. So, not so paradoxically, testicular tumors needing removing one testicle and blood tumor (including Hodgkin's lymphoma, non-Hodgkin's lymphoma and leukemia) causing fever both resulted in lower sperm concentrations than other cancers.

Cancer patients choose to use their cryopreserved sperm for fertility will result in good chances of fatherhood. Nevertheless, studies report that the rates of frozen semen usage vary between 7–30%, with about 50% of them being successful at in vitro fertilization and intracytoplasmic sperm injection [22, 23]. Over the course of 10 years, 3.8% of the patients used their samples in our facility. The time elapsed between sperm freezing and follow up is the fundamental factor that determines the utilization rate. Without this information it is not possible to evaluate the importance of the result. Even if the observation time is 10 years, it is possible that the last recruited patients had too short a follow up time to be evaluated.

Limitations of our study include inability to follow up patients who were natural conception and not showing the reason patients electively discarded. In future, we will follow up all cancer who came our cohort for sperm banking for their reproductive outcomes and inquire about the reason why patients selected electively discarded to improve patients' storage time.

In contrast to Europe and the USA, there are no practice guidelines advocating fertility preservation for cancers in China. One obstacle to sperm banking is inadequate communication between physician and patients about the risk of post-treatment infertility [24]. Specialists in male reproduction or oncologists

should be discussed with cancer patients about sperm preservation as early as possible. All ways of oncological therapy, including surgical procedures, chemotherapy and radiotherapy have some risk to fertility [4]. We are all talking about adults that can cryopreserve sperm by masturbation. To date, for the prepubertal male patients especially the patients that cannot masturbate to obtain sperm, cryopreservation of sperm is impossible. When it is not possible to obtain sperm, testicular tissue containing spermatogonia stem cells (SSCs) can be obtained by biopsy. Exciting basic science work is under way that addresses the unmet fertility preservation and may expand fertility options for men in the future.

## Conclusion

In conclusion, our study supports studies that sperm banking is an effective way of fertility preservation in cancer patients. Fertility preservation utilization is very low among Chinese male cancer patients. Therefore, reproductive physicians and oncologists are required to discuss fertility preservation before gonadotoxic therapy with all male cancer patients. Sperm cryopreservation is a quick and effective technique that can preserve fertility. Development of local clinical guidelines and the organization of conference to promote the fertility preservation should be encouraged.

## Abbreviations

ART: assisted reproductive treatments; WHO: World Health Organization; SSCs: spermatogonia stem cells; EGTC, extragonadal germ cell tumors; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, Chronic myelocytic leukemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; NPC, nasopharyngeal carcinoma; ICSI, intracytoplasmic sperm injection; IVF, In vitro fertilization; IUI, intrauterine insemination.

## Declarations

### Acknowledgements

Not applicable.

### Author Contributions

Xiao Liu wrote the article and prepared the tables and figures. Bo Liu, Xiao Xiao, Li Wang, Xiaofang Zhu and Bizhen Shu verified all data collected. Wenrui Zhao carried out the data analysis. Xiao Liu, Bo Liu, Shasha Liu and Yang Xian freeze semen. Min Jiang and Bin Zhou provide technical support. Fuping Li designed the study and edited the article. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University. Informed written consent was obtained from all patients prior to their enrollment in this study.

### Conflict of Interest

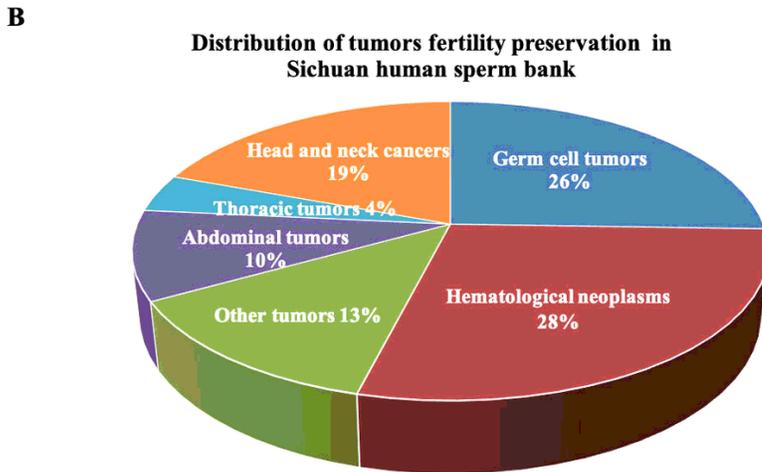
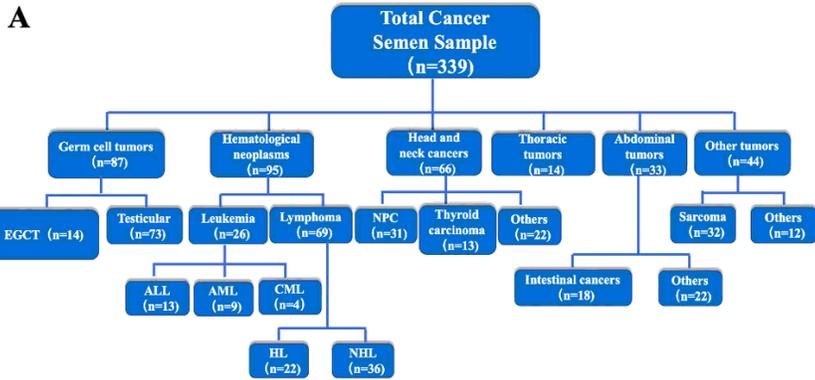
The authors declare that they have no competing interest.

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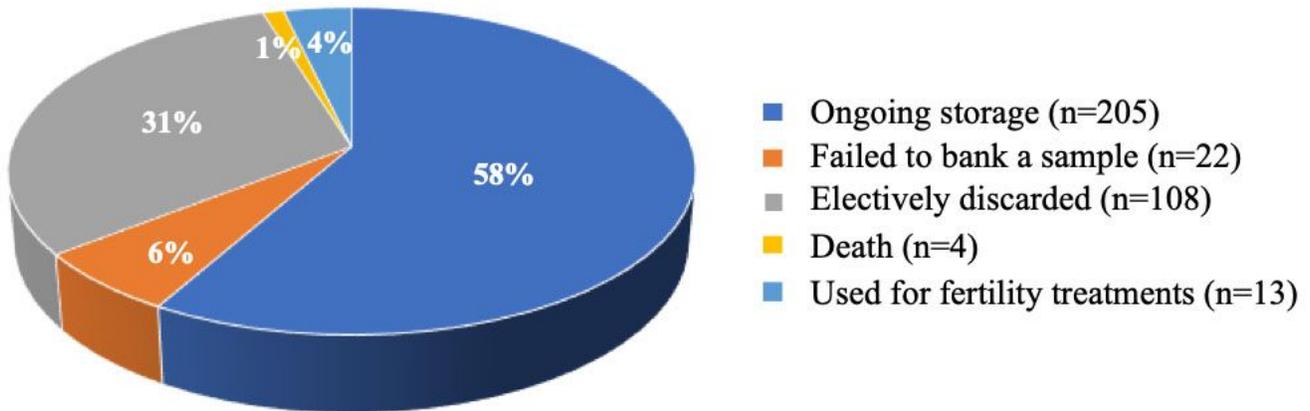
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## Figures



**Figure 1.** Flow diagram of cancer men who presented to Sichuan human sperm bank from 2010 to 2019 for sperm cryopreservation. Abbreviations: EGTC, extragonadal germ cell tumors; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, Chronic myelocytic leukemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; NPC, nasopharyngeal carcinoma.

**Figure 1**  
Flow diagram



**Figure 2.** Outcomes for the cancer patients who attempted banking

**Figure 2**  
Outcomes for the cancer patients who attempted banking