FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68589
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20919693

Title: A Modified Co-Culture System for Understanding Granulosa-Theca Cell Interactions in the Bovine Ovary

Authors and Affiliations:
Anja Baufeld, Jens Vanselow

Research Institute for Farm Animal Biology (FBN)

Corresponding Authors:
Jens Vanselow vanselow@fbn-dummerstorf.de
Email Addresses for All Authors:
Anja Baufeld baufeld@fbn-dummerstorf.de
Jens Vanselow vanselow@fbn-dummerstorf.de

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**

   Videographer: Please film the following SCOPE shots with a SCOPE KIT

   SCOPE: 2.5.2 / 2.6.1 / 2.6.2/ 2.7.1-2.7.2

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: 20
Number of Shots: 46

Introduction

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

1.1. **Anja Baufeld:** The co-culture of bovine theca and granulosa cells serves as a reliable foundation to analyze paracrine signaling and substrate transport between the steroidogenic cells of the follicle.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

What advantage does your protocol offer compared to other techniques?

1.2. **Anja Baufeld:** The model enables the compartmentalization of theca and granulosa cells similar to the *in vivo* environment and the usage of commercially available inserts allows a reproducible and standardized cell culture.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.4*

What new scientific questions have your results paved the way for?

1.3. **Anja Baufeld:** We could create a physiological relevant environment to investigate granulosa-theca cell interactions in more detail like substrate exchange or follicular dynamics during the process of folliculogenesis.

1.3.1. 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.

Protocol

2. Isolation and Enzymatic Digestion of Bovine Theca Cells

Demonstrator: Maren Anders and Veronica Schreiter

2.1. To begin, obtain bovine ovaries **[1].** Wash the bovine ovaries 3 times in PBS supplemented with antibiotics to remove blood and residues from the surface **[2]**.

2.1.1. WIDE: Talent holding a dish containing bovine ovaries.
AUTHORS: Please note that JoVE requires the opening shot to be a wide, non-screen capture. This shot was added specifically to meet that requirement

2.1.2. Talent adding PBS to the dish with the bovine ovaries.

2.2. Place the ovaries in a tumbler **[1].** Fill it with PBS just enough to cover the ovaries **[2]** and discard the solution then add more PBS **[3-TXT]**.

2.2.1. Talent placing ovaries in a tumbler.

2.2.2. Talent filling it with phosphate-buffered saline.
AUTHOR/VIDEOGRAPHER NOTE: 2.2.1-2.2.2 were filmed together

2.2.3. Talent pouring out the liquid and adding new PBS. **TXT: Repeat rinse 3 x until ovaries are clean**

2.3. Place one ovary in a glass dish **[1].** Use a ruler to measure and select follicles that are between 5 to 11 millimeters in diameter for dissection **[2]**.

2.3.1. Talent placing an ovary in a glass dish.

2.3.2. Talent measuring follicles using a ruler.

2.4. Now aspirate the follicular fluid by puncturing a selected follicle with an 18-gauge needle attached to a 3-milliliter syringe **[1]**. Discard the follicular fluid immediately **[2]**.

2.4.1. Talent inserting the needle into the follicle and aspirating fluid.

2.4.2. Talent discarding the aspirated fluid into a waste container.

2.5. Transfer the follicles under a binocular microscope **[1].** With a pair of scissors, cut the follicle open at the puncture site **[2].**

2.5.1. Talent placing the dish with follicles under a binocular microscope.

2.5.2. SCOPE: An incision is being made at the puncture site using scissors.
Videographer: Please record this shot with a SCOPE kit

2.6. Using tweezers, grasp the theca interna layer from the inner surface of the opened

follicle **[1]**. Peel off the theca interna gently from the inner follicular wall **[2]**.
Videographer: Please record this step with a SCOPE kit

2.6.1. SCOPE: The follicle is being held open and tweezers are being used to grip the theca interna.

2.6.2. SCOPE: Talent carefully peeling the membrane layer from the follicle wall.

2.7. Transfer the theca cell layer into a dish containing PBS supplemented with antibiotics to wash off remaining granulosa cells **[1]**. Then use a scalpel to gently scrape the granulosa cells off the membrane surface **[2]**.

2.7.1. SCOPE Talent placing the theca membrane into a dish with buffer.

2.7.2. SCOPE Talent scraping off granulosa cells from the membrane using a scalpel.

2.8. Place the membrane into a new dish with fresh PBS for washing **[1]**. Rinse the membrane by swirling it gently in the buffer to remove any residual cells **[2]**.

2.8.1. Talent moving the membrane to a fresh dish of buffer.

2.8.2. Talent gently swirling the theca layer to rinse the membrane.

2.9. Transfer the theca interna into a prepared digestion solution within a well of a 12-well plate **[1].** Use a scalpel to cut it into 1 to 3-millimeter sized pieces **[2]**.

2.9.1. Talent placing the membrane in a digestion solution.

2.9.2. Talent cutting it into small fragments.

2.10. Then transfer the tissue pieces into a prepared 1.5-milliliter reaction tube **[1]**. Incubate the tubes in a thermos-shaking incubator **[2-TXT]**.

2.10.1. Talent adding tissue fragments to a labeled reaction tube.

2.10.2. Talent placing the tubes into a shaking incubator. **TXT: Incubation: 800 rpm, 37 °C, 45 - 50 min**

2.11. After 30 minutes of incubation, vortex the tubes for 3 to 5 seconds before returning them to the incubator **[1]**. Repeat this vortexing step 3 more times over the remaining incubation period **[2]**.

2.11.1. Talent briefly vortexing a tube after 30 minutes.

2.11.2. Talent repeating the vortexing and placing tubes back in the incubator.

2.12. When incubation is complete, use a pipette to resuspend the digested cells **[1]**. Pass the solution through a 100-micrometer cell strainer placed on a 50-milliliter tube to remove any undigested tissue **[2-TXT]**.

2.12.1. Talent pipetting the digested cell mixture to resuspend it thoroughly.

2.12.2. Talent filtering cell suspension through a cell strainer into a tube. **TXT: Proceed immediately for cell counting and cryopreservation**

3. Establishment of Theca and Granulosa Cell Co-Culture Using Inverted Inserts

Demonstrator: Veronica Schreiter

3.1. Prepare the inoculation chamber for culturing theca cells **[1]**. Place the coated insert upside down into a larger plate, such as a 12-well plate **[2]**. Place the cut and autoclaved tube on top of the inverted insert, ensuring it fits securely to prevent media leakage **[3]**.

3.1.1. Talent assembling components for the inoculation chamber at a biosafety cabinet.

3.1.2. Talent placing the insert upside down inside a 12-well plate.

3.1.3. Talent positioning the cut autoclaved tube on the inverted insert with a firm press.

3.2. Thaw the cryopreserved theca cells quickly in a 37 degrees Celsius water bath for 3 to 5 minutes **[1]**. Immediately transfer the thawed cell suspension into pre-warmed media **[2]**.

3.2.1. Talent submerging a cryovial in a water bath.

3.2.2. Talent pipetting the thawed cell suspension into a tube containing warm media.

3.3. Centrifuge the theca cell suspension at 500 *g* for 3 minutes at approximately 20 degrees Celsius **[1]**. Discard the supernatant **[2]**. Then resuspend the pellet in supplemented alpha minimum essential medium **[3].**

3.3.1. Talent placing tubes in the centrifuge and setting parameters.

3.3.2. Talent removing the supernatant.

3.3.3. Talent pipetting in new media to resuspend cells.

3.4. Seed 200 microliters of the cell suspension into the inoculation chamber **[1-TXT]**. ~~Ensure at least three technical replicates are included for each condition **[2].**~~

3.4.1. Talent seeding cells into multiple inserts using a micropipette. **TXT: Seeding density: 1.7 - 1.8 x 10^5 cells per insert; Include 3 replicates for each condition**

3.4.2. ~~Talent labeling and arranging inserts to show biological replicates.~~ AUTHOR/VIDEOGRAPHER NOTES: Shot deleted

3.5. To close the cell culture dish, increase the distance between the plate and the lid using cut and autoclaved caps from 1.5-milliliter reaction tubes placed in each corner of the dish **[1]**. Close the well plate carefully without displacing the inserts **[2]**. Then gently transfer the closed plate into a humidified incubator **[3-TXT]**.

3.5.1. Talent placing cut reaction tube caps at each corner of the culture plate.

3.5.2. Talent carefully positioning the lid over the plate.

3.5.3. Talent transferring the sealed plate into the incubator. **TXT: Incubation: 37 °C, 5% CO_2, 3 days**

3.6. Fill a 24-well plate with 500 microliters of media in each desired well **[1]**.

3.6.1. Talent pipetting media into each well of a 24-well plate.

3.7. ~~With a pipette, remove all media from the inoculation chamber **[1].** Then~~ Gently remove the chamber from the insert using tweezers **[2]**. Place the insert containing attached theca cells into the 24-well plate with the theca-facing side down **[3]**.

3.7.1. Talent using a pipette to remove media.
AUTHOR/VIDEOGRAPHER NOTE: Shot deleted

3.7.2. Talent using tweezers to lift the chamber.

3.7.3. Talent carefully placing the insert into the 24-well plate.

3.8. For seeding granulosa cells, pipette 250 microliters of the cell suspension inside each insert **[1-TXT]**. Incubate the co-culture at 37 degrees Celsius with 5 percent carbon dioxide for 6 days **[2]**. Perform a media exchange every 48 hours after granulosa cell seeding by replacing two-thirds of the media volume **[3-TXT]**.

3.8.1. Talent pipetting granulosa cells into the center of each insert. **TXT: Seeding Density: 1.0 x 10^5 cells per insert**

3.8.2. Talent placing the co-culture plate inside the incubator.

3.8.3. Talent pipetting out two-thirds of the media from an insert. **TXT: This reduces the stress of the procedure**

Results

4. Results

4.1. Theca cells expressed CYP17A1 *(C-Y-P-Seventeen-A-One)* exclusively **[1]**, while granulosa cells predominantly expressed CYP19A1 **[2]**.

4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the red bar labeled "CYP17A1" showing signal*

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the blue bar labeled "CYP19A1"*

4.2. After 3 days in culture, theca cells exhibited a flattened and elongated morphology on the collagen-coated membrane, indicating successful attachment **[1]**. When cultured alone for 9 days, theca cells proliferated and reached confluence by day 9 **[2]**. Granulosa cells cultured alone for 6 days developed a fibroblast-like morphology and formed clusters, characteristic of *in vitro* behavior **[3]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight A*

4.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight B*

4.2.3. LAB MEDIA: Figure 3. *Video editor: Highlight C*

4.3. In the co-culture system, no morphological differences were observed in either cell type compared to their respective mono-cultures **[1]**.

4.3.1. LAB MEDIA: Figure 3D.

4.4. Estradiol levels were similar between granulosa cell mono-cultures and co-cultures, while theca cell mono-cultures produced only negligible amounts **[1]**. CYP17A1 expression remained confined to theca cells under both mono- and co-culture conditions **[2]**. CYP19A1*(C-Y-P-Nineteen-A-One)* expression remained confined to granulosa cells under both mono- and co-culture conditions **[3]**.

4.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight the tall blue bar (GC) and the red bar (TC & GC) in 4A*

4.4.2. LAB MEDIA: Figure 4. *Video editor: Highlight the bars for TC (red and blue) in 4B*

4.4.3. LAB MEDIA: Figure 4. *Video editor: Highlight the tall blue bar (GC) and the red bar (GC)in 4C*

Pronunciation Guide:

1. Granulosa

Pronunciation link:
https://www.merriam-webster.com/dictionary/granulosa
IPA: /ˌgrænjəˈloʊsə/
Phonetic Spelling: gran-yuh-loh-suh

---

2. Theca

Pronunciation link:
https://www.merriam-webster.com/dictionary/theca
IPA: /ˈθiːkə/
Phonetic Spelling: thee-kuh

---

3. Folliculogenesis

Pronunciation link:
https://www.merriam-webster.com/dictionary/folliculogenesis
IPA: /fəˌlɪkjəloʊˈdʒɛnəsɪs/
Phonetic Spelling: fuh-lik-yuh-loh-jen-uh-sis

---

4. Bovine

Pronunciation link:
https://www.merriam-webster.com/dictionary/bovine
IPA: /ˈboʊvaɪn/
Phonetic Spelling: boh-vine

---

5. Ovaries

Pronunciation link:
https://www.merriam-webster.com/dictionary/ovary
IPA: /ˈoʊvɛriz/
Phonetic Spelling: oh-vuh-reez

---

6. Aspirate (verb, as in "aspirate the follicular fluid")

Pronunciation link:
https://www.merriam-webster.com/dictionary/aspirate
IPA: /ˈæspəˌreɪt/
Phonetic Spelling: ass-puh-rayt

7. Binocular (as in "binocular microscope")

Pronunciation link:
https://www.merriam-webster.com/dictionary/binocular
IPA: /baɪˈnɑːkjələr/
Phonetic Spelling: bai-nah-kyuh-lur

8. Interna (as in "theca interna")

Pronunciation link:
No confirmed link found
IPA: /ɪnˈtɜrnə/
Phonetic Spelling: in-tur-nuh

9. Cryopreservation

Pronunciation link:
https://www.merriam-webster.com/dictionary/cryopreservation
IPA: /ˌkraɪoʊˌprɛzərˈveɪʃən/
Phonetic Spelling: cry-oh-preh-zur-vay-shun

10. Inoculation

Pronunciation link:
https://www.merriam-webster.com/dictionary/inoculation
IPA: /ɪˌnɑːkjəˈleɪʃən/
Phonetic Spelling: in-nah-kyuh-lay-shun

11. Centrifuge

Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛntrəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj

12. Estradiol

Pronunciation link:
https://www.merriam-webster.com/dictionary/estradiol
IPA: /ˌɛstrəˈdaɪˌɔl/
Phonetic Spelling: ess-truh-dai-awl

13. CYP17A1

Pronunciation link:

No confirmed link found (gene/protein designation)
IPA: /siː waɪ piː ˈsɛvənˈtiːn eɪ wʌn/
Phonetic Spelling: see-why-pee seventeen-A-one

14. CYP19A1

Pronunciation link:

No confirmed link found (gene/protein designation)
IPA: /siː waɪ piː ˈnaɪntiːn eɪ wʌn/
Phonetic Spelling: see-why-pee nineteen-A-one