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Case report

In vitro maturation and cryopreservation of oocytes retrieved from intra-operative aspiration during second enucleation for ovarian tumor: A case report

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1. Introduction

There are currently a variety of methods of fertility preservation, including cryopreservation of ovarian tissue (Segers et al., 2015), mature oocytes (Walls et al., 2015), and embryos. For unmarried women with breast cancer or malignant blood disorders, it is not rare to choose the resection and cryopreservation of ovarian tissue. At the same time, many studies have reported retrieving immature oocytes from resected ovaries and then maturing these oocytes using in vitro maturation (IVM). A report in 2014 described a live birth resulting from cryopreserved embryos obtained from the resected ovaries of a patient with ovarian cancer (Prasath et al., 2014). It is common to select oophorectomy in cases of malignant tumors, even if the patients are young. On the other hand, we must also consider cases of benign recurrent ovarian tumor in women with a single ovary. What is the best way to preserve fertility in patients who do not currently require oophorectomy but in whom recurrence is expected in the near future? To the best of our knowledge, there have been no previous reports of fertility preservation involving intra-operative retrieval of immature oocytes and the subsequent cryopreservation of the oocytes after IVM in a patient with a single ovary with past enucleation for ovarian tumor. Here we describe a case in which intra-operative aspiration of the non-stimulated ovary was performed to retrieve immature oocytes, and cryopreservation of one mature oocyte was successful following IVM.

2. Methods

2.1. Patient presentation

The patient was an unmarried, 25-year-old woman with a left ovarian tumor. At age 19 she underwent emergent surgery consisting of right oophorectomy due to torsion of a right ovarian mucinous borderline tumor, and left ovarian enucleation for mucinous cystadenoma. No treatment was administered postoperatively, and she was followed by ultrasound sonography at intervals of several months at the hospital where the operation was performed. Two years postoperatively, multiple cysts with a total diameter of 4 cm were detected in the left ovary. Because there were no reproductive endocrinologists at the hospitals in which she was followed, there was no opportunity to receive recommendations regarding fertility preservation options. Two years later the left ovarian tumor had grown to 5 cm. She was referred to our hospital for fertility preservation.

When she first presented at our institution she was 23 years old and had not been married. After we consulted with her, we decided to avoid multiple surgeries if possible in order to preserve ovarian function, and instead conducted careful follow-up to monitor the ovarian tumor size. During this follow-up we presented the patient with the option of cryopreservation of unfertilized oocytes. She declined, due to the difficulty of frequent visits to our hospital and the high cost of this approach given the lack of public subsidy in Japan. After two more years, the tumor grew to over 7 cm. Its preoperative appearance on magnetic resonance imaging is shown in Fig. 1A. Since we were worried about tumor torsion, which might require oophorectomy of the left ovary, we chose to treat the tumor by performing a second enucleation. The patient's pre-operative level of anti-müllerian hormone (AMH) was low at 1.26 ng/mL, probably because of her first operation at age 19. We discussed with the patient the possibility of retrieving oocytes, primarily via trans-vaginal aspiration after controlled ovarian hyperstimulation (COH) a few months after this surgery. In addition, we obtained consent to collect immature oocytes during the current surgery in order to maximize the overall number of oocytes retrieved.

2.2. Intra-operative retrieval of oocytes, in vitro maturation, and cryopreservation

We chose not to perform COH before this surgery in order to preserve the ability to distinguish between the multiple mucinous cysts of the ovarian tumor, which were to be removed, and growing follicles. Only human chorionic gonadotropin (HCG) 5000 IU (Gonadotropin; ASKA Pharmaceutical, Tokyo, Japan) was administered to the patient 24 h prior to the surgery, to increase the maturation rate of the collected

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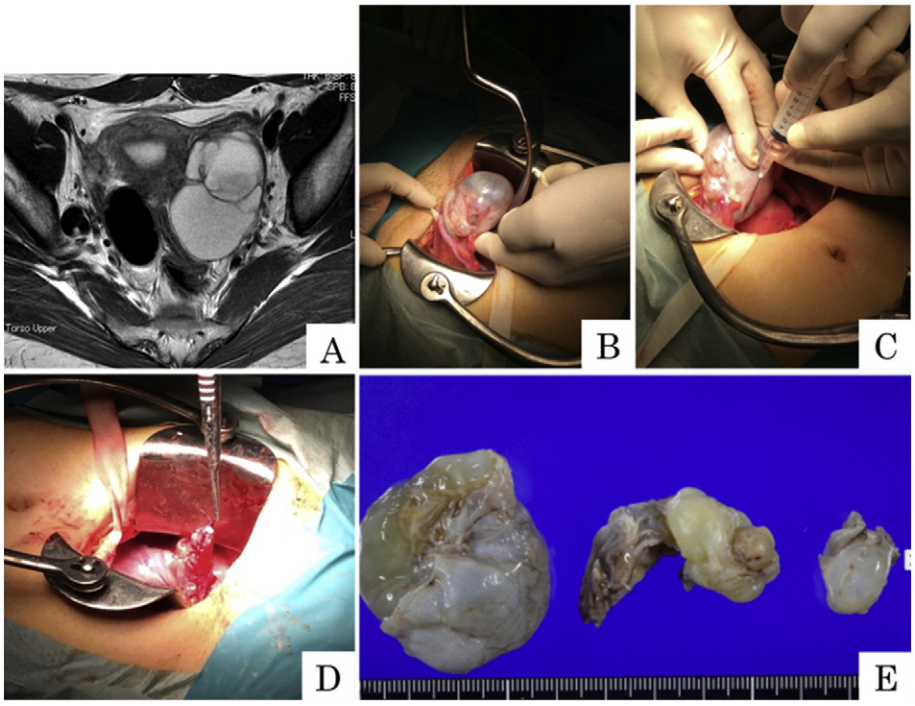

Fig. 1. Information on patients and operation. A; Magnetic resonance image before surgery, B; appearance of ovarian cyst, C: aspiration of visible follicles, D; ovary after enucleation, E; tissue of resected ovarian tumor.

oocytes (De Vos et al., 2011). Most commonly, HCG 5000 or 10,000 IU is administered about 36 h prior to the collection of immature oocytes (De Vos et al., 2011; The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013), but due to the timing of our patient's hospitalization, she received HCG 24 h before the surgery. Fig. 1B shows the left ovarian tumor during the operation. We punctured the normal ovarian cortex at the location of what appeared to be a normal follicle about 10 times using a 19-gauge needle. Prior to puncturing the follicle, we transferred 1 mL of oocyte washing medium (Sage IVM Media; Cooper Surgical, Trumbull, CT, USA), warmed to 37°, into a 10-mL syringe. Fig. 1C shows the aspirated follicular fluid. After aspiration we quickly transported the syringe to the laboratory near the operating room. We microscopically examined and retrieved three immature oocytes from the follicular fluid; these are shown in Fig. 2. All three oocytes had multiple layers of cumulus cells. After washing the oocytes in oocyte washing medium, we placed them in oocyte maturation medium (Cooper Surgical) with 75 IU of FSH and 75 IU of LH (HMG Injection Teizo; Asuka Pharmaceutical, Tokyo, Japan) according to the data sheet. We cultured the oocytes for 24 h at 37° in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. After the incubation, we checked the maturation stage and morphology of each oocyte. Fig. 3 shows the oocytes following IVM. Extrusion of the first polar body was identified in only one oocyte, while the other two oocytes remained immature. We vitrified the mature oocyte using Cryotop (Kitazato BioPharma, Shizuoka, Japan), and placed the two immature oocytes back into the oocyte maturation medium for another 24 h. Despite the additional IVM, the maturation stage of these oocytes did not change.

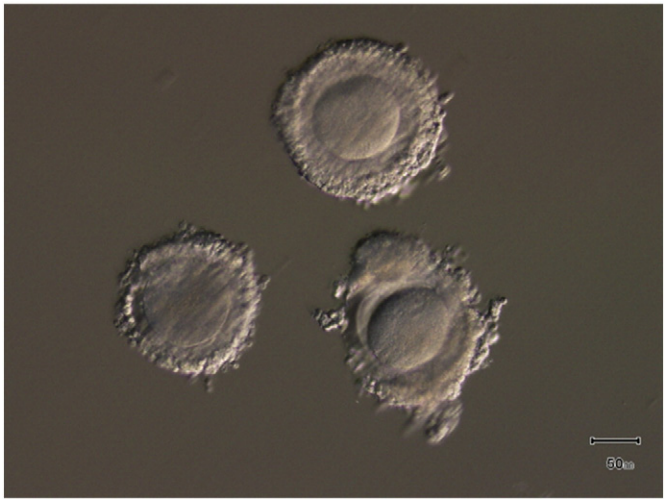

Fig. 2. Immature oocytes before in vitro maturation. Scale bar, 50 μm.

Fig. 1D shows the left ovary after enucleation at the end of the operation, while Fig. 1E shows the resected ovarian tumor. The pathological diagnosis of this patient was mucinous cystadenoma. We are planning further retrieval of oocytes under COH and hope to vitrify enough mature oocytes before recurrence of the ovarian cysts.

2.3. Ethics approval and patient consent

This case received the ethics approval of the institutional review board of Akita University (No. 1214). We obtained written informed consent for IVM and cryopreservation, as well as for publication of the case report.

3. Discussion

In this case we performed intra-operative oocyte collection as requested by the patient. The main reason for this decision was our concern that the patient's ovarian function would be further reduced postoperatively and that even subsequent COH would not yield sufficient numbers of oocytes. Her AMH level (1.26 ng/mL) was markedly low even before surgery. It is known that AMH level and the number of collected oocytes are correlated in in vitro fertilization (Kedem et al., 2013). In addition, we had to consider the risk of ovarian tumor recurrence in the remaining ovary. It is possible that the patient would have chosen to undergo oocyte collection under COH before the

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