

Comparative Study on Development of Mouse Embryos in Three Commercial Media and Hatching Rates of Mouse Embryos with/without Pronase

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Objectives: The purpose of this present study was to compare mouse embryo development in 3 commercial media and hatching competence of mouse embryo with or without enzymatic treatment.

Methods: Collected 375 mouse embryos were divided into three groups, and then cultured in IVF-20 (G2), Medicult IVF (M3), P-1 (blastocyst M), respectively. Three day mouse morulae were cultured in G2 media treated with pronase. The results were analyzed using Chi-square test, and considered statistically significant when $p < 0.01$.

Results: The developmental rate of 2 cell mouse embryo after 72 hours was highest in IVF-20 (G2) among conventional 3 media. The hatching rate of mouse morulae was low when cultured in G2 media without pronase during 48 hours. However, it was higher when cultured in media treated with 1 $\mu\text{g/ml}$, 2.5 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$ pronase, respectively.

Conclusions: Using good media and digestion of zona pellucida with enzymatic treatment improve development and hatching rate of embryo. Therefore, implantation and pregnancy rate could be improved.

Key Words: Mouse, Media, Pronase

가 . 2 3 가 .
2 3 2 2가 가 .
4~8 가
5~30% 가
10~20% 가

(sub-optimal culture condition)

가 가

4~6 2 가 ,
4~8 3
90~99

(morula e stage)

(early blastocyst

(seru m free culture systems) ,

stage)

4~6

(somatic helper cells)

8

(oviductal envi-

5~6 1~3

ronment) 70~75

20~24

IVF programs

8-10

1

가

가

4

가

6

7

(Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine, 1998).

.¹¹

가

zona lysin

(cleavage stage embryo)

가

vivo blastocyst)

50%

(human in

De Felice¹²

II

(metaphas e II oocytes)

가

가

가

가

(zona hardening)

가

(assisted-hatching)

Jones⁴

.³

(hole)

4~8

genome

.¹¹

가

가

(embryo hatching)

pronase

(enzymatic treatment)가

(digestion)

가

.^{6,7}

Table 1. Development of mouse embryos in conventional 3 media during 24 hours

Media	No. of embryo	2 cell	4 cell	Morula	Blastocyst	Exp. blastocyst	Hatching	Deg.
IVF-20	122	48 (39.3%)	74 (60.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Medicult IVF (M3)	123	52 (42.3%)	70 (56.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)
P-1 (Blastocyst-st M.)	130	37 (28.5%)	55 (42.3%)	38 (29.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Exp.: expanded, Deg.: degeneration

Table 2. Development of mouse embryo in conventional 3 media during 48 hours

Media	No. of embryo	2 cell	4 cell	Morula	Blasocyst	Exp. blastocyst	Hatching	Deg.
IVF-20 (G2)	122	31 (25.4%)	23 (27.0%)	68 (55.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Medicult IVF (M3)	123	37 (30.1%)	35 (28.5%)	45 (36.6%)	2 (1.6%)	0 (0%)	0 (0%)	4 (3.3%)
P-1 (blastocyst M)	130	31 (23.8%)	8 (6%)	52 (40.0%)	32 (24.6)	1 (0.8%)	0 (0%)	6 (4.6%)

3.
1.
ICR
2.
PMSG (pregnant mare's serum gonadotropin, Sigma Co.) 5 IU (1), 48
hCG (human chorionic gonadotropin, Sigma Co.) 5 IU (3), 4
가
5 가
30-G
2
3.
1)
Ham's F-10 HPLC
(Baxter Co.) , 0.4%
BSA 가
2)
6~8
1, 2 IVF-20 (Vitrolife, IVF Science Scandinavia) , 3
G2 . 2 1,
2 P-1 Medium (Irvine Scientific Company, USA)
, 3 Blastocyst
Medium
3 1, 2 IVF Medium (Medicult company, Denmark) , 3 M3
Medium
3)
pronase (1~100 µg/ml)
3

Table 3. Development of mouse embryo in conventional 3 media during 72 hours

Media	No. of embryo	2 cell	4 cell	Morula	Blastocyst	Exp. blastocyst	Hatching	Deg.
(IVF-20) G2	122	27 (22.1%)	9 (7.4%)	14 (11.5%)	63 (51.6%)	0 (0%)	1 (0.8%)	8 (6.6%)
(Medicult IVF) M3	123	34 (27.6%)	28 (22.8%)	27 (22.0%)	20 (16.3%)	7 (5.7%)	0 (0%)	7 (5.7%)
(P-1) blastocyst M	130	23 (17.7%)	4 (3.1%)	6 (4.6%)	18 (13.8%)	42 (32.3%)	8 (6.2%)	29 (22.3%)

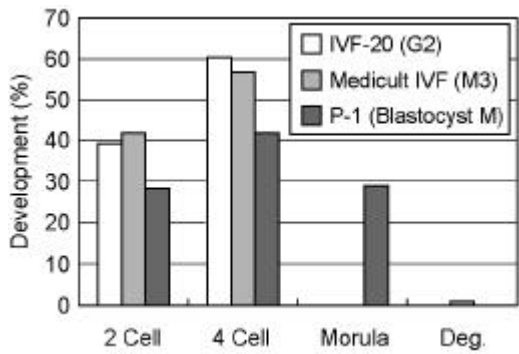


Figure 1. Development of mouse embryos conventional 3 media during 24 hours.

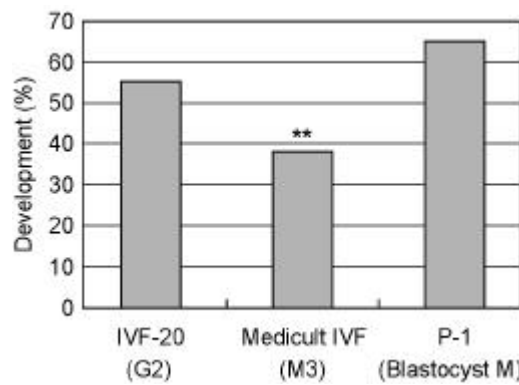


Figure 2. Development over morula in conventional 3 media during 48 hours. ** p<0.01

3

1.

가

3

IVF-20 (G2), Medicult IVF (M3), P-1 (blastocyst medium)

24

(Table 1, Figure 1) IVF-20 60.7% 가 4 56.9% 가 4 42.3% 가 4 , Medicult IVF P-1 , 29.2% 가 가

48

(Table 2) IVF-20 55.7% 가 , Medicult IVF 36.6% 가 , 1.6% 가 , Medicult IVF 38.2% . P-1 40.0% 가 , 24.6% 가 , 0.8% 가 , P-1 65.4% . Medicult IVF (38.2%), IVF-20 (55.7%) P-1 (65.4%) (p<0.01, Figure 2).

72

(Table 3), (IVF-20) G2 51.6% 가 0.8% 가 . (Medi-

Table 4. Development of mouse embryo in conventional 3 media during 96 hours

Media	No. of embryo	2 cell	4 cell	Morula	Blastocyst	Exp. blastocyst	Hatching	Deg.
(IVF-20) G2	122	14 (11.5%)	3 (2.5%)	1 (0.8%)	29 (23.8%)	27 (22.2%)	19 (15.6%)	28 (23.4%)
(Medicult IVF) M3	123	20 (16.3%)	14 (11.4%)	4 (3.3%)	6 (4.9%)	34 (27.6%)	16 (13.0%)	36 (29.3%)
(P-1) blastocyst M	130	12 (9.2%)	0 (0%)	0 (0%)	2 (1.5%)	30 (23.1%)	28 (21.6%)	58 (44.6%)

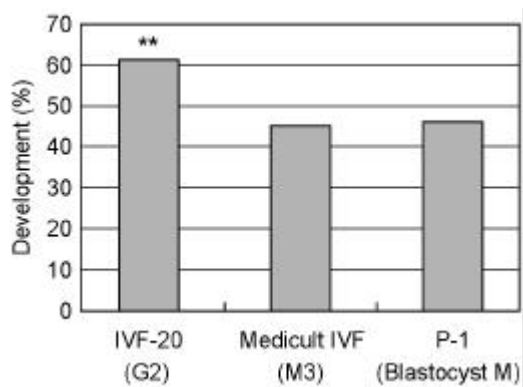


Figure 3. Development of blastocyst in conventional 3 media during 96 hours. **p<0.01

(IVF-20) G2 11.5%
 , 22.2% 가
 , 15.6% 가
 (Medicult IVF) M3 4.9%
 , 27.6% 가
 , 13.0% 가
 (P-1) blastocyst 1.5% 가
 , 23.1% 가
 , 21.5% 가

(Medicult IVF) M3 45.5% 가
 (P-1) blastocyst medium 46.1% 가
 (IVF-20) G2 61.5% 가
 (p<0.01, Figure 3).
 120
 (Figure 4) (IVF-20) G2 11.5%
 가 , 14.8% 가 , 22.1% 가
 (Medicult IVF) M3 4.1% 가
 , 3.3% 가 , 11.4% 가
 (P-1) blastocyst medium 19.2% 가
 (IVF-20) G2 36.9% 가
 , (Medicult IVF) M3 14.7% 가
 , (P-1) blastocyst medium 19.2% 가
 (IVF-20) G2 (p<0.01).
 2
 IVF-20 (G2), Medicult IVF (M3), P-1 (blastocyst medium)

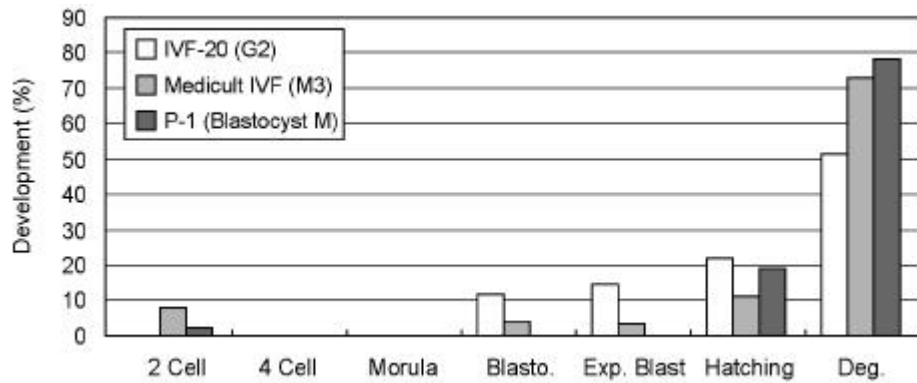


Figure 4. Development of mouse embryo in conventional 3 media during 120 hours.

Table 5. Hatching of mouse morula treated with/without pronase in G2 medium during 48 hours (96 hours after 2 cell culture beginning)

Con. of pronase (µg/ml)	No. of embryo	Morula	Blastocyst	Exp. blastocyst	Hatching	Deg.
0	65	0(0%)	5 (7.7%)	18 (27.7%)	19 (29.2%)	23 (35.4%)
1	51	0(0%)	4 (7.8%)	6 (11.8%)	31 (60.8%)*	10 (19.6%)
2.5	40	0(0%)	5 (12.5%)	4 (10%)	31 (77.5%)*	0 (0%)
5	58	0(0%)	5 (8.6%)	19 (32.8%)	24 (41.4%)	10 (17.2%)
10	60	7 (11.7%)	10 (16.7%)	21 (35%)	11 (18.3%)	11 (18.3%)
100	32	0(0%)	3 (9.4%)	4 (12.5%)	0 (0%)	5 (78.1%)

2. (zona pellucida) pronase

(Table 5)

(glycoprotein units) (dissulfide bonds) 48 (29.2% 가, 96)
 (high rate of early pregnancy wastage) 가 가
 (micromanipulator) 16% 가 100 µg/ml
 (IVF-20) G2 pronase 가 (10~100 µg) (1~2.5 µg) pronase
 2 48 pronase

?

Krebs-Ringer's bicarbonate
 Tyrode's solution 가
 2가 Ham's F-10
 Gardner Lane^{16,17} 가
 Jones¹⁸ 가
 (human tubal fluid) Earle's HTF
 (Hoppe and pitts, Tyrode's,
 Ham's F10 and Earle's)
 1가¹³ 가
 pH, 가
 pH 가
 가 Jones¹⁴ 가
 (fomulation)
 가
 pH 가
 (IVF/ET)
 (formulation)
 가
 ions 가 가
 T6 HTF¹⁹
 Na⁺ K⁺ 가 T6 HTF (fully expanded blastocysts) 4
 1060 293 (human ovi-^{20,21}
 duct fluid) Na⁺/K⁺ ions 가 18 Quinn (lysins,)
¹³ Na⁺/K⁺ ions Ca²⁺/MG²⁺
 가
 IVF (glycoprotein)
 (species)
 (serum free culture system)²²
 5 6 가

가 ,

23 (acid Tyroide's solution) (zona drilling) 33

가 (Cohen^{34,35})

23,24 가 , 가

(assist hatching) 가

25~30%

70~75% 1가

가 (suboptimal culture condition) 가 IVF-20 (G2), Medicult IVF (M3), P-1 (blastocyst medium)

가 (micromanipulation) pronase

(acid Tyroide's solution) 48 2

25,26 (micromanipulator) IVF-20 (G2) 55.7%, P-1 (blastocyst medium) 65.4% Medicult M3 38.2% (p<0.01) 72

(mechanical partial zona dissection, PZD)^{27,28} laser 29 (IVF-20) G2 52.4%, (P-1) blastocyst medium 52.3% (Medicult IVF) M3 22% (p<0.01). 96

30 (PZD) , (Medicult IVF) M3 45.5%, (P-1) blastocyst medium 46.1% 가 , (IVF-20) G2 61.5% 가 가 (p<0.01). 120

가 . (Medicult IVF) M3 14.7%, (P-1) blastocyst medium 19.2% , (IVF-20) G2 36.9% 가 가 (p<0.01).

31 (blastomeres) IVF-20 (G2) 3 5~6

32 (initial compaction) 가 , 3 4

가
5, 6
가
nase
2
48
pronase
29.2%
2.5 µg/ml, 5 µg/ml pronase
(p<0.01),
27,36
lazer
가
37,38
pronase
가

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