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OP-IVM: Combining In Vitro Maturation After Oocyte Retrieval with Gynecological Surgery

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Corresponding Author:	Jie Yan, Ph.D. Peking University Third Hospital Beijing, Beijing CHINA
Corresponding Author's Institution:	Peking University Third Hospital
Corresponding Author E-Mail:	yanjiebjmu@bjmu.edu.cn
Order of Authors:	Tao Liu
	Xueling Song
	Xiaoying Zheng
	Liying Yan
	Caihong Ma
	Rong Li
	Jie Yan, Ph.D.
	Jie Qiao
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TITLE:

OP-IVM: Combining In Vitro Maturation After Oocyte Retrieval with Gynecological Surgery

AUTHORS AND AFFILIATIONS:

Tao Liu[1,2,3,4,5*], Xueling Song[1,2,3,4,5*], Xiaoying Zheng[1,2,3,4,5*], Liying Yan[1,2,3,4,5], Caihong Ma[1,2,3,5], Rong Li[1,2,3,4,5], Jie Yan[1,2,3,4,5], Jie Qiao[1,2,3,4,5]

[1]Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing 100191, China
[2]National Clinical Research Center for Obstetrics and Gynecology, Beijing 100191, China
[3]Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing 100191, China
[4]Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproduction, Beijing 100191, China
[5]Research Units of Comprehensive Diagnosis and Treatment of Oocyte Maturation Arrest, Chinese Academy of Medical Sciences, Beijing 100191, China

[*]These authors contributed equally

Email Addresses of Co-authors:

Tao Liu	(taoliu1017@qq.com)
Xueling Song	(sxldxx@263.net)
Xiaoying Zheng	(zheng_xiaoying@126.com)
Liying Yan	(yanliyingkind@aliyun.com)
Caihong Ma	(macaihong123@aliyun.com)
Rong Li	(roseli001@sina.com)
Jie Yan	(yanjiebjmu@bjmu.edu.cn)
Jie Qiao	(jie.qiao@263.net)

Corresponding authors:

Jie Yan	(yanjiebjmu@bjmu.edu.cn)
Jie Qiao	(jie.qiao@263.net)

KEYWORDS:

IVM, fertility preservation, transvaginal retrieval, endoscopic gynecological procedures, PCOS

SUMMARY:

In vitro maturation (IVM) before gynecological operation (OP-IVM) combines IVM following oocyte retrieval with routine gynecological surgery and serves as an extension of conventional IVM applications for fertility preservation.

ABSTRACT:

The use of in vitro maturation (IVM) before gynecological operation (OP-IVM) is an extension of conventional IVM that combines IVM following oocyte retrieval with routine gynecological surgery. OP-IVM is suitable for patients undergoing benign gynecological surgery who have the need for fertility preservation (FP) or infertility treatments such as in vitro fertilization and embryo transfer (IVF-ET). In the operating room, patients undergoing benign gynecological surgery are first anesthetized and receive ultrasound-guided immature follicle aspiration

(IMFA) treatment. As the subsequent gynecological surgery is performed, the cumulus-oocyte complexes (COCs) are examined, and the immature COCs are transferred into the IVM medium and cultured for 28–32 h in the IVF laboratory. After assessment, mature oocytes in the MII stage will be selected and cryopreserved in liquid nitrogen for FP or fertilized by intracytoplasmic sperm injection (ICSI) for IVF-ET. By combining IVM with gynecological surgery, immature oocytes that would have been discarded can be saved and used for assisted reproductive technology (ART). This article describes the procedure, significance, and critical aspects of OP-IVM.

INTRODCUTION:

IVM is an ART in which human immature oocytes are cultured in vitro to maturation for IVF-ET or FP. In IVM, ovulation induction medications are not used, thus reducing pain, financial burden, and complications such as ovarian hyperstimulation syndrome (OHSS)[1,2]. In addition, IVM is particularly suitable for the FP of cancer patients and the infertility treatment of hormone-sensitive patients who may be unable to or have no time to receive ovulation induction therapy[3]. Therefore, although the number of oocytes retrieved, clinical pregnancy rate (CPR), and live birth rate (LBR) are lower than those of IVF[4,5], IVM has its own unique advantages.

Infertile patients with endometrial lesions, hydrosalpinx, or ovarian cysts usually have gynecological surgery before ART treatment, and their oocytes are usually immature. OP-IVM uses guided transvaginal ultrasound to retrieve the immature oocytes and grow them in vitro until maturation for IVF-ET or FP. OP-IVM combines IVM after oocyte retrieval and gynecological surgery, thereby reducing complications that are common to controlled ovarian hyperstimulation cycles and saving time and money. For fertile patients, OP-IVM could serve as a “fertility insurance” while undergoing routine gynecological surgery.

Furthermore, damage caused by gynecological surgeries, such as electrocautery[6,7] and ovarian tumor resection, could be reduced through oocyte retrieval before gynecological surgery. Therefore, compared with routine gynecological surgery, OP-IVM could reduce the number of operations during infertility treatment and prevent the loss of functional oocytes during ovarian surgery.

A previous study has shown that the additional procedure of oocyte retrieval would neither increase surgical complications and adverse pregnancy outcomes, nor prolong the hospital stay[8]. Some patients have given live birth through OP-IVM[8], indicating the feasibility of this method. This paper describes characteristics of patients who may benefit from OP-IVM as well as procedures and critical points of OP-IVM and discusses the evaluation of human oocyte maturity.

PROTOCOL:

NOTE: Studies related to the OP-IVM method have been approved by the institutional review board (IRB) of Peking University Third Hospital and the Ethics Committee of Peking University (2014S2004). A summary of OP-IVM is shown in **Figure 1**. The step-by-step procedure will be introduced in the following section.

1. Introduction of OP-IVM to appropriate patients

1.1. Identify potential patients who may benefit from OP-IVM such as those described in steps 1.1.1–1.1.3.:

1.1.1. Polycystic ovarian syndrome (PCOS) patients with clomiphene resistance who need laparoscopic ovarian drilling surgery.

1.1.2. Infertile patients who need benign gynecological surgeries, such as hysteroscopic myomectomy, polypectomy, transcervical resection of septum and laparoscopic tubal surgery, and oophorocystectomy, before ART treatment.

1.1.3. Patients with cancer or hematological disease who are receiving chemoradiotherapy or radiotherapy.

2. Informed consent

2.1. Provide full information to patients, including why OP-IVM may be beneficial, its procedure, uses of IVM oocytes (IVF-ET or cryopreservation), estimated CPRs and LBRs, and possible complications. Ask patients to give informed consent by signing the consent form.

3. Prepare labels and IVM oocyte medium

3.1. Print identification (ID) labels with the patient's name and dates for culture dishes and tubes.

3.2. Add 0.5 mL of IVM oocyte medium supplemented with 0.075 IU/mL of follicle-stimulating hormone (FSH) and 0.075 IU/mL of luteinizing hormone (LH) to each well of a 4-well plate. Cover the medium with oil.

NOTE: Perform all these procedures in laminar flow clean benches.

3.3. Prewarm the 4-well plate with IVM oocyte medium at 37 °C in humidified air containing 5% CO_2 and 5% O_2 at least 6 h before use.

4. Administeranesthesia in an operating room

4.1. Check the name of the patient before administering anesthesia.

4.2. Instruct the anesthesiologist to intravenously anesthetize the patient.

5. Perform oocyte retrieval

5.1. Tag the labels with name, date, and ID on the culture dishes and tubes.

5.2. Place the patient in the bladder lithotomy position; constantly disinfect, drape, and scrub the vagina with warm saline.

5.1. Place the ultrasound probe inside the vagina; scan and record the number of follicles in both the ovaries. Find the location closest to the ovary as the puncture site, and avoid the intestine, bladder, and large blood vessels.

5.2. Aspirate follicular fluid.

5.2.1. Wash the needle with a pH-stable handling medium before puncturing (see **Table of Materials**).

5.2.2. Inject the 19 G, single-lumen aspiration needle into the ovaries under the guidance of ultrasound.

5.2.3. Puncture larger follicles with clear boundaries closest to the probe. At a low position, quickly inject the rinse solution supplemented with 25 U/mL of heparin. Aspirate follicular fluid with the needle under a pressure of 80–90 mmHg, rotating the needle slightly to aspirate as much follicular fluid as possible. Puncture other follicles from the near to far side on this plane.

NOTE: During aspiration, follicular fluid containing oocytes will flow into a sterile 10 mL test tube under negative pressure. Heparin can reduce the follicular fluid viscosity to facilitate the aspiration process.

5.2.4. Remove the needle from the ovary (keep the needle in the vaginal wall). Adjust the direction of the ultrasound probe, and puncture the remaining follicles on other planes. Try to aspirate all the follicles with a diameter of ~5–9 mm.

NOTE: Adjust the probe's position to keep it closest to the ovaries at all times. Press the vaginal fornix with appropriate force to reduce injury and bleeding.

5.2.5. Pull out the needle after finishing in one ovary, wash the needle with the handling medium, and puncture the other side using the same method.

NOTE: Complete follicle aspiration within 25–30 min.

5.3. Transfer the follicular fluid from the operation room to the IVF laboratory within a few minutes after confirming the patient's name and ID.

NOTE: Transfer aspirated follicular fluid to the IVF laboratory bench as soon as possible to prevent coagulation.

5.4. Detect active hemorrhage in the pelvic cavity with B-mode ultrasound after puncturing all the follicles. Insert a speculum, and point the tip at the posterior fornix to detect active bleeding at the puncture site.

NOTE: No active bleeding should be observed at the vaginal puncture site if the ovary position is normal, and oocyte retrieval is performed carefully. For light bleeding that continues even

after compression, keep a sterile gauze compress in the vagina for 2–4 h. Control excessive bleeding from small arteries using clamps with vascular forceps for 2–4 h. Bleeding in the pelvic cavity or ovary, which seldom happens, should be controlled by electrocoagulation by laparoscopic surgery.

6. Gynecological surgery

6.1. Based on the need and condition of the patient, perform appropriate gynecological surgery after oocyte retrieval.

6.1.1. Perform laparoscopic ovarian drilling surgery for polycystic ovarian syndrome (PCOS) patients with clomiphene resistance.

6.1.2. Perform benign gynecological surgery for infertile patients before ART treatment, such as hysteroscopic myomectomy, polypectomy, transcervical resection of septum and laparoscopic tubal surgery, and oophorocystectomy.

6.1.3. Perform ovariectomy for fertility cryopreservation of patients with cancer or hematological disease who need to receive chemoradiotherapy.

NOTE: These gynecological surgeries are basic and standardized clinical operations. Operation guidelines in various countries and hospitals should be relatively similar.

7. Perform IVM

NOTE: Perform the whole process of IVM on a 37 °C homothermal flat.

7.1. Filter the aspirated follicle fluid with a 70 µm nylon cell strainer. Repeatedly rinse the culture tube and strainer with pre-warmed pH-stable handling medium. Ensure all the immature COCs are completely transferred to the culture dish. Collect the filtered fluid , rinse, and culture in a 100 x 15 mm Petri dish.

7.2. Examine the COCs (**Figure 2**) under the stereoscope with 40x magnification. Quickly transfer the immatures into a pre-warmed IVM oocyte medium.

NOTE: Choose an appropriate magnification depending on the operator's habit.

7.1. Record the number of cultured immature COCs.

7.2. Inform the patient about the number of cultured COCs. Discuss the collection of semen with the patients and their partners. Perform sperm extraction according to the normal procedure.

7.3. Culture the immature COCs at 37 °C in humidified air containing 5% CO2 and 5% O_2 for 28–32 h.

7.4. Assess the oocyte maturity.

7.4.1. Denude the cumulus cells by repeated pipetting using a glass Pasteur pipette under the stereoscope with 40x magnification. Examine the extrusion of the polar body (PB) to identify the developmental stage of the oocytes. See **Figure 2** for representative images of oocytes in COCs, metaphase II (MII), metaphase I (MI), and germinal vesicles (GVs) with clear morphological characteristics.

NOTE: Use a vertical flame (e.g., of a Bunsen burner) to adjust the diameter of the glass Pasteur pipette to the size of an oocyte.

7.4.2. Count MII oocytes and record the number.

7.6.3 Choose mature oocytes for IVF or vitrification.

NOTE: Collect sperm on the day the oocytes mature for IVF, but not for oocyte vitrification.

7.6.4. Culture the GV and MI stage immature oocytes in the original IVM culture medium with cumulus cells for another 10–14 h. Repeat steps 7.6.2–7.6.3.

8. Perform ICSI or oocyte vitrification

8.1. Follow the standard procedure of the reproductive center[8].

9. Culture embryos and perform embryo cryopreservation

9.1. Follow the standard procedure of the reproductive center[8].

REPRESENTATIVE RESULTS:

Until December 2019, OP-IVM was used for fertility preservation of 274 patients. Embryological and reproductive outcomes of 158 patients between 2014 to 2016 were published in a previous paper[8]. The following example discusses the procedure followed for a PCOS patient receiving OP-IVM in 2016. The patient is a 28-year-old diagnosed with primary infertility, left adnexal cyst, and PCOS. She received laparoscopic cystectomy and OP-IVM on September 28th, 2016; 27 immature COCs were obtained. After a 28 h culture, 7 oocytes in the MII stage were selected and fertilized by ICSI. The number of good-quality 2 pro-nuclei (2PNs) on day 1 and embryos on day 3, and day 5 were 6, 6, and 2, respectively. The blastocysts obtained on day 5 (October 4th, 2016) were frozen. On February 14th, 2017, one blastocyst was thawed. This thawed blastula was alive and transferred into the uterus on February 14th, 2017. Results of a blood human chorionic gonadotropin (hCG) test were positive, and ultrasonography showed intrauterine early pregnancy. The patient delivered a healthy girl by cesarean section at 40 weeks on November 2nd, 2017.

FIGURE AND TABLE LEGENDS:

Figure 1: OP-IVM flow chart. Before gynecological surgery, under transvaginal ultrasound guidance, follicle fluid is aspirated through IMFA. The obtained follicle fluid is transferred into the IVF laboratory, filtered, and rinsed. Immature COCs are transferred into a prewarmed IVM

culture solution and cultured for 28–32 h. Mature oocytes with PB are used for cryopreservation or IVF-ET. Abbreviations: IMFA = immature follicle aspiration; IVF = in vitro fertilization; IVF-ET = IVF and embryo transfer; COCs = cumulus-oocyte complexes; IVM = in vitro maturation; OP-IVM = IVM before a gynecological operation; GV = germinal vesicle; MI = metaphase I; ICSI = intracytoplasmic sperm injection; PB = polar body; PN = pro-nucleus; D1 = day 1.

Figure 2: Morphological characteristics of representative images of oocytes. (**A**) COCs, (**B**) MII, (**C**) MI, (**D**) GV stage. Scale bars = 100 μm. Abbreviations: COCs = cumulus-oocyte complexes; GV = germinal vesicle; MI = metaphase I; MII = metaphase II.

DISCUSSION:

The OP-IVM method described in this article extends to conventional IVM applications and combines IVM after oocyte retrieval with routine gynecological surgery. Oocytes that would have been lost in the gynecological surgery can now be used for IVF-ET or FP without additional surgical risks. OP-IVM was first used to retrieve oocytes before ovarian drilling in PCOS patients. Its application soon expanded to include infertile patients who need benign gynecological surgery and cancer or hematological disease patients who need chemoradiotherapy. Because of the potential risk of metastasis, OP-IVM is not suitable or recommended for patients with malignant tumors. Based on OP-IVM, a new Chinese mode of egg bank for the FP of infertile patients and cancer patients has been established at this institution.

For OP-IVM, both the urgency of gynecological surgery and the potential of obtaining a higher number of oocytes should be considered. In general, IVM outcome is positively correlated with the number of immature oocytes obtained during oocyte retrieval[9]. Dominant follicles may inhibit the growth of surrounding smaller follicles and cause atresia[10], resulting in a reduced number of retrieved immature oocytes. Therefore, if the gynecological surgery is not urgent, the best window for OP-IVM is when a greater number of small follicles with few dominant follicles are observed by B-mode ultrasonography. In addition, anti-Mullerian hormone (AMH) can be used as a parameter to predict ovarian reserve as the numbers of retrieved oocytes are significantly positively correlated with AMH[11,12]. In cases where gynecological operation has been prioritized, as many immature oocytes should be retrieved as much as possible. In emergency operations, oocyte retrieval and IVM should be carefully evaluated based on the patient's condition and will.

IMFA under transvaginal ultrasound guidance is a method used to retrieve oocytes that can reduce damage to ovarian function[13,14]. Laparoscopic ovarian puncture to aspirate immature oocytes is also used in some studies[15]. However, compared with laparoscopy, IMFA under transvaginal ultrasound takes up less time, is less invasive, is easier to perform[16,17], and can achieve more targeted follicle aspiration. Therefore, to minimize the impact on ovarian reserve and increase aspiration accuracy, IMFA under transvaginal ultrasound guidance is recommended as a better method for oocyte retrieval in OP-IVM. However, ICSI is recommended for fertilization. Previous studies have shown that the zona pellucida will harden after being cultured in vitro for long periods, resulting in a reduced rate of successful fertilization[18]. ICSI has been shown to effectively improve the fertilization rate of IVM oocytes in natural cycles[19,20]. Although recent studies have shown similar fertilization rates for ICSI

and IVF[21,22], ICSI holds the advantage of using natural cycles instead of ovarian stimulation. Collected embryos are frozen because of unprepared endometrium, and embryo transfer can be performed later according to the standard procedure at each center.

The purpose of ART is to treat infertility and deliver healthy babies. Previous research about the reproductive outcomes of OP-IVM showed that the numbers of retrieved oocytes and oocyte maturation rates are comparable with those observed for conventional IVM cycles using FSH and hCG. However, OP-IVM has a lower CPR and a lower LBR than conventional IVM[8,23–25]. This may be explained by constrained oocyte maturation due to the poor development potential of immature oocytes in natural cycles. However, hormone stimulation is not an option for some patients who are unable to or who do not have the time to receive the treatment. In addition, vitrification may damage the oocytes' developmental potential, resulting in low CPRs and LBRs[26,27].

Moreover, gonadotropins may enlarge the ovaries and increase the risk of bleeding during oocyte retrieval, adversely affecting the exposure of operative fields in laparoscopy. The LBR of the OP-IVM method is relatively low. More studies are needed to examine the best operative timing and modify the IVM culture system to improve OP-IVM outcomes. Overall, OP-IVM combines IVM with gynecological surgery so that immature oocytes that would have been damaged or discarded can be saved and used for ART. OP-IVM has the advantages of avoiding complications caused by ovarian stimulating medications and reducing the number of operations for infertility treatment. Therefore, OP-IVM should be considered as a potentially valuable treatment method for certain patients and studied more in depth to better understand its effects and outcomes.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

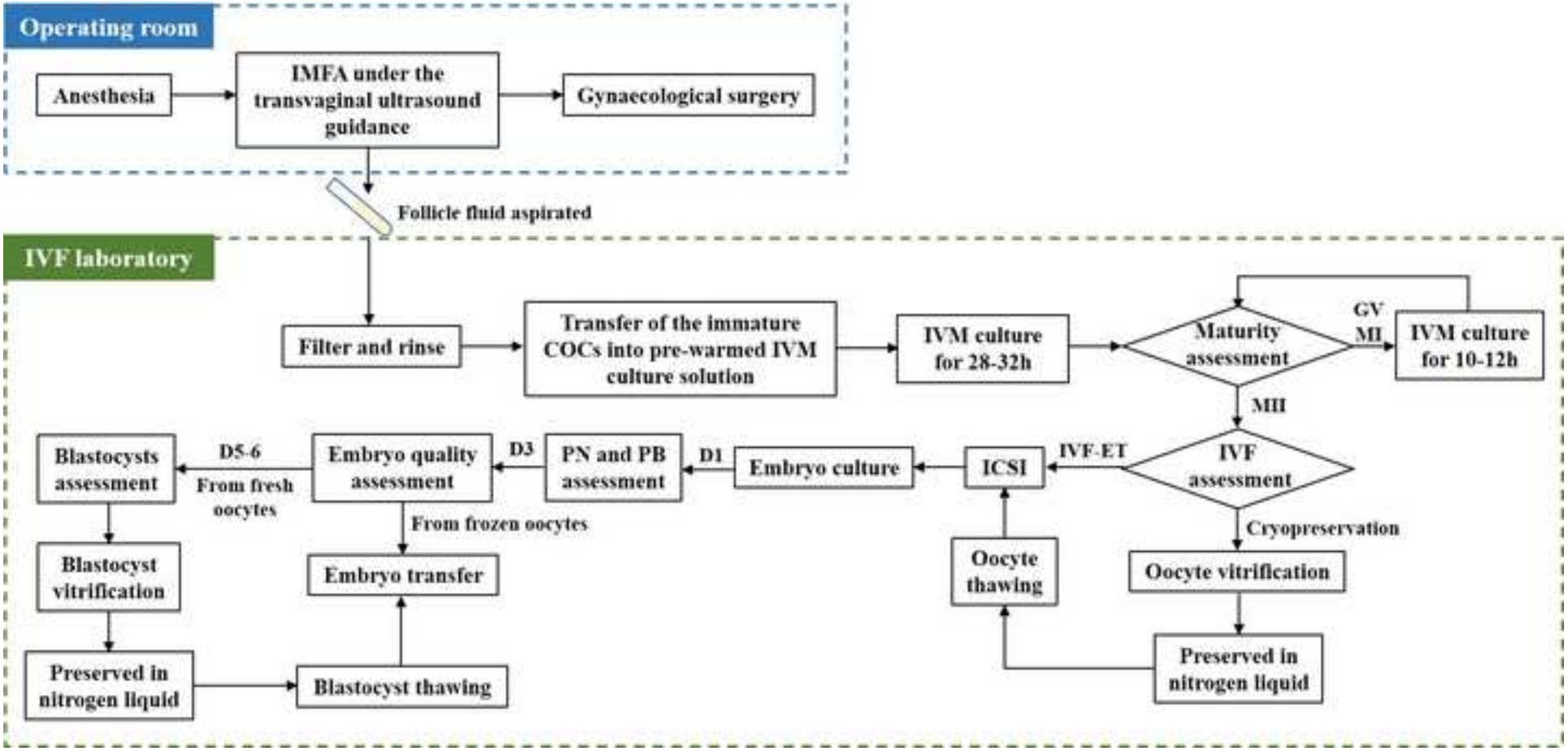

Figure 2

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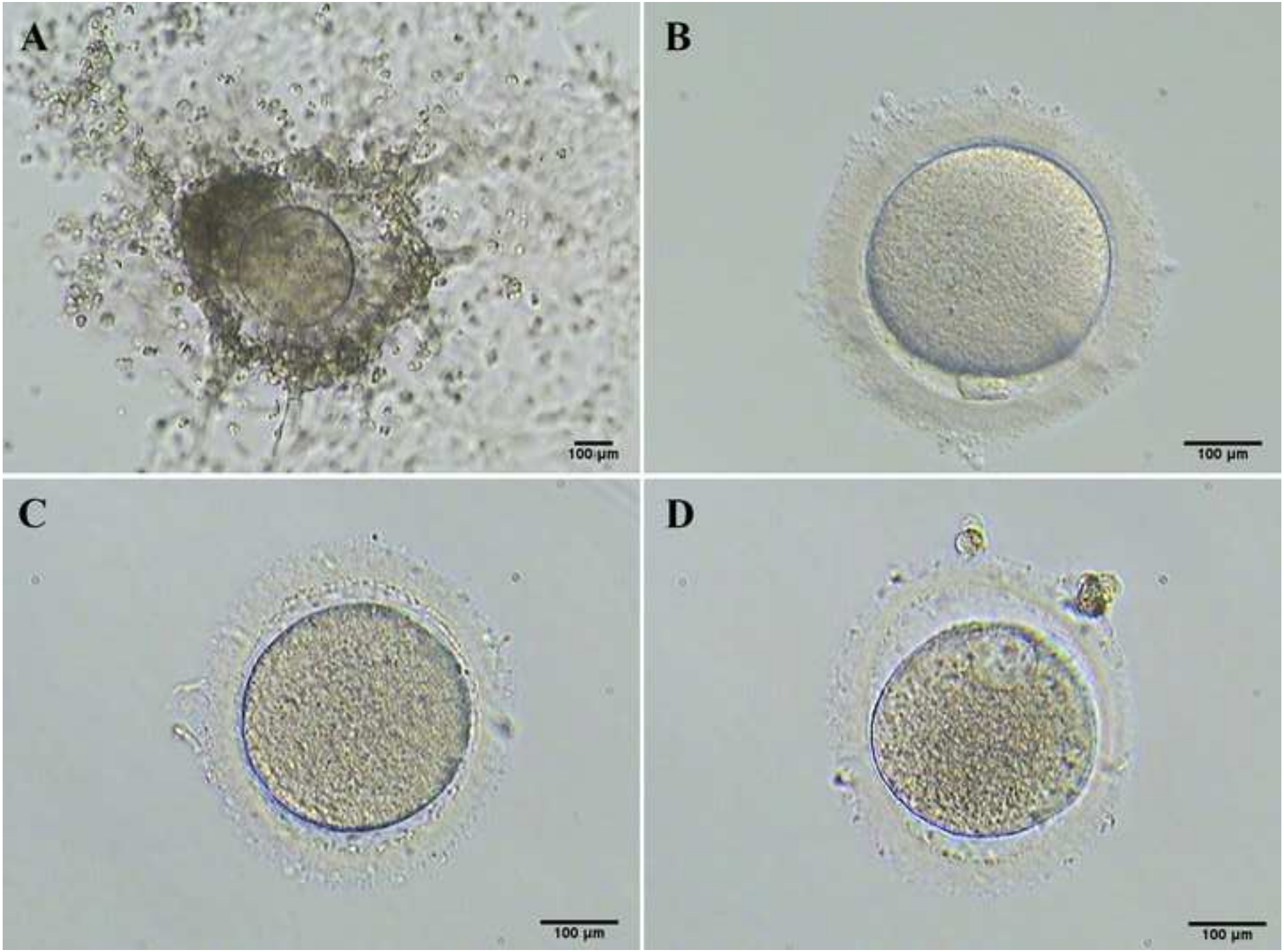

Table of Materials

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Name of Material/Equipment	Company	Catalog Number
19 G single-lumen aspiration needles	Cook, Australia	K-OPS-7035-REH-ET
4-well plate	Corning	
70 µm nylon cell strainer	Falcon, USA	352350
CO_2 Incubator	Thermo	
Culture oil	Vitrolife, Sweden	10029,OVOIL
FSH & LH	Ferring Reproductive Health, Germany	MENOPUR®
Glass Pasteur pipette	Hilgenberg GmbH, Germany	3154102-26
G-MOPS medium		
IVM medium	Origio, Denmark	ART-1600-B
Laminar Flow Clean Benches	ESCO	
Petri dish	Thermo Fisher Scientific, Denmark	263991
pH stable handing media designed to support the handling and manipulation of oocytes and embryos outside the incubator	Vitrolife, Sweden	10130, G-MOPS PLUS
Rinse solution	Cook, Australia	K-SIFB-100
Stereoscope	Nikon	

Comments/Description

Step 3.2.

pH-stable handling medium for washing the needle before puncturing

Step 7.1.

Dear Editor，

Thanks for giving us these comments on structure and content of our manuscript. Here we submit a third version of our manuscript with the code JoVE61647, which has been revised according to the editor and reviewers' suggestions. Efforts were also made to correct the mistakes and improve the English of the manuscript.

The following is a line-by-line response to the editorial and reviewer comments.

Editorial comments:

Thanks for the editor's detailed and specific suggestions on language and structure of this manuscript. The following changes were made according to these comments.

Comment 1: 1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: Spelling and grammar of this manuscript had been checked carefully again to remove all the errors.

Comment 2: Please revise the title for conciseness: Combining in Vitro Maturation After Oocyte Retrieval and Gynecological Operation for Fertility Preservation.

Response: Based on the comments of the editor and reviewer 5, we changed the title into "OP-IVM: A New Technology Combining in Vitro Maturation After Oocyte Retrieval with Gynecological Surgery".

Comment 3: Please provide citations for step 6, 8, and 9.

Response: Citation for step 8 and 9 has been provided. Gynecological surgery process in step 6 is various in different kind of diseases. Therefore, it is difficult for us to provide specific citations for this step. Instead, the appropriate surgery for three kinds of potential patients who may benefit from OP-IVM are described in this step.

Comment 4: Please do not abbreviate journal titles.

Response: Journal titles in reference list has been revised.

Reviewer #2: Thanks for the reviewer's suggestions on the usage of OP-IVM technology and description of laboratory procedures.

Major Concerns: The manuscript still strongly lacks comprehensive and detailed descriptions of ovarian puncture of immature follicles and then subsequent IVM of immatures oocytes with numerous tricks and recommendation to succeed OP-IVM.

Representative images and results of these two innovative techniques are still lacking in the manuscript (such as laparoscopic view of the ovaries showing how to identify and aspirate small antral follicles, how to handle bloody and viscous follicular fluid of immature follicles, pictures of immature cumulus-oocyte complexes bathing in follicular fluid, culture conditions during IVM, how to successfully denude immature COCs, etc.).

Response: In this version, detailed operative process of oocyte retrieval is described in step 5, including the method of aspirating as much follicular fluid as possible under the guidance of ultrasound and bleeding control in oocyte retrieval. The video attached to this article will show the process of oocyte retrieval and gynecological surgery as well as the laparoscopic view of the ovaries related with the points mentioned in the comments.

Reviewer #3: Thanks for the reviewer's concerns on this article. Next are the explanations of these

questions. We sincerely hoped that these words would be helpful to understand this manuscript.

Manuscript Summary: As stated previously this manuscript describes only one case report yet presented as a full manuscript. The authors present approach for handling one case only and I see no reason why one case report be published as a full manuscript.

Major Concerns:

What the authors claim to be a novel approach has been previously reported

Minor Concerns:

The authors compare their results with unrelated techniques.

It is not a case report. Actually totally 274 patients received OP-IVM technology until December 2019, which figure is added into "Representative Results" section (Line 238-240). The case carefully described in "Representative Results" section is the treatment process of one in 274 patients. We hope that the description of this case will be helpful for readers to understand the operation process of OP-IVM because it provides more comprehensive and clear demonstrations of this technology.

We are not sure that "the approach that has been previously reported" and "the unrelated techniques" refer to the method of the article published on *Human Reproduction* (Song, X.L., et al.: Enhancing the scope of in vitro maturation for fertility preservation: transvaginal retrieval of immature oocytes during endoscopic gynaecological procedures. *Human Reproduction*. 2020). If they are, we have to explain that the work published on *Human Reproduction* is completed by the authors' team and OP-IVM, the technology detailedly described in the manuscript submitted to *JoVE,* is actually the method adopted in the work on *Human Reproduction*. Also, the results used to prove the feasibility and safety of OP-IVM (Line 74-78) are from the article published on *Human Reproduction*.

Reviewer #4: We sincerely appreciate the recognition of this reviewer on OP-IVM. Spelling and grammar of this manuscript had been checked carefully again to remove all the errors.

Reviewer #5: We sincerely appreciate the comments of this reviewer on the article. Next are the revisions we made according to these comments.

Minor Concerns:

The grammar and writing still needs some work.

Title - consider changing to "OP-IVM: A New Technology Combining in Vitro Maturation After Oocyte Retrieval with Gynecological Surgery"

Line 28 - I recommend taking out "the" so that it reads "The technology of in vitro maturation (IVM) before gynecological surgery"

Line 42 - remove either detailed or described

Line 109 - I'm not sure what "ask the patient to sign up" means. I know you aren't getting enrollment at this point, because you wouldn't do that (right before anesthesia!) and you already mention getting consent.

Line 145 - consider revising the phrase "inform her husband" to "inform her partner (if applicable)"

Line 204 - I am still not sure what "a new China mode 'Egg Banking'" means - do they mean "a new Chinese model of egg banking"?

Response: Based on the comments of the editor and reviewer 5, we changed the title into "OP-IVM: A New Technology Combining in Vitro Maturation After Oocyte Retrieval with Gynecological Surgery". Error in Line 28, 42 and 145 has been corrected. The words "ask the patient to sign up"

has been removed in this version and we actually don’t do that in this step. “a new China mode Egg Banking” refer to the egg bank that stores the oocyte after IVM from patients undergoing natural cycles. Most of these oocytes were obtained through OP-IVM. The words have been changed into “a new Chinese mode of egg bank” (Line274-275) to make the expression clear.

Dear Editor,

We would like to submit the revised manuscript entitled "A New Technology Combines in Vitro Maturation After Oocyte Retrieval and Gynecological Operation for Fertility Preservation" for the publication in *Journal of Visualized Experiments*. The work described has not been submitted elsewhere for publication, in whole or in part, and all the authors listed have approved the manuscript that is enclosed.

The technology of in vitro maturation (IVM) before the operation (OP-IVM) introduced in this article is a comprehensive technology system that combines IVM technology following oocyte retrieval with routine gynecological surgery. The advantage of this technology is the full utilization of the immature oocytes that would have been discarded in surgery through the conjunction of IVM and gynecological surgery, which have been mentioned in the first submission. According to the comments given by the editor and reviewers, protocol in the current manuscript focuses on how to combine oocyte retrieval and gynecological surgery as well as the process of IVM, which are the innovation points of OP-IVM technology, while the detailed descriptions of the routine clinical applications like oocyte vitrification and IVF-ET are simplified to one step. Besides, spelling and grammar of this manuscript are also checked carefully to remove all the errors. We sincerely hope that OP-IVM technology will be popularized through this article.

Thank you very much for your time and consideration.

Sincerely yours,

Professor Jie Yan
Peking University Third Hospital, Beijing, China
E-mail: yanjiebjmu@bjmu.edu.cn