[8]Taniguchi Department of Ophthalmology and Genecology,

[9]Yamaguchi University,

[10]Tokyo Rosai Hospital,

[11]Fukuda Women's Clinic

Corresponding author:

Abstract

Purpose: Interest in fertility preservation therapy has increased due to improved survival rates and life expectancies attributed to cancer treatments. However, the conditions of facilities that conduct cryopreservation are unclear, and disparities in culture techniques among such facilities are concerning. The status of embryo freezing for the fertility preservation of cancer patients in Japan was clarified via a nationwide survey of embryologists at fertility preservation treatment institutions.

Methods: This study included 622 institutions registered by the Japan Society of Obstetrics and Gynecology for in vitro fertilization and embryo transfer. An online survey regarding the status of embryo freezing activities was completed by embryologists.

Results: Embryo freezing for general assisted reproductive is performed by all 352 institutions that responded, though only 178 (50.6%) perform embryo freezing for the purpose of fertility preservation. Forty-one institutions (23.0%) reported using different criteria or personnel when performing

cryopreservation for the purpose of fertility preservation versus for fertility treatment. Twenty-seven institutions (15.2%) reported freezing pronuclear stage embryos, 150 (84.3%) reported freezing cleavage stage embryos, and 165 (92.7%) reported freezing blastocyst stage embryos. All institutions in this study used the vitrification freezing method, and more than 90% followed the manufacturer's recommended protocol for freezing and thawing embryos.

Conclusions: Embryo freezing is not actively implemented as a fertility preservation therapy in Japan. Data regarding the current state of fertility preservation therapy for patients with cancer and treatment and results following such therapy are insufficient. Further studies are needed to ensure that patients with cancer have the opportunity to preserve their fertility without major concerns, ultimately improving their quality of life after treatment.

Keywords:

Introduction

Recent advances in medical technology have led to significant improvements in the survival rate and life expectancy of patients with cancer, resulting in increased interest in fertility preservation therapy and awareness regarding the psychological impact[1] of initiating cancer treatment after fertility preservation therapy. Efforts to ensure a high quality of life after cancer treatment have also increased. Fertility preservation options for women include unfertilized egg freezing, embryo freezing, and ovarian tissue freezing. Embryo freezing is a crucial assisted reproductive technology (ART). It is considered a well-

established treatment, as thawed embryos have implantation rates similar to those of fresh embryos.[2] Therefore, when married women pursue fertility preservation, embryo freezing is the most common method used. In Japan, the incidence of cancer among women of reproductive age[3] is increasing, and the number of patients with cancer who desire embryo freezing for the purpose of fertility preservation is expected to continue to increase.

Embryologists play a major role in ART and cryopreservation techniques. The skill of the embryologist significantly affects the outcome of fertility preservation therapy. However, the present state of embryo freezing for fertility preservation ART in Japan is unclear. For example, the appropriate developmental stages for embryo freezing, freezing and thawing methods, embryo transfer methods after thawing, and culture techniques and methods for patients with cancer are controversial. Therefore, there may be treatment result disparities between regions and facilities. Previous reports regarding fertility preservation in Japan include a clinical study aimed at establishing clinical application techniques for the cryopreservation of unfertilized eggs that was conducted by the Japan Association of Private Assisted Reproductive Technology Clinics and Laboratories from 2007 to 2015.[4] In 2014, the Japanese Society of Clinical Oncology conducted a survey to formulate clinical practice guidelines for fertility preservation in patients with cancer[5] and reported the actual conditions of fertility preservation in Japan. However, no studies regarding embryo freezing as a technique for preserving fertility among patients with cancer have been conducted in Japan. Therefore, the status of embryo freezing for fertility preservation in Japan is

poorly understood.

Therefore, a survey of embryologists working at fertility preservation facilities throughout Japan was conducted to investigate the current status of embryo freezing for fertility preservation in patients with cancer in Japan and to establish fertility preservation techniques and a medical environment that will enable the provision of uniform and advanced fertility preservation therapies in the 47 prefectures of Japan.

Materials and Method

This study was approved by the Bioethics Committee of St. Marianna University School of Medicine (approval number 5093). The online survey was distributed to 622 facilities that conduct in vitro fertilization (IVF) and embryo transfer registered with the Japanese Society of Obstetrics and Gynecology (ART-registered facilities). The respondents were individual embryologists working at each facility. A letter of invitation and a QR code for the primary survey were sent to the hospital director or medical department manager of each facility, and the survey was conducted online. Macromill Co., Ltd. (Tokyo, Japan) was commissioned to design the online survey system. The response period was open from February 26, 2021 to March 24, 2021. The questionnaire included 21 items regarding the background of the institution, the culture solution and devices used for the cryopreservation of embryos, the freezing method, and the problems encountered while implementing fertility preservation therapy. All respondents

86 provided consent prior to beginning the survey. The survey also allowed respondents to withdraw their
87 consent at any time during the survey. Privacy protection was ensured by setting and managing access
88 restrictions via the assignment of individual identifications and passwords.

89 **Results**

90 A total of 352 Japan Society of Obstetrics and Gynecology ART-registered institutions responded to the
91 survey (response rate: 56.6%). Embryo freezing is performed for general patients undergoing ART at all
92 responding institutions (100%). However, only 50.6% (178/352) of instructions reported performing
93 embryo freezing for the purpose of preserving fertility in patients with cancer (Fig. 1), of which 83.7%
94 (149/178) were medically endorsed by the Japan Society of Obstetrics and Gynecology.

95 The target age for embryo cryopreservation for patients with cancer was restricted by 55.4% of
96 institutions (93/168), including 51.2% (53/91) with an upper limit and 41.8% (38/91) with both upper and
97 lower age limits (Fig. 2). The median upper limit was 45 years (range: 39-50 years), and the median lower
98 limit was 16 years (range: 0-20 years).

99 Forty-one institutions (23.0%) reported using an embryo freezing method that differed from that used
100 for general patients undergoing ART (Fig. 3), including 31 (75.6%) that reported lowering the grade of
101 the embryos to be frozen and cryopreserving more embryos; 14 that reported assigning more experienced
102 embryologists for patients with cancer undergoing embryo freezing; and nine (22.0%) that reported

103 actively implementing Split-intra-cytoplasmic sperm injection (ICSI), a combination of conventional IVF
104 and ICSI during the same cycle, to obtain more embryos based on the risk of non-fertilization when
105 conventional IVF was conducted (Fig. 4).

106 Most institutions (79.2%; 141/178) employed five or fewer embryologists who were capable of
107 freezing and thawing embryos for patients with cancer. Twenty-eight institutions (15.7%) employed 6-10
108 capable embryologists, three (1.7%) employed 11-15, four (2.2%) employed 16-20, and two (1.1%)
109 employed 21 or more (Fig. 5).

110 The developmental stage at which the embryo was frozen was the pronuclear stage at 15.2% (27/178)
111 of institutions, the cleavage stage at 84.3% (150/178), and the blastocyst stage at 92.7% (165/178) (Fig.
112 6).

113 All 178 institutions (100%) reported using the vitrification method, and one institution (X%) reported
114 using the slow freezing method. The majority of institutions reported using cryopreservation devices from
115 company A (87.1%; 155/178), while 3.4% (6/178) used devices from company B, 23.0% (41/178) from
116 company C, and 0.6% (1/178) from company D. Two institutions (1.1%) reported using homemade
117 cryopreservation devices, and one (0.6%) used devices from another source (Fig. 7). Most institutions
118 (89.3%; 159/178) reported using an open type device, 5.1% (11/178) reported using a closed type device,
119 and 3.9% (7/178) reported using open and closed type devices. The type of device was unknown or not
120 reported in one institution (0.6%) (Fig. 8). In addition, most institutions (83.7%; 149/178) reported using

121 cryopreservation solution from Company A, 0.6% (1/178) from Company B, 22.5% (40/178) from
122 Company C, and 5.1% (9/178) from Company D. Five institutions (2.8%) reported using homemade
123 cryopreservation solution (Fig. 9). Most institutions (81.5%; 145/178) reported using thaw solution from
124 Company A, 0.6% (1/178) from Company B, 21.9% (39/178) from Company C, and 5.6% (10/178) from
125 Company D. Seven institutions (3.9%) reported using homemade thaw solution, and one institution (0.6%)
126 used thaw solution obtained from another source (Fig. 10).

127 Embryo freezing was implemented according to the manufacturer's recommended protocols by 98.4%
128 (60/61) of institutions for pronuclear stage embryos, 95% (151/159) for cleavage stage embryos, and 93.2%
129 (165/177) for blastocyst stage embryos (Fig. 11). Modifications to the protocols included performing
130 artificial shrinkage treatments before freezing, changing the volume of the freezing culture medium,
131 changing the duration of immersion in the freezing culture medium depending on the developmental stage
132 and condition of the embryo, and changing the dish used (Table 1). Thawing was conducted in accordance
133 with the manufacturer’s recommended protocols at 93.8% (61/65) of institutions for pronuclear stage
134 embryos, 93.1% (149/160) for cleavage stage embryos, and 91.5% (162/177) for blastocyst stage embryos
135 (Fig. 12). Modifications to the protocol included changing the duration of immersion in the culture medium
136 according to the developmental stage and condition of the embryo, changing the osmotic pressure of the
137 culture medium, and changing the volume of the culture medium (Table 2).

138 A total of 45 institutions responded to an open-ended question regarding problems embryologists face

when freezing embryos for patients with cancer, including 13 (31.1%) who expressed concerns regarding the management of frozen embryos after fertility preservation therapy, including the storage period, cost, renewal procedures, and protocol in the event of hospital closures. Thirteen respondents (28.9%) addressed concerns regarding the criteria for freezing embryos, including the stage and grade of embryo development and the number of embryos required. Eight institutions (17.8%) reported concerns regarding communication with patients, such as monitoring the treatment status of the primary diseases, changes of address, divorce or bereavement, and loss of communication. Six institutions (13.3%) wrote about securing storage space for preservation tanks. Another six (13.3%) reported other concerns, including an inability to perform egg retrieval due to prioritizing the treatment of the primary disease, difficulties explaining the expected outcomes without knowing the impact of the primary disease on embryonic development, the pressure of failure, the protocol for responding to a failure, the protocol for transferring embryos after the primary disease was treated, and differences between fertility preservation protocols for patients with cancer and general patients undergoing ART.

Discussion

Cryopreservation technologies for reproductive cells such as unfertilized eggs, sperm, embryos, and ovarian tissue are essential for fertility preservation in oncofertility. Among these, embryo freezing can be performed using the same methods used for general patients undergoing ART. The results of this survey indicate that embryo freezing is performed for general patients undergoing ART at all of the institutions

157 that responded to the survey. However, approximately half of the respondents conduct embryo freezing for
158 the purpose of fertility preservation. Oncofertility treatment requires close collaboration between
159 oncologists who treat the primary disease and reproductive medicine specialists who perform fertility
160 preservation. Inter-facility collaboration is being conducted at the national level in the United States and
161 Europe.[6] However, in Japan, most patients undergoing ART are treated in private clinics, highlighting the
162 importance of a network to foster collaboration between facilities that provide cancer treatment. At present,
163 the formation of this network is being led by the Japan Society for Fertility Preservation.[7] The small number
164 of embryo cryopreservation facilities for fertility preservation may be a consequence of regional
165 characteristics and specific conditions at each facility; however, this concern is not within the scope of this
166 study. As approximately 20,000 women in their 20s and 30s are diagnosed with cancer each year in Japan,[8]
167 the treatment environment for fertility preservation therapy is underdeveloped.

168 In addition, to perform fertility preservation therapy according to the medical indications, the
169 Recommended Medical Indications for Freezing and Preserving Unfertilized Eggs, Embryos (Fertilized
170 Eggs), and Ovarian Tissue was enacted by the Japanese Society of Obstetrics and Gynecology in April
171 2014. These recommendations require accreditation for fertility institutions. However, in this survey, 29
172 facilities were conducting embryo freezing for the preservation of fertility without accreditation. As part
173 of a national research promotion project, financial support for these institutions was initiated in April
174 2021, though institutions must be accredited as a medical institution for fertility preservation therapy by

the Japanese Society of Obstetrics and Gynecology to receive such funds. This research promotion project is expected to establish data regarding fertility preservation therapy for pediatric, adolescent, and young adult patients with cancer.

Several facilities set age restrictions for embryo freezing for patients with cancer. The lower age limit is set due to the need for vaginal manipulation for egg retrieval and sperm for fertilization, while the upper age limit is set in consideration of the perinatal risk during pregnancy, as the thawed embryo transfer that follows fertility preservation therapy occurs after treatment of the primary disease is finished.

Furthermore, fertility preservation therapy for patients with cancer must be performed within the limited time before treatment for the primary disease and cannot delay the primary treatment. Therefore, some institutions change the patient criteria, fertilization methods, developmental stage during embryo freezing, and grade of frozen embryos. The risk of failure is lowered as experienced embryologists often conduct embryo cryopreservation for fertility preservation in patients with cancer. Approximately 80% of the respondents in this study employ five or fewer embryologists capable of freezing and thawing embryos for patients with cancer, suggesting that the number of embryologists involved in cancer reproductive medicine is relatively small.

Vitrification was used as the freezing method at all of the institutions , and nearly all of the institutions used cryopreservation devices, freezing solutions, and thawing solutions from two companies, Company

192 A and Company C. Announced in 2000, the Cryotop method,[9] which became popular worldwide due to
193 its high survival rate after freezing and thawing, was subsequently improved by various companies that
194 developed products for vitrification freezing. A recent study reported that the addition of fatty acids to the
195 thawing solution improves the developmental capacity of embryos after thawing.[10] Further improvement
196 to embryo freezing methods are expected in the future.

197 Two types of freezing devices are used: open, in which the embryo is in direct contact with liquid
198 nitrogen, and closed, in which there is no direct contact between the embryo and liquid nitrogen. In
199 Western countries, closed-type devices are used to avoid viral contamination[11] though most facilities in
200 Japan use open-type devices. Cryopreservation for fertility preservation may require a long preservation
201 period, indicating that significant reviews of the safety, techniques, and cost of cryopreservation and the
202 development of safer methods are necessary.

203 Though there have been no major changes in the freeze-thaw protocols provided by each manufacturer
204 since the development of cryopreservation supplies, the survival rate of the embryos is high, leading to
205 most institutions using the manufacturer’s recommended protocols. However, several institutions based
206 their protocols on veteran embryologists' experiences in culture work to modify the protocols, ultimately
207 improving treatment outcomes. The establishment of more effective protocols will require studies
208 regarding the details of these modifications and their effects on treatment outcomes.

The open-response comments regarding problems in fertility preservations provided insight into the challenges of fertility preservation therapy, indicating that several institutions put embryologists in charge of administrative tasks, including direct communication with patients, which is traditionally not a role of embryologists. As patients who require fertility preservation therapy are typically struggling with a variety of health concerns before, during, and after fertility treatment, it is important for embryologists who interact with these patients to improve their social skills, including their bedside manner and word choice.

The results of this survey indicate that embryo freezing for fertility preservation therapy by embryologists in Japan is not being actively implemented despite its similarities to the procedure used for general patients undergoing ART. Embryologists expressed concern regarding the patients' primary disease, leading to doubts and apprehensions regarding the success of cryopreservation. These concerns may be due to a lack of information regarding the current status of fertility preservation therapy for patients with cancer, current treatments, and outcomes after fertility preservation. This is the first Japanese survey of embryologists regarding the status of fertility preservation therapy, and it has provided insight into the status of embryologists' work on fertility preservation therapy in Japan. However, more detailed investigations, including those regarding treatment results, are necessary. For patients with cancer to be able to concentrate on treating their primary disease and improving their quality of life after treatment, more research regarding fertility preservation is necessary.

The findings of this survey study will enable embryologists to actively engage in fertility preservation therapy and create an environment in which patients with cancer can receive a high standard of treatment anywhere in Japan.

Acknowledgements: None

Disclosures

Conflicts of interest: The authors declare no Conflicts of Interest for this article.

Human/Animal Rights: This article does not contain any studies with human and animal subjects performed by any of the authors.

Approval by ethics committee: This study was approved by the Bioethics Committee of St. Marianna University School of Medicine (approval number 5093).

References

1) Gonçalves V, Ferreira PL, Saleh M, Tamargo C, Quinn GP. Perspectives of Young Women with Gynecologic Cancers on Fertility and Fertility Preservation: A Systematic Review. Oncologist. 2022 Mar 11;27(3):e251-65.

2) Yu H, Zhang Y, Han M. Comparison of Clinical Pregnancy Outcomes after Fresh and Frozen Embryo Transfer. Comput Math Methods Med. 2022 Oct 3;2022:6498356.

3) Tanaka S, Palme M, Katanoda K. Trends in cervical cancer incidence and mortality of young and middle adults in Japan. Cancer Sci. 2022 May;113(5):1801-7.

4) Kato K, Ochi M, Nakamura Y, Kamiya H, Utsunomiya T, Yano K. A multi-centre, retrospective case series of oocyte cryopreservation in unmarried women diagnosed with haematological malignancies. Hum Reprod Open. 2021 Jan 16;2021(1):hoaa064.

5) Okamoto S, Fujii N, Yoshioka N, Harada M, Tanimoto M, Maeda Y, et al. Nationwide survey of fertility preservation in patients with hematological malignancies in Japan. Int J Clin Oncol. 2021 Feb;26(2):438-42.

6) von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, et al. Fertility-preservation counselling and treatment for medical reasons: date from a multinational network of over 5000 women. Reprod Biomed Online. 2015 Nov;31(5):605-12.

7) Suzuki N. Oncofertility in Japan: advances in research and the roles of oncofertility consortia. Future Oncol. 2016 Oct;12(20):2307-11.

8) Katanoda K, Shibata A, Matsuda T, Hori M, Nakata K, Narita Y, et al. Childhood, adolescent and young adult cancer incidence in Japan in 2009-2011. Jpn J Clin Oncol. 2017 Aug 1;47(8):762-71.

9） Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 2007 Jan 1;67(1):73-80.

10） Ohata K, Ezoe K, Miki T, Kouraba S, Fujiwara N, Yabuuchi A, et al. Effects of fatty acid supplementation during vitrification and warming on the developmental competence of mouse, bovine and human oocytes and embryos. Reprod Biomed Online. 2021 Jul;43(1):14-25.

11） Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C. Viral contamination of embryos cryopreserved in liquid nitrogen. Cryobiology. 2000 Mar;40(2):110-6.

Tables

Figure legends