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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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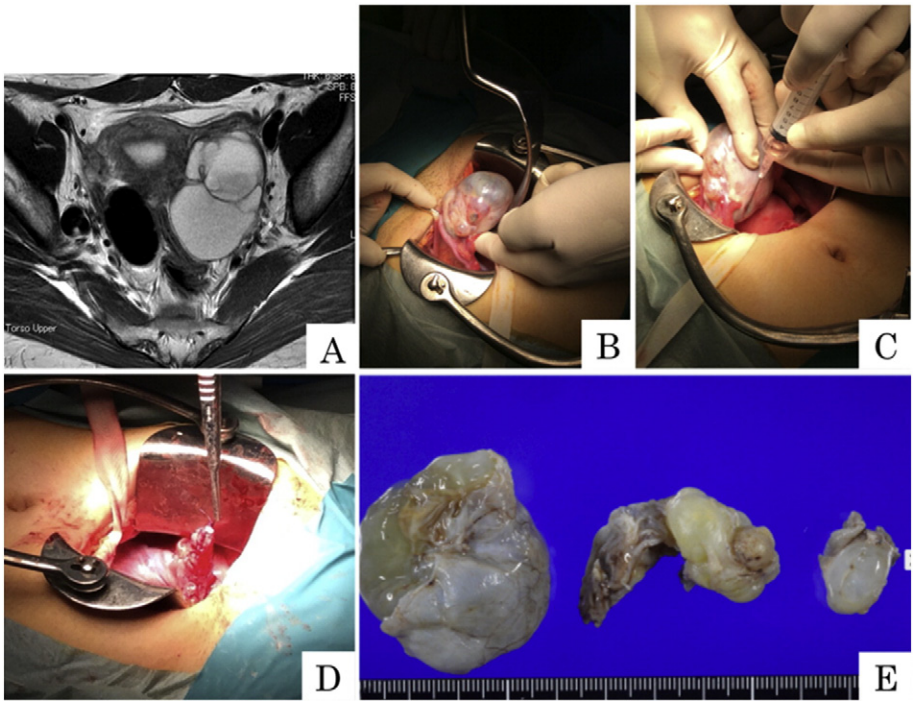

Fig. 1. Information on patients and operation. A; Magnetic resonance image before surgery, B; appearance of ovarian cyst, C: aspiration of visible follicles, D; ovary after enucleation, E; tissue of resected ovarian tumor.

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.

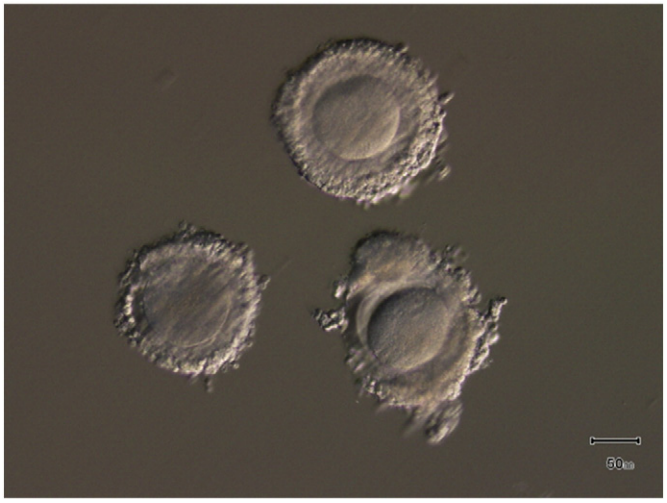

Fig. 2. Immature oocytes before in vitro maturation. Scale bar, 50 μm.

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

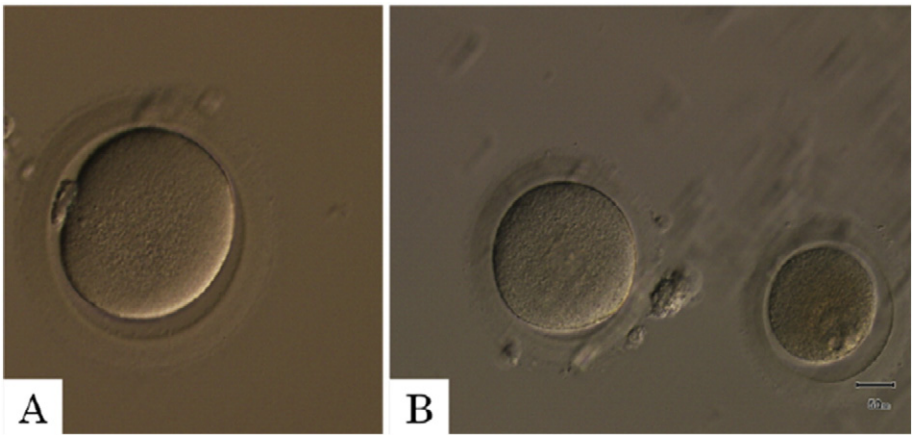

Fig. 3. Oocytes after in vitro maturation for 24 h. A; matured oocyte, B; immature oocytes; scale bar, 50 μm.

recurrence of the ovarian tumor if reproductive endocrinologists had been involved in her care soon after her first surgery at age 19. It is getting currently common in Japan for fertility preservation techniques such as cryopreservation of unfertilized oocytes to be performed in patients with malignant tumor (Suzuki, 2016). Patients wanting fertility preservation should ideally be referred to a reproductive endocrinologist as early as possible.

There are several reports concerning oocyte retrieval from resected ovaries and the outcomes of IVM in patients with various malignancies, such as breast cancer, endometrial carcinoma, and cervical cancer (Walls et al., 2015; Lavery et al., 2011; Takae et al., 2015). On the other hand, few studies have reported the intra-operative retrieval of immature oocytes in vivo from stimulated ovaries (Kim et al., 2015). One study did attempt to collect immature oocytes by aspirating normal ovaries during cesarean section (Pongsuthirak, 2014). The present study is the first in which immature oocytes were retrieved from a patient who had a single ovary, which was not being stimulated, during the patient's second enucleation for recurrent ovarian tumor.

We should consider the present consensus about IVM. The committee opinion of the American Society for Reproductive Medicine (ASRM) on IVM (The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013) is that the procedure should be limited to patients with polycystic ovary syndrome (PCOS) to minimize ovarian hyperstimulation syndrome (OHSS) and patients who have limited time to undergo ovarian stimulation for collecting oocytes. On the other hand, in recent years there have been reports of IVM of immature human oocytes collected from resected non-stimulated ovaries and cryopreservation of mature oocytes after IVM (Walls et al., 2015; Prasath et al., 2014). Consistent with these prior reports, in the present case we chose to perform IVM and cryopreservation of the mature oocytes. We should carefully consider the indications for these procedures, taking into account that the ASRM opinion considers IVM to be an experimental technique (The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013).

Three oocytes were retrieved in this case. This low number is understandable given the patient's low AMH level. We were only able to cryopreserve one mature oocyte. Pongsuthirak et al. reported that IVM led to the maturation of 60% of immature oocytes (Pongsuthirak, 2014). Because the number of immature oocytes was small in our case, an assessment of the results of IVM is difficult. Prior to the surgery we performed only HCG priming. Fadini et al. reported that HCG increased the maturation rate of immature oocytes, but priming with both HCG and follicle stimulating hormone (FSH) had a more significant effect in IVM (Fadini et al., 2009). They also found that clinical pregnancy rates were higher in patients who were primed with both HCG and FSH than in those who received no priming, HCG alone, or FSH alone (Fadini et al., 2009). In light of this previous study, we think that priming with both HCG and FSH is recommended in IVM when the condition of the patient permits. We expect to see improved IVM results following trans-vaginal oocyte retrieval under COH in the future.

It is known that oncofertility treatment requires about 20 matured oocytes (meiosis II stage) for one child (Yin et al., 2016), so the number of oocytes collected in this case was insufficient. Intra-operative retrieval of oocytes may be considered in addition to postoperative oocyte retrieval. On the other hand, one study reported a live birth resulting from four immature oocytes collected from a resected ovary in a case of ovarian cancer (Prasath et al., 2014). Even one oocyte may be important for patients desiring fertility preservation. No clinical trials thus far have compared the results of conventional IVF with IVM. Regarding the fertility preservation potential of one matured oocyte, future studies are needed to compare patients receiving IVM and those undergoing conventional IVF.

Also, we should consider the risks involved in oocyte retrieval by intra-operative aspiration during enucleation for ovarian tumor. In this case, the final pathology result was benign mucinous cystadenoma. In cases where the ovarian tumor is malignant, aspiration carries the risk of surgical spill as International Federation of Gynecology and Obstetrics (FIGO) stage IC1. Adequate informed consent is needed prior to intraoperative oocyte retrieval. If ovarian cancer is more suspected, resection of the ovary may be necessary. Even in such cases, retrieval of oocytes from resected ovaries may be beneficial for fertility preservation (Segers et al., 2015; Walls et al., 2015; Prasath et al., 2014).

Recently, assisted reproductive technology has involved the use of various COH strategies. In general, IVM is selected as a way to reduce the risk of OHSS in patients with PCOS (The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013). It was reported that the risk of OHSS was reduced by using gonadotropin-releasing hormone (GnRH) antagonists for ovarian stimulation or employing GnRH agonists to trigger oocyte maturation (Fertil. Steril., 2016). Recently, random-start controlled ovarian stimulation with letrozole for patients with estrogen-sensitive cancers was reported (Cakmak et al., 2013). The optimal method of ovarian stimulation should ideally be chosen based on the details of each case.

In conclusion, we report a rare case of retrieval of oocytes during surgery for second enucleation of benign ovarian cysts in a patient with a single ovary and low AMH level. We performed IVM and cryopreservation for fertility preservation. We think that collecting immature oocytes during surgery is a potential fertility preservation option that is

both less expensive and time-consuming for patients than other options. When evaluating strategies for preserving fertility it is important to consider each patient's age, medical disorders, and personal preferences.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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