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**The Effects of Human Follicular Fluid on Embryonal Development  
of Mouse in In Vitro Culture**

**Bu.Kie. Min, Ki.WooK. Choi, Kie.Suk. Kim, Hee.Sub. Lee, Ki.Yeon. Hong,  
Bong.Ju. Lee, Sun.Young. Lee and Seung.Teak. Park\***

*Department of Obstetrics and Gynecology, Department of Anatomy\*,  
School of Medicine, Won Kwang University, Iksan, Korea*

## **=Abstract=**

The follicular fluid (FF) of ovary contains various biological active products which affected on the growth of follicles and the fertilization of oocyte in physiological reproductive process of mammals.

This study was designed to determine the effects of human FF on fertilization of oocyte and embryonal development in in vitro culture. The FF was prepared as clear without blood contamination by needle aspiration from mature follicles of human at the time of oocytes retrieval for in vitro fertilization (IVF). As the medium for culture in vitro of embryonal cells, human tubal fluid (HTF) supplemented with follicular fluids at concentrations of 10%, 40% and pure FF were used. These effects were compared to control group of cultured embryos in HTF supplemented with 0.4% BSA (bovine serum albumin). For IVF, 64 eggs in control group, 67 eggs in 10% FF, 57 eggs in 40% FF and 64 eggs in pure FF were respectively allocated. And the rates of fertilization were almost similar in all groups as resulting 82.81% in control, 85.07% in 10% FF, 87.71% in 40% FF and 81.25% in pure FF. On the examination for embryonal cleavage from fertilized eggs, the rates of developing to 4 cell stage was similar in all groups, as results 98.11% in control, 98.27% in 10% FF and 98% in 40% FF but 78.84% in pure FF. And the rates of developing to 8-16 cell stage were significantly reduced as 44% in 40% FF and 44.23% in pure FF ( $p < 0.05$ ) compare to 71.69% in control media. As likewise, the rates of developing to morular stage were also significantly reduced to 36% ( $p < 0.05$ ) and 21.15% ( $p < 0.01$ ) respectively in 40% FF and pure FF. And the rates to blastocystic stage of embryo was lowest as 7.69% in pure FF (Table 1).

The quality of embryonal cells on cleavage to the 8-16 cell stage was poorer, higher concentrations of FF. The rates of grade 1 in pure FF, as 23.07%, was lowest compare to those of other groups, in which the rates of grade 1 in control, 10% FF and 40% FF were 58.49%, 47.36% and 34% respectively. And on the contrary, the rate of grade 4 in pure FF was highest as 23.07%, while those were 5.66% in control, 8.77% in 10% FF and 20% in 40% FF (Table 2).

On the viability of embryos, the rate of embryonal cell death was more rise, at the higher concentrations as well as longer exposure in the follicular fluid. At 48 hours after in vitro culture of embryos, the rate of survival embryos in pure FF was markedly lowered as 44.23%, compare to that of control ( $p < 0.05$ ). But there was not significant difference between the rates of survival embryos in each group beside the pure FF, which the rates were 77.35% in control, 70.17% in 10% FF and 60% in 40% FF respectively. And at 72 hours after in vitro culture, the rates of survival embryos were also significantly dropped to 21.15% in pure and 36% in 40% at concentration of FF compare to 62.26% in control ( $P < 0.05$ ,  $P < 0.01$ ). Finally, the rate of embryonal death at 96 hours after in vitro culture was highest as 82.69% in pure FF among all groups which those were 35.84 in control, 56.14% in 10% FF and 64% in 40% FF respectively (Fig. 1, 2, 3).

In conclusion, this study suggests that the FF has no effects, in particular, to the in vitro fertilization of oocytes but exerted a bad effect to the cleavage, quality and viability of the embryonal cells during in

vitro culture. However, the FF is harmful on embryonal development at conditions in higher concentration and especially on the embryos after 8-16 cell stage.

Key words: Follicular fluid/ Human tubal fluid/ In vitro culture of embryo/  
Embryonal development/ Embryonal cell quality/ Embryo viability.

#

가

가

가

mucopolysaccharide

<sup>1</sup> Blumenfeld <sup>2</sup> Lambert <sup>3</sup> 가

가

(acrosome reaction) <sup>4-6</sup>

가 <sup>7</sup>

Laroca <sup>8</sup> Kim <sup>9</sup>

Hemmings <sup>10</sup> 10% 가

10% 가

Delf Aquila <sup>11</sup>

가

가

10%, 40%,

가

가

1.

18mm  
 56 30  
 0.2 μm microfilter 20  
 hCG 34  
 1000xg 10

2.

10 B6C3F1 PMSG(pregnant mare serum gonadotrophin) 5 IU  
 48 HCG(human chorionic gonadotrophin) 5 IU  
 HCG 15

0.4% BSA(bovine serum albumin) 가 10%, 40%  
 가  
 30x30x10 mm (Falcon) 10%, 40% 가 ,  
 0.4% BSA 300 μℓ oil  
 37 , 5% CO2 30  
 10 B6C3F1  
 10%, 40% 가 ,  
 0.4% BSA 300 μℓ  
 oil 37 , 5% CO2 1 가  
 15  
 96

3.

10% 가 67 , 40% 가 57 , 64 ,  
 64 2  
 96 , ,  
 200  
 Salha 12 grade 1:

가 , 10% , grade 2: 가 , 20% ,  
grade 3: , , grade 4:  
12 ,  
12

4.

Student t-test

P>0.01, P>0.05

BSA 82.81%, 10%, 40% 가 85.07%, 87.71%, 0.4%  
81.25%

4 0.4% BSA , 10%,  
40% 가 98.11%, 98. 27%, 98%  
78.84% . 8-16 0.4% BSA  
10% 71.69%, 63.15% 40%  
44% 0.4% BSA (P<0.05),  
44.23% . 0.4% BSA 10%  
62.26%, 56.14% 40% 36% 21.15%  
(P<0.05, P<0.01)  
가 7.69% 가 ( .1).  
16  
0.4% BSA grade 1 grade 4  
58.49%, 5.68% 가  
가 grade 1 23.07% 가 grade 4  
23.07% 가 ( .2).

, , 48  
0.4% BSA , 10%, 40% 가  
77.35%, 70.17%, 60% 가 44.23%  
0.4% BSA (P <0.05)  
가 ( .1). 72  
62.26% 40% 36% (P<0.05)  
21.15% (P<0.01).  
가 가 96  
( .2, 3).

, hormone,  
 가 13,14 . Hemmings 10  
 insulin like growth factor(IGF), transforming growth factor, platelet derived  
 growth factor IGF-1  
 15 IGF-1 가 가  
 가  
 4~6  
 Funahashi 16 25% 가  
 . 10%, 40%,  
 가  
 17 .  
 albumin  
 albumin 가 가  
 10 . Bayer 1 Hemmings 10 가  
 가 450nm 가  
 가 17,000Da.  
 가  
 albumin . Romero 18  
 purine, adenosine, hypoxanthine  
 Serta 14 10% 가  
 .  
 10% 가 ,  
 0.4%  
 . 40% 가  
 48 ,  
 가  
 가 ,  
 , 가 가  
 가 가 가



Dell Aquila <sup>11</sup>

가

inhibin

19

gene

8-10

Serta <sup>14</sup>

가

albumin,

inhibin

interleukin, IGF-1  
activin

20,21

22

가

가  
가  
가  
40%  
가  
4  
8-16  
40%  
가  
가  
. 10%

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Table 1. The effects of follicular fluid in various concentrations on the cell cleavage of embryonal development during 96 hours of incubation.

medium embryo cleav.	0.4% HTF Em/ O (%)	10% FF Em/ O (%)	40% FF Em/ O (%)	pure FF Em/O (%)
fertilization	53/64(82.81)	57/67(85.07)	50/57(87.71)	52/64(81.25)
<4 cells	52/53(98.11)	56/57(98.27)	49/50(98.00)	41/52(78.84)
<8-16 cells	38/53(71.69)	36/57(63.15)	22/50(44.00) †	33/52(44.23)
morula	31/53(62.26)	32/57(56.14)	18/50(36.00) †	11/52(21.15) ‡
blastocyst	13/53(24.52)	12/57(21.50)	6/50(12.00)	4/51( 7.69)

Em; embryos

O; oocytes

† ; P < 0.05 vs control of 0.4% BSA HTF

‡ ; P < 0.01 vs control of 0.4% BSA HTF

Table 2. The effects of follicular fluids in various concentration and human tubal fluid on the cell quality at the 8-16 cell stage of embryonal development

Medium cell grade	0.4% HTF Em/Fe. egg (%)	10% FF Em/Fe. egg (%)	40% FF Em/Fe. egg (%)	pure FF Em/ Fe. egg (%)
grade 1	31/53(58.49)	27/57(47.36)	17/50(34.00)	12/52(23.07)
grade 2	7/53(13.20)	11/57(19.29)	5/50(10.00)	11/52(21.15)
grade 3	12/53(22.64)	14/57(24.56)	18/50(36.00)	17/52(32.69)
grade 4	3/53(5.66)	5/57(8.77)	10/50(20.00)	12/52(23.07)

Em; Embryos

Fe. egg; Fertilized egg

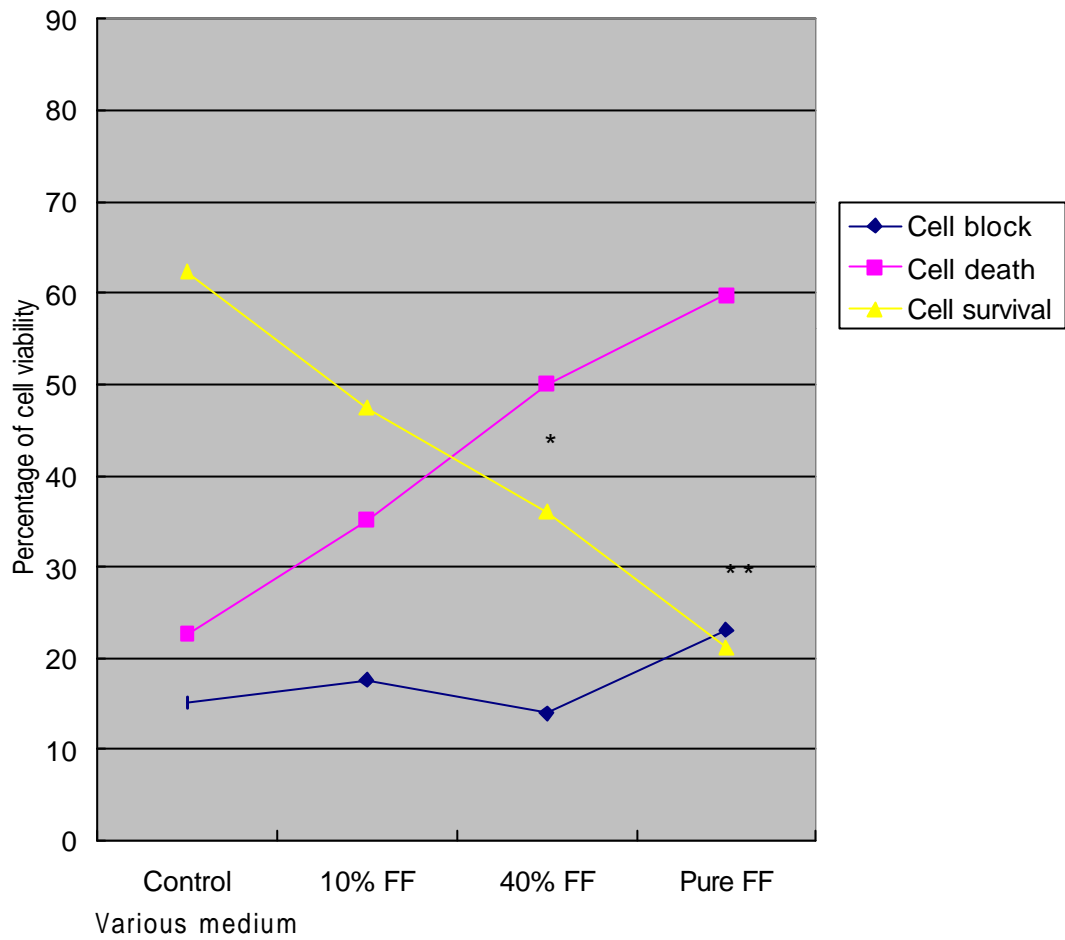


Fig.1. The effects of FF in different concentrations on the cell viability at 48 hours after in vitro culture of embryos. \* P<0.05

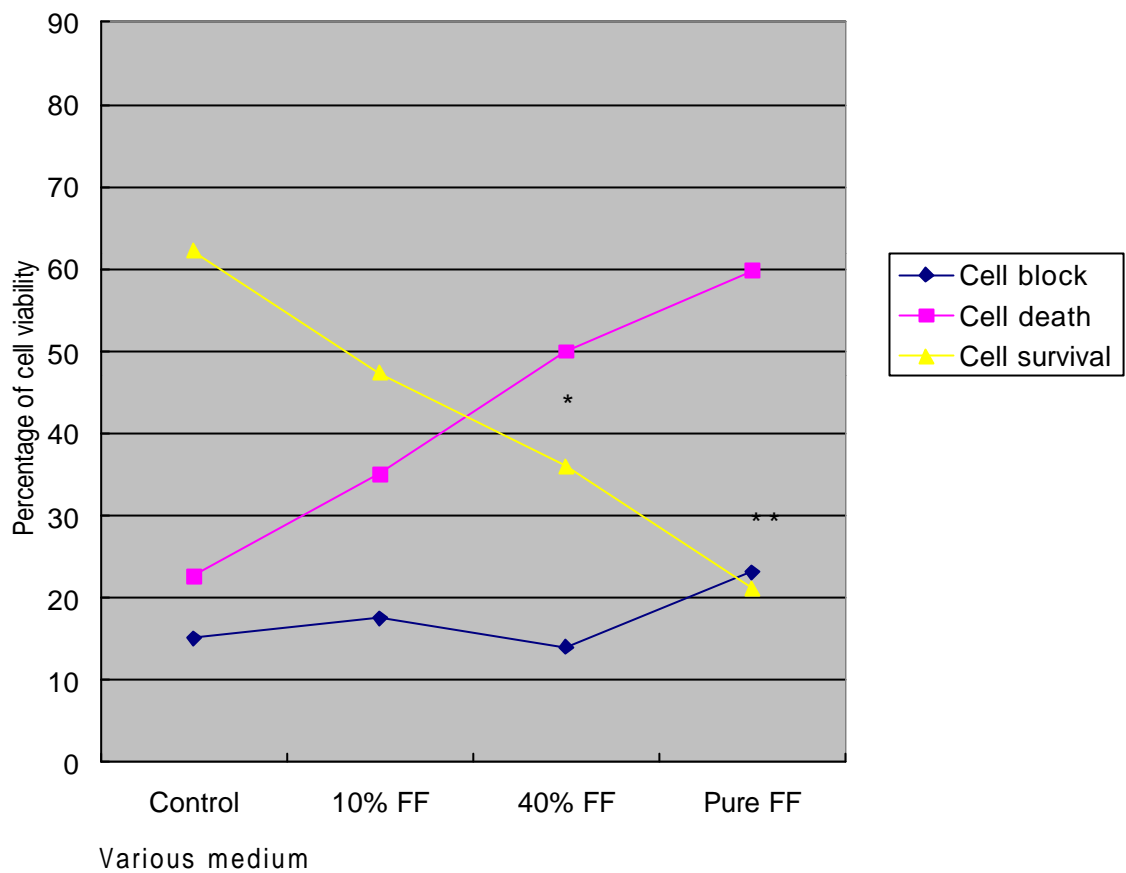


Fig.2. The effects of FF in different concentrations on the cell viability at 72 hours after in vitro culture of embryos. \*P<0.05, \*\*P<0.01.



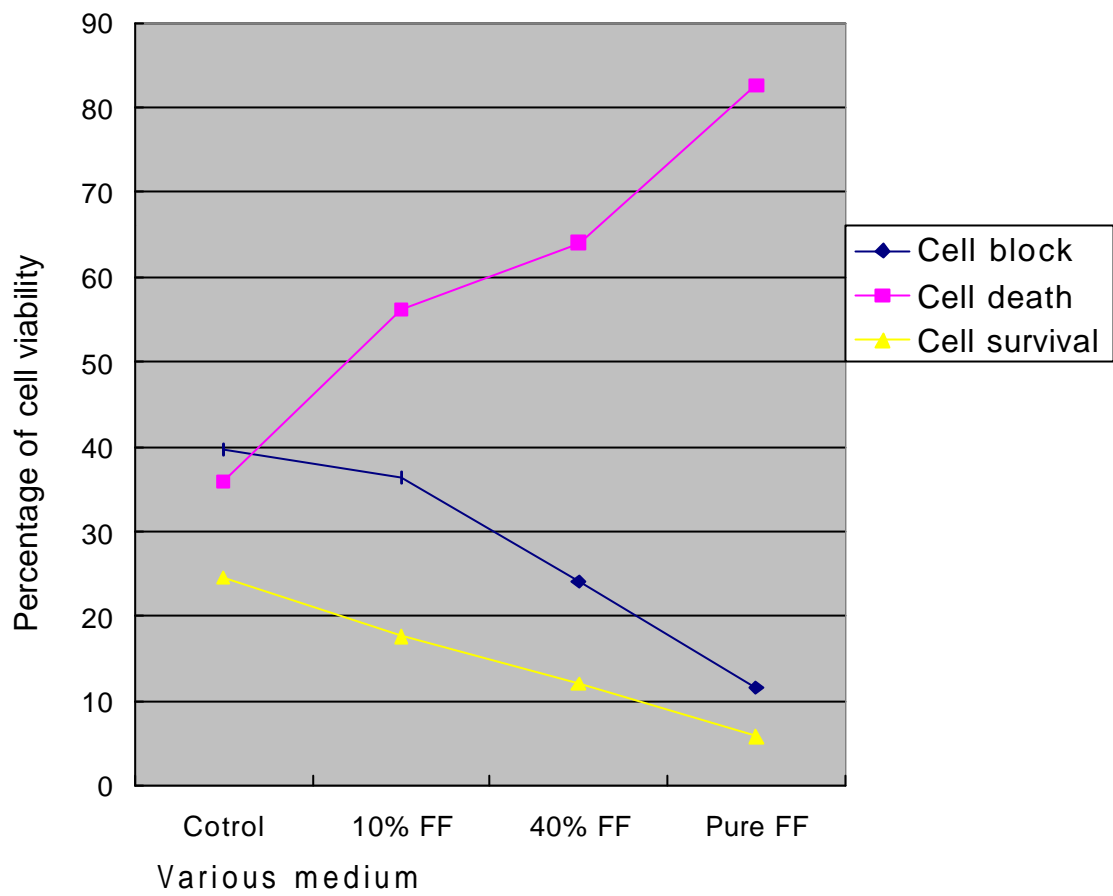


Fig.3. The effects of FF in different concentrations on the cell viability at 96 hours after in vitro culture of embryos.

