

Apoptotic Gene

1, 2
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Expression of Apoptotic Genes in Mouse Preimplantation Embryo Development

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Objective: The aim of this study was to evaluate the influence of three different media on preimplantation embryo development and the expression of *Bcl-2*, *Mcl-1*, *Bax*, and *Bok* in mouse.

Materials and Methods: Two-cell embryos were retrieved from ICR female mice (4 weeks old) at 48 hr after hCG injection and cultured in Ham's F-10, HTF, and G1.2 media. The developmental rate of 2-cell embryos was evaluated from 24 hr to 72 hr after culture. RT-PCR was performed for the detection of *Bcl-2*, *Mcl-1*, *Bax*, and *Bok* gene expression.

Results: The rates of morula and blastocyst in HTF and G1.2 media (88%, 98.1%) were significantly higher than those in Ham's F-10 media (39.6%) at 48 hr. Likewise, the rates of hatching and hatched blastocyst in HTF and G1.2 media (21.9%, 52.9%) were higher than those in Ham's F-10 media (3.5%) at 72 hr. *Bcl-2* and *Bax* mRNAs were highly detected in embryos cultured in Ham's F-10 when compared in embryos cultured in HTF and G1.2. In contrast, the expression of *Mcl-1* and *Bok* was not significantly different.

Conclusion: These results show that HTF and G1.2 culture media increase the rate of blastocyst formation and stimulate *Bcl-2* and *Bax* gene expression in mouse preimplantation embryos.

Key Words: Blastocyst, Culture medium, Apoptotic gene, Preimplantation embryo

120 96~ 15~50% 30~50%
가
1
(frag- 15%
mentation), (arrest) (dying) 34
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5-7 가

Gardner^{9,10} 가

45.5% mRNA genome activation

programmed cell death¹¹ genome activation

*Bcl-2*¹¹ *Bcl-2*^{12,13}

Bcl-2

1. F-10 Nutrient Mixture Medium (Ham's F-10, Gibco, USA), Human Tubal Fluid (HTF) G1.2 (IVF Science, Sweden) Ham's F-10 1 mM Ca-lactate (Calbiochem, Germany), 20 mM NaHCO₃ (Sigma, USA) 0.075 g anti-biotics (Streptomycin sulfate; Penicillin-G, Sigma, USA) 가 HTF 101 mM NaCl, 4.7 mM KCl, 0.37 mM KH₂PO₄, 0.2 mM MgSO₄ · 7H₂O, 25 mM NaHCO₃, 2 mM CaCl₂ · 2H₂O 21.4 mM Sodium lactate, 0.33 mM Sodium pyruvate, 2.8 mM Glucose, 1 mM Glutamine, 0.1 mM EDTA, 0.025 g Streptomycin sulfate 0.025 g Penicillin-G 가 G1.2 , Dulbecco's phosphate buffered saline (DPBS, Gibco, USA) 0.1 g CaCl₂ (Gibco, USA) 가 37 , 5% CO₂

2. ICR 4~6 , 5 IU pregnant mare's serum gonadotropin (PMSG, Sigma, USA) 48 5 IU human chorionic gonadotropin (hCG, Sigma, USA) . hCG 48

3. 2 DPBS 2 가 Ham's F-10, HTF G1.2 24 2~3 4~8 72 가 37 5% CO₂, 100%

4. Total RNA RT - PCR 2 24 가 72 가

Table 1. Primers designed for amplification of the target mRNA in RT-PCR reaction

Name of the gene product	Forward (F) and reverse (R) primer sequences	RT-PCR product size	Gene Bank accession No.	Reference
<i>Anti-apoptotic</i>				
<i>Bcl-2</i>	F 5'-ACTTTGCAGAGATGTCCAGT-3' R 5'-CGGTTTCAGGTAAGTCAATCAT-3'	217 bp	U34964	(14)
<i>Mcl-1</i>	F 5'-TTAAAAACGAGGACGATGTT-3' R 5'-CCTTCTAGGTCCTGTACGTG-3'	268 bp	AF115380	(15)
<i>Pro-apoptotic</i>				
<i>Bax</i>	F 5'-CGGCGAATTGGAGATGAACTG-3' R 5'-GCAAAGTAGAAGAGGGCAACC-3'	160 bp	L22473	(16)
<i>Bok</i>	F 5'-TCTTCTCAGCAGGTATCACA-3' R 5'-CTGTGCTGACCACACTT-3'	207 bp	AF027954	(17)

Table 2. In vitro development of mouse 2-cell embryos cultured in different media for 24 hr

Media	Total No. of 2-cell embryos	Developmental stage (%)	
		2~3-cell	2~3-cell
Ham's F-10	366	254 (68.2)	112 (31.2)
HTF	760	83 (10.1)*	677 (89.9)*
G1.2	504	65 (15.2)*	439 (84.8)*

* p<0.01, when compared to embryos in Ham's F-10

2 ml eppendorf tube -70 . PCR .

 RNeasy mini kit (Qiagen, USA) PCR cycle 95 , 10 de-

 total RNA . Reverse transcrip- naturation , primer annealing

 tion DEPC-DW 20 ?l total *Bcl-2* 55 , *Mcl-1* 50 ,

 RNA 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM *Bax* 61 , *Bok* 50 5 anne-

 dNTP, 1 unit RNase inhibitor, 2.5 ?M Random Ha- aling 72 extension

 xamers 2.5 unit MuLV reverse transcriptase . PCR product 0.5 ?g/ml ethi-

 60 ?l . dium bromide 가 1.8% agarose gel

 . UV . Band density GelDoc

 RT 65 10 RNA denatura- 2000 (Bio-Rad, Italy) .

 tion , 42 60 RNA

 cDNA , 99 5 MuLV

 reverse transcriptase

 . PCR 10 mM Tris-HCl, pH 8.3, 50

 mM KCl, 1.5 mM MgCl₂, 1 mM dNTP, 1 unit Taq

 polymerase (Takara, Japan), 10 pmol 5' 3' primer

 RT product 가 Roche PCR machine Pearson's Chi

5. Primer Pairs

RT-PCR *Bcl-2*, *Mcl-1*, *Bax*
Bok cDNA primer pairs Table 1

6.

Table 3. In vitro development of 2~3-cell and 4~8-cell embryos cultured in different media for 48 hr

Media	Morula (%)		Blastocyst (%)	
	2~3-cell	4~8-cell	2~3-cell	4~8-cell
Ham's F-10	5 (2.2)	32 (35.5)	0	4 (4.1)
HTF	10 (11.5)	480 (67.7)*	0	114 (20.3)*
G1.2	11 (9.9)	210 (46.7)*	1 (0.7)	222 (51.4)*

* p<0.01, when compared to embryos in Ham's F-10

Table 4. In vitro development of 2~3-cell and 4~8-cell embryos cultured in different media for 72 hr

Media	Blastocyst (%)		Hatching & Hatched Blast. (%)	
	2~3-cell	4~8-cell	2~3-cell	4~8-cell
Ham's F-10	8 (3.8)	30 (31.5)	0	4 (3.5)
HTF	2 (0.7)	404 (60.4)*	0	135 (21.9)*
G1.2	7 (12.5)	172 (43.5)*	3 (2)	249 (52.9)*

* p<0.01, when compared to embryos in Ham's F-10

square test, p = 0.01. hatching hatched blastocyst Ham's F-10 HTF G1.2 3.5% (4/112) 21.9% (135/677), 52.9% (249/439) Ham's (Table 4).

1. Ham's F-10, HTF G1.2 2 F-10 Bcl-2 ICR 2 Ham's F-10, HTF G1.2 2 24 24 4 72 2~3 4~8 72 가 total RNA Bcl-2 가 . 24 Mcl-1, Bax Bok cDNA primer RT-PCR . Bcl-2 (439/504) , Ham's F-10 31.2% , Table 2 Bax (112/366) 2 (Table 2). 72 hatching hatched blasto Bax 24 4~8 Ham's cyst 3.5% Ham's F-10, HTF G1.2 48 39.6%, 88% (Figure 1). Bax 2.5 Bcl-2 Ham's F10 HTF G1.2 98.1% , 2~3 2.2%, 11.5% 10.6% 2 . , Bok (Table 3). Ham's F-10, HTF G1.2 72 Mcl-1 Bax Bcl-2

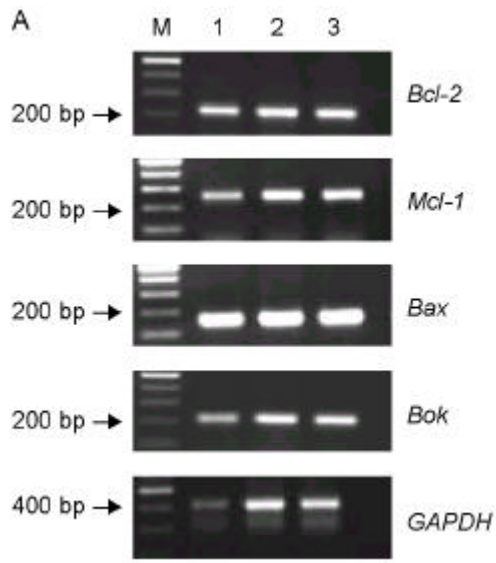
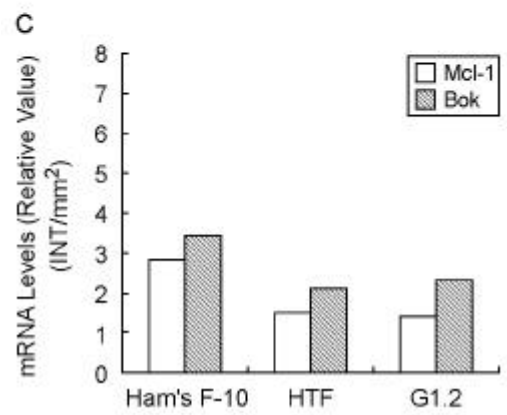
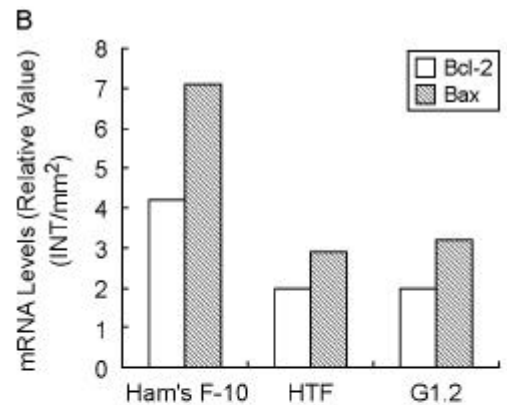


Figure 1. Expression of Bcl-2 family members in mouse preimplantation embryos. (A) Total RNA extracted from 4-cell embryos cultured in Ham's F10 (lane 1), HTF (lane 2), and G1.2 (lane 3) for 72 hr were used for RT-PCR. The expression of GAPDH was used as an internal standard. M, MW marker. Densitometric analysis of relative mRNA levels of *Bcl-2/Bax* (B) and *Mcl-1/Bok* (C). Data represent mean value from two independent experiments.



Ham's F10 HTF G1.2

20

가 Ham's

F-10, HTF G1.2 2

48

G1.2 HTF

97.2%, 88%

18

Ham's F10 (39.6%)

가

72

RNA

Bcl-2, Mcl-1, Bax *Bok*

, Ham's F-10

HTF

G1.2

Bax

2.5

Bcl-2, Bax

219

50%

가

2~3

15
Bok Mcl-1
Bcl-2

17 24
 4 Ham's F-10,
 72

가 가 가 HTF G1.2
 89 Quinn 8
 HTF
 가 ,
 F-10 *Bcl-2 Bax* Ham's
 2 2.5

21,22 , Gardner 9 2 F-10 HTF G1.2 Ham's
 G1 3 G2 *Bcl-2 Bax* ,
 45.5% 96.4% 72 82.3%
 가 . *Mcl-1 Bok*

Ham's F-10 HTF
 G1.2 2 72
 . 24
 가 HTF G1.2
 Ham's F-10 *Bcl-2 Bax*
 F-10 . Sano 26 가 Ham's
 가 HTF G1.2 *Bcl-2* *EAT/*
 Ham's F-10 *mcl-1* 2
 . 24 8
 (2~3) (4~8) . Kim 27
) 72 가 *Bcl-2*가
 , (4~8) , 가 *Bax*가
 , , 가 *Bcl-2*

23,24 , Fenwick
 23 25 2 ,
 32.2%가
 , 25 2 *Bax Bok*
 16.6%

2가
 . 25

, *Bcl-2 Bax* RT-PCR
 25
Bcl-2 Mcl-1 , ,

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