

### 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).

.<sup>11</sup>

가

zona lysin

(cleavage stage embryo)

가

vivo blastocyst)

50%

(human in

De Felice<sup>12</sup>

II (metaphas e II oocytes)

가

가

가

가

(zona hardening)

가

(assisted-hatching)

Jones<sup>4</sup>

.<sup>3</sup>

(hole)

4~8

genome

.<sup>11</sup>

가

가

(embryo hatching)

pronase

(enzymatic treatment)가

(digestion)

가

.<sup>6,7</sup>

**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. 1)

ICR (Baxter Co.) Ham's F-10 HPLC 0.4%

2. BSA 가

6~8 2)

PMSG (pregnant mare's serum gonadotropin, Sigma Co.) 5 IU ( 1 ), 48 1 1, 2 IVF-20 (Vitrolife, IVF Science Scandinavia) , 3

hCG (human chorionic gonadotropin, Sigma Co.) 5 IU G2 . 2 1,

( 3 ), 4 2 P-1 Medium (Irvine Scientific Company, USA) Blastocyst Medium

5 가 3 1, 2 IVF Medium (Medicult company, Denmark) , 3 M3 Medium

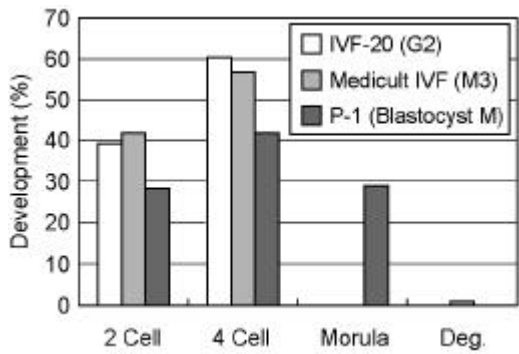
30-G 3)

pronase (1~100 µg/ml)

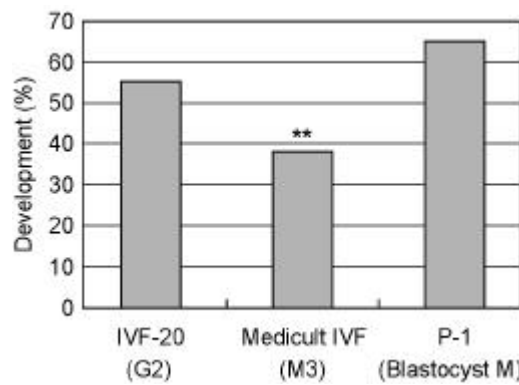
, 2 3

**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.



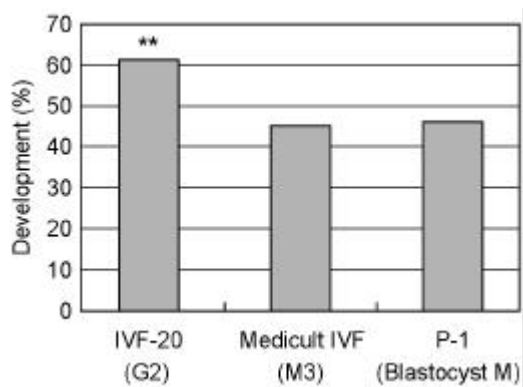
**Figure 2.** Development over morula in conventional 3 media during 48 hours. \*\* p<0.01

3  
 1. 3  
 가  
 3  
 IVF-20 ( G2 ), Medicult  
 IVF ( M3 ), P-1 ( blastocyst medium ) 2  
 24  
 (Table 1, Figure 1) IVF-20  
 60.7% 가 4 , Medicult IVF  
 56.9% 가 4 , P-1  
 42.3% 가 4 , 29.2% 가  
 가

48  
 (Table 2) IVF-20 55.7% 가  
 , Medicult IVF  
 36.6% 가 , 1.6% 가 , Medi-  
 cult 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%  
 (Medicult IVF) M3 4.1%  
 (P-1) blastocyst medium 1.5%  
 (IVF-20) G2 15.6%  
 (Medicult IVF) M3 13.0%  
 (P-1) blastocyst medium 21.6%  
 (IVF-20) G2 23.4%  
 (Medicult IVF) M3 29.3%  
 (P-1) blastocyst medium 44.6%

(Medicult IVF) M3 45.5%  
 (P-1) blastocyst medium 46.1%  
 (IVF-20) G2 61.5%  
 (p<0.01, Figure 3).  
 120  
 (Figure 4) (IVF-20) G2 11.5%  
 , 14.8%  
 (Medicult IVF) M3 4.1%  
 , 3.3%  
 (P-1) blastocyst medium 1.5%  
 , 11.4%  
 (IVF-20) G2 15.6%  
 (Medicult IVF) M3 13.0%  
 (P-1) blastocyst medium 21.6%  
 (IVF-20) G2 23.4%  
 (Medicult IVF) M3 29.3%  
 (P-1) blastocyst medium 44.6%

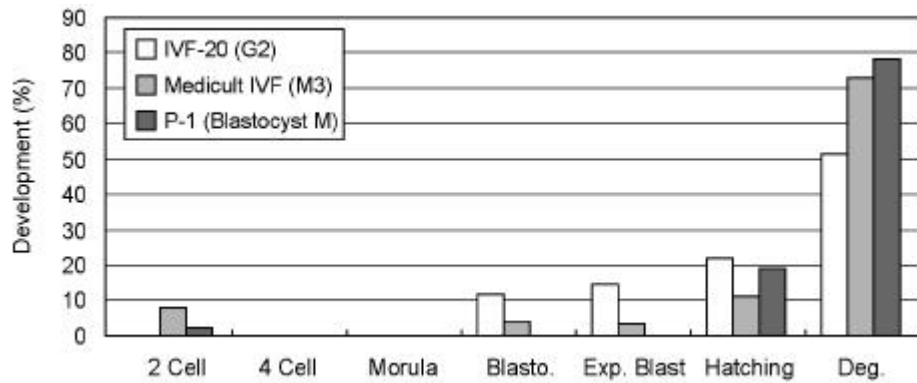


Figure 4. Development of mouse embryo in conventional 3 media during 120 hours.

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

?

Gardner Lane<sup>16,17</sup> Jones<sup>18</sup>

Krebs-Ringer's bicarbonate 가 가

Tyrode's solution 가 가 .

2가 . Ham's F10 , HTF

(human tubal fluid) Earle's

.4 (Hoppe and pitts, Tyrode's, ,

Ham's F10 and Earle's)

1가 .<sup>13</sup> 가

pH, 가 가

pH 가 가 (fomulation)

가 . Jones<sup>14</sup> .

가 .

pH ,

가 .

(IVF/ET)

(formulation)

가 ,

ions 가 가 ,

T6 HTF .<sup>19</sup>

Na<sup>+</sup> K<sup>+</sup> 가 T6 HTF (fully expanded blastocysts) 4

1060 293 . (human ovi- ,<sup>20,21</sup>

duct fluid) Na<sup>+</sup>/K<sup>+</sup> ions 가 18 .Quinn (lysins, )

<sup>13</sup> Na<sup>+</sup>/K<sup>+</sup> ions Ca<sup>2+</sup>/MG<sup>2+</sup> .

가 .

IVF (glycoprotein)

(species)

(serum free culture system) .<sup>22</sup>

5 6 가 .

가 ,

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

가 (Cohen<sup>34,35</sup>)

23,24 가 , 가

(assist hatching) 가

25~30%

70~75% 1가

가 (suboptimal culture condition) 가 (G2), Medicult IVF (M3), P-1 (blastocyst medium) 3 IVF-20

가 (micromanipulation) 가 pronase ,

(acid Tyroide's solution) 48 2 ,

25,26 (micromanipulator) IVF-20 (G2) 55.7%, P-1 (blastocyst medium) 65.4% Medicult M3

(mechanical partial zona dissection, PZD)<sup>27,28</sup> 38.2% (p<0.01) . 72

laser laser<sup>29</sup> (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 IVF-20 (G2) 3

(blastomeres) 5~6 ,

32 (initial 가 , 3

compaction) 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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