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

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

Yes

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Interview statements: Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one.**



Interviewees self-record interview statements. JoVE can provide support for this option.

4. Filming location: Will the filming need to take place in multiple locations? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Jie Yan:** OP-IVM technology is an extension of conventional IVM that combines IVM following oocyte retrieval with routine gynecological surgery. It is suitable for infertile women who need to receive gynecological surgery before ART treatment.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.2. **Tao Liu:** By combining gynecological surgery and IVM technology, OP-IVM could reduce the waste of immature oocytes in surgery and the number of operations during infertility treatment.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

- 1.3. **Tao Liu:** This technique can benefit polycystic ovarian syndrome patients with clomiphene resistance who need laparoscopic ovarian drilling surgery, infertile patients who need benign gynecological surgeries before ART treatment, and patients with cancer or hematological disease who are receiving chemoradiotherapy or radiotherapy.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

- 1.4. Procedures involving human subjects have been approved by the Institutional Review Board (IRB) of Peking University Third Hospital and the Ethics Committee of Peking University.

Protocol

2. Oocyte retrieval

- 2.1. Place the ultrasound probe inside the vagina to scan and record the number of follicles in both ovaries. Find the location closest to the ovary as the puncture site, avoiding the intestine, bladder, and large blood vessels [1].
 - 2.1.1. LAB MEDIA: Clip 1_Oocyte retrieval.mp4. 0:08 – 0:24
- 2.2. Wash the 19-gauge, single-lumen aspiration needle with a pH-stable handling medium, then inject the needle into the ovaries under the guidance of ultrasound [1].
 - 2.2.1. LAB MEDIA: Clip 1_Oocyte retrieval.mp4. 0:25 – 0:42
- 2.3. Aspirate follicular fluid with the needle under a pressure of 80 to 90 millimeters of mercury, rotating the needle slightly to aspirate as much follicular fluid as possible [1].
 - 2.3.1. LAB MEDIA: Clip 1_Oocyte retrieval.mp4. 0:47 – 1:00.
- 2.4. Use heparin to reduce follicular fluid viscosity during the aspiration. Adjust the probe's position to keep it as close as possible to the ovaries at all times and press the vaginal fornix with a proper force to reduce injury and bleeding. Make sure to complete follicular fluid aspiration within 25 to 30 minutes [1].
 - 2.4.1. LAB MEDIA: Clip 1_Oocyte retrieval.mp4. 1:09-1:25. *Video Editor: Make sure to show the needle and the fluid being collected in the tube.*
- 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 [1].
 - 2.5.1. LAB MEDIA: Clip 1_Oocyte retrieval.mp4. 2:06 – end.

3. IVM

- 3.1. Working in a 37-degree Celsius homothermal flat, prepare the handling media, Petri dish and cell strainer. Rinse the culture tube and strainer with pre-warmed pH stable handling media [1].
 - 3.1.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 0:05 – 0:30.
- 3.2. Filter the aspirated follicle fluid with a 70-micrometer nylon cell strainer [1], repeatedly rinsing the culture tube and strainer with pre-warmed pH-stable handling medium. Ensure that all immature COCs are completely transferred to the culture dish [2].
 - 3.2.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 0:31 – 0:55.
 - 3.2.2. LAB MEDIA: Clip 2_In vitro maturation.mp4. 0:56 – 1:20.

- 3.3. Transfer the blood clot into clean handling media **[1]**.
 - 3.3.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 1:21 – 1:35.
- 3.4. Then, transfer the cell strainer to a new dish and wash it **[1]**. Aspirate and transfer the handling media with the COCs, granulosa cells, and tissues to a new dish **[2]**.
 - 3.4.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 1:36 – 1:50.
 - 3.4.2. LAB MEDIA: Clip 2_In vitro maturation.mp4. 2:05 – 2:20.
- 3.5. Examine the COCs under the stereoscope with 40x magnification **[1]**. Quickly transfer the immatures into a pre-warmed IVM oocyte medium **[2]**.
 - 3.5.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 3:05 – 3:40.
 - 3.5.2. LAB MEDIA: Clip 2_In vitro maturation.mp4. 3:41 – 4:25.
- 3.6. Next, examine the COCs in the blood clot **[1]** and in the cell strainer **[2]**.
 - 3.6.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 4:25 – 4:35.
 - 3.6.2. LAB MEDIA: Clip 2_In vitro maturation.mp4. 5:20 – 5:30.
- 3.7. Transfer the COCs in handling media into pre-warmed IVM oocyte medium **[1]**.
 - 3.7.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 6:20 – 6:30.
- 3.8. Culture the immature COCs for 28 to 32 hours at 37 degrees Celsius in humidified air containing 5% carbon dioxide and 5% oxygen **[1]**.
 - 3.8.1. LAB MEDIA: Clip 2_In vitro maturation.mp4. 7:00 – end.

Results

4. Results: OP-IVM flow chart

4.1. By December 2019, OP-IVM had been used for fertility preservation of 274 patients. One example was a 28-year-old patient who was diagnosed with primary infertility, left adnexal cyst, and PCOS. She received laparoscopic cystectomy and OP-IVM in September 2016 and 27 immature COCs were obtained [1].

4.1.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize the operating room section.*

4.2. After 28 hours in culture, 7 oocytes in the MII ('M-2') stage were selected and fertilized by ICSI [1]. After 5 days, 2 blastocysts were frozen [2]. One was thawed in February 2017 and transferred into the uterus. The patient delivered a healthy girl in November [3].

4.2.1. LAB MEDIA: Figure 2 B.

4.2.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize the steps from "ICSI" to "preserved in nitrogen liquid".*

4.2.3. LAB MEDIA: Figure 1. *Video Editor: Emphasize "Blastocyst thawing" and "Embryo transfer" steps.*

Conclusion

5. Conclusion Interview Statements

5.1. **Tao Liu:** OP-IVM was first used to retrieve oocytes for infertile women with PCOS. Its application soon expanded to patients who need benign gynecological surgery and cancer or hematological disease with chemoradiotherapy.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.2. **Tao Liu:** This method makes it possible to expand the application range of IVM technology by combining it with other traditional gynecological operations.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

