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The Effects of Glutamine on Blastulation of Human Embryos on Vero Cells *In Vitro*

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=Abstract=

This study was conducted to investigate the effects of Tissue Culture Medium 199 (TCM) and Dulbecco's Modified Eagle Medium (DMEM) on the blastulation and grade of human oocytes on Vero cells *in vitro*.

A cohort of 79 and 93 oocytes in metaphase II stage were used in TCM 199 and DMEM respectively. No differences were found in the number of oocytes showing two-pronuclei between TCM (82.3%) and DMEM (86.0%). The number of fertilized oocytes reaching the blastocyst was not significant in TCM (60.0%) and DMEM (63.1%). A total of 89 blastocysts were categorized into the four grades (BG1, BG2, BG3 and early) depending on their morphology. The number of embryos achieving the blastocyst grade 1 (BG1) was significantly higher ($P < 0.05$) in DMEM than TCM, at 50.8% (33 out of 65) and 15.0% (12 out of 80) respectively.

It is concluded that cultured oocytes in DMEM with glutamine on Vero cells should be significantly increased BG1.

Key words : Tissue Culture Medium 199 (TCM), Dulbecco's Modified Eagle Medium (DMEM), Two-pronuclei, Blastulation, Blastocyst, Blastocyst grade 1 (BG1)

(cavitated) (blastocyst)가 fallopian tube 가
 (Schillaci , 1994; Gardner Lane, 1997). (IVF cycle)
 2 3
 (Dokras , 1993; Turner and Lenton, 1996),
 degeneration 가 가 (Dokras ,
 1993; Huisman , 1994). (Schillaci 1994)
 , 가
 . glucose pyruvate
 lactate , glucose가
 (Gardner Lane, 1997). Hamster
 glycolysis "Crabtree effect" (Crabtree, 1929; Koobs, 1972)
 가 glucose (Seshagiri
 Bavister, 1989; Barnett Bavister, 1996). Glutamine mouse metabolic source
 , glutamine transaminase 가 .
 grade . grade BG1, BG2
 BG3 , hatching attachment , attachment hCG
 grade , grade
 (Dokras , 1993; Turner Lenton,
 1996).

TCM DMEM

grade

1.

F-10 Nutrient Mixture Medium (F-10, 11550-043, Gibco, USA) 0.8 mM
Ca-lactate (276044X, BDH, UK), 5.1 mM KHCO_3 (P-9144, Sigma, USA) 20 mM NaHCO_3
(S-5761, Sigma, USA) 가 . TCM (11150-059, Gibco,
USA) DMEM (11966-025, Gibco, USA) , Vero cell TCM
. 0.5% antibiotics (Streptomycine sulfate, S-9137; Penicillin-G, P-3032, Sigma,
USA) 가 (Osmomat 030, Gonatec, Germany) 280
mOsmol/kg . 0.2 μm (Minisart 17597, Sartorius,
Germany) 14 ml tube (2001, Falcon, USA) 4 .
95% , 37 5% CO_2 (3154, Forma, USA) 6

2.

gonadotrophin releasing hormone agonist (GnRHa)
. Mid-luteal phase 300 μg Buserelin (Suprefact, Hoechst, Germany)
, follicular phase 3 225 IU human menotrophins [human menopausal
gonadotropin (hMG, Pergonal, Serono, Italy)] / follicle stimulation hormones (FSH,
Urofollotropin, Metrodin, Serono, Italy) GnRHa 4 . oestradiol
, menotrophins
. 6-8 oestradiol
. 18 mm 1 oestradiol 가
900 pg/ml 10,000 IU human chorionic gonadotrophin (hCG, Profasi, Serono, Italy)
. hCG 36-38
. 37 5% CO_2 (IVF chamber, Iljin, Korea)
(Veck, 1991) , 10%

human follicular fluid (hFF) 가 TCM DMEM (3260, Costar, USA)
5 가

3. (hFF, human follicular fluid)

hFF

2 (30 , 10) hFF (3,500 rpm)
56 35 0.2 μm
-20 가 2

4.

(masturbation)

World Health Organization Criteria (WHO, 1993)

, 10% hFF가 가 F-10 1,500 rpm 2 (5 , 3)

pellet 1 ml F-10 가 1

5 ml tube (2003, Falcon, USA)

200,000 가 가 2 ml

, 37 5% CO₂ syringe

needle (320310, Becton Dickinson, USA)

가

5. Vero cell

Vero cell Ouhibi (1989) cell 2-3X10⁶ cell

flask 4 (6-8X10⁶ cell) , trypsin cell suspension 3

flask , ,

monolayer 2 ml 200,000 cell

3 . 10% fetal bovine serum (FBS, 26140-079, Gibco, USA) 가
TCM . Vero cell Ouhibi (1989) .

6. Vero cell monolayer

Vero cell .
20% hFF가 가 TCM DMEM , 2-3
가

7. grade

grade (BG) Dokras (1993) , BG1 early cavitation
expanded cavity (ICM trophectoderm layer)가 . BG2 initial
cavitation 1-2 BG1 (“late” “slow” developer) ,
initial cavitation 1-2 5
(early blastocyst) . BG3 vacuole de generative
foci가 , vacuolated morula vacuole cavity ,
BG3 ICM trophectoderm .

8.

TCM DMEM
5-7 Vero cell
grade

SAS (Statistical Analysis System, 1988) package

t-test , 5%

21 172 (Metaphase II) TCM (n=93, 11 cycle)
 DMEM (n=79, 10 cycle) . 172
 145 (TCM: 80; DMEM: 65) Vero cell 5-7
 .
 TCM 86.0% (80/93) DMEM 82.3% (65/79)
 가 (Table 1).
 TCM 60.0% (48/80) DMEM 63.1% (41/65)
 가 (Table 2). BG1, BG2 BG3 early
 grade , 가 BG1
 DMEM 50.8% (33/65) TCM 15.0% (12/80) (P<0.05) (Table
 2). BG2 BG3 가 (early
 blastocyst) TCM 35.0% (28/80) DMEM 7.7% (5/65) (P<0.05) .
 가 BG1
 DMEM TCM .

Table 1 2

가 . grade , 가
 가 BG1 DMEM (50.8%) TCM (15.0%) (P<0.05)
 . (early blastocyst) TCM (35.0%) DMEM
 (7.7%) (P<0.05). DMEM glutamine
 TCM glucose
 Barnett Bavister (1996) 1 mM glutamine 0.1-0.25mM pyruvate가 HECM
 glucose/inorganic phosphate (Gu/Pi, 5 mM/0.35 mM) 가 hamster 2
 가 , 가 (75%) 가 (36%) (P<0.05)
 Glu/Pi가 Glu/Pi action
 glycolysis , glycolysis가 cytosolic metabolism mitochondrial
 metabolism oxidative phosphorylation
 (Crabtree effect; Crabtree, 1929; Koobs, 1972).
 Seshagiri Bavister (1989) hamster 8 glucose
 , hamster 8 energy
 glycolysis "Crabtree effect" (Crabtree, 1929; Koobs, 1972)가
 . "Crabtree effect" 가 oxidative
 potential Krebs cycle catabolism
 (Seshagiri Bavister, 1989; Barnett Bavister,
 1996). Glutamine glutamine transminase 2-oxoglutarate
 (Hornsby, 1982), mouse embryo metabolic source glutamine (Nasr-Esfahani ,
 1992). Gardner Lane (1997) pyruvate, lactate, glucose 가
 oviduct (0.32, 10.5, 0.50mM) uterus (0.10, 5.87, 3.15) , oviduct가
 uterus glucose 가 가
 glucose .

Dokras (1993) Turner Lenton (1996) BG 1, BG2

BG3 3가 , grade .

가 BG1 , BG1 1-2 6-7 BG1 가

BG2 BG1

. BG2

BG1 가 BG3

BG1 BG2 . 가

hCG 10- 11 (Dokras , 1993). BG 1

BG2 hCG pattern 가 , BG 1 8 9 BG2

(Dokras , 1993). grade

(developmental potential) (Dokras , 1993, Turner

Lenton, 1996).

가 ,

glutamine 가 DMEM glucose glutamine 가 TCM

BG1 , TCM 가

glucose가 .

5-7

(Gardner Lane, 1997), human ampullary cells (Bongso , 1989),

bovine uterine fibroblasts (Wiemer , 1989) monkey Vero cells (Schillaci , 1994; Turner

Lenton, 1996; Gardner Lane, 1997) feeder cell .

, Vero cells

glutamine 가 DMEM

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Table 1. Effects of different culture media on *in vitro* fertilization of human oocytes.

Treatment	No of cycles examined	No of embryos cultured	Fertilization (%) ^a
DMEM	10	79	65 (82.3)
TCM	11	93	80 (86.0)

^aNo significant differences

Table 2. Effects of different culture media on development of *in vitro* fertilized oocytes.

Treatment	No of cycles examined	No of embryos cultured	Blastocyst (%)				
			Total	BG 1	BG2	BG3	Early
DMEM	10	65	41 (63.1)	33 (50.8) ^a	4 (6.2)	1 (1.5)	5 (7.7) ^a
TCM	11	80	48 (60.0)	12 (15.0) ^b	4 (5.0)	4 (5.0)	28 (35.0) ^b

^{a-b}Within columns treatments with different superscripts are significantly different (P<0.05).