

Submission ID #: 71053

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21671213>

Title: Mouse Oocyte *In Vitro* Maturation, Fertilization, and Culture of Preimplantation Embryos

Authors and Affiliations:

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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, all done!**

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

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

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 24

Number of Shots: 52

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Debao Wang:** Our research focuses on controlled *in vitro* maturation and fertilization of mouse oocytes, and early embryonic development of pre-implantation embryos.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Debao Wang:** Existing methods often separate *in vitro* maturation from *in vitro* fertilization and embryo culture; our protocol integrates these steps and supports efficient developmental rates.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Debao Wang:** Stable temperature, optimal pH, and careful handling are critical for successful embryo development under controlled *in vitro* conditions.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Debao Wang:** Embryos generated using this protocol can be utilized for imaging, gene expression analysis, vitrification, or embryo transfer.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Debao Wang:** Researchers can use this protocol to evaluate genetic, pharmacological, or culture-condition effects on embryonic development.

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

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. **Jingcheng Zhang, Professor, Northwest A&F University:** (authors will present their testimonial statements live)

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

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Jingcheng Zhang, Professor, Northwest A&F University:** (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Northwest A&F University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Kathyayani Sridharan) for queries regarding lab media.

2. Ovary Collection for Cumulus–Oocyte Complexes Isolation and Culture

Demonstrator: Xueting Li

- 2.1. To begin, place the euthanized female mouse in a supine position to isolate the ovary after 48 hours of pregnant mare serum gonadotropin, or PMSG (*P-M-S-G*) injection [1]. Spray the abdomen with 70 percent ethanol to wet the fur and prevent it from entering the abdominal cavity [2].
 - 2.1.1. WIDE: Talent placing the euthanized mouse in a supine position. **TXT: C57BL/6 mice; Age: 8 to 10 weeks old.** *Videographer: Please obtain CU shot as well for this shot*
 - 2.1.2. Talent spraying the abdomen with 70 percent ethanol.
- 2.2. Use fine dissecting scissors to make a small lower abdominal skin incision [1] and gently open the skin with gloved hands to expose the peritoneal membrane [2].
 - 2.2.1. Talent making a small lower abdominal skin incision with fine dissecting scissors.
 - 2.2.2. Talent opening the skin with gloved hands to expose the peritoneal membrane.
- 2.3. After opening the peritoneal membrane and gently moving the intestines aside, locate the uterine horn and follow it toward the ovary [1]. Identify the ovary near the kidney, typically enclosed by a fat pad [2].
 - 2.3.1. Talent locating the uterine horn and the ovary.
 - 2.3.2. CU: Shot of the ovary near the kidney within the fat pad.
- 2.4. Use fine dissecting scissors to separate the ovary from the uterine horn [1] and trim away excess connective tissue and fat [2].
 - 2.4.1. CU: Shot of ovary being separated from the uterine horn.
 - 2.4.2. CU: Shot of excess connective tissue and fat being removed from the ovary.
- 2.5. Immediately transfer the ovaries to pre-warmed M2 (*M-two*) medium supplemented with isobutylmethylxanthine, or IBMX (*I-B-M-X*) [1]. Also, collect the contralateral ovary and transfer it to the same dish [2].
 - 2.5.1. Talent transferring the ovary to pre-warmed M2 medium supplemented with

IBMX.

- 2.5.2. Talent transferring the contralateral ovary to the same dish.
- 2.6. Place the dish under a stereomicroscope [1] and visualize the ovary prior to follicle puncture and cumulus-oocyte complexes, or COCs (*C-O-sees*) collection [2].
 - 2.6.1. Talent placing the dish under a stereomicroscope.
 - 2.6.2. LAB MEDIA: 2.6.2.mp4. 0:00 – 0:04
- 2.7. For COCs collection, gently puncture visible antral follicles with a sterile syringe needle [1] to release COCs in the M2 medium [2].
 - 2.7.1. LAB MEDIA: 2.7.1-2.7.2.mp4. 0:00 – 0:15
 - 2.7.2. LAB MEDIA: 2.7.1-2.7.2.mp4. 0:27 – 0:33.
- 2.8. Use a finely pulled glass Pasteur pipette to collect the released COCs [1]. Transfer them to a clean 35-millimeter dish containing 2 milliliters of pre-warmed M2 medium supplemented with 0.2 millimolar IBMX [2].
 - 2.8.1. LAB MEDIA: 2.8.1.avi. 00:00:00 – 00:00:13; 00:00:16 – 00:00:20
 - 2.8.2. LAB MEDIA: 2.8.2.avi. 00:00:00 – 00:00:07; 00:00:17 – 00:00:26
- 2.9. Wash the COCs by serial transfer to a clean area of the 35-millimeter dish [1] to remove detached granulosa cells and tissue debris [2], leaving debris behind at each transfer [3].
 - 2.9.1. LAB MEDIA: 2.9.1-2.9.2-2.9.3.mp4. 00:00 – 00:15
 - 2.9.2. LAB MEDIA: 2.9.1-2.9.2-2.9.3.mp4. 00:16 – 00:20
 - 2.9.3. LAB MEDIA: 2.9.1-2.9.2-2.9.3.mp4. 00:21 – 00:26
- 2.10. After debris removal, transfer the COCs to the *in vitro* maturation, or IVM (*I-V-M*) wash dish [1] and wash them by sequential transfer through three 100-microliter drops of IVM wash medium [2].
 - 2.10.1. LAB MEDIA: 2.10.1-2.10.2.mp4. 00:03 – 00:07; 00:20 – 00:40
 - 2.10.2. LAB MEDIA: 2.10.1-2.10.2.mp4. 00:41 – 00:49; 01:05 – 01:09; 01:10 – 01:15
- 2.11. Transfer the washed COCs into 100-microliter drops of IVM medium, with 20 to 30 COCs per drop [1]. Culture them at 37 degrees Celsius in 5 percent carbon dioxide for 14 hours [2].
 - 2.11.1. LAB MEDIA: 2.11.1.mp4. 00:00 – 00:06; 00:18 – 00:23
 - 2.11.2. Talent placing the dish in the incubator.

3. Sperm Collection and Preparation

- 3.1. Remove the cauda epididymides from a euthanized DBA/2 (*D-B-A-two*) male mouse at 10 to 16 weeks age, while avoiding as much fat, blood, and tissue fluid as possible [1]. Transfer both cauda epididymides into a 300-microliter drop of pre-equilibrated G-IVF (*G-I-V-F*) medium for sperm release and capacitation [2].
 - 3.1.1. Talent removing the cauda epididymides while avoiding fat, blood, and tissue fluid.
 - 3.1.2. Talent transferring both cauda epididymides into a 300-microliter drop of pre-equilibrated G-IVF medium.
- 3.2. Immobilize the cauda epididymis using a pair of forceps [1], make five to seven cuts in the cauda epididymis with a sterile syringe needle [2], and shred the tissue to allow the release of concentrated sperm into the media [3]. Incubate the dish at 37 degrees Celsius in 5 percent carbon dioxide for 1 hour to allow sperm capacitation [4].
 - 3.2.1. Talent immobilizing the cauda epididymis with a pair of forceps.
 - 3.2.2. LAB MEDIA: 3.2.2-3.2.3.avi. 00:00:00–00:00:14
 - 3.2.3. LAB MEDIA: 3.2.2-3.2.3.avi. 00:00:36–00:00:50
 - 3.2.4. Talent placing the dish in the incubator.

4. *In vitro* Fertilization and Embryo Culture

- 4.1. After 14 hours of *in vitro* maturation, wash the COCs by sequential transfer through three 100-microliter drops of *in vitro* fertilization, or IVF (*I-V-F*) wash medium [1]. Transfer the washed COCs into the IVF insemination drops, with 20 to 30 COCs per 100-microliter drop [2].
 - 4.1.1. LAB MEDIA: 4.1.1-4.1.2.avi. 00:00:03 – 00:00:08; 00:00:19 – 00:00:23; 00:00:38 – 00:00:45; 00:00:54 – 00:01:00
 - 4.1.2. LAB MEDIA: 4.1.1-4.1.2.avi. 00:01:20 – 00:01:25
- 4.2. Preincubate the fertilization dish containing COCs at 37 degrees Celsius in 5 percent carbon dioxide for 30 minutes before insemination [1]. After capacitation, determine the sperm concentration using a hemocytometer [2].
 - 4.2.1. Talent placing the fertilization dish containing COCs in the incubator.
 - 4.2.2. Talent placing the sperm suspension on a hemocytometer for counting the sperm cells.
- 4.3. Based on the sperm concentration, calculate the required inoculum volume [1]. Add capacitated sperm to each IVF drop to achieve a final concentration of 4×10^5 to 1×10^6 sperm cells per milliliter [2].
 - 4.3.1. Representative shot of talent calculating the required inoculum volume based on the sperm concentration on a spreadsheet.

- 4.3.2. LAB MEDIA: 4.3.2.mp4. 00:00 – 00:06; 00:16 – 00:26; 00:32 – 00:35
- 4.4. Incubate the IVF dishes containing COCs and sperm at 37 degrees Celsius in a 5 percent carbon dioxide incubator for 4 to 6 hours [1]. After incubation, aspirate the presumptive zygotes with a finely pulled Pasteur pipette in minimal volume [2]. Transfer them into the IVF wash drops [3].
- 4.4.1. Talent placing the IVF dishes containing COCs and sperm in the incubator.
- 4.4.2. LAB MEDIA: 4.4.2-4.4.3..avi. 00:00:00 – 00:00:08
- 4.4.3. LAB MEDIA: 4.4.2-4.4.3..avi. 00:00:19 – 00:00:24.
- 4.5. Gently pipette within each drop to remove excess sperm and debris [1]. Wash the presumptive zygotes by sequential transfer through three IVF wash drops [2].
- 4.5.1. LAB MEDIA: 4.5.2-4.5.1.mp4. 0:03 – 0:12; 0:19 – 0:25
- 4.5.2. LAB MEDIA: 4.5.2-4.5.1.mp4. 0:53–01:00; 01:05 –01:12; 01:31 –01:36; 01:42–01:46; 01:52–02:00
- 4.6. Transfer the washed presumptive zygotes to the *in vitro* culture, or IVC (*I-V-C*) wash dish [1] and wash them by sequential transfer through three pre-warmed embryo culture medium wash drops, minimizing carryover of IVF medium [2].
- 4.6.1. LAB MEDIA: 4.6.1-4.6.2.mp4. 0:04 – 0:12
- 4.6.2. LAB MEDIA: 4.6.1-4.6.2.mp4. 0:28–0:37; 0:49–0:59; 01:13–01:20
- 4.7. Place the presumptive zygotes into embryo culture medium, with 20 to 30 embryos per 100-microliter drop [1]. Culture them at 37 degrees Celsius in 5 percent carbon dioxide [2].
- 4.7.1. LAB MEDIA: 4.7.1.avi. 00:00:06–00:00:12
- 4.7.2. Talent placing the dish in the incubator.
- 4.8. After 6 to 8 hours, assess fertilization by scoring the presence of male and female pronuclei [1] and discard oocytes that retain only one polar body and show no visible pronuclei [2].
- 4.8.1. LAB MEDIA: 4.8.1..avi. 00:00:00– 00:00:06. *Video Editor: Please zoom-in and emphasize the cells on the left side*
- 4.8.2. LAB MEDIA: 4.8.2..avi. 00:00:06– 00:00:12
- 4.9. After 24 hours, check and count the number of two-cell embryos [1] and calculate the two-cell rate as the number of two-cell embryos divided by the number of inseminated oocytes [2].
- 4.9.1. LAB MEDIA: 4.9.1.mp4. 0:00–0:03
- 4.9.2. Close-up shot of the Excel sheet showing the formula and calculations.

4.10. Next, remove and discard uncleaved embryos [1]. Continue to culture the embryos under the same incubation conditions until 96 hours post-insemination [2]. Assess blastocyst formation at 96 hours post-insemination based on morphological criteria [3].

4.10.1. LAB MEDIA: 4.10.1.avi. 00:00:00–00:00:05

4.10.2. Talent placing the embryo culture dish in the incubator.

4.10.3. LAB MEDIA: 4.10.3.avi.00:00:00–00:00:07. *Video Editor: Please emphasize the bubble-like cells on the right side and show the central hollow-like space.*

Results

5. Results

- 5.1. After 14 hours of *in vitro* maturation, COCs typically exhibit cumulus expansion and intact morphology, allowing quantification of meiotic maturation by denuding a designated cohort and scoring first polar body, or PB1 (*P-B-one*) extrusion [1]. The average maturation rate based on PB1 extrusion rates across three independent replicates was 81.3 percent [2]. Following insemination, fertilization was assessed after 6–8 hours by scoring the formation of male and female pronuclei [3].
 - 5.1.1. LAB MEDIA: Figure 6A. *Video Editor: Please emphasize the cell with the label 'First polar body'.*
 - 5.1.2. LAB MEDIA: LAB_MEDIA_BarChart_PB1_Two-cell_Blastocyst_rates.pdf. *Video Editor: Please emphasize the last row "Mean $\pm$ SEM" in the "PB1 extrusion rate" column.*
 - 5.1.3. LAB MEDIA: Figure 6B. *Video Editor: Please emphasize the cell showing 'Male pronucleus', 'Female Pronucleus' and 'Secondary polar body'.*
- 5.2. Cleavage was evaluated 24 hours after insemination using the two-cell rate [1]. The average two-cell rates across three independent replicates were 87.6 percent [2]. The developmental competence was evaluated by further culturing embryos *in vitro* and by assessing blastocyst formation at 96 hours post-insemination [3]. The average blastocyst formation rate across three independent replicates was 52.6 percent [4].
 - 5.2.1. LAB MEDIA: Figure7.pdf. *Video Editor: Please highlight panel A.*
 - 5.2.2. LAB MEDIA: LAB_MEDIA_BarChart_PB1_Two-cell_Blastocyst_rates.pdf. *Video Editor: Please emphasize the last row "Mean $\pm$ SEM" in the "Two-cell rate" column.*
 - 5.2.3. LAB MEDIA: Figure7.pdf. *Video Editor: Please emphasize the annotated "Blastocyst" cell in panel B.*
 - 5.2.4. LAB MEDIA: LAB_MEDIA_BarChart_PB1_Two-cell_Blastocyst_rates.pdf. *Video Editor: Please emphasize the last row "Mean $\pm$ SEM" in the "Blastocyst rate" column.*
- 5.3. Poor-quality cleavage outcomes include embryos that fail to cleave, fragmented embryos, or embryos with unevenly sized blastomeres [1]. Suboptimal developmental outcomes during extended culture included arrest at the two-cell stage, poorly expanded blastocysts, or embryos with obvious fragmentation and abnormal morphology [2].

- 5.3.1. LAB MEDIA: Figure8.pdf. *Video Editor: Please emphasize the annotated “Uncleaved” cells in panel A.*
- 5.3.2. LAB MEDIA: Figure8.pdf. *Video Editor: Please emphasize the annotated “two-cell arrest” and “fragmented embryo” cells sequentially in panel B.*

Pronunciation Guide:

1. Ovary
Pronunciation link: <https://www.merriam-webster.com/dictionary/ovary>
IPA: /'oʊvəri/
Phonetic Spelling: oh·vuh·ree
2. Cumulus
Pronunciation link: <https://www.merriam-webster.com/dictionary/cumulus>
IPA: /'kju:mjələs/
Phonetic Spelling: kyoo·myuh·luhs
3. Oocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/oocyte>
IPA: /'oʊəˌsaɪt/
Phonetic Spelling: oh·uh·syte
4. Gonadotropin
Pronunciation link: <https://www.merriam-webster.com/dictionary/gonadotropin>
IPA: /ˌɡoʊnədə'troʊpən/
Phonetic Spelling: goh·nuh·duh·troh·pin
5. Peritoneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/peritoneal>
IPA: /ˌperətə'ni:əl/
Phonetic Spelling: peh·ruh·tuh·nee·uhl
6. Uterine
Pronunciation link: <https://www.merriam-webster.com/dictionary/uterine>
IPA: /'ju:tərən/
Phonetic Spelling: yoo·tuh·rin
7. Isobutylmethylxanthine
Pronunciation link: No confirmed link found
IPA: /ˌaɪsoʊˌbju:təlˌmɛθəl'zænθi:n/
Phonetic Spelling: eye·soh·byoo·tuhl·meth·uhl·zan·theen
8. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /ˌsteri.oʊˈmaɪkrəˌskoʊp/
Phonetic Spelling: steh·ree·oh·my·kruh·skohp
9. Follicles
Pronunciation link: <https://www.merriam-webster.com/dictionary/follicle>
IPA: /'fɑ:lɪkəl/
Phonetic Spelling: fah·luh·kuhl

10. Granulosa
Pronunciation link: <https://www.merriam-webster.com/medical/granulosa>
IPA: /ˌgrænjəˈlooʊsə/
Phonetic Spelling: gran-yuh-loh-suh
11. Epididymides
Pronunciation link: <https://www.merriam-webster.com/dictionary/epididymis>
IPA: /ˌepəˈdɪdəməˌdiːz/
Phonetic Spelling: eh-puh-did-uh-muh-deez
12. Capacitation
Pronunciation link: <https://www.merriam-webster.com/medical/capacitation>
IPA: /kəˌpæsəˈteɪʃən/
Phonetic Spelling: kuh-pass-uh-tay-shuhn
13. Hemocytometer
Pronunciation link: <https://www.merriam-webster.com/medical/hemocytometer>
IPA: /ˌhiːməsaɪˈtəːmətər/
Phonetic Spelling: hee-moh-sy-tom-uh-ter
14. Inoculum
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculum>
IPA: /ɪˈnoːkjələm/
Phonetic Spelling: ih-naak-yuh-luhm
15. Zygotes
Pronunciation link: <https://www.merriam-webster.com/dictionary/zygote>
IPA: /ˈzaɪˌɡoʊt/
Phonetic Spelling: zy-goht
16. Pronuclei
Pronunciation link: <https://www.merriam-webster.com/medical/pronucleus>
IPA: /proʊˈnuːkliəɪ/
Phonetic Spelling: proh-noo-kee-eye
17. Blastocyst
Pronunciation link: <https://www.merriam-webster.com/dictionary/blastocyst>
IPA: /ˈblæstəˌsɪst/
Phonetic Spelling: blas-tuh-sist
18. Morphological
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphological>
IPA: /ˌmɔːrfəˈlɑːdʒɪkəl/
Phonetic Spelling: mor-fuh-loj-ih-kuhl
19. Embryo
Pronunciation link: <https://www.merriam-webster.com/dictionary/embryo>
IPA: /ˈembri.oʊ/
Phonetic Spelling: em-bree-oh
20. Contralateral
Pronunciation link: <https://www.merriam-webster.com/medical/contralateral>

IPA: /ˌkɑːntɹəˈlætərəl/

Phonetic Spelling: kon·truh·lat·uh·ruhl