

가 Pre-antral Follicles

1, 2
1,2 1 2 1 2

Antrum Formation and Outgrowth In Vitro of Mouse Pre-antral Follicles

Cultured in Media without Hormones

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Objective: Mouse pre-antral follicles require the addition of gonadotropins (Gns) to complete maturation and ovulation of oocyte and antrum formation in vitro. However, we tried examination of in vitro growth of mouse pre-antral follicles in medium without Gns and/or physiological factors. And also, pre-antral follicles were isolated from ovaries by mechanical method. Our present studies were conducted to evaluated on the growth of follicles and intra-follicular oocytes and antrum formation in vitro of mouse pre-antral follicles.

Methods: Pre-antral follicles (91-120 μm) were isolated mechanically by fine 30G needles not using enzymes from ovaries of 3-6 week-old female ICR mice. Isolated pre-antral follicles were cultured in 20 μl droplets of TCM (n=17; follicles: $107.8 \pm 1.58 \mu\text{m}$; oocytes: $57.9 \pm 1.2 \mu\text{m}$) or MEM (n=12; follicles: $109.3 \pm 2.53 \mu\text{m}$; oocytes: $55.4 \pm 1.6 \mu\text{m}$) under mineral oil on the 60 mm culture dish. All experimental media was supplemented with 10% FBS without Gns and/or physiological factors. Pre-antral follicles were individually cultured for 8 days. Antrum formation and outgrowth of pre-antral follicles and intra-follicular oocytes were evaluated using precalibrated ocular micrometer at $\times 200$ magnifications during in vitro culture. Results were analyzed using combination of Student's t-test and Chi-square, and considered statistically significant when $P < 0.05$.

Results: Antrum formation had started in two culture media on day 2. On day 8, antrum formation had occurred in 58.3% (7/12) of pre-antral follicles cultured in DMEM, but only in 23.5% (4/17) of those cultured in TCM ($P = 0.0364$). Outgrowth of pre-antral follicles and intra-follicular oocytes were observed on day 4 and 8. On day 4, follicular diameter was similar ($P = 0.1338$) in TCM ($119.4 \pm 2.58 \mu\text{m}$) and MEM ($125.4 \pm 4.52 \mu\text{m}$). However, on

day 8, diameters of pre-antral follicle cultured in MEM ($168.9 \pm 17.29 \mu\text{m}$) were significantly bigger ($P=0.0248$) than that in TCM ($126.7 \pm 4.28 \mu\text{m}$). On day 4 and 8, diameters of intra-follicular oocytes were similar in TCM (67.1 ± 1.3 and $72.4 \pm 0.9 \mu\text{m}$) and MEM (65.2 ± 1.7 and $73.3 \pm 1.5 \mu\text{m}$), respectively.

Conclusions: We can conform that medium without Gns and/or physiological factors can be used for in vitro antrum formation and outgrowth of pre-antral follicles and intra-follicular oocytes in mouse. In conclusion, MEM supplemented with FBS can be used for growth in vitro of mouse pre-antral follicles isolated mechanically.

Key words: Mouse pre-antral follicles, Intra-follicular oocytes, Media, In vitro growth, Antrum formation

pre-antral follicles

pre-antral follicles

.¹

가

, 가

oocyte-granulosa cell

,

가

pre-antral follicle

.

Eppig Schroeder²가

granulosa-oocyte

collagen membranes 10

. , Torrance

³ Carroll ⁴

collagen gels

, Eppig

Schroeder²

.

5-12

pre-antral follicle

.

(4-6) , membrane

,⁵

transpose

.⁷⁻¹²

pre-antral follicles

6,10,13

,

serum / FSH 가가

LH

.⁶

, , growth factors(ITS, EGF), gonadotropins(FSH, LH

) hypoxanthine, dibutyryl-cyclic AMP (dbcAMP) 3-isobutyl-1-

methyxanthine (IBMX)

가

.^{2,4,8,9,14,15,16}

pre-antral follicle

가

가

가

가

가

가

1.

pre-antral follicle 3-6 ICR

(20-22) (12 hrs : 12 hrs)

가

2.

Ham's F-10 10% fetal bovine serum (FBS; 26140-079, Gibco, USA) 가 Tissue Culture Medium 199

(TCM; 11150-059, Gibco, USA) Dulbecco's Modified Eagle Medium (MEM; 11966-025, Gibco, USA) 10% FBS 가

0.5% antibiotics (Streptomycine sulfate, S-9137; Penicillinine-G, P-3032, Sigma, USA) 가 , (Osmomat 030, Gonatec, Gemany)

280 mOsmol/kg , 0.2 μ m (SLGV R25 LS, Millipore, France)

14 M \varnothing tube (2001, Falcon, USA) 4 (3682, Forma, Japan) 가 4 가

6 37 , 5% CO₂ (3158, Forma, USA)

3.

1M washing medium watch glass
 forceps 30G syringe needle(320310, BD, USA)

, Fig. 1A

pre-antral pasteur pipette washing medium 2-well
 dish 3 , 17

4.

(1)

91-120 μm

60 mm culture dish (3002, Falcon, USA) 20 μl
 mineral oil (M-8410, Sigma, USA)

8

(2)

theca cell

2

4

8

($\times 200$)

micrometer

(3)

48

1/2 10 μl

()

5.

Student's t-test

Chi-square (χ^2 -test)

,

Chi-square (χ^2 -test)

5%

.

\pm SEM

.

Pre-antral follicle

Table 1

, TCM 2 (n=2), 3 (n=1) 4 (n=1), MEM
2 (n=2), 3 (n=2), 4 (n=1) 5 (n=2) , 가
pre-antral follicle , 2-5 (Table 1).
, TCM 17 4 (23.5%)가, MEM 12
7 (58.3%)가 , MEM TCM (P=0.0364) . ,
pre-antral follicle

pre-antral follicle 4 8

Figure 2

pre-antral follicle TCM 107.8 ± 1.58μm, MEM
109.3 ± 2.53μm . 4 pre-antral follicle , TCM 119.4
± 2.58μm, MEM 125.4 ± 4.52μm (P=0.1338).
8 , TCM 126.7 ± 4.28μm, MEM 168.9 ± 17.29μm
MEM TCM
(P=0.0248). TCM 58 ± 1.2μm, MEM
55 ± 1.6μm . 4 8 TCM 67.1 ± 1.3μm 72 ±
0.9μm MEM 65.2 ± 1.7μm 73 ± 1.5μm , 가 .

Roy Greenwald^{14,15} hamster small primary secondary

FSH LH가 , Katska Ry ka¹⁶ FSH, ITS(insulin / transferrin / selenium), L-glutamine sodium pyruvate가

serum

FSH 가 , LH 가 .⁷ Wright

¹⁸ 10% human serum -minimum essential medium(-MEM), Waymouth's, Earle's balanced salt solution(EBSS)

10 -MEM initiation 가 , -MEM

가 300 mIU/M² FSH가 , atresia

serum 가 human serum

albumin(HSA) ITS가 가 , FBS

가 pre-antral follicle (Fig. 1C) (Fig. 2A)

FSH ITS 가

가

Roy Treacy¹⁹가 pre-antral follicle

. Abir²⁰

collagen gels . rat,^{21,22} hamster,^{15,23}

²⁴ pre-antral follicle ,

E_2 theca layer가
^{25,26}
 gel
 theca-free antral agar gel²⁷ matrigel²⁸
 Collagen gels pre-antral ²⁹ oocyte
³⁰ 2 granulosa cells 가 human follicles
 collagen gels ²⁰ 17
 pre-antral follicle
 가 ,
 mincing
 (Fig. 1A). (Fig. 1B)
 11 가
 metaphase II granulosa cell
 (Fig. 1D), ^{31,32} ³³ ³⁴ ³⁵ 가 가
 가 , 가
 Cortvrindt Smitz³⁶ F1 hybrid (C57blxCBACa)
 pre-antral follicle (100 ~ 130 μ m) 5% FCS, 10 μ g/M ℓ transferrin, 5 μ g/M ℓ insulin 100
 mIU/M ℓ rFSH가 가 -MEM 가 56.5 \pm 4.4 μ m
 67 \pm 4.1 μ m 16 () 72.5 \pm 3.2 μ m

, (55.4 ± 1.6 ~ 57.9 ± 1.2 μm)

4 (65.2 ± 1.7 ~ 67.1 ± 1.3 μm) 8 (72.4 ± 0.9 ~ 73.3 ± 1.5 μm)

, MEM FBS 가

(Fig. 2B).

pre-antral follicle growth

FSH가 ,³⁷ FSH

가 ⁵ 가 가 가

가가

가 (Fig 1C).

granulosa cell cumulus cell . Pre-antral follicle granulosa

cell layer (quality)

, ()

.

, pre-antral follicle

가 ,

2-5 8 가

. ,

FBS 가 MEM .

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Table 1. Formation of antrum-like cavity of mouse pre-antral follicles following in vitro growth day.

Viable	Media		P - value*
	TCM	MEM	
No. of examined pre-antral follicles	17	12	
Formation of antrum-like cavity			
On day 1	-	-	
2	2 (11.8)	2 (16.7)	0.4492
3	1 (5.9)	2 (16.7)	0.8988
4	1 (5.9)	1 (8.3)	0.6511
5	-	2 (16.7)	0.2526
6	-	-	-
7	-	-	-
8	-	-	-
Total	4 (23.5)	7 (58.3)	
0.0364			

* χ^2 -test