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Title: Proteolytically Degraded Alginate Hydrogels and Hydrophobic Microbioreactors for Porcine Oocyte Encapsulation

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes**

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

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Nicon SMZ1000

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**

Current Protocol Length

Number of Steps: 17
Number of Shots: 45

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Malgorzata Duda:** This protocol for encapsulation of porcine oocytes makes it possible to maintain spherical organisation of COCs. This prevents their flattening and consequent disruption of gap junctions between the oocyte and surrounding follicular cells.

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

1.2. **Malgorzata Duda:** The main advantages of the two described protocols are that they are user-friendly and allow for effective control of both oocyte and cumulus cells survival.

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

OPTIONAL:

1.3. **Gabriela Gorczyca:** Both 3D culture systems are valuable tools for basic research in reproductive biology and may have clinical relevance for improved infertility treatment. They can be also applied for developing novel biotechnology methods and for livestock improvement.

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 animal subjects have been approved by the Animal Welfare Committee at the Institute of Zoology and Biomedical Research at Jagiellonian University.

Protocol

2. Isolation of Porcine Cumulus-oocyte Complexes (COCs)

2.1. After rinsing the ovaries, transfer them to a beaker filled with HM medium **[1]** and store them in an incubator at 38 degrees Celsius during all subsequent manipulations **[2]**.

2.1.1. WIDE: Establishing shot of talent putting ovaries in a beaker.

2.1.2. Talent putting the beaker in the incubator and closing the door.

2.2. Aspirate the follicular fluid from large porcine follicles **[1]** and centrifuge it at 100 *x g* for 10 minutes at room temperature **[2]**. Then, filter the supernatant using a sterile syringe attached to a 0.2-micrometer membrane pore filter **[3]** and snap freeze it at negative 80 degrees Celsius **[4]**.

2.2.1. Talent aspirating the fluid.

2.2.2. Talent putting the fluid in the centrifuge and closing the lid.

2.2.3. Talent filtering the supernatant.

2.2.4. Talent snap-freezing the filtrate.

2.3. To isolate the COCs from medium-sized follicles, transfer 2 to 3 ovaries to a sterile 10-centimeter diameter Petri dish filled with HM **[1]**. Gently cut the surface of the protruding ovarian follicles with a sterile surgical number 15 blade, which will cause the follicular fluid and the COCs to flow out into the Petri dish **[2]**.

2.3.1. Talent transferring the ovaries to the Petri dish.

2.3.2. Talent cutting the ovarian follicle.

2.4. Aspirate the follicular content with a 28-gauge needle attached to a disposable syringe and transfer it into another Petri dish **[1]**. To prepare the IVF Petri dishes, add 1 milliliter of HM to the central wells **[2]** and place 3 to 4 drops of HM in the outer rings **[3]**.

2.4.1. Talent aspirating the follicular content.

2.4.2. Talent adding HM to the central wells.

2.4.3. Talent placing HM drops in outer rings.

2.5. Then, use a polycarbonate micropipette to move the undamaged COCs **[0]** to drops of HM in the outer rings and briefly rinse them 3 to 4 times **[1]**. When finished, individually transfer them into the central well **[2]**. Store the IVF plates in the incubator **[3]**.

2.5.0. Added shot: SCOPE: Image showing cell suspension and COCs immediately after isolation from ovarian follicles.

2.5.1. SCOPE: Talent putting the COCs into a drop.

2.5.2. SCOPE: Talent transferring COCs into the central well.

2.5.3. Talent putting the plates in the incubator and closing the door.

3. Encapsulation in Fibrin-alginate Hydrogel Beads

3.1. Prepare thrombin and fibrinogen solutions as described in the text manuscript **[1]**. On the day of the procedure, slowly thaw the fibrinogen solution on ice and bring it to room temperature right before use **[2]**.

3.1.1. Thrombin and fibrinogen solutions in tubes, with the tubes labeled.

3.1.2. Talent putting the fibrinogen solution on ice.

3.2. Mix 0.5% alginate solution and the fibrinogen solution at a 1 to 1 ratio for a final volume of 2 milliliters **[1]**, then gently vortex the mixture **[2]**. Prepare incubation chambers by applying thin strips of paraffin films to glass microscope slides **[3]**. *Videographer: This step is important!*

3.2.1. Talent mixing the fibrinogen and alginate.

3.2.2. Talent vortexing the tube.

3.2.3. Talent applying paraffin film to a slide.

3.3. Pipette 7.5-microliter drops of the FA mixture onto the paraffin film coated glass slide with separating spacers **[0]**, placing 8 to 10 drops arranged in 2 rows **[1]**. Use a micropipette to transfer 3 to 5 COCs to the center of the FA drop, along with a minimum volume of maturation medium **[2]**. *Videographer: This step is important!*

3.3.0. Added shot: Talent putting separating spacers on a glass slide covered with paraffin film

3.3.1. Talent putting drops on the slide.

3.3.2. SCOPE: Talent putting COCs into a drop.

3.4. Add 7.5 microliters of thrombin solution on top of each FA drop to cover it. There is no need to mix them because the gel forms almost instantaneously **[1]**. Cover the incubation chamber with a previously prepared glass slide **[2]**, then turn the chamber upside down and place it in a 100-millimeter Petri dish lined with moist filter paper **[3]**. *Videographer: This step is important!*

3.4.1. Talent adding thrombin solution on top of the FA drop.

3.4.2. Talent covering the chamber with a slide.

3.4.3. Talent turning the chamber upside down and placing it in the Petri dish.

3.5. Put the Petri dish in the 5% carbon dioxide, 38-degree Celsius incubator for 5 to 7 minutes **[1]**. After the incubation, transfer each FA capsule into a well of a 96-well plate containing 100 microliters of maturation medium **[2]**. *Videographer: This step is difficult!*

3.5.1. Talent putting the dish in the incubator and closing the door.

3.5.2. Talent transferring a capsule to a well.

3.6. Every 2 days replace half of the medium with fresh, pre-equilibrated maturation medium **[1]**. Image COCs using an inverted light microscope at 10x magnification **[2]**.

3.6.1. Talent refreshing the maturation medium.

3.6.2. Talent at the microscope, imaging the COCs.

3.7. After culturing the COCs for 4 days, remove the maturation medium from the wells and add 100 microliters of 10 IU per milliliter alginate lyase in DMEM **[1]**. Leave the culture plate in the incubator for 25 to 30 minutes **[2]**.

3.7.1. Talent removing the maturation medium from a well and replacing it with alginate lyase.

3.7.2. Talent putting the plate in the incubator and closing the door.

3.8. Remove the COCs from the dissolved capsules **[1]**, wash them in DMEM several times **[2]**, then transfer them to the inner ring of an IVF dish containing PBS **[3-TXT]**.

3.8.1. SCOPE: Talent removing the COCs from the capsules.

3.8.2. SCOPE: Talent washing the COCs.

3.8.3. SCOPE: Talent putting the COCs in the inner ring of the IVF dish. **TEXT: 5-10 COCs per dish**

4. Encapsulation in Super-hydrophobic Fluorinated Ethylene Propylene (FEP) Microbioreactors

4.1. Make a bed of 5 grams of FEP powder in a 30-millimeter Petri dish **[1-TXT]**. Distribute a single droplet of maturation medium containing 3 to 5 COCs onto the FEP powder **[2]**. Rotate the plate gently in a circular motion to ensure that the particles completely cover the surface of the liquid drop and form liquid marbles, or LMs **[3]**. *Videographer: This step is important!*

4.1.1. Talent adding FEP powder to a dish, with the FEP container in the shot. **TEXT: average particle size of 1 µL**

4.1.2. Talent putting the drop of maturation medium and COCs into the FEP bed.

4.1.3. Talent rotating the plate and LM forming. *Videographer: Obtain multiple usable takes, this will be reused in 4.4.2.*

4.2. Prepare several 60-millimeter IVF Petri dishes and add 3 to 4 milliliters of sterile water to the outer rings to create a humidity chamber **[1]**. Pick up the formed LM with a 1,000-microliter pipette **[2]** and place it into the central well **[3]**. Incubate the marbles for 4 days at 38 degrees Celsius in a 5 % carbon dioxide incubator **[4]**. *Videographer: This step is difficult and important!*

4.2.1. Talent adding water to the outer rings of an IVF dish.

4.2.2. Talent picking up the LM.

4.2.3. Talent putting the LM into the central well. *Videographer: Obtain multiple usable takes, this will be reused in 4.4.3.*

4.2.4. Talent putting the dish in the incubator and closing the door.

4.3. To change the medium, apply 30 microliters of maturation medium onto each LM, which will cause it to spread **[1-TXT]**. When the marble content dissolves, transfer the COCs released from the bioreactor to a drop of fresh maturation medium in the Petri dish **[2]**.

4.3.1. SCOPE: Talent adding medium to the LM and the LM dissolving. **TEXT: Change the medium daily**

4.3.2. SCOPE: Talent transferring the COCs to a fresh drop of maturation medium.

4.4. After 3 to 4 washes in maturation medium, transfer the COCs, along with 30 microliters of fresh medium, onto the FEP powder bed **[1]**. Gently rotate the plate in a circular motion to ensure that the powder particles completely cover the surface of the liquid drop and form a new LM **[2]**, then transfer the LM to the IVF Petri dish **[3]**.

4.4.1. SCOPE: Talent putting the COCs into the FEP powder bed.

4.4.2. *Use 4.1.3.*

4.4.3. *Use 4.2.3.*

Results

5. Results: Morphology and Viability of COCs after Encapsulation

5.1. Both in vitro maturation systems produced COCs with granulosa cells that were tightly adhered to each other and intact layers of cumulus cells **[1-TXT]**.

5.1.1. LAB MEDIA: Figure 1 A and B. *Video Editor: Label A "***FAB***" and B "***LM***".*

5.2. The COC viability analysis confirmed that optimal growth conditions were achieved in both encapsulation systems **[1]**. In both groups, only high-viability oocytes were observed **[2]**.

5.2.1. LAB MEDIA: Figure 2.

5.2.2. LAB MEDIA: Table 1.

5.3. The oocytes were imaged with transmission electron microscopy **[1]**. The mitochondria were evenly distributed and had a shell-like shape. Only a few of them were elongated and their clustering was sporadically observed **[2]**.

5.3.1. LAB MEDIA: Figure 1 C and D. *Video Editor: Label C "FAB" and D "LM".*

5.3.2. LAB MEDIA: Figure 1 C and D. *Video Editor: Emphasize the structures labeled M.*

5.4. Endoplasmic reticulum was either associated with mitochondria or free in the oocyte cytoplasm **[1]**. Lipid droplets appeared as small dark round structures **[2]** and Golgi apparatus were emerged with dilated cisternae **[3]**.

5.4.1. LAB MEDIA: Figure 1 C and D. *Video Editor: Emphasize the structures labeled ER.*

5.4.2. LAB MEDIA: Figure 1 C and D. *Video Editor: Emphasize the structures labeled Lp.*

5.4.3. LAB MEDIA: Figure 1 C and D. *Video Editor: Emphasize the structures labeled G.*

Conclusion

6. Conclusion Interview Statements

6.1. **Gabriela Gorczyca:** It is important to be precise and careful when transferring FA capsules from incubation chambers into culture plates and when picking up and transferring formed LM into the IVF Petri dish.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.2 and 4.2.2 – 4.2.3.*