Tracing ovarian follicles growth *in vitro* by three-dimensional culture system

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Method Article

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Abstract

In female mammals, the basic reproductive unit in the ovaries is the follicle, which is composed by the oocyte and surrounding somatic granulosa cells (GCs). The development of follicles is a highly programed progress and is regulated by both the paracrine and endocrine factors. To culture the follicle *in vitro*, various approaches were established in different groups including the 2-dimensional culture and 3-dimensional culture. In this study, we improved a three-dimensional Matrigel system to culture the mouse follicles from secondary stage. In this system, the 3D culture guaranteed spherical structures of the follicles, which was utilized to trace the difference of growth dynamics of follicles in mutant and wild type females.

Introduction

Reagents

MEM α , nucleosides, GlutaMAX™ Supplement (Gibco, 32-571-036)

Fetal Bovine Serum (Gibco, 10-099-141)

ITS Liquid Media Supplement (100×) (Sigma-Aldrich, 13146)

10 ng/ml FSH (ovine Follicle Stimulating Hormone, NHPP)

100 U/ml penicillin and 100 μ g/ml streptomycin (Thermo Fisher Scientific, 15140122)

Matrigel (BD, 354234)

PBS (Gibco, 20-012-027)

Equipment

Sterile 1 mL syringe (BD, 300841)

Omnican U-40 Insulin syringe (B.Braun, 9161635S)

Leica bright field dissection microscope (MZ10)

35 mm culture dish (Nest, 706001)

6 Well Culture Plate (Nest, 723001)

Incubator at 37°C (20% O₂ and 5% CO₂, Memmert INCO2/153)

Nikon Eclipse Ti digital fluorescence microscope

Mouth pipette for transferring follicle

Procedure

1. Preparation of the follicle culture medium:

The basic medium to isolate the follicles was the MEM α medium. To culture the follicles, the MEM α medium was supplied with 10% FBS, 1% insulin-transferrin-selenium (ITS; Sigma-Aldrich), 10 ng/mL FSH (ovine Follicle Stimulating Hormone, NHPP) and 0.1% penicillin and streptomycin (Thermo Fisher Scientific) and was pre-warmed to 37°C before using.

2. Preparation of the Matrigel microdroplet

The precooling liquid Matrigel was mixed with culture medium (3:1), and then added to 6 well culture plate to make the microdroplet (20 μ L per droplet, 6 droplets per well) on ice by pipette (Tip: Make sure the tips were precooled to avoid the coagulation of gel). Gently putting the plate into the 37°C incubator. After around 20 min, the phase of Matrigel droplets changed from liquid to solid.

3. Ovarian follicle culturing:

- (1) Collecting the mouse ovaries from the C57BL/6 females at postnatal day (PD) 21, washing the ovaries with PBS to remove the blood and then put in 35 mm culture dish with 2 mL basic medium.
- (2) Tearing the ovary to the small pieces, and gently isolating the ovarian follicles (diameter 120-160 μ m) by Omnican U-40 Insulin syringe in the medium. Choosing the follicles with intact theca layer to culture.
- (3) Poking the solid Matrigel microdroplet to make a hole in the center of droplet by mouth pipette, and transfer one isolated follicle and seed it into the hole of Matrigel microdroplet by mouth pipette.
- (4) Repeating step 3 to seed one follicle into each microdroplet.
- (6) Adding the 2 μ L liquid Matrigel to seal the hole and put the plate into incubator at 37°C for 10 min to make the gel solid. After this step, the cultured follicles were completely surrounded by the gel.
- (7) Adding 1 mL the culture medium to each well, culturing the ovarian follicle in incubator at 37°C (5% CO₂, saturation humidity) for 8 days and replaced half of the culture medium every other day.

4. Tracing the follicle growth:

(1) In ovarian follicle diameter tracing experiment, the developmental dynamics of follicles were recorded by the increase of the diameter in bright field with a Nikon Eclipse Ti digital fluorescence microscope. The survival rate of the follicles was around 350 μm after 8 days cultured.

Troubleshooting

Time Taken

About 1 week

References

1. Higuchi, CM., Maeda, Y., Horiuchi, T., & Yamazaki, Y. A Simplified Method for Three-Dimensional (3-D) Ovarian Tissue Culture Yielding Oocytes Competent to Produce Full-Term Offspring in Mice. *PLoS One* **10**, e0143114 (2015).

Figures

Figure 1

Illustration of the strategy of the process of ovarian follicle culture