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FERTILITY PRESERVATION

Radiofrequency identification tag system improves the efficiency of closed vitrification for cryopreservation and thawing of bovine ovarian tissues

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Abstract

Purpose A radiofrequency identification (RFID) tag system was designed to streamline cryopreservation and thawing procedures. This study evaluated the usefulness of the RFID tag system for improving the efficiency of cryopreserving/thawing bovine ovarian tissue by the closed vitrification protocol.

Methods Six participants carried out closed vitrification and thawing of bovine ovarian tissues procedures using either the conventional or the new RFID tag method, and the time required to perform each step of the respective methods was measured. After normality of data was confirmed by the Shapiro-Wilk test, the significance of differences was assessed by the unpaired *t* test.

Results When closed vitrification was performed, the time required for each step showed a significant difference between the two methods ($t(4) = 2.938$, $p = 0.042$, $d = 2.40$), and the total cryopreservation time was 11 min shorter using the RFID tag system. When thawing was performed, the time required for each step also showed a significant difference between the two methods ($t(4) = 2.797$, $p = 0.049$, $d = 2.28$), and the total thawing time was 2 min shorter using the RFID tag system.

Conclusion The RFID tag system tested in this study seems to be suitable for managing biological samples stored in liquid nitrogen. Adoption of an RFID tag system by fertility centers may not only improve the efficiency of cryopreserving/thawing reproductive tissues but could also reduce human error.

Keywords Infertility treatment · Cryopreservation · Thawing · Ovarian tissue closed vitrification · Frozen samples · RFID tag · Storage system

Introduction

Advances in medical treatment have led to an increase of patients surviving cancer, resulting in more healthcare providers recognizing the importance of fertility preservation for cancer patients. It is important to offer cancer patients the option to preserve their reproductive tissues (oocytes, sperm, embryos, or ovaries) before initiation of treatment since many anticancer therapies reduce fertility [1–9]. Cryopreservation of reproductive tissues can be performed by fertility centers until a cancer survivor is ready to start a family, but storage time of the reproductive tissues may be as long as decades in the case of children with cancer [10]. Accordingly, meticulous management of the stored reproductive tissues from many patients over a prolonged period is required and this is a challenge for every fertility centers.

Conventional methods for managing cryopreserved tissues have several flaws. Since most steps in the cryopreservation process are carried out manually, human error can be a significant problem, as emphasized by several reported cases (Kagawa, Japan, in 2008, Singapore in 2010, and Rome, Italy, in 2013). In recent years, two-dimensional barcode

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systems marketed by Merk or Oligio have improved the efficiency of managing cryopreserved tissues by minimizing manual data entry, but practical use of such systems is challenging for samples stored in liquid nitrogen (LN2) because frost forms on cryogenic vials and interferes with readability. Therefore, it is necessary to wipe the label dry before reading it with a reader, which leads to a risk of damaging the sample if its temperature rises to about − 135 °C. In addition, the current design of storage systems contributes to low efficiency in tracking and retrieving patient samples. The need for an efficient storage system that allows easy tracking/retrieval of cryopreserved tissues and reduces manual data entry has never been stronger in the fertility preservation field. We have designed a new storage system and a new device for preserving ovarian tissues that combines the use of two-dimensional barcode labels with radiofrequency identification (RFID) tags. The major advantages of using RFID tags are to eliminate manual data entry and allow readability when samples are stored in LN2. Patient information is transferred to the RFID tag from a computer, and by using an RFID reader, the same information can then be retrieved from the tag attached to sample devices that are stored in LN2. The new storage system has many sub-compartments for samples to maximize storage space in the LN2 tank. In the present study, we evaluated the influence of this RFID tag system on the efficiency of closed vitrification and thawing of bovine ovarian tissue.

Materials and methods

In total, 6 participants were asked to carry out the procedures of closed vitrification and thawing of bovine ovaries by either the conventional or the RFID tag method. Three participants were randomly selected by computer to perform the conventional method while the other 3 participants perform the RFID tagged method. Each participant processed 1 bovine ovary. The conventional method involved manual processing, while the RFID tag method employed a combination of two-dimensional barcode labels and RFID tag technology.

The following eight components were used in the RFID tag method:

1. Two-dimensional barcode label (Fig. 1A)

Both vertical and horizontal matrices are used in the barcode label, making it possible to read more information than provided by the original Universal Product Code. The code also has an error correction function that restores data if part of the code is missing.

2. RFID tags (Fig. 1B and C)

RFID uses electromagnetic radiation to wirelessly read and capture information stored in tags attached to objects. While a laser bar code scanner can only scan bar codes within direct view, an RFID reader can read multiple tags up to several feet away and does not need direct line-of-sight access to the tags. Another advantage of RFID is that it can identify tags in soiled or non-translucent bags. Each RFID tag stores data in the embedded IC chip and transmits data at a frequency of 13.56 MHz. We designed a round RFID tag (16 mm in diameter and 1 mm in thickness) for ovarian tissue cryopreservation devices by taking the size of the device and the data storage limit into consideration (Fig. 1B). Each device holds one ovarian tissue sample and the round RFID tag attached to the device can function after being stored in LN2 (− 196 °C). To prevent the slowing of the electrical circuit at low temperatures, which sometimes causes RFID tags to become unresponsive, we redesigned the circuit and improved the sensitivity of the tag. The RFID tag thus obtained was more sensitive and functioned better after storage in LN2 (Fig. 1C).

3. RFID reader (Fig. 1D)

The RFID reader uses radiofrequency waves to read information stored in the RFID tags. In our new RFID system, the antenna of the reader was modified to increase sensitivity for reading data from tags that had been stored in LN2. The modifications included lengthening the antenna, using copper wire, and increasing the magnetic field density by narrowing the antenna edge.

4. Device box (Fig. 1E)

The device box was made from SUS304 stainless steel containing chromium and nickel, with the metal inert gas welding method being used to seal any gaps so that the samples remained in LN2 after being taken out of the storage tank. Each cuboidal device box was designed to store up to 20 ovarian tissue cryopreservation devices from one patient, since an average of approximately 17 ovarian tissue samples are harvested per patient.

5. Storage box (Fig. 1F)

The storage box was also made from SUS304 stainless steel by spot welding and was large enough to contain 6 device boxes (120 cryopreservation devices with ovarian tissue samples from 6 patients).

6. Canister (Fig. 1G)

The cylindrical canister was used for the conventional method. Each canister could store samples from 4 patients. In the RFID tag system, each cuboidal canister had an

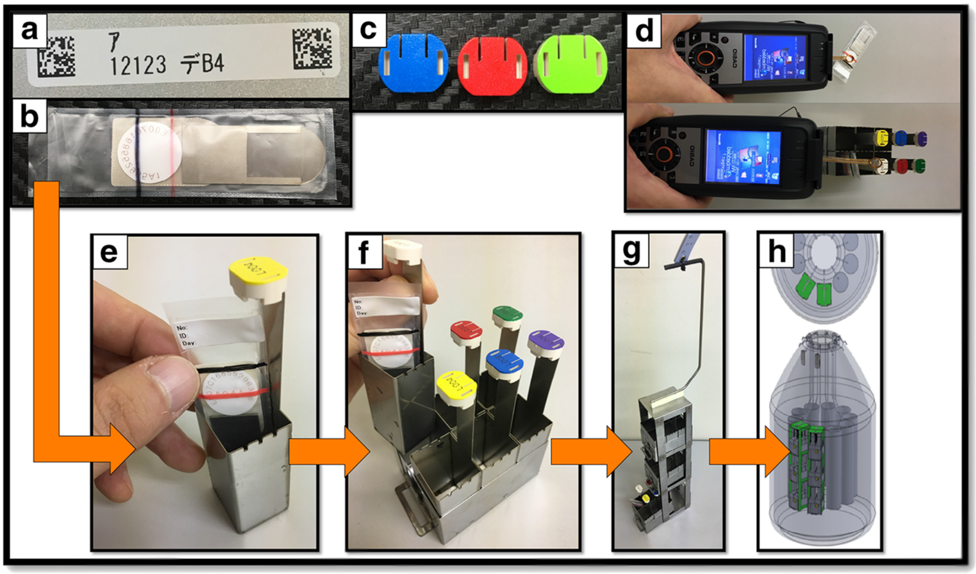

Fig. 1 The RFID tag system. (A) A two-dimensional barcode label. (B) An ovarian tissue cryopreservation device with a round RFID tag. (C) RFID tag for the device box. Tags of different colors are available for easy identification. (D) RFID reader (top). The reader shows patient information in the monitor (bottom). (E) Device box. Each device box holds 20 cryopreservation devices. (F) Storage box. Each storage box holds 6 device boxes. (G) Cuboidal canister. Each canister holds 3 storage boxes. (H) LN2 tank. Each tank holds 10 canisters

RFID tag attached to the handle and held 3 storage boxes, which could store up to 360 cryopreservation devices with ovarian tissues from 18 patients.

7. LN2 tank (Fig. 1H)

The tanks used in this study were the HC35 (Taylor Wharton) and KD32-10 LN2 (Kitazato). Each LN2 tank held 10 canisters storing samples from 40 patients (conventional method) or 180 patients (RFID tag method).

8. Computer

A computer was used to manage storage efficiently, with the computer screen showing the location of each RFID tag and indicating empty space in the tank.

9. Pretest

The Uchida-Kraepelin (U-K) test is a questionnaire that requires intense concentration by the participants and is used to assess mental agility, long-term attention, and recovery from fatigue. Each participant was asked to perform the U-K test prior to carry out the closed vitrification and thawing procedures. All participants achieved similar U-K test scores, indicating a similar ability to follow instructions irrespective of their professional background.

10. Steps in closed vitrification procedure

Conventional method (cryopreservation of bovine ovarian tissue; Fig. 2):

Step 1.The patient's hospital card was swiped through a card reader to obtain patient information. Then the patient information sheet (name, date of birth, hospital ID number, diagnosis, date of operation) was filled in manually.

Step 2.The resected ovary was placed in a cup filled with PBS and the cup was labeled (patient's name, hospital ID number, and date of birth).

Step 3.The same patient information was written on a Petri dish.

Step 4.The ovary was transferred from the cup to the Petri dish and cut into small pieces.

Step 5.Each ovarian tissue piece was assigned a number and photographed with the number.

Step 6.Patient information was written on 3 dishes containing graduated concentrations of cryoprotectant and the ovarian tissue samples were processed through the dishes.

Step 7.Each ovarian tissue sample was placed in a cryopreservation device (one per device). Then each device was placed in a plastic pouch and patient information (name, hospital ID number, date of birth, and ovarian tissue sample number) was written on the pouch.

Step 8.The labeled device was placed in a metal holder and successively into a canister and LN2 tank.

RFID tag method (cryopreservation of bovine ovarian tissue; Fig. 2):

Step 1.The patient's hospital card was swiped through a card reader to print out two-dimensional barcode labels with patient information (name, hospital ID number, and date of birth).

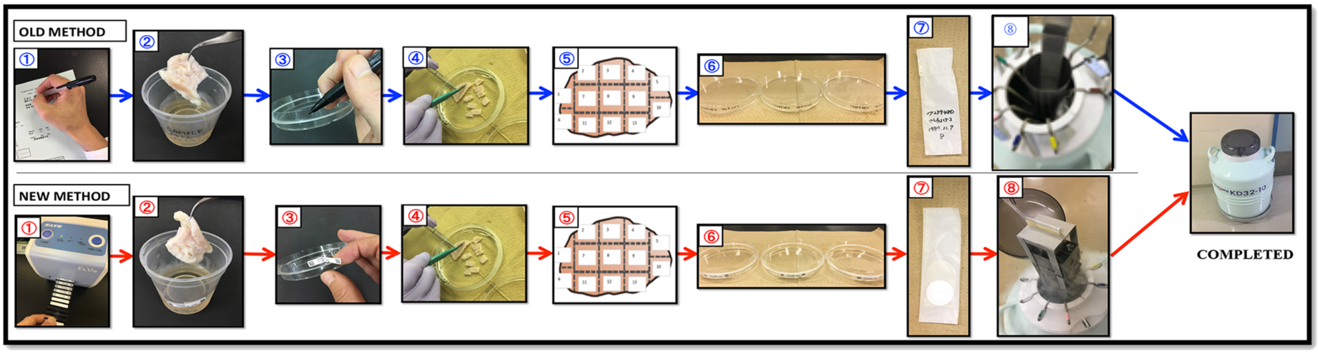

Fig. 2 Conventional and RFID tag methods for cryopreservation of ovarian tissue samples

Step 2.The resected ovary was placed in a cup filled with PBS and a barcode label was attached to the cup.

Step 3.A barcode label was attached to a Petri dish.

Step 4.The ovary was transferred from the cup to the Petri dish and cut it into small pieces.

Step 5.Each ovarian tissue piece was assigned a number and a single photograph was taken of that included all of the numbered tissue pieces. The camera was linked to a computer, allowing automatic recognition of the numbered tissues.

Step 6.Labels were attached to 3 dishes containing graduated concentrations of cryoprotectant and the ovarian tissue samples were processed through the dishes.

Step 7.Each ovarian tissue sample was placed in a cryopreservation device with an RFID tag, which stored patient information (name, hospital ID number, date of birth, and ovarian tissue sample number).

Step 8.The cryopreservation device was placed in a device box with an RFID tag and then successively in a storage box, canister, and LN2 tank.

11. Steps in thawing procedure

Conventional method (thawing bovine ovarian tissue; Fig. 3):

Step 1.The patient's information sheet was identified in a ledger.

Step 2.The ovarian tissue sample number and location in the LN2 tank were obtained from the patient information sheet.

Step 3.The cryopreservation device containing the ovarian tissue sample of interest was retrieved from the LN2 tank and compared with the handwritten information in the ledger.

Step 4.Patient information was written on a Petri dish.

RFID tag method (thawing bovine ovarian tissue; Fig. 3):

Step 1.Patient information was retrieved on a computer by swiping the patient's hospital card through a card reader and two-dimensional barcode labels were printed.

Step 2.The ovarian tissue sample number and location in the LN2 tank were obtained from the computer.

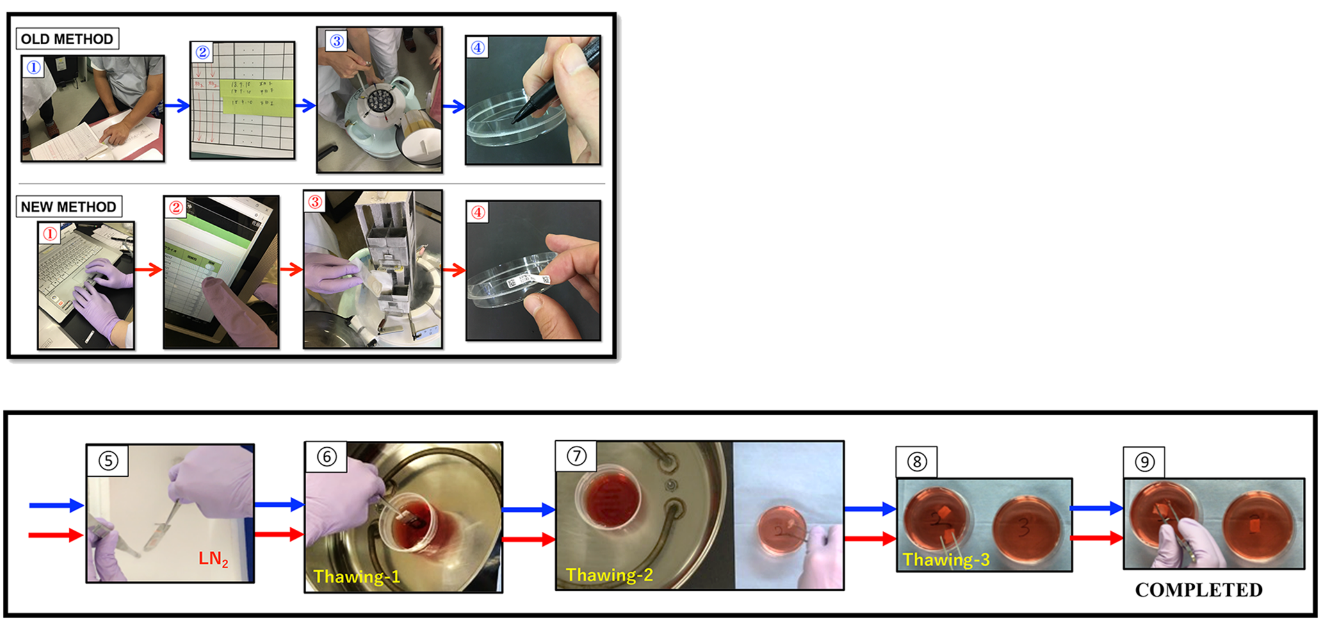

Fig. 3 Conventional and RFID tag methods for thawing ovarian tissue samples

Step 3.The cryopreservation device containing the ovarian tissue sample of interest was retrieved from the LN2 tank and its RFID tag was scanned with an RFID reader to confirm the patient information stored in the tag.

Step 4.A barcode label was attached to a Petri dish.

The remaining steps were the same for both methods and were not timed in this study.

Step 5 – The cryopreservation device with the sample of interest was removed from the plastic pouch in LN2.

Step 6.The cryopreservation device was placed in thawing solution 1.

Step 7.The cryopreservation device was transferred to thawing solution 2.

Step 8.The cryopreservation device was transferred to thawing solution 3.

Step 9.The ovarian tissue sample was removed from the cryopreservation device.

Statistical analysis

The time required to perform each step of the two methods was measured in seconds. Normality of data was determined by using the Shapiro-Wilk test. After normality was confirmed, the time for each step was treated as the dependent variable and the unpaired t test was performed without logarithmic transformation.

Results

All 6 participants underwent the Uchida-Kraepelin (U-K) test before performing the closed vitrification and thawing procedures by either the conventional or RFID tag method. The U-K test scores indicated that all participants had a similar ability to follow instructions and none was particularly slow or fast, irrespective of their professional background.

Performance with the two methods

When the RFID tag method was used for closed vitrification [11], the total time required for ovarian tissue cryopreservation was significantly shorter than that for the conventional method ($t(4) = 2.938$, $p = 0.042$, $d = 2.40$). The mean time required for cryopreservation was 1412.33 (± 130.5) seconds by the RFID tag method versus 2073.0 (± 367.0) seconds by the conventional method, and the use of the RFID tag method reduced the cryopreservation time by a mean of 11 min. A similar result was obtained with the thawing procedure, and the total time required for thawing samples was significantly shorter with the RFID tag method than the conventional method ($t(4) = 2.797$, $p = 0.049$, $d = 2.28$). The mean time required thawing ovarian tissue samples was 260.67 (± 38.7) seconds by the RFID tag method versus 405.0 (± 80.6) seconds by the conventional method, and using the RFID tag method reduced the thawing time by a mean of 2 min. The time differences between the two methods were calculated for each step. During closed vitrification procedure, the largest time difference was observed in step 3, where additional time was taken to correct patient information after incorrect information was written on the cup when performing the conventional method. During the thawing procedure, the largest time difference was noted when locating and retrieving the device with the target ovarian tissue sample. In the conventional method, retrieving the cryopreservation device took longer due to time required to confirm the handwritten information on the device pouch matched the patient information in the ledger. Also, when participants were searching for the target cryopreserved device, some incorrect devices were removed from the LN2 storage. In contrast, locating and retrieving the RFID-tagged cryopreserved device was performed efficiently, since the computer system showed the exact location of the device and the RFID reader was used to confirm patient information stored in the tag before removing the device from the LN2 tank.

Discussion

In Japan, the need for cryopreservation of ovarian tissue is increasing. The Japanese Ministry of Health, Labour and Welfare has conducted research in this field and launched a program to promote fertility preservation for adolescents and young adults with cancer in 2016 [12]. To date, 201 patients have undergone ovarian tissue cryopreservation [12]. The Obstetrics and Gynecology Department of St. Marianna Medical University is the one of the major fertility preservation centers in Japan and co-operates with The Ministry of Health, Labour and Welfare to support adolescents and young adults with cancer who require fertility preservation. In this study, we evaluated the usefulness of the RFID tag method for improving the efficiency of the closed vitrification protocol because our group has achieved 3 live births using vitrified/thawed ovarian tissues. Two of these live births have been reported already, although the third case has not been reported (unpublished observations) [13, 14]. Slow freezing is currently the standard method of ovarian tissue cryopreservation, but there is ongoing debate as to which, slow freezing or vitrification, is superior [15, 16]. Automatic vitrification systems have been developed for oocytes and embryos. However, an automatic system has not yet been developed for vitrification or slow freezing of ovarian tissue samples [17, 18].

Against this background, we aimed to develop a system that could streamline the cryopreservation and thawing procedures, and we performed this study to evaluate the usefulness of our RFID tag method in closed vitrification and thawing of bovine ovarian tissue samples. We found that application of the RFID tag system improved the efficiency of both procedures, with the total time required for cryopreservation being reduced by 11 min when using the RFID tag system and the thawing time being reduced by 2 min.

Efficiency and control of human error are closely associated. The key advantages of utilizing an RFID tag system at fertility centers are improved efficiency and reduced the risk of human error. When the RFID tag system is integrated into a hospital network, swiping the hospital ID card transfers patient information to the IC chip on an RFID tag without manual entry, thus minimizing the risk of typing or writing errors associated with manual procedures. For tracking patient samples, the computer shows the exact location of each cryopreserved tissue sample in the LN2 tank and the RFID reader identifies the cryopreservation device with the target sample while it remains under LN2. The new round RFID tag that we developed has been tested to confirm readability while under LN2. If the IC chip in the RFID tag is damaged, the identification number etched on the surface of the device can still be used to retrieve patient information. In the future, we hope to focus on total elimination of human error and devising a backup system, such as cloud technology, to support this RFID tag method.

The RFID tag method can be applied to other forms of fertility preservation. The devices that are used for cryopreservation depend on the type of tissue being cryopreserved and the method of cryopreservation adopted. For example, the cryotop and the cryovial with a screw cap are usually employed for cryopreservation of oocytes, embryos, and sperm by vitrification, while the cryovial with metal pins attached to the screw cap is used for cryopreservation of ovarian tissue by open vitrification and a flat metal device (Fig. 1B) is used for cryopreservation by closed vitrification. The RFID tag manufacturer (KRD in Japan) can modify the tag size and sensitivity to fit the needs of each device.

Conclusion

Combining two-dimensional barcode labels with an RFID tag system improved the efficiency of cryopreservation and thawing by closed vitrification and reduced human error.

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Authors' roles Y.S., T.S., N.S., T.S., and H.K. participated in study conception and design; Y.S., T.S., and H.K. developed the device; T.S, Y.S., Y.S., M.K., S.F., and U.A. performed the study; T.K. performed the statistical analysis; M.A., T.S., and H.K. built the computer system; N.S. edited the English manuscript; N.S, A.T., and N.S. gave suggestions for this research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Green DM, Liu W, Kutteh WH, Ke RW, Shelton KC, Sklar CA, et al. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. The Lancet Oncology. 2014;15(11):1215–23.
2. Thomson AB, Campbell AJ, Irvine DC, Anderson RA, Kelnar CJ, Wallace WH. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. Lancet. 2002;360(9330):361–7.
3. Aubier F, Flamant F, Brauner R, Caillaud JM, Chaussain JM, Lemerle J. Male gonadal function after chemotherapy for solid tumors in childhood. J Clin Oncol. 1989;7(3):304–9.
4. Williams D, Crofton PM, Levitt G. Pediatr Blood Cancer. 2008;50(2):347–51.
5. Lampe H, Horwich A, Norman A, Nicholls J, Dearnaley DP. Fertility after chemotherapy for testicular germ cell cancers. J Clin Oncol. 1997;15(1):239–45.
6. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2010;28(2):322–9.
7. Howell SJ, Shalet SM. Testicular function following chemotherapy. Hum Reprod Update. 2001;7(4):363–9.
8. Shalet SM, Tsatsoulis A, Whitehead E, Read G. Vulnerability of the human Leydig cell to radiation damage is dependent upon age. J Endocrinol. 1989;120(1):161–5.
9. Fisch B, Abir R. Female fertility preservation: past, present and future. Reproduction. 2018;156(1):F11–27.
10. Kristensen SG, Andersen CY. Cryopreservation of ovarian tissue: opportunities beyond fertility preservation and a positive view into the future. Front Endocrinol (Lausanne). 2018;9:347.
11. Sugishita Y, Okamoto N, Uekawa A, Yamochi T, Nakajima M, Namba C, et al. Oocyte retrieval after heterotopic transplantation of ovarian tissue cryopreserved by closed vitrification protocol. J Assist Reprod Genet. 2018;35(11):2037–48.
12. Suzuki N. Research on the effectiveness of cancer and reproductive health care for young cancer patients (fertility preservation), Degree the Ministry of Health, Labor and Welfare of children, Child care support promotion research project. 2018 (in Japanese)
13. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. Proc Natl Acad Sci U S A. 2013;110(43):17474–9.
14. Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, et al. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. Hum Reprod. 2015;30(3):608–15.
15. Amorim CA, Curaba M, Van Langendonckt A, Dolmans MM, Donnes J. Vitrification as an alternative means of cryopreserving ovarian tissue. Reprod BioMed Online. 2011;23(2):160–86.

16. Abir R, Fisch B, Fisher N, Samara N, Lerer-Serfaty G, Magen R, et al. Attempts to improve human ovarian transplantation outcomes of needle immersed vitrification and slow-freezing by host and graft treatments. J Assist Reprod Genet. 2017;34(5):633–44.
17. Arav A, Natan Y, Kalo D, Komsky-Elbaz A, Roth Z, Levi-Setti PE, et al. A new, simple, automatic vitrification device; preliminary results with murine and bovine oocytes and embryos. J Assist Reprod Genet. 2018;35(7):1161–8.
18. Roy TK, Brandi S, Tappe NM, Bradley CK, Vom E, Henderson C, et al. Embryo vitrification using a novel semi-automated closed system yields in vitro outcomes equivalent to the manual Cryotop method. Hum Reprod. 2014;29(11):2431–8.