Sperm cryopreservation for fertility preservation in male patients with cancer at a single-center in Japan

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Abstract
Advanced cancer treatments such as chemotherapy and radiation have improved the prognosis of cancer survivors. Currently, the 5-year survival rates of cancers in most types have exceeded 70% in Japan[1]. Therefore, the peritreatment preservation of fertility in adolescent and young adult populations is very important. For male patients, sperm cryopreservation before the initiation of treatment for cancer is currently the most effective method for the preservation of fertility. We performed a retrospective cohort analysis of a sperm cryopreservation protocol at the Yokohama City University Medical Center between 2012 and 2017. A total of 235 men were referred and attempted sperm cryopreservation during this period. The most prevalent diseases were hematological malignancies (38.7%; leukemia, malignant lymphoma, and multiple myeloma), testicular cancer (32.3%, including extragonadal germ cell tumor), bone and soft tissue sarcoma (8.5%), lung cancer (4.7%), prostate cancer (4.3%), and brain tumor (2.6%) in descending order. The median age of patients was 31 (range: 13–65) years, mean sperm density and motility were 28.54 ± 30.25 106/mL, and 20.38 ± 20.62%, respectively. Sperm cryopreservation was successfully performed in 195 patients (83.0%). In those with cryopreservation failure (36 patients; 15.3%), the primary causes were azoospermia or poor semen quality. In the remaining 4 patients (1.7%), testicular spermatozoa were successfully cryopreserved through surgical extraction. In the cryopreservation failure group (n = 36), 23 patients (63.9%) were referred after initiation of treatment. Of those, 17 patients were referred from the departments of hematology and oncology. Moreover, sperm density was significantly lower in patients who under gone treatment than in those of the pretreatment group (P = 0.003). Cryopreserved sperm from 18 patients was used in 23 in vitro fertilization cycles, resulting in a clinical pregnancy rate of 56.5% per cycle. It is important to inform other departments regarding the option for sperm cryopreservation before initiating treatment in patients with cancer.

Keywords: Sperm cryopreservation, Fertility preservation, Adolescent and young adults, Cancer treatment, Testicular cancer, Hematological malignancies

Advanced cancer treatments such as chemotherapy and radiation have improved the prognosis of cancer survivors. Currently, the 5-year survival rates of cancers in most types have exceeded 70% in Japan[1]. The available treatments for cancer have been diversified because of the development of new therapeutic options. On the other hand, Saito et al[2] reported that 70% of young patients with cancer wanted to have a child after cancer chemotherapy. However, these treatments may adversely affect the fertility of these patients. Therefore, peritreatment preservation of fertility in adolescent and young adult populations is very important. For male patients, sperm cryopreservation before the initiation of treatment for cancer is currently the most effective method for the preservation of fertility. We performed a retrospective cohort analysis of a sperm cryopreservation protocol at the Yokohama City University Medical Center between 2012 and 2017. A total of 235 men were referred and attempted sperm cryopreservation during this period. The most prevalent diseases were hematological malignancies (38.7%; leukemia, malignant lymphoma, and multiple myeloma), testicular cancer (32.3%, including extragonadal germ cell tumor), bone and soft tissue sarcoma (8.5%), lung cancer (4.7%), prostate cancer (4.3%), and brain tumor (2.6%) in descending order. The median age of patients was 31 (range: 13–65) years, mean sperm density and motility were 28.54 ± 30.25 106/mL, and 20.38 ± 20.62%, respectively. Sperm cryopreservation was successfully performed in 195 patients (83.0%). In those with cryopreservation failure (36 patients; 15.3%), the primary causes were azoospermia or poor semen quality. In the remaining 4 patients (1.7%), testicular spermatozoa were successfully cryopreserved through surgical extraction. In the cryopreservation failure group (n = 36), 23 patients (63.9%) were referred after initiation of treatment. Of those, 17 patients were referred from the departments of hematology and oncology. Moreover, sperm density was significantly lower in patients who under gone treatment than in those of the pretreatment group (P = 0.003). Cryopreserved sperm from 18 patients was used in 23 in vitro fertilization cycles, resulting in a clinical pregnancy rate of 56.5% per cycle. It is important to inform other departments regarding the option for sperm cryopreservation before initiating treatment in patients with cancer.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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or immediately after the treatment of cancer. The objective of this study was to report the current status and feasibility of peritreatment sperm cryopreservation in patients with cancer and raise awareness regarding its importance before the treatment of cancer.

**Materials and methods**

**Patients**

This study retrospectively assessed the medical records of all patients who attempted sperm cryopreservation at the Reproduction Center of Yokohama City University Medical Center between April 2012 and December 2017. The cases in which samples were delivered from other hospital, and cases with noncancer diseases were excluded. The following information was extracted from the medical records: age, type of cancer, timing of consultation (the status before or immediately after treatment), treatment to be performed or ongoing treatment, prefreeze semen parameters, success or failure of cryopreservation, maintenance status, and utilization of thawed sperm. Because we examined maintenance status of cryopreservation in freezable group, subjects of this study were patients whose follow-up durations were $>1$ year.

All patients provided written informed consent before their participation. The study design was approved by the institutional review board of Yokohama City University Medical Center.

**Semen samples for cryopreservation**

Semen samples were provided through masturbation at our hospital with various intervals of sexual abstinence. Analyses of semen were performed according to the recommendations of the World Health Organization (WHO)[11], using the Sperm Motility Analyzing System (SMAS; DITECT Corp., Tokyo, Japan) after 30 minutes of liquefaction at 37°C. Samples in which semen analysis revealed azoospermia or necrozoospermia were not cryopreserved. We termed the successful sperm cryopreservation group “freezable group,” whereas the sperm cryopreservation failure group was termed “nonfreezable group.” The freezing procedure was performed as follows: (1) dilution of semen sample in freezing medium (Sperm Freeze, Kitazato, Co. Ltd, Tokyo, Japan) after 30 minutes of liquefaction at 37°C. Samples in which semen analysis revealed azoospermia or necrozoospermia were not cryopreserved. We termed the successful sperm cryopreservation group “freezable group,” whereas the sperm cryopreservation failure group was termed “nonfreezable group.” The freezing procedure was performed as follows: (1) dilution of semen sample in freezing medium (Sperm Freeze, Kitazato, Co. Ltd, Tokyo, Japan) in equal amounts and transfer into a straw tube; (2) sealing of both edges of the straw tube using a sealer; (3) placement of straw tubes together in 1 column; and (4) suspension of the column in vapor-phase nitrogen 5 minutes before storage in liquid nitrogen until required for analysis.

**Onco-testicular sperm extraction (TESE) procedures**

For patients whose sperm could not be cryopreserved, we proposed the option of onco-TESE. The onco-TESE procedure allows sperm to be obtained before cancer treatment from the normal testicular tissues of patients who do not ejaculate motile sperm. The procedure is as follows: (1) incision on the scrotum and tunica dartos under anesthesia; (2) half-circumferential incision on the tunica albuginea; (3) observation and retrieval of whitish and dilated seminiferous tubules using a microscope; and (4) confirmation of motile sperm and cryopreservation by an embryologist. In patients with testicular cancer, TESE from the affected testicle simultaneously with high orchiectomy as a backbench procedure may be the most reasonable option.

**Maintenance of cryopreservation**

Each year, the patients were asked whether they wished to maintain their cryopreserved sperm specimens in storage or discard them. The straw tubes were destroyed following patient’s request or death.

**Utilization of cryopreserved sperm**

After thawing in a water bath at 37°C, cryopreserved sperm specimens were used for assisted reproductive technologies. In our hospital, all sperm specimens were used for intracytoplasmic sperm injection (ICSI). The preparation of semen samples was performed as follows: (1) 2-layer density gradient centrifugation using 90% and 45% SpermGrad (Vitrolife, Gothenburg, Sweden) and (2) swim-up technique. For ovarian stimulation, both the gonadotropin-releasing hormone agonist and antagonist protocol were used. The procedure for ICSI was performed as previously described. The selection of sperm for ICSI was based on the criteria of normal morphologic sperm defined by the World Health Organization (WHO) in 2010. Blastocysts were vitrified on day 5. In frozen embryo transfer cycles, frozen-thawed blastocyst embryos were transferred into the uterine cavity after preparation of the endometrium. Determination of clinical pregnancy was performed by the presence of the gestational sac and fetal heartbeat at 6 weeks of gestation.

**Statistical analysis**

Statistical analysis of the data were performed using the JMP Pro 12 (SAS Institute Inc., Cary, NC). All data, except patient age, were reported as mean±SD. Comparisons between 2 groups were performed using unpaired t test, whereas those between >3 groups were performed using nonrepeated measures analysis of variance. The comparison between observed frequency and expected frequency was analyzed using the $\chi^2$ test. In all cases, $P<0.05$ was considered statistically significant.

**Results**

**Study population**

A total of 248 patients were referred and attempted sperm cryopreservation in Yokohama City University Medical Center.
from April 2012 to December 2017 (Fig. 1). Of those, 235 met the inclusion criteria for our study. The remaining 13 patients were excluded as cases samples were transferred from other hospitals. The median age was 31 years (range: 13–65 y). The most prevalent original diseases in our cohort were hematological malignancies (38.7%; including leukemia, malignant lymphoma, and multiple myeloma), testicular cancer (32.3%; including extragonadal germ cell tumor), bone and soft tissue sarcoma (8.5%), lung cancer (4.7%), prostate cancer (4.3%), and brain tumor (2.6%) (Table 1, Fig. 2). Of 235 patients who attempted sperm cryopreservation, 195 patients (83.0%) could successfully cryopreserved, while 36 patients (15.3%) failed. The primary causes of sperm cryopreservation failure were azoospermia or poor quality of semen (such as necrozoospermia). In the remaining 4 patients (1.7%), sperm cryopreservation initially failed because of azoospermia or inability to ejaculate. However, testicular sperm was successfully retrieved through onco-TESE and cryopreserved in all cases.

**Timing of consultation**

In the freezable group, 169 of 195 (86.7%) patients consulted the clinic before the treatment of cancer versus 26 patients (13.3%) who were referred after initiation of treatment. In the nonfreezable group, these numbers were 13 of 36 patients (36.1%) and 23 patients (63.9%), respectively. The rate of patients consulting the clinic after initiation of treatment was significantly higher in the nonfreezable group versus the freezable group (63.9% vs. 13.3%; P < 0.001) (Table 2).

**Semen parameters**

The mean sperm density and sperm motility in the 235 patients included in this study were 28.54 ± 30.25 million/mL and 20.38 ± 20.62%, respectively. Among the diseases, the sperm density of patients with testicular cancer was significantly lower than that reported in patients with other diseases (Table 1). Notably, the average sperm concentration (32.40 ± 29.84 vs. 10.03 ± 25.52 million/mL; P < 0.001) and sperm motility (27.88 ± 22.92% vs. 18.26 ± 18.26% vs. 22.01 ± 0.077; P < 0.001) were significantly higher in the freezable group than in the nonfreezable group (Table 2). In the freezable group, the sperm density was significantly lower in patients who had initiated treatment at the time of sperm cryopreservation than in those of the pretreatment group (17.02 ± 15.85 vs. 34.93 ± 30.95 million/mL, respectively; P = 0.003).

**Onco-TESE cases**

Among the patients who failed to cryopreserve the sperm in the ejaculate, 4 patients underwent semiemergent onco-TESE. Three patients were azoospermic with testicular tumors, and one was unable to ejaculate with acute myeloid leukemia. In all cases, testicular motile sperm was successfully retrieved and cryopreserved.

**Maintenance status**

In the freezable group, mean follow-up duration was 45.6 months (15–83 mo). Of 195 patients, 120 (61.5%) patients visited our hospital to maintain cryopreservation. Forty-four (22.6%) patients did not want to extend cryopreservation for various reasons (ie, patients’ deaths, sperm regeneration in the ejaculate, and not hoping for children) and wished to discard. Thirty one (15.9%) patients did not visit to maintain cryopreservation and were unable to contact.

**Utilization of cryopreserved sperm**

The cryopreserved sperm of 18 patients (9.2%) was used, with 23 ICSI cycles performed. The most prevalent diseases among these 18 patients were testicular cancer (7 patients; 38.9%), hematological malignancies (6 patients; 33.3%), and prostate cancer (3 patients; 16.7%). The mean duration from cryopreservation
to utilization was 17.1 (3–39) months. Clinical pregnancies in 13 ICSI cycles (56.5%), and live births in 10 cycles (43.5%) among them were confirmed, respectively. Thereafter, the cryopreserved sperm of 5 patients was discarded because of patient death (1 patient), live birth (3 patients), and other reasons (1 patient), and the straw tubes were destroyed accordingly.

### Discussion

This retrospective study investigated the status and feasibility of pretreatment sperm cryopreservation in patients with cancer in a single institution. Since the first sperm cryopreservation established in 1953, this system has been refined and standardized. There have been many reports regarding sperm cryopreservation for the preservation of fertility\(^{122–15}\). In all these reports, testicular cancer and hematological malignancies (ie, leukemia and malignant lymphoma) accounted for the majority of cases.

As demonstrated in our study, the sperm density in patients with testicular cancer was significantly low (Table 1). Testicular cancer is a relatively rare disease, of which incidence in Japan has been thought to be 1.1–2.7 per 1,000,000 population per year\(^{16}\). It occurs more frequently in patients of reproductive age. In the previous study evaluating 764 male cancer patients prior to cancer treatment, patients with testicular cancer and extragonadal germ cell tumor had significantly lower sperm count than other malignancies\(^{17}\). And other studies involving preoperative semen analysis reported azoospermia and oligozoospermia in 10%–15% and > 50% of patients with testicular cancer, respectively\(^{18,19}\). This may be attributed to testicular dysgenesis syndrome, as hypothesized by Skakkebaek\(^{20}\). Testicular dysgenesis syndrome results from disruptions that impair normal testicular development, leading to defective sperm production and higher rates of testicular cancer. Moreover, Haddad et al\(^{21}\) identified tumor toxicity, high temperature, mass effect, and paracrine effects (eg, interleukin-1, interferon-γ, and leukemia-inhibiting factor) as factors affecting testicular tissue, leading to defective spermatogenesis. This may explain the relatively high number of patients with testicular cancer belonged to nonfreezable group despite pretreatment.

Moreover, chemotherapy plays a central role in the treatment of cancer, especially in patients with testicular cancer and hematological malignancies. Numerous anticancer drugs cross the blood-testis barrier, damaging germ cells\(^{22,23}\). The degrees of gonadotoxicity vary depending on the type of drug, dosage, and treatment cycles\(^{24}\).

Advanced germ cell tumors are curable and treated with multiple cycles of bleomycin/etoposide/cisplatin (BEP) chemotherapy following high orchiectomy. The gonadotoxic effect of cisplatin is considered moderate\(^{25}\). However, in the group treated with 5–6 cycles of BEP, regeneration of spermatogenesis was significantly delayed and none of the patients reestablished spermatogenesis within 2 years\(^{24}\). Retroperitoneal lymph node dissection for para-aortic lymph node metastasis may result in injury of the sympathetic nerve system and cause an ejaculatory disorder\(^{25}\). Therefore, in cases with advanced testicular cancer, presurgical sperm cryopreservation should be promptly attempted regardless of the pathologic type. Moreover, in cases with azoospermia or inability to cryopreserve, onco-TESE simultaneously with high orchiectomy should be performed\(^{26}\). In this cohort, we performed onco-TESE in 3 patients with testicular cancer and were able to cryopreserve their testicular sperm\(^{27}\).

Hematological malignancies, including leukemia and malignant (Hodgkin or non-Hodgkin) lymphoma, are diseases in which complete remission may be expected following treatment. In the case of leukemia (acute/chronic and myeloid/lymphoblastic), immediate remission induction therapy may be required and the treatment may be shifted to hematopoietic stem cell transplantation according to the course of the disease\(^{28}\). Before hematopoietic stem cell transplantation, administration of a large dosage of an alkylating agent or total body irradiation may be performed\(^{29,30}\). Alkylating agents, including cyclophosphamide, busulfan, and melphalan, have been classified into highest risk group of prolonged azoospermia according to the guideline of ASCO in 2013\(^{31}\). They induce cellular apoptosis by impairing DNA synthesis and RNA transcription. And they are often used in combination with other anticancer drugs or irradiation as a pretreatment before hematopoietic stem cell transplantation for leukemia and other hematological malignancies, which have severe gonadotoxic effects irreversibly\(^{32}\). Meistrich\(^{33}\) reported that a total dose of cyclophosphamide exceeding 7.5 g/m\(^2\) resulted in prolonged or permanent azoospermia. Total body irradiation may also affect the testes; doses of 9–10 Gy have been shown to produce gonadal dysfunction. Regarding malignant lymphoma, the standard regimen for the treatment of Hodgkin lymphoma is Adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) therapy, whereas and for non-Hodgkin lymphoma, the standard regimen is cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) therapy. Bujan and colleagues reported that patients already had altered sperm characteristics before the treatment of lymphoma and sperm density and motility decreased after

### Table 2

|                | Pretreatment | Posttreatment | Pretreatment | Posttreatment | P    |
|----------------|--------------|---------------|--------------|---------------|------|
| N              | 169          | 26            | 13           | 23            | <0.001† |
| Sperm density (million/mL) | 32.40 ± 29.84* | 10.03 ± 25.52* | 3.66 ± 9.36  | 11.31 ± 29.13 | <0.001‡ |
| Sperm motility (%) | 27.88 ± 19.68* | 1.24 ± 4.50*  | 22.84 ± 23.86 | 1.50 ± 3.80   | <0.001‡ |

*Data were expressed as mean ± SD.
†Comparison between 2 groups were performed using χ\(^2\) test.
‡Comparison between 2 groups were performed using unpaired t test.
*P<0.003.
‡P=0.008.
treatment, with the lowest values observed at 3 and 6 months after treatment[34,35]. They also reported that, 12 months after treatment, the sperm density recovered to that reported before treatment. However, this effect was only observed in patients treated with ABVD therapy—not in those treated with CHOP therapy[36]. Furthermore, at 24 months, 7% of the patients treated with ABVD therapy remained azoospermic. Afterward, > 90% of patients treated with ABVD therapy recovered to normal sperm count versus 61% of those treated with CHOP therapy. Therefore, patients with hematological malignancies should attempt sperm cryopreservation before treatment as soon as possible. In the case of inability to ejaculate or azoospermia after initiation of treatment, the onco-TESE approach may be considered[36].

For adolescent and young adult cancer survivors, preservation of fertility before treatment of cancer is of crucial importance in both sexes. Therefore, oncologists should present this option to patients prior to initiating treatment[36]. According to the clinical guidelines established by the American Society of Clinical Oncology (ASCO) updated in 2018, health care providers caring for adult and pediatric patients with cancer should address the possibility of infertility as early as possible before initiation of treatment and refer to reproductive specialists those who express an interest[36]. However, the treatment of cancer is the top priority, and the principle of fertility preservation is that treatment should be performed without delay.

Our study demonstrated that sperm cryopreservation was successful in 83.0% of patients with cancer, a finding that is consistent with those of a previous report[12–15]. The majority of these patients (86.7%) were referred prior to the initiation of treatment. Notably, many of those in whom cryopreservation failed (63.9%) were referred after initiation of treatment. Hematological malignancies, including leukemia, in young patients require prompt treatment, and it is considered that many miss the opportunity for the preservation of fertility prior to treatment. According to a previous report, the factors impeding pretreatment sperm cryopreservation were scheduled hematopoietic stem cell transplantation, patient age, requirement for urgent treatment, disease stage, and disease type[10]. Yumura et al[37] reported that many hematologists did not have adequate information regarding the preservation of fertility, and few institutions performed sperm cryopreservation and a considerable burden was required for hematologists. Therefore, cooperation between oncologists and reproductive specialists is indispensable for sperm cryopreservation prior to treatment. For this purpose, establishing systems that enhance such cooperation and introduce patients to sperm cryopreservation is vital.

On the contrary, utilization rate of cryopreserved sperm was as low as 5.1%, which was a little lower than previously reported. One of the reasons was that the patients who were younger at the time of sperm cryopreservation might not have hope for having children yet because the follow-up duration was short.

The limitation of this study was the outcome of short follow-up duration and small number of cases from single-center in Japan. For a short follow-up duration, number of cases were relatively large. The number of cancer patients whose sperm were cryopreserved for fertility preservation was top-class in a single-center in Japan.

One of the strengths of this study was that we usually proposed the option of semiemergent onco-TESE for patients whose sperm in the ejaculate could not be cryopreserved (ie, those who have never ejaculated, or whose semen analysis revealed azoospermia, severe oligozoospermia, or necrozoospermia). However, onco-TESE is not always feasible in a timely manner. In order not to miss the opportunity for the preservation of fertility, it is important to establish a system enabling patients to consult facilities able to perform onco-TESE.

Other strength was that this study evaluated the maintenance status of sperm cryopreservation for fertility preservation for the first time. Maintenance rate was not good at all, and there were a lot of patients who had not visited medical institution their sperm were cryopreserved, so it was considered vital to establish a network to encourage them to visit there.

Conclusions
Sperm cryopreservation ahead of gonadotoxic treatment is less-invasive and reliable to preserve fertility for the patients with cancer. For patients who were unfortunately unable to cryopreserve ejaculated sperm, onco-TESE should be recommended if the timing is right. In order to carry out fertility preservation of patients with cancer smoothly, close cooperation between oncologists and reproduction specialists is considered to be essential.

Conflict of interest statement
The authors declare that the present research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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