

Fas, Fas-ligand, Bax, Bcl-2

1, 2, 3,
4, 5, 6,
1,3 . 1,4 . 2 . 3 . 4 . 5 . 6 . 6

The Study on Apoptosis and Expression of *Fas, Fas-ligand, Bax, and Bcl-2* in Human Fragmented Embryos

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Objective: The present study was performed to investigate whether apoptosis occur in human embryos by annexin staining and detect the expression of *Fas, Fas-ligand (FasL), Bax, and Bcl-2* in human fragmented embryos derived from IVF-ET by immunofluorescence and Western blot analysis.

Materials and Methods: Using annexin staining, immunofluorescence and Western blot analysis on normal and fragmented embryos, we were able to detect apoptosis and apoptotic gene products in fragmented embryos.

Result: Phosphatidylserine (PS) translocation, the marker for apoptosis, were detected frequently in fragmented embryos. *Bcl-2* and *Bax* protein were detected in both fragmented and non-fragmented embryos. When fragmented embryos compared to normal embryos, immunofluorescent intensity of *Bcl-2* tended to be lower in fragmented embryos. *Bax* gene expression increased in the fragmented embryos compared to the normal embryos. This result supports a model in which the molar ratio of *Bcl-2* to *Bax* determines whether apoptosis induced or inhibited in human embryo. *Fas* was highly expressed in human preimplantation embryos but not *FasL*. It suggests that embryo may undergo apoptosis by binding with *FasL* produced by follicular or immune cells.

Conclusion: The over expression of *Bax* and *Fas* will trigger apoptosis to lead embryo fragmentation and change embryo to be nonviable.

Key Words: Apoptosis, *Fas, Fas-ligand (FasL), Bax, Bcl-2*, Human fragmented embryos

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가
 . Jurisicova¹⁴ 가
 75%가 가
 .
 .1 (programmed cell
 death, PCD)
 (fragmentation) 가
 ,² (fragment) .15
 .3,4 mRNA
 50% ,⁵ DNA 가
 ,⁶ .16 DNA
 가 가
 .7 가
 .17
 .18,19
 가 (apop-
 tosis)가⁸
 가
 (growth factor)
 (survival factors)
 ,⁹ (reac-
 tive oxygen species, ROS)가
 가¹⁰
 DNA ,
 .11 Bax가 가 Bcl-2²⁰
 ,²¹ Fas, Fas
 Bcl-2
 , Bax, Fas,
 Bcl-2
 .22
 Bcl-2
 ,²³
 Bax 가
 Bcl-2
 .19,24
 Bax Fas 가²⁵
 Fas
 ,
 가 FasL
 .13 .26 Fas

Bcl-2 가 .²⁰ rono) GnRHa (GnRHa long protocol)
 가 21 GnRHa
 3 FSH FSH hMG
 mRNA (hormones), estradiol (E₂)
 가 (growth factor), (cytokine) . 18 mm
 가 3 10,000 IU human chorionic gonadotropin (hCG, Profasi, Serono)
²⁷ . hCG 36 (Single Lumen Ovum Pick-Up Needle Set, COOK)
 37 5% CO₂ (SZH10, Olympus) (oocytes cumulus complex, OCC) OCC가
 annexin 가 가 (germinal vesicle, GV) . , 1 . ,
 Blotting Western Fas, FasL, Bax, Bcl-2
 10% synthetic serum substrate (SSS, Irvine) 가 P 1 (Preimplantation 1) (metaphase II, MII)
 1. 3~4 , (metaphase I, MI)
 2001 9 2002 5 8~10 , (immature, GV) 20
 37 ~48 CO₂ 3~5
 가
 (n=46, Figure 1a) , 2)
 F-10 Nutrient Mixture Medium (Ham's F-10, Gibco) 0.5% antibiotics (Penicillin-G, Streptomycin sulfate) 가
 P 1
 2. (2.04 mM CaCl₂ · 2H₂O, 101.6 mM NaCl, 4.69 mM KCl, 0.2 mM MgSO₄, 25 mM NaHCO₃, 0.33 mM Na pyruvate, 21.4 mM Na-lactate, 0.05 mM Taurine, 0.15 mg/l Na-citrate, 10 ? g/l Gentamicin, 0.005 g/l Phenol red) Sigma
 1) gonadotropin releasing hormone agonist (GnRHa, Lucrine subQ, ABBOT) ,
 follicle stimulating hormone (FSH, Metrodin, Serono) 95%
 human menopausal gonadotropin (hMG, Pergonal, Se , 37 5% CO₂ (CO₂ incuba-

tor, Heraeus) 10 50%

3) (human follicular fluid, hFF) 가 G4, 가 가 G5
. 29 G1~G3 3~5
3500 rpm 가 50% G4 G5 gra
30 de
56 35 (heat 가
inactivation) 0.22 ? m (Sterivex GV, G1 G2 grade
Millipore) -20

4) 3.
(masturbation) 1) Annexin V
37 20~30 5 Annexin V FITC Ap-
(WHO)²⁸ phosphate buf-
10% fered saline (PBS, Gibco) annexin V
가 Ham's F-10 2 FITC 20 PBS propi-
pellet 1 ml dium iodide (PI) UV
Ham's F-10 5% CO₂ DAPI/FITC/Rhodamine triple filters가
30 (swim (E600, Nikon)
up) (insemination) 2) acid tyrode solution pH 2.1
18~22 (fertilization) ~2.5)
(male pronuclear) (female
pronuclear) 10% NBF (neutral buffered formalin, Sigma) 10
, (polor body)가 PBS
. 2% BSA가 가 PBS
1 가 4~5 10 *Fas, FasL, Bax*
. (*Bcl-2* (Table 1, BD PharMingen)
. , PBS
. FITC
. 1
. PBS mounting medium
. (Sigma)
5) 3) Western blot
5% CO₂ 37 10% SSS가 Lysis
가 P 1 72 가 buffer (125 mM Tris-HCl, pH 6.8, 10% SDS, 1 mM PM-
. 가 Grade 1 (G1), SF (phenylmethylsulphonyl fluoride), 125 mM dithioth-
가 가 reitol, 20% glycerol, 0.002% bromophenol blue)
G2, 가 20%
. G3,
. -80

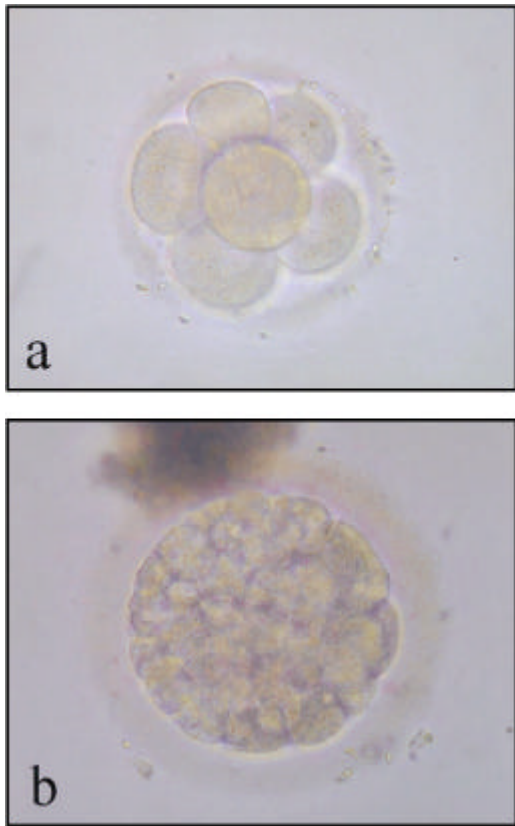


Figure 1. Light microscopy of normal and fragmented human embryos collected at the 8 cell stage. All embryos were collected at approximately 72 hours after post-insemination. **a.** Control group, 8 cell embryo with non-fragmentation derived from 3 pronuclei embryo (3PN), **b.** Study group, embryo with fragmentation derived from 2 pronuclei embryo (2PN).

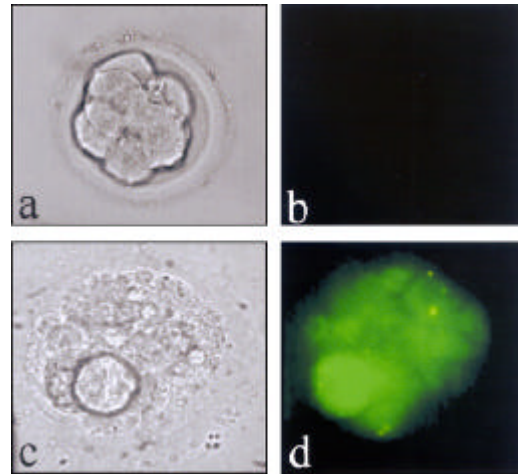


Figure 2. Detection of apoptosis in a human embryo by annexin V staining. Normal and fragmented embryos were stained with PharMingen annexin V-FITC apoptosis detection kit. After staining, pictures were taken under bright field (**a**, **c**) and under UV light with FITC filter (**b**, **d**). (**b**) Non-fragmented embryo negatively stained but (**d**) fragmented embryo positively stained for annexin V staining.

12% SDS-Polyacrylamide gel
 transfer buffer (48 mM Tris, 39 mM glycine,
 0.037% SDS, 20% methanol)
 (Hybond ECL, Amersham pharmacia biotech)
 5%
 TBS-T (Tris buffered saline, 10 mM Tris, 0.2 M NaCl,
 0.1% Tween-20) 1 TBS-T
 . *Fas*, *FasL*, *Bax*, *Bcl-2*
 (BD PharMingen) 1
 TBS-T , HRP (horseradish per-
 oxidase) 1
 . TBS-T ECL (enhanced che-
 mi-luminescence) (Amersham pharmacia biotech)

1 X-ray (Hyperfilm, Amersham pharmacia biotech)

1. Annexin V

5 annexin V-FITC

Figure 2). 가 (Fi

(Figure 2d)

가

PI

2.

42

21

Table 1. Primary antibodies used in immunofluorescence staining

Primary antibody	Source	Dilution	Company
<i>Fas</i>	mouse anti-human <i>Fas</i>	1 : 100	BD PharMingen, USA
<i>FasL</i>	mouse anti-human <i>FasL</i>	1 : 100	BD PharMingen, USA
<i>Bax</i>	rabbit anti-human <i>Bax</i>	1 : 100	BD PharMingen, USA
<i>Bcl-2</i>	mouse anti-human <i>Bcl-2</i>	1 : 100	BD PharMingen, USA

Table 2. Frequency of positive staining of *Fas*, *FasL*, *Bax*, and *Bcl-2* in fragmented and non-fragmented embryos

	Frequency of positive staining (+/n) ^a			
	<i>Bcl-2</i>	<i>Bax</i>	<i>Fas</i>	<i>FasL</i>
Non-fragmented embryos	5/5	3/5	4/5	0/6
Fragmented embryos	3/4	5/5	6/6	1/6

a+/n, Number of samples with positive staining / of total samples

Table 2 . *Bcl-2* *Bax*
Bcl-2 *Bax*
Bcl-2 8

(Figure 3). *Fas* *FasL*
Fas
FasL
(Figure 4).

3. Western blot

Western blot 5
40 가 *Bcl-2*
Bax
Fas
FasL
(Figure 5).

14,30 2
4
8
31,32 8
가
33 Aoki 34 1
(embryonic activation)

DNA
35 DNA
(endonuclease)
DNA 180~200 bp
gel DNA
ladder-like
DNA TUNEL (terminal deoxynucleo-
tidyl transferase (TdT)-mediated dUTP-dogoxigenin nick
end-labbling) 36
DNA
DNA
7
가
ph-
osphatidylserine (PS)
DNA

mRNA

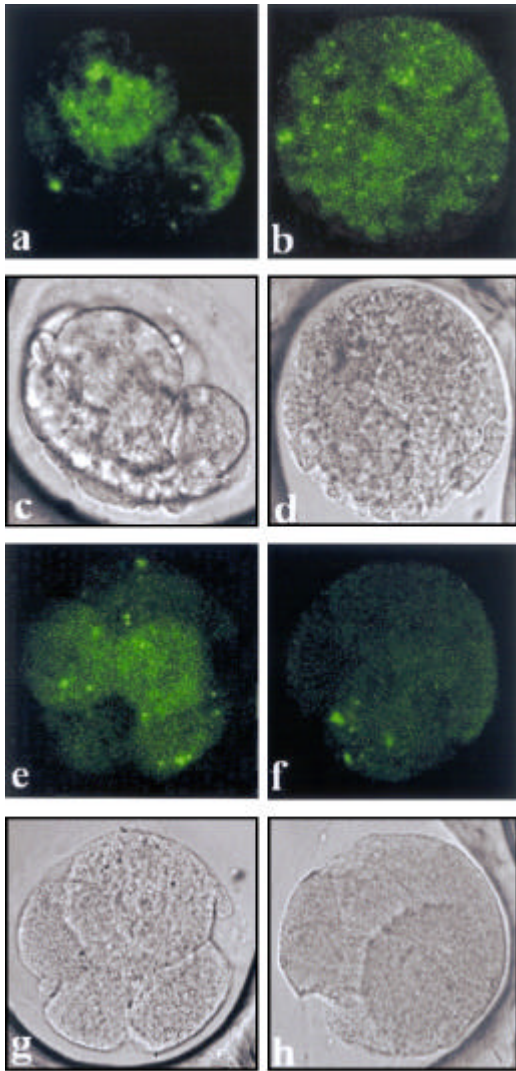


Figure 3. Immunofluorescent detection of *Bcl-2* and *Bax* in fragmented and non-fragmented embryos by confocal image analysis. Fragmented embryos (a~d) and non-fragmented embryos (e~h) were collected at 72 hours after post-insemination. The presence of green stain on the embryos to show that expression of *Bcl-2* (a, e) and *Bax* (b, f) during embryogenesis. (a, b, e, f) The upper pannel shows pictures were taken with confocal microscope and (c, d, g, h) lower pannel shows picture taken in bright field.

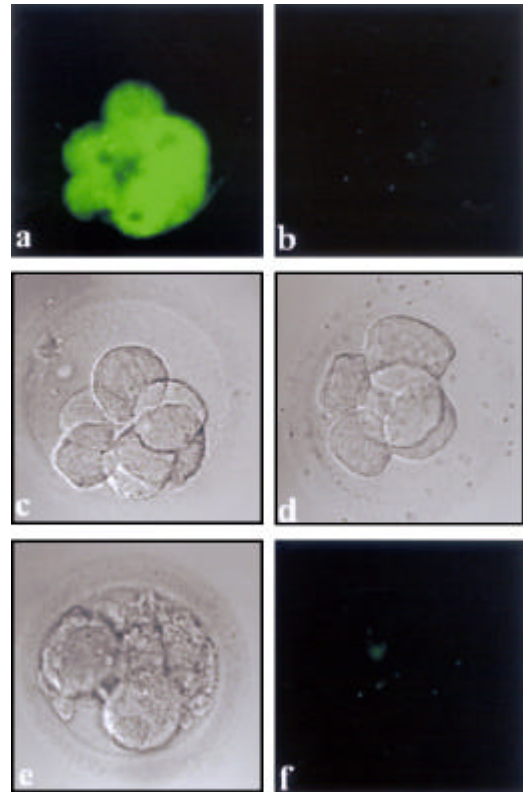


Figure 4. Immunofluorescent detection of *Fas* and *FasL* in fragmented and non-fragmented embryos by confocal image analysis. The presence of green stain on the embryos to show that expression of *Fas* during embryogenesis (a, c). *FasL* was not detected in both group (b, d). (a, b) The upper pannel shows pictures were taken with confocal microscope and (c, d) lower pannel shows picture taken in bright field. (e, f) Negative control.

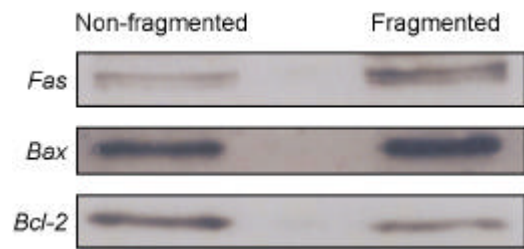


Figure 5. Western blot analysis of *Fas*, *FasL*, *Bax*, and *Bcl-2* in normal and fragmented embryos.

37
 PS가
 annexin V가,
 propidium iodide (PI)가

가 PS
 FITC
 annexin V가

PI *Bax*

Bcl-2 *Bax*

PI

(necrosis)

PI가 DNA *Bcl-2* *Bax* 가

annexin V PI *Bcl-2*

annexin V PI

annexin V PI ⁴⁰ *Bcl-2* 가

Figure 2 ⁴¹ 가

annexin V 가 *Bcl-2*가

가 가 ²¹ *Bax* *Bcl-2* 가

가 가 45% *Bax* *Bcl-2*

가 가 ⁴² hete-

(Figure 2d). 가 rodimer ⁴³ Ginger ⁴⁴ *Bax* *Bcl-2* *Bax*가

PI 가 ³⁸ *Bcl-2* gene family, *Fas*, Liu ²⁵ *Bax* 가

가 *Fas ligand (FasL)* ³⁹ *Bax* 가

Bcl-2

²² 72 8 (7

stern blot We-) *Bcl-2* 가

Bax *Bcl-2* genomic DNA가 7

(Figure 3). 가 *Bcl-2* 가

*Bcl-2*가 , 가

*Bax*가

(Figure 3b, e). *Bcl-2*

Fas FasL (Figure 4).

Fas FasL (Figure 4a).

Fas FasL

Fas FasL

Tumor Necrosis Factor/Nerve Growth Factor (TNF/NGF)²⁶

mi-

tochondria

*Fas FasL*⁴⁵

Fas FasL

FasL ^{25,46}

FasL *Fas* ⁴⁷

2 *Fas*

Fas

Fas

Fas

Fas

가

Western blot

Bcl-2

Bax

Fas

Fas

FasL 가 (Figure 5).

Fas FasL

Fas 가

Fas 가

FasL 가

FasL 가

Bax

가

가

가

가

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