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**Influence of ovulation induction medicine on the nuclear maturation of mouse immature oocytes and developement of mouse 2-cell embryo in various culture media.**

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**( Influence of ovulation induction medicine on the nuclear maturation of mouse immature oocytes and development of mouse 2-cell embryo in various culture media. )**

**= Abstract =**

Purpose of the present study was to find the optimal ovulation induction medicine for the maturation and development of immature oocytes and culture media for 2-cell embryos in the mouse model. ICR female mouse aged 6 to 8 weeks, were stimulated with 5 IU PMSG injection. At 47 to 50 hour post-PMSG injection, ovaries were dissected out and oocytes-cumulus complexes were punctured. The oocyte-cumulus complexes were cultured in media containing various ovulation induction medicine, CC, HMG and Metrodin for 18 hours.

Female ICR mice were stimulated with 5 IU PMSG and 48 hours later were injected 5 IU of hCG, then female and male mice were mated. At 48 hour post-hCG injection, oviducts were dissected out and 2-cell embryos were flushed. The 2-cell embryos were cultured in various media, Ham's F-10 media of milli-Q water(3°), Ham's F-10 media of HPLC(high performance liquid chromatography, Baxter) water, Medicult media, HTF(human tubal fluid) media for 96 hours.

The results were as follows.

1. When the oocytes-cumulus complexes were cultured in  $10^{-9}\mu\text{g}/\text{Ml}$   $10^{-8}\mu\text{g}/\text{Ml}$  of CC, those were suppressed in meiotic maturation(28.2 33.7%). Whereas the oocytes-cumulus complexes were cultured in  $10^{-7}\mu\text{g}/\text{Ml}$   $10^{-4}\mu\text{g}/\text{Ml}$ , these were not effected in meiotic maturation(54.5 72.7%).
2. When the oocytes-cumulus complexes were cultured in  $10^{-4}\mu\text{g}/\text{Ml}$   $10^{-1}\mu\text{g}/\text{Ml}$  of Metrodin, those were suppressed in meiotic maturation(35.7 41.5%). Meanwhile the oocytes-cumulus complexes were cultured in  $10^{-7}\mu\text{g}/\text{Ml}$   $10^{-5}\mu\text{g}/\text{Ml}$ , those were not effected in meiotic maturation (54.2 70.3%).
3. When the oocytes-cumulus complexes were cultured in  $10^{-5}\mu\text{g}/\text{Ml}$   $10^{-4}\mu\text{g}/\text{Ml}$  of HMG, those were suppressed in meiotic maturation (48.2 50.4%). As being cultured in  $10^{-7}\mu\text{g}/\text{Ml}$   $10^{-6}\mu\text{g}$

/MØ, increased in meiotic maturation(75.8 80.7%).

4. When the 2-cell embryos were cultured in Ham's F-10 media of milli-Q water(3°), Ham's F-10 media of HPLC(high performance liquid chromatography, Baxter) water, Medicult media, HTF(human tubal fluid) media, developmental rates to blastocyst and hatching for 96 hour were 50.0%, 45.2%, 71.5% and 95.6%, respectively.

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**Key words** : Ovulation induction, Maturation of immature oocytes, Culture media.

1978 Steptoe Edwards

가

(IVF)

aromatase enzyme system

4mm

가

5

7

가

estradiol(E2), inhibin,

가

2

가

estradiol

가

LH surge

24

estrogen peak가

, estrogen peak 24

LH peak가

10 12

clomiphene citrate(CC) , human menopausal

gonadotropin (HMG),

" threshold requirement "

가

3

40 44

(HT6, T6,

Earle's, Ham's F-10, Hoppe & Pitts)

. (Medicult, HTF · Haman Tubal Fluid, GPM · Gamate Preparation

Medium, Serono).

2

72

70 90%

가

가

1.

ICR

2.

6 8 PMSG(pregnant mare's serum gonadotropin, Sigma Co.) 5 IU

가

가

(zoom stereo- microscope, Olympus, model SZH-131)

가

3.

PMSG 5 IU

( 1 ), 48

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

( 3 ), 4

(vaginal plug)

가

. 5 가

30 gauge needle

. , 2

4.

: 3mM hypoxanthine -

Brinster paraffin drop method . 60 × 15mm 1

(Falcon Co.) paraffin oil(Sigma Co.) 7Mℓ 50μℓ

1 37 5% CO<sub>2</sub>가 (Forma Scientific Co. Model 3037)

3mM hypoxanthine

가 3mM hypoxanthine 0.4% bovine

serum albumin (BSA, Sigma Co.) 가 1

. 30 18

5.

: Brinster paraffin drop method . 60 × 15mm 1

paraffin oil 1

. 2 96 . 24

, 48 , 72 , 96 (Olympus, model IMT - 2)



: Ham's F-10 (Ham's F-10 powder,  $Ca^{++} \cdot lactate \cdot 2H_2O$   
0.2452g/ ,  $NaHCO_3$  2.106g/ , penicillin 0.075g/ , streptomycin 0.075g/ ) , -

BSA(Sigma Co.) 0.4% 가 .

osmolarity 280 300milliosmol/kg , pH 7.2 7.4 (Fisher, Accumet 15)

. membrane filter paper(Millipore Co. 0.22 $\mu$ m)

. CC(Sigma Co. U.S.A.), HMG(IBSA,Swiss), Metrodin(Laboratories Serono S.A. Swiss)

가 .

: Ham's F-10 milli Q(3 ) HPLC (Baxter)

0.4% BSA 가 2 . Medicult

(Medicult, Denmark) HTF(Irvine scientific, U.S.A.) 2 .

1.

가

3mM hypoxanthine( , 1994) 1

(30 ) 가 가 18 .

1) CC : CC( $10^{-9} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$ )가 가 -

. I 3.7% ,  $10^{-9} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$

17.0%, 17.5%, 5.3%, 3.1%, 5.9%, 2.3% , 가 I

33.7%,  $10^{-9} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$  53.4%, 53.2%, 40.2%, 24.3%, 28.2% ,

35.1% . II 62.6%  $10^{-9} \mu\text{g}/\text{Ml}$

33.7%,  $10^{-8} \mu\text{g}/\text{Ml}$  28.2%,  $10^{-7} \mu\text{g}/\text{Ml}$  54.5% ,  $10^{-6} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$  62.9

72.7% . CC ( $10^{-8}$   $10^{-9} \mu\text{g}/\text{Ml}$ ) CC

II (28.2 33.7%)

$10^{-7} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$

(54.5 72.7%) (Table 1, Fig. 1).

2) Metrodin : Metrodin( $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-1} \mu\text{g}/\text{Ml}$ ) 가 -

. 가 I 3.7% ,  $10^{-7} \mu\text{g}$

$/\text{Ml}$   $10^{-1} \mu\text{g}/\text{Ml}$  7.8%, 12.7%, 0%, 0%, 5%, 0%, 0% , 가 I

33.7%,  $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-1} \mu\text{g}/\text{Ml}$  21.9%, 29.9%, 45.8%, 58.5% ,

45.5%, 62.6%, 65.5% . 가 II 62.6% ,

$10^{-7} \mu\text{g}/\text{Ml}$  70.3%,  $10^{-6} \mu\text{g}/\text{Ml}$  57.4%,  $10^{-5} \mu\text{g}/\text{Ml}$  54.2%,  $10^{-4} \mu\text{g}/\text{Ml}$  41.5%,  
 $10^{-3} \mu\text{g}/\text{Ml}$  49.4%,  $10^{-2} \mu\text{g}/\text{Ml}$  37.4%,  $10^{-1} \mu\text{g}/\text{Ml}$  34.5% .

Metrodin II  $10^{-4} \mu\text{g}/\text{Ml}$   
 (34.5% 41.5%)  $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-5} \mu\text{g}/\text{Ml}$

(Table 2, Fig 2).

3) HMG : HMG( $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$ ) -

. 가 I 3.7%,  $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$   
 1.5%, 0%, 2.8%, 0% , 가 I 33.7%,  $10^{-7} \mu\text{g}/\text{Ml}$   
 $10^{-4} \mu\text{g}/\text{Ml}$  22.7%, 19.3%, 46.8%, 51.8% . 가 II  
 62.6% ,  $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-6} \mu\text{g}/\text{Ml}$   
 75.8%, 80.7% ,  $10^{-4} \mu\text{g}/\text{Ml}$   $10^{-5} \mu\text{g}/\text{Ml}$  48.2%, 50.4%

(Table 3, Fig 3). HMG II  $10^{-5}$

$\mu\text{g}/\text{Ml}$   $10^{-4} \mu\text{g}/\text{Ml}$   $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-6} \mu\text{g}/\text{Ml}$

가 .

CC ( $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-4}$

$\mu\text{g}/\text{Ml}$ ) , Metrodin

( $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-5} \mu\text{g}/\text{Ml}$ )

. HMG ( $10^{-7} \mu\text{g}/\text{Ml}$   $10^{-6} \mu\text{g}/\text{Ml}$ )

가 HMG가

가 (Fig. 4).

2.

가

Milli Q(3 ) Ham 's F- 10 : milli Q(3 )

Ham 's F- 10 BSA 0.4% 가 2 (Table 4). 24

66.7%가 3 4 , 16.7%가 5 8 . 48 16.7%가 5 8

, 23.3%가 , 16.7%가 . 72 30%가 , 16.7%가

. 96 46.7%가 , 3.3%가 .

Milli Q(3 ) Ham 's F- 10 2 96

50.0%가 .

HPLC (Baxter) Ham 's F- 10 : HPLC (Baxter) Ham 's F- 10

2 BSA 0.4% 가 2

(Table 5). 24 57.1%가 3 4 14.3%가 5 8 . 48

11.9%가 5 8 , 40.5%가 . 72 16.7%가 , 21.4%가

. 96 9.5%가 , 28.6%가 , 7.1%가 .

HPLC (Baxter) Ham 's F- 10 2 96

45.2%가 .

Medicult : Medicult 2

(Table 6). 24 57.1%가 3 4 , 4.8%가 5 8 , 2.4%가

. 48 14.3%가 5 8 , 42.9%가 , 23.8%가 .

72 19.0%가 , 14.3%가 42.9%가 . 96 14.3%

가 , 16.7%가 , 40.5%가 .

Medicult 2 96 71.5%가

HTF : HTF 1% human serum  
 albumin(HSA) 가 (Table7). 24 69.6%가 3 4 , 21.7%가  
 5 8 8.7%가 . 48 65.2%가 , 17.4%가 , 13.0%  
 가 . 72 13.0%가 , 69.6%가 . 96  
 4.3%가 , 17.4%가 , 73.9%가 . HTF 2  
 96 95.6%가

2 96 Medicult HTF  
 , HTF 2 가

(Fig. 5).

Trounson CC

hCG

가

CC E2 (E2-binding receptors)

antiestrogen

가

estrogen agonist-antagonist .

30 CC

CC

CC

가

CC LH pulse

LH pulse

가

CC nonsteroidal estrogen diethylstilbestrol(DES)

nonsteroidal

agent , estrogen

estrogen antiestrogen . CC

catecholaminergic neuron system CC가

estrogen estrogen

(GnRH) . 가 GnRH

가

가

가 , , ,

CC

. CC가

1

가

CC

가가

CC

HMG Gmezell

Garcia

FSH LH가

FSH LH

1:1

75 IU

(Menotropin). HMG

가

,

CC

가

Human Urinary FSH(Metrodin) LH

FSH , 75 IU FSH 1 IU LH

가

. FSH HMG

가

HMG

MCD 3

FSH

MCD 2 . Protocol

HMG

LH

2

LH

FSH HMG

Mg )

가 (Metrodin HMG  $10^{-4} \mu\text{g}/$

가 (CC  $10^{-8} \mu\text{g}/\text{Mg}$  )

, CC

$10^{-7} \mu\text{g}/\text{Mg}$   $10^{-4} \mu\text{g}/\text{Mg}$

. Metrodin

$10^{-7} \mu\text{g}/\text{Mg}$   $10^{-5} \mu\text{g}/\text{Mg}$

HMG

$10^{-7} \mu\text{g}/\text{Ml}$   $10^{-6} \mu\text{g}/\text{Ml}$

가

가

가

Ham 's

F - 10

Medicult

, HTF

. Milli Q(3 )

Ham 's F - 10

2

96

50.0%

. HPLC (Baxter)

Ham 's F - 10

96

45.2%

. Medicult

96

71.5%

, HTF

95.6%



1. CC - (10<sup>-9</sup> μg/Mℓ 10<sup>-8</sup> μg/Mℓ)  
 (28.2 33.7%) (10<sup>-7</sup> μg/Mℓ 10<sup>-4</sup> μg/Mℓ)  
 (54.5 72.7%).

2. Metrodin (10<sup>-4</sup> μg/Mℓ 10<sup>-1</sup> μg/Mℓ)  
 (34.5% 41.5%) (10<sup>-7</sup> μg/Mℓ 10<sup>-5</sup> μg/Mℓ)  
 (54.2% 70.3%).

3. HMG (10<sup>-4</sup> μg/Mℓ 10<sup>-5</sup> μg/Mℓ)  
 (48.2% 50.4%), (10<sup>-7</sup> μg/Mℓ 10<sup>-6</sup> μg/Mℓ)  
 가 (75.8% 80.7%).

4. 2 96 milli Q(3 ) Ham 's F - 10  
 50.0%, HPLC (Baxter) Ham 's F - 10 45.2%, medicult  
 71.5%, HTF 95.6% .

- HMG  
 가 . 2

Medicult HTF .

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Table 1. Effect of CC on the rate of maturation of cumulus-cell enclosed oocytes<sup>a</sup>.

Group ( $\mu\text{g}/\text{Ml}$ )	Total	No. of oocytes(percent)		
		Prophase I	Metaphase I	Metaphase II
Control	91	4( 3.7 $\pm$ 2.2) <sup>b</sup>	30(33.7 $\pm$ 3.8)	57(62.6 $\pm$ 4.0)
10 <sup>-9</sup>	45	6(17.0 $\pm$ 8.8)	24(53.4 $\pm$ 1.7)	15(33.7 $\pm$ 1.5)**
10 <sup>-8</sup>	39	7(17.5 $\pm$ 6.4)	21(53.2 $\pm$ 1.6)	11(28.2 $\pm$ 0.7)**
10 <sup>-7</sup>	33	2( 5.3 $\pm$ 3.1)	13(40.2 $\pm$ 3.5)	18(54.5 $\pm$ 2.9)
10 <sup>-6</sup>	57	2( 3.1 $\pm$ 3.1)	14(24.3 $\pm$ 7.3)	41(72.7 $\pm$ 6.1)
10 <sup>-5</sup>	52	3( 5.9 $\pm$ 2.4)	15(28.2 $\pm$ 4.9)	34(65.9 $\pm$ 4.5)
10 <sup>-4</sup>	46	1( 2.3 $\pm$ 2.0)	16(35.1 $\pm$ 1.3)	29(62.9 $\pm$ 2.7)

<sup>a</sup> The oocyte - cumulus complexes(occ) were preincubated for 1 hour in the medium containing 3mM hypoxanthine and cultured for 30 minutes in plain medium, and then transferred to the CC medium (10<sup>-9</sup>  $\mu\text{g}/\text{Ml}$  - 10<sup>-4</sup>  $\mu\text{g}/\text{Ml}$ ), cultured further for 18 hours.

<sup>b</sup> Values are means  $\pm$  SE.

\*\* P < 0.01

CC on the rate of maturation of cumulus cell-enclosed oocytes.

\*\* P < 0.01

Fig. 1. Effect of

Table 2. Effect of Metrodin on the rate of maturation of cumulus cell-enclosed oocytes<sup>a</sup>.

Group ( $\mu\text{g}/\text{Ml}$ )	Total	No. of oocytes(percent)		
		Prophase I	Metaphase I	Metaphase II
control	91	4(3.7 $\pm$ 2.2) <sup>b</sup>	30(33.7 $\pm$ 3.8)	57(62.6 $\pm$ 4.0)
10 <sup>-7</sup>	28	2(7.8 $\pm$ 4.2)	6(21.9 $\pm$ 5.9)	20(70.3 $\pm$ 3.0)
10 <sup>-6</sup>	25	3(12.7 $\pm$ 6.4)	8(29.9 $\pm$ 5.0)	14(57.4 $\pm$ 6.3)
10 <sup>-5</sup>	24	0(0)	11(45.8 $\pm$ 4.2)	13(54.2 $\pm$ 4.2)
10 <sup>-4</sup>	26	0(0)	15(58.5 $\pm$ 5.2)	11(41.5 $\pm$ 5.2)**
10 <sup>-3</sup>	28	2(5.0 $\pm$ 5.0)	12(45.6 $\pm$ 8.5)	14(49.4 $\pm$ 6.8)
10 <sup>-2</sup>	32	0(0)	20(62.6 $\pm$ 1.4)	12(37.4 $\pm$ 1.4)**
10 <sup>-1</sup>	23	0(0)	15(65.5 $\pm$ 2.7)	8(34.5 $\pm$ 6.0)**

<sup>a</sup> The oocyte-cumulus complexes(occ) were preincubated for 1 hour in the medium containing 3mM hypoxanthine and cultured for 30 minutes in plain medium, and then transferred to the HMG medium (10<sup>-7</sup>  $\mu\text{g}$  10<sup>-1</sup>  $\mu\text{g}/\text{Ml}$ ), cultured further for 18 hours.

<sup>b</sup> Values are means  $\pm$  SE.

\*\* P < 0.01



Fig. 2. Effect of Metrodin on the rate of maturation of cumulus cell-enclosed oocytes.

\*\* P < 0.01

Table 3. Effect of HMG on the rate of maturation of cumulus cell-enclosed oocytes<sup>a</sup>.

Group ( $\mu\text{g}/\text{Ml}$ )	Total	No. of oocytes(percent)		
		Prophase I	Metaphase I	Metaphase II
control	91	4(3.7 $\pm$ 2.2) <sup>b</sup>	30(33.7 $\pm$ 3.8)	57(62.6 $\pm$ 4.0)
10 <sup>-7</sup>	47	1(1.5 $\pm$ 1.47)	11(22.7 $\pm$ 4.9)	35(75.8 $\pm$ 5.3) <sup>*</sup>
10 <sup>-6</sup>	46	0(0)	10(19.3 $\pm$ 1.86)	36(80.7 $\pm$ 3.3) <sup>**</sup>
10 <sup>-5</sup>	40	1(2.8 $\pm$ 2.8)	19(46.8 $\pm$ 1.86)	20(50.4 $\pm$ 2.6) <sup>**</sup>
10 <sup>-4</sup>	48	0(0)	25(51.79 $\pm$ 5.0)	23(48.2 $\pm$ 5.0) <sup>**</sup>

<sup>a</sup> The oocyte-cumulus complexes(OCC) were preincubated for 1 hour in the medium containing 3mM hypoxanthine and cultured for 30 minutes in plain medium, and then transferred to the HMG medium (10<sup>-7</sup>  $\mu\text{g}/\text{Ml}$  - 10<sup>-4</sup>  $\mu\text{g}/\text{Ml}$ ), cultured further for 18 hours.

<sup>b</sup> Values are means  $\pm$  SE.

\* P < 0.05

\*\* P < 0.01

Fig. 3.  
Effect of

HMG on the rate of maturation of cumulus cell-enclosed oocytes.

\*  $P < 0.05$

\*\*  $P < 0.01$

Fig. 4. Comparison of various ovulation induction medicine on the metaphase II oocytes.

\*\* P < 0.01

Table 4. Development of embryos from 2-cell stage during culture with Ham's F-10 media of milli Q(3°) water.

Stage	2 cell	3-4 cell	5-8 cell	Morula	Blastocyst	Expanded B.	Hatching	Fragment	Irregular
Hours	No.(%)								
24	30	4 (13.3± 8.6)	20 (66.7± 5.6)	5 (16.7± 2.7)					1 (3.3± 2.3)
48	30	2 (6.7± 4.8)	10 (30.0± 13.6)	5 (16.7± 10.2)	7 (23.3± 10.2)	5 (16.7± 13.8)			1 (3.3± 2.3)
72	30	10 (3.3± 5.1)	7 (23.0± 13.6)		3 (10.0± 5.3)	10 (30.0± 14.4)	5 (16.7± 8.2)	3 (10.0± 5.4)	1 (3.3± 2.3)
96	30					14 (46.7± 7.0)	1 (3.3± 2.8)	15 (50.0± 1.1)	

B : blastocyst

Table 5. Development of embryos from 2-cell stage during culture with Ham's F-10 media of HPLC water.

Stage	2 cell	3-4 cell	5-8 cell	Morula	Blastocyst	Expanded B.	Hatching	Fragment	Irregular
Hours	No.(%)								
24	42	12	24	6					
		(28.6± 10.0)	(57.1± 11.3)	(14.3± 4.1)					
48	42	8	12	5	7			5	2
		(19.0±8.1)	(28.6± 3.9)	(11.9± 4.1)	(40.5± 6.7)			(11.9± 4.3)	(4.8± 3.9)
72	42	7	7	2	7	9		5	2
		(16.7±7.7)	(16.7± 6.5)	(4.8± 2.3)	(16.7± 12.2)	(21.4± 5.0)		(11.9± 4.3)	(4.8± 3.9)
96	42	2	2		4	12	3	19	
		(4.8± 3.9)	(4.8± 3.9)		(9.5± 4.9)	(28.6± 4.0)	(7.1± 5.9)	(45.2± 11.4)	

HPLC : high performance liquid chromatography

B : blastocyst

Table 6. Development of embryos from 2-cell stage during culture with media of Medicult.

Stage	2 cell	3-4 cell	5-8 cell	Morula	Blastocyst	Expanded B.	Hatching	Fragment	Irregular
Hours	No.(%)								
24	42	15 (35.7 ± 14.3)	24 (57.1 ± 23.8)	2 (4.8 ± 3.9)	1 (2.4 ± 6.7)				
48	42	2 (4.8 ± 1.7)	5 (11.9 ± 4.9)	6 (14.3 ± 18.7)	18 (42.9 ± 23.8)	10 (23.8 ± 9.5)		1 (2.4 ± 6.1)	
72	42	2 (4.8 ± 8.0)	4 (9.5 ± 5.8)	2 (4.8 ± 6.2)	8 (19.0 ± 6.1)	6 (14.3 ± 9.0)	18 (42.9 ± 28.9)	1 (2.4 ± 6.1)	1 (2.4 ± 3.9)
96	42				6 (14.3 ± 5.2)	7 (16.7 ± 28.2)	17 (40.5 ± 16.4)	12 (28.6 ± 17.4)	

B : blastocyst

Table 7. Development of embryos from 2-cell stage during culture with media of HTF.

Stage	2 cell	3-4 cell	5-8 cell	Morula	Blastocyst	Expanded B.	Hatching	Fragment	Irregular
Hours	No.(%)								
24	23	16 (69.6± 16.7)	5 (21.7± 12.8)	2 (8.7± 5.8)					
48	23	1 (4.3± 6.6)		15 (65.2± 23.4)	4 (17.4± 14.8)	3 (13.0± 6.7)			
72	23	1 (4.3± 6.7)		2 (8.7± 5.8)	3 (13.0±7.1)	16 (69.6± 23.2)		1 (4.3± 6.7)	
96	23				1 (4.3± 6.7)	4 (17.4± 11.6)	17 (73.9±23.8)	1 (4.3±6.7)	

HTF : human tubal fluid

B : blastocyst



Fig. 5. Development of embryos in various culture media for 96hr(Blastocyst ~ hatching).

\*\* P < 0.01

- A. Ham's F-10 media of mili Q(3 °) water
- B. Ham's F-10 media of HPLC
- C. Medicult media
- D. HFT media