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REVIEW ARTICLE

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In vitro maturation of human immature oocytes for fertility preservation and research material

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Abstract

Aim: In recent years, the importance of fertility preservation (FP) has increased. In vitro maturation (IVM), an important technique in FP, has started to be used in the clinic, but controversies persist regarding this technique. Here, a survey of IVM for FP is provided.

Methods: Based on a literature review, the applications of FP, methods of FP, IVM of oocytes that had been collected in vivo and ex vivo, maturation of oocytes after IVM for FP, cryopreservation of oocytes for FP, explanation of the procedures to patients, and recent research on FP using IVM were investigated.

Results: Although IVM for FP remains controversial, the application of FP is expected to expand. Depending on the age and disease status of the patient, various methods of oocyte collection and ovarian stimulation, as well as various needle types and aspiration pressures, have been reported. The maturation rate of IVM in FP ranges widely and requires optimization in the future. In regard to cryopreservation for matured oocytes, the vitrification method is currently recommended.

Conclusion: Regarding FP for patients with cancer, the treatment of cancer is prioritized; thus, the time and use of medicines are often constrained. As several key points regarding IVM remain unclear, well-designed and specific counseling for patients is necessary.

KEYWORDS

assisted reproductive technology, cryopreservation, fertility preservation, in vitro maturation, oncofertility

1 | INTRODUCTION

In recent years, the importance of the in vitro maturation (IVM) of human immature oocytes has increased greatly and has various advantages in reproductive medicine, being widely applied to immature oocytes that have been collected from patients with polycystic cystic ovarian syndrome (PCOS). Reproductive endocrinologists also need to deepen their knowledge, not only in regard to the application of IVM to PCOS, but also in regard to its application to fertility preservation (FP); for example, oncofertility. As the survival rate of patients with cancer improves, the demand for FP is increasing. However, several controversies persist regarding the use of IVM in the field of FP. In this review, the knowledge regarding IVM that should be familiar to reproductive endocrinologists providing FP to patients with cancer in daily clinical practice is outlined. Furthermore, although the use of human oocytes in reproductive medicine is essential, there are several obstacles to research on human oocytes and embryos due to ethical and legal limitations in many countries. In vitro maturation has the potential to obviate the ethical problems pertaining to research on human oocytes and embryos. This review also outlines recent basic research on the clinical use of IVM for human oocytes.

2 | OUTLINE OF IN VITRO MATURATION

The IVM of immature oocytes was first reported in rabbit in 1935.[1] Subsequently, the technique was applied to humans. In 1991, the first live human birth following IVM was reported.[2] Currently, IVM is widely applied, mainly to patients with PCOS.[3] As IVM procedures are not difficult, even common clinics can offer this procedure. It is used clinically primarily to avoid ovarian hyperstimulation syndrome (OHSS), as well as to decrease the costs that are associated with ovarian stimulation, when performing in vitro fertilization.[4] Over 4000 babies have been born by assisted reproductive technology (ART) by IVM and these children have manifested no health problem.[5,6] The incidence of anomalies is not elevated among babies born by IVM, although the possibility must be considered that oocyte methylation is altered during the maturation process.[7] In addition, the IVM procedure facilitates the salvage of immature oocytes.[8] More than 30 years have passed since the first live birth using cryopreserved human oocytes[9] and securing cryopreserved oocytes by IVM is also useful for FP. This approach also can be applied to patients with irregular menstrual cycles[10] and to avoid the risk of delaying cancer treatment. Recently, various stimulation methods in order to avoid OHSS also have been developed.[7] We must consider the opinion of the American Society for Reproductive Medicine (ASRM) that IVM should only be applied for patients with PCOS in order to avoid OHSS.[11] Although the IVM of human oocytes has a long history, reproductive endocrinologists must recognize that several points remain to be elucidated and that the ASRM defines IVM as an experimental technique. Nonetheless, in current clinical practice, IVM has become an indispensable technique for promoting FP.

3 | APPLICATION TO FERTILITY PRESERVATION

Currently, many cancers have survival rates of 80%.[12] In addition, 20% of gynecologic cancers occur in women of reproductive age who are undecided about family planning.[13] However, survivors of cancer who undergo chemotherapy and radiotherapy often experience a loss of ovarian function. Therefore, it is important to present patients with cancer with choices of FP.[14] In recent years, patients with breast cancer, blood cancer, brain cancer, gynecological cancer etc. have been provided with FP.[10,12,15,16] In particular, breast cancer is the most common cancer in women and one-third of cases affect women of reproductive age. Accordingly, breast cancer is the main target for FP. The preservation of gametes and embryos from patients with cancer for FP is termed "oncofertility."[17] Several studies have shown that ovarian function declines due to the original disease in patients with cancer.[18,19] Specifically, there are concerns regarding decreases in the number and quality of the oocytes that are collected.[19,20] In addition, the patients who adopt FP may vary from country to country. A case report described oocyte collection and cryopreservation as a method of FP for patients who have undergone a hysterectomy.[21] As of 2017, in Japan, neither surrogate mothers nor host mothers are allowed to undergo this procedure, but in some countries, FP also may be indicated for patients who have undergone a hysterectomy. For reproductive endocrinologists providing FP, it is important to design a strategy for reproductive treatment that does not delay the treatment of cancer.[22] It is also necessary to take into account the possibility of a low ovarian response due to immaturity of the hypothalamus, pituitary gland, and ovary; also, age is an important factor.[22] In addition to patients with cancer, IVM and FP are useful in patients with autoimmune disease or prothrombotic syndrome[23,24] and the application of FP is expected to expand in the future.

4 | METHODS OF FERTILITY PRESERVATION

Various methods of FP have been developed: the main methods in current use are embryo cryopreservation, mature oocyte cryopreservation after IVM, immature oocyte cryopreservation, and ovarian tissue cryopreservation (OTC). These techniques are performed either alone or in combination. The protection of ovarian function by gonadotropin-releasing hormone agonist during chemotherapy has been reported, but remains controversial.[25] In patients with cervical cancer who are receiving radiation therapy, ovarian transposition surgery also has been considered as a means of preserving ovarian function.[26] In this review, the authors mainly focus on FP by using IVM. In order to avoid a delay in the treatment of the original cancer, it is necessary to select a method of ovarian stimulation for FP that does not cause OHSS. Fertility preservation for patients with breast cancer often is administered within a period of 2-6 weeks between surgery and adjuvant chemotherapy.[15] If there is sufficient time, conventional controlled ovarian stimulation (COH), which requires both expense and time, is possible. The use of neoadjuvant chemotherapy has increased in recent years, so it is also necessary to consider treatment for patients with no time delay. Controlled ovarian stimulation is costly and time-consuming and thus it is unacceptable for some patients.[27] Recently, OTC has been considered for patients with breast cancer who are undergoing neoadjuvant chemotherapy and strongly desire FP.[28] More than 60 successful births from ART using OTC were reported in 2015[29] and a total of >80 births in 2016.[30] Although OTC has been spreading in ART in recent years, it remains an experimental method; therefore, it is important to recognize that surgery is necessary and that the economic burden is significant.[7] Also, when considering COH, attention should be paid to estrogen-sensitive diseases, such as breast and endometrial cancer, as COH raises the level of serum estrogen >10-fold above normal levels,[31] which is of concern in cases of hormone-sensitive breast cancer, even after surgery or before adjuvant chemotherapy.[15] For patients with breast cancer who cannot undergo COH, the combination of OTC and IVM might be the best option.[32] In contrast, in the case of blood cancers, OTC might not be recommended because of concerns about recurrence.[12] The methods of FP are summarized in Table 1.

TABLE 1 Methods of fertility preservation and problems

Method	Problem
Embryo cryopreservation	A partner is required
Matured oocyte cryopreservation with or without IVM	The live birth rate per oocyte is a few percent
Immature oocyte cryopreservation	Significance is controversial
Ovarian tissue cryopreservation	High cost and experimental method
GnRHa during chemotherapy	Significance is controversial
Ovarian transposition surgery	Invalid for chemotherapy

GnRHa, gonadotropin-releasing hormone agonist; IVM, in vitro maturation.

5 | IN VITRO MATURATION OF OOCYTES COLLECTED IN VIVO OR EX VIVO

As with OTC, the IVM of immature oocytes that are collected from resected ovarian tissue is an important procedure in FP. The transvaginal collection of immature oocytes is performed in conventional ART, but ex vivo oocyte collection is often selected for FP. For example, in FP for patients with ovarian cancer, ex vivo oocyte collection has the advantage that the dissemination of the cancer into the peritoneal cavity can be prevented.[20] In 2003, IVM first was reported for the collection of immature oocytes during cryopreservation of the ovarian cortex.[33] In 2004, the same authors reported the IVM of immature oocytes that had been collected from the resected ovaries of a patient with an endometrial adenocarcinoma.[34] Such ex vivo oocyte collection has been widely clinically applied over the past decade. In 2012, a case report was published of the first embryo transfer following IVM and cryopreservation of ex vivo-collected immature oocytes.[35] Similarly, in 2014, the first live birth due to FP was reported for a patient with a borderline ovarian tumor.[36] Live births also have been achieved by using the IVM of immature oocytes that were obtained from resected ovaries.[27] Immature oocytes usually are collected from the ovarian cortex, although the recently reported collection of immature oocytes from the ovarian medulla has the potential as an add-on method of FP.[12,37] Future research should investigate whether the immature oocytes that have been collected from the ovarian cortex and the immature oocytes that have been collected from the ovarian medulla have equivalent developmental potential and safety.

Recently, several studies have reported in vivo oocyte collection from ovaries during surgery and the performance of the IVM of immature oocytes. In vivo immature oocyte collection also has been reported during Cesarean section for research[38] and during ovarian enucleation for FP.[39] Several reports described cases in which COH was performed before surgery and the mature oocytes were collected in vivo[40] or ex vivo[41,42] for FP. If possible, COH should be considered in order to increase the number of collected oocytes and improve IVM's performance. However, COH delays the treatment of cancer by an average of 30 days, an interval that cannot be ignored.[43] In FP for oncofertility, the time between the patient's first visit to reproductive endocrinologists and the execution of FP is often limited, ranging from several days to several weeks.[19] Fertility preservation was reported for six patients with gynecologic cancer for whom only 24 hours had elapsed between the FP consultation and surgery.[20] Considering the results of IVM, priming using follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) before the collection of immature oocytes is preferable.[44-46] However, as mentioned above, in many cases there is no spare time and it is necessary to collect immature oocytes from unstimulated ovaries, followed by IVM.

When actually using IVM in the clinic, the procedure can differ depending on the facility and the purpose. These points are summarized in Tables 2 and 3. As shown in Table 2, various oocyte collection methods have been reported, depending on the purpose of oocyte collection. In FP, transvaginal aspiration,[10,15,47] intraoperative aspiration from the ovaries,[39] and collection from resected ovarian tissue[12,16,19,20,48-51] have been reported with the record of a needle gauge. In IVM for patients with PCOS, transvaginal aspiration is the common method.[52-57] In contrast, oocyte collection for FP mainly is performed from resected ovarian tissue because the combined method in which both IVM and OTC are done at the same time is relatively common. In IVM for FP, the methods of oocyte collection are decided on a case-by-case basis, taking into account the adoption of OTC and COS. Transvaginal aspiration is difficult in patients before puberty and in patients without a history of sexual intercourse and oocyte collection from resected ovarian tissue and OTC are considered as FP. The aspiration needles that are used for this purpose also vary, from 17 to 23 gauge, as summarized in Table 2. Single-lumen needles are relatively common. Whether suction pressure is applied depends on the facility and the specific report; when applied, the suction pressure also varies from 40 to 85 mm Hg. In summary, the methods for collecting oocytes for FP are various; thus, it is recommended that the collection of oocytes be performed in a manner that is familiar to the facility.

6 | MATURATION OF OOCYTES AFTER IN VITRO MATURATION FOR FERTILITY PRESERVATION

In many studies, the maturation rate for IVM is evaluated by the ratio of oocytes reaching meiosis II (MII), as determined by the extrusion of the first polar body. In Table 3, the maturation rate in FP from 13 recent studies[10,12,15,16,19,20,47-51,58,59] is summarized. In FP, the age of the patients ranges widely, from 0 to 45 years old. The number of collected oocytes per patient also ranges from 0 to 58. Therefore, when the maturation rate of FP is evaluated, the patient's age, as well as whether ovarian stimulation (eg FSH or hCG) was performed, must be considered. In FP, it is not rare to omit ovarian stimulation (ie both FSH and hCG), as shown in Table 3. The menstrual phase on the day of oocyte collection was ignored in some reports. Patients

TABLE 2 Reports about aspiration in oocyte collection for in vitro maturation

Reference	Year	Purpose of collection	Oocyte collection method	Number of patients	Number of oocytes	Needle gauge	Aspiration pressure	Needle type
Creux, Monnier, Son, Tulandi & Buckett[47]	2017	FP	TV	164	ND	19	7.5 kPa (56 mm Hg)	Single
Shirasawa, Kumazawa, Sato, Ono & Terada[39]	2017	FP	Intraoperatively	1	3	19	ND	ND
Abir, Ben-Aharon, Garor, et al.[48]	2016	FP	ROT	42	395	21	ND	ND
Grynberg, Poulain, le Parco, Sifer, Fanchin & Frydman[15]	2016	FP	TV	248	ND	19	7.5 kPa (56 mm Hg)	ND
Park, Lee, Yang, et al.[20]	2016	FP	ROT	6	53	18	ND	ND
Sonigo, Simon, Boubaya, et al.[16]	2016	FP	TV and ROT	340	3369	19	7.5 kPa (56 mm Hg)	Single
Safian, Khalili, Karimi-Zarchi, Mohsenzadeh, Ashourzadeh & Omidi[49]	2015	FP	ROT	26	61	21	40-50 mm Hg	ND
Wilken-Jensen, Kristensen, Jeppesen & Yding Andersen[50]	2014	FP	ROT	61	334	23	80 mm Hg	ND
Fasano, Moffa, Dechene, Englert & Demeestere[51]	2011	FP	ROT	57	266	18	ND	ND
Maman, Meirow, Brengauz, Raanani, Dor & Hourvitz[10]	2011	FP	TV	18	ND	19	7.5 kPa (56 mm Hg)	Single
Spits, Guzman, Mertzanidou, et al.[52]	2015	PCOS	TV	16	239	21	ND	ND
Vitek, Witmyer, Carson & Robins[53]	2013	PCOS	TV	18	334	19	80 mm Hg	Single
Junk & Yeap[54]	2012	PCOS	TV	66	844	16	ND	ND
Zheng, Wang, Zhen, Lian, Liu & Qiao[55]	2012	PCOS	TV	82	1155	19	80 mm Hg	Single
Guzman, Ortega-Hrepich, Albuz, et al.[56]	2012	PCOS	TV	44	967	17	70 mmHg	Single
Liu, Jiang, Feng, Ma, Li & Li[57]	2010	PCOS	TV	ND	2296	17	7.5 kPa (56 mm Hg)	Double
Pongsuthirak, Songveeratham & Vutyavanich[38]	2015	Research	Intraoperatively	89	1032	22	ND	ND
Imesch, Scheiner, Xie, et al.[14]	2013	Research	ROT	7	63	19	85 mm Hg	Single

FP, fertility preservation; ND, no data; PCOS, polycystic cystic ovarian syndrome; ROT, resected ovarian tissue; TV, transvaginal.

TABLE 3 Reports of in vitro maturation for fertility preservation

Reference	Year	Number of patients	Average age of patients (years): Phase or range	Oocyte collection method	Oocytes collected per patient (N):Phase or range	Maturation rate (%):Phase	Ovarian stimulation	Menstrual phase of oocyte collection
Creux, Monnier, Son, Tulandi & Buckett[47]	2017	164	29.5 (early F), 31.0 (late F), 30.0 (L)	TV	8.5 (early F), 8.0 (late F), 7.0 (L)	53.5 (early F), 58.0 (late F), 50.0 (L)	hCG	Early F, late F, L
Yin, Jiang, Kristensen & Andersen[12]	2016	36	26.0 (8-41)	ROT	10.9 (0-43)	29.2	None	ND
Abir, Ben-Aharon, Garor, et al.[48]	2016	42	12.0 (2-18)	ROT	9.4 (0-42)	30.6	None	ND
Park, Lee, Yang, et al.[20]	2016	6	29.0 (19-39)	ROT	10.6 (0-19)	67.9	None	ND
Grynberg, Poulain, le Parco, Sifer, Fanchin & Frydman[15]	2016	248	31.5 (19-39)	TV	9.3 (F), 11.1 (L)	66.7 (F), 64.5 (L)	hCG	F or L
Sonigo, Simon, Boubaya, et al.[16]	2016	340	31.8 (18-41)	TV, ROT	9.5	65.0	hCG	F or L
Segers, Mateizel, Van Moer, et al.[58]	2015	34	25.2 (0-38)	ROT	14.7 (0-58)	36.1	None	ND
Hourvitz, Yerushalmi, Maman, et al.[59]	2015	113	ND (10-41)	TV, ROT	12.3 (AIVM), 7.0 (OTIVM) (0-31)	58.6 (AIVM), 34.7 (OTIVM)	FSH, hCG	ND
Safian, Khalili, Karimi-Zarchi, Mohsenzadeh, Ashourzadeh & Omidi[49]	2015	26	34.0 (21-45)	ROT	2.3	30.2	None	ND
Wilken-Jensen, Kristensen, Jeppesen & Yding Andersen[50]	2014	61	26.5	ROT	11.2 (1-32)	3.1	None	ND
Escriba, Grau, Escrich, Novella-Maestre & Sanchez-Serrano[19]	2012	33	31.5 (15-38)	ROT	3.3	36.1	None	F or L
Fasano, Moffa, Dechene, Englert & Demeestere[51]	2011	57	26.0 (8-35)	ROT	4.0	27.9 (F), 39.5 (L)	None	F or L
Maman, Meirow, Brengauz, Raanani, Dor & Hourvitz[10]	2011	18	24.1 (F), 23.4 (L)	TV	17.3 (F), 12.8 (L)	57.8 (F), 48.6 (L)	FSH, hCG	F or L

AIVM, in vitro maturation of aspirated oocytes; F, follicular phase; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; L, luteal phase; ND, no data; OTIVM, in vitro maturation of ovarian tissue oocytes; ROT, resected ovarian tissue; TV, transvaginal.

with cancer sometimes underwent FP before puberty and OTC was scheduled emergently in the majority of patients; thus, the menstrual phase was not recorded in detail in some studies. As shown in Table 3, the maturation rate in FP is ~30%-60%. In past reports that compared the maturation rate between the follicular and luteal phases, no statistical difference was observed.[15,18,47,51] In recent reports, a commercial maturation medium has been used primarily for IVM in FP, as in patients with PCOS. However, the reported maturation rate of FP was lower than that for the patients with OHSS, which was ~50%-80%.[54,56,57] A low patient age and a lack of ovarian stimulation are considered to be associated with a relatively low maturation rate, as shown in Table 3. A recent study reported the effect of supplementation with epidermal growth factor, brain-derived neurotrophic factor, and insulin-like growth factor-1 in order to improve the maturation rate.[60] Future research is necessary in order to further improve the maturation rate in FP.

For oocyte maturation after IVM, it is important to evaluate the quality of the oocyte at various points; however, several aspects of this process remain to be elucidated. Nuclear maturation can be evaluated based on the form of the germinal vesicle (GV) and extrusion of the first polar body, but evaluation of cytoplasmic maturation is difficult.[61] As with nuclear maturation, it is not clear whether the cytoplasm changes dramatically in the IVM process.[7,62] Furthermore, it is not known whether oocytes that have been matured by IVM are equivalent to oocytes that have developed in vivo by conventional ART and there is some evidence that the IVM oocytes might be inferior, from the standpoint of their developmental potential.[11,63] In particular, in IVM for FP in the context of oncofertility, there is not much time for ovarian stimulation. However, the methods of ovarian stimulation by drugs, such as FSH and hCG, yield higher rates of pregnancy and live births than IVM without ovarian stimulation.[11,15,64] Future studies should evaluate the influence of IVM in the process of spindle formation and the arrangement of the chromosomes in meiosis.[65] In IVM, immature oocytes usually are collected from 10 to 14 mm follicles.[47,66,67] However, in the case of IVM for FP, the oocytes often are collected from small follicles (6-10 mm) in resected ovaries and the safety of these oocytes should be considered. One study found no difference between the oocytes that had been collected from follicles of 6 mm and those that had matured in vivo[68] and another study reported live births resulting from the IVM of immature oocytes that had been collected from such small follicles.[56] Therefore, the optimal diameter of the follicles for the safe collection of oocytes needs to be investigated in the future.

7 | CRYOPRESERVATION OF OOCYTES FOR FERTILITY PRESERVATION

It is not uncommon for patients with cancer to lack a partner at the time when they undergo cancer treatment.[13] If the patient has a partner, cryopreservation of the blastocysts is recommended after IVM and artificial fertilization, although cryopreservation of the oocytes or OTC is considered if there is no partner. In one study, 301 of 340 patients with cancer opted for oocyte cryopreservation after counseling, whereas cryopreservation of the blastocysts and OTC was chosen at a rate of 11 and 14%, respectively.[16] Currently, cryopreservation of the oocytes is the most important procedure, especially in the context of FP for oncofertility. However, the procedure of cryopreservation is often controversial. It is necessary to consider the effects of ice crystal formation and osmotic effects, which can damage the oocytes during cryopreservation.[69] Previously, it was thought that the freezing of oocytes at the GV stage decreased the extent of freezing damage.[70] When considering the results of cryopreservation of the oocytes in IVM, it is necessary to pay attention to whether the reference literature performed the slow-freezing method[71] or vitrification.[72] Recent reports have not concluded whether it is preferable to cryopreserve immature oocytes at the GV or MII stage.[61,73-77] Vitrification results in more normally formed spindles than the slow-freezing method.[78] The maturation rate following thawing and IVM after vitrification at the GV stage is 50.8%, significantly lower than the rate of 70.4% for the IVM of fresh material.[79] Several reports have yielded consistent results;[80,81] therefore, it is common to cryopreserve matured oocytes when using the vitrification method.

8 | EXPLANATION TO PATIENTS

In the last decade, techniques related to FP, such as OTC, have been provided clinically to patients with cancer. In these cases, oncologists and reproductive endocrinologists share the responsibility to fully explain to patients who desire FP before the treatment of malignant disease that their condition could reduce their fertility potential. However, many controversies persist regarding these techniques, as described above. It is necessary to explain to patients the current limitations and the necessity for future research. Fertility preservation, including OTC, is expensive and the burden on patients is large.[27] It is also desirable to explain the likelihood of a live birth from FP that the patient can reasonably expect at the present time. For example, at least 8-10 MII oocytes are required to obtain a live birth, depending on the age of the patient.[82] In addition, the live birth rate from each mature oocyte in patients aged 23-37 years is 4.47%[83] and this information would be useful to patients. Another study of patients with cancer reported that the pregnancy rate per oocyte was 1.52%.[22] It is also necessary to explain the possibility that cryopreserved oocytes might not be obtained, even if FP is performed.[47] For example, it was reported that oocytes cannot be collected from 2.8% of patients with cancer and that the antral follicle count and anti-Müllerian hormone levels are useful for predicting the number of cryopreserved oocytes after IVM.[16] Furthermore, the rate of chromosomal abnormality is higher in blastocysts that are obtained from IVM than in blastocysts that are obtained from conventional ART.[84] As mentioned above, babies born following IVM are reported to be healthy, but long-term follow-up is also necessary.[5,6]

9 | RECENT RESEARCH ON FERTILITY PRESERVATION USING IN VITRO MATURATION

As mentioned above, IVM is a very important technique in FP. Accordingly, research using human oocytes is indispensable for further improving the performance of IVM in the future. However, due to ethical and legal hurdles in individual countries, it is not easy to obtain human oocytes for research purposes. In order to obtain immature human oocytes for research purposes, a method has been developed for receiving volunteer immature oocytes at the time of intracytoplasmic sperm injection for ART.[85] In addition, the authors reported a method for obtaining immature oocytes from the resected ovaries of surgical patients with endometrial cancer for use in research.[86] In Figure 1, the resected ovaries of a patient with an endometrioid adenocarcinoma are shown, as well as the collected immature oocytes and mature oocytes after IVM. However, it is difficult to fertilize mature oocytes that have been obtained after IVM due to legal and ethical concerns.[12,14,61] In addition, if the fertilization process or the development of the blastocyst cannot be evaluated, it is difficult to fully evaluate the oocyte's potential. This is a limitation of research on IVM and it is necessary to recognize that there is also a limitation on the provision of information to patients who are undergoing FP. Recently, as a substitute for the fertilization process, several researchers have studied parthenogenetic embryonic development in oocytes after IVM.[61,75] Currently, it is necessary to evaluate fertilization and the development of blastocysts by simulation, to the extent that this is possible. Moreover, resected ovarian tissue and oocytes often cannot be evaluated when they are transported from satellite hospitals to a central ART hospital for FP (eg in ovary banks).[49,50,87-89] The influence of the transportation of tissue in relation to FP by IVM needs to be more carefully considered in the future. Moreover, the IVM process allows studies of human oocytes at various stages. For example, IVM makes it possible to study the expression of the proteins that are related to the aging of oocytes, such as cohesin protein,[90] in human

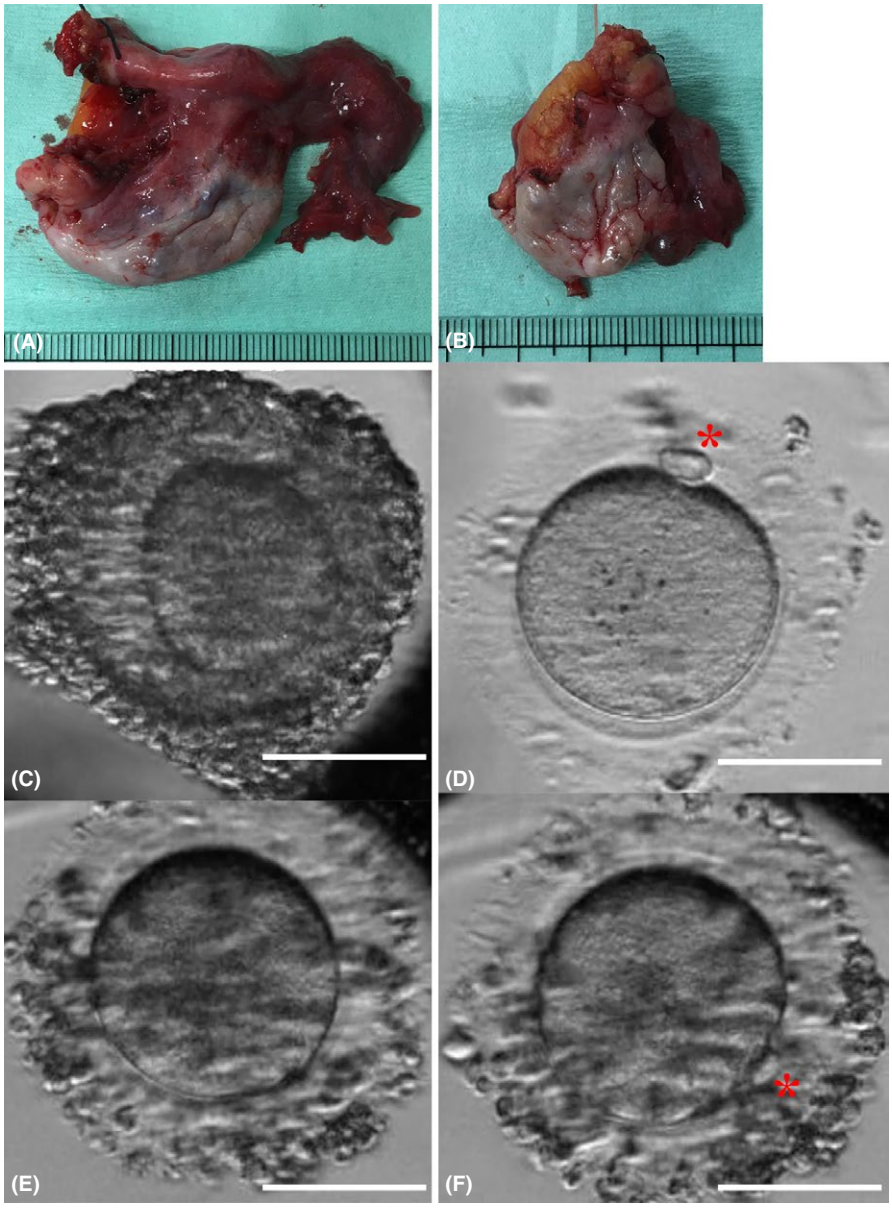

FIGURE 1 Resected ovaries and collected oocytes. A and B, Appearance of the resected ovaries of a patient with an endometrioid adenocarcinoma. C, A collected immature oocyte from the resected ovaries. D, A matured oocyte after the in vitro maturation (IVM) of the oocyte in (C). E, A collected immature oocyte from the resected ovaries. F, A matured oocyte after the IVM of the oocyte in (E). The asterisk indicates the first polar body. Scale bar: mean 100 μm

oocytes of various stages. Using new technology like electric-field mixing technology for efficient immunofluorescence staining,[91] research on the aging of oocytes with IVM will advance the field further.

10 | CONCLUSION

It is now possible to present various methods of FP to patients with cancer and other diseases. A wide range of choices is desirable, given considerations regarding the patient's quality of life. However, it must be emphasized that many aspects of the technologies that are related to FP, including IVM technology, remain unclear. By switching from manually made to commercial maturation medium, the maturation rate of IVM has improved.[58] In the future, further improvements can be expected in FP performance due to changes in culture medium and additives. Especially in oncofertility, reproductive endocrinologists and oncologists need to pursue a rapid, low-cost, uninvasive method of FP for patients facing cancer treatment.

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DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human and Animal Rights*: This study of oocyte collection from the resected ovaries of patients with endometrioid adenocarcinoma was approved by the Ethical Review Board of Akita University (No. 787, June, 2011). This article does not contain any study with animal participants that has been performed by any of the authors.

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