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**Effect of Human Cord Serum  
on Oocyte Maturation and Cumulus Cell Expansion**

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## **Effect of Human Cord Serum on Oocyte Maturation and Cumulus Cell Expansion**

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**=Abstract=**

This study was performed to investigate the stimulating effect on oocyte maturation and cumulus cell expansion in TC199 media by human cord serum (HCS) supplementation. Immature mouse oocyte cumulus complexes (OCCs) were cultured in TC199 media supplemented with bovine serum albumin (BSA), HCS and human chorionic gonadotropin (hCG) instead of luteinizing hormone (LH) respectively, and the expression of cumulus expansion and oocyte maturation were observed. After 4hr and 24hr culture with or without OCCs, media containing 0.4% BSA, 10% HCS and 10 IU hCG respectively were collected and analyzed for changing concentrations of estradiol (E<sub>2</sub>), progesterone (P<sub>4</sub>), testosterone (T) and PGF<sub>2</sub>. There were no elevation of E<sub>2</sub>, T and PGF<sub>2</sub> by OCCs culture, but minute elevation of P<sub>4</sub> level by 24hr OCCs culture in hCG supplementation (p=0.048). The stimulating pattern of cumulus expansion of OCCs by HCS and hCG supplementation was similar to our previously report using Ham's F-10 media, however oocyte maturation rates after 24hr OCCs culture in all media were increased by 20-30% compared to Ham's F-10 media. These results suggest that LH in HCS induce cumulus expansion probably by P<sub>4</sub> secretion of OCCs, and TC199 is efficient media for immature mouse oocyte maturation.

**Key Words:** Oocyte maturation, Cumulus expansion, Human cord serum, Oocyte-cumulus complex.

가 . 가  
(Fukui & Ono, 1989), (Das *et al.*, 1992; Bar-Ami *et al.*, 1993),  
(Ashwood *et al.*, 1989 ; Benadiva *et al.*, 1989; Khan *et al.*, 1991)

가 . (Bavister, 1981)

가 (Tanikawa, 1994) RNA protein  
(George & Johnson, 1993)  
(Lopata & Oliva, 1993) .  
(1995) (1997)  
Ham's F- 10 가 -

가  
- , -  
TC199 가  
- estradiol (E<sub>2</sub>), progesterone (P<sub>4</sub>), testosterone (T),  
prostaglandin (PGF<sub>2</sub> ) .

1.

(Burdick & Jackson) 1ℓ TC199 (Gibco #31100-035) 9.9g Penicilline G 0.075g (Sigma), Streptomycin 0.075g (Sigma), Calcium lactate 0.2452g (Calbiochem), NaHCO<sub>3</sub> 2.2g (Sigma) 가  
 TC199 . 280mOsm/kg 가  
 4 2 . 4  
 0.4% (bovine serum albumine; BSA)  
 가 , 10% (human cord serum; HCS) 가 ,  
 10 IU (human chorionic gonadotropin; hCG) 가 ,  
 HCS 10 IU hCG 가  
 37 , 5% 15 .

2. HCS

HCS 1000rpm 30  
 0.22μm 가 56 1  
 1ml -70 .

3.

3 4 ICR 5 6 ,  
 5 IU pregnant mare serum gonadotropin (Sigma) 45 48

2 3

(Nikon, 200X)

4.

-

4 가 25 , 4

(Nunclon ®, Denmark) 0.4% BSA 가 , 10% HCS 가

10 IU hCG 가 1ml 2 , -

4 , 24 .

LH -

0.4% BSA 가 10%

HCS 가 LH 10 IU hCG 가 , 10% HCS 10

IU hCG 가 . 25 µl drop

1ml mineral oil 15 37 , 5% CO<sub>2</sub>

20 - 4 , 8 , 24

.

5.

4 24 E<sub>2</sub>, P<sub>4</sub>, T, PGF<sub>2</sub>

RIA .

6.

Ball (1984)

, (-), (+),

(++) . -

metaphase (M )

1 가 metaphase (M ) .

7.

one-way analysis of variance(ANOVA) multiple-range test

p<0.05 .

1. HCS hCG가 - PGF<sub>2</sub>  
(Table 1, 2)

HCS LH가 - PGF<sub>2</sub>  
- HCS가 가 , LH  
LH hCG가 가 4 , 24 ,  
BSA가 가 E<sub>2</sub>, P<sub>4</sub>, T, PGF<sub>2</sub>  
, HCS가 가 E<sub>2</sub>, P<sub>4</sub>, T, PGF<sub>2</sub> 가 -  
가 (p>0.05). hCG 가  
- 24 -  
P<sub>4</sub> 가 (p=0.048).

2. - HCS hCG  
(Table 3)

- (energy source) BSA 가  
(Fig. 1)  
(Fig. 2). 10 IU hCG 가 8  
100% (Fig 3), 24  
100% (Fig. 4). HCS 가  
- 8 53.9%가  
(Fig. 5), 24 hCG  
가 61.1% (Fig. 6)  
(p<0.05), hCG 가  
HCS hCG 가 8 92.0%  
가 (Fig. 7), 24 95.7%가  
hCG HCS 가  
(Fig. 8). 4

75-85.7% 1 4 가 24

TC199

HCS hCG HCS hCG Ham's F-10  
 ( , 1997)

TC199 24 1 가  
 75 85.7% Ham's F-10 45 54.6%  
 가 가 TC199

gap junction

가 1 가 (Wassarman &  
 Albertini, 1994) LH 가 가  
 LH cAMP가 가 PKA가  
 (Dekel & Galiani, 1989) microfilament가 (Allworth &  
 Albertini, 1993) hyaluronic acid (Dekel & Beers,  
 1980 ; Sutovsky *et al.*, 1995). gap  
 junction PKC가  
 P34<sup>cdc2</sup> (Aberdam & Dekel, 1985 ;  
 Wassarman & Albertini, 1994) (Larsen *et al.*, 1986 ; Larsen *et al.*,  
 1987 ; Racowsky & Satterlie, 1987; Racowsky & Baldwin, 1989 ; Racowsky *et al.*,  
 1989).

Allworth (1993) - 2  
 FSH, LH, E<sub>2</sub> 가  
 , Amstrong (1996) FSH  
 (0.04μg/Ml) LH  
 LH

가 hCG - HCS 가

HCS LH가

FSH LH

(Chen *et al.*, 1994).

Prostaglandin PGE PGF<sub>2</sub> 가 isoform 가

(Ainsworth *et al.*, 1975; Bauminger *et al.*, 1975; Ainsworth *et al.*, 1984) (Holmes *et al.*, 1983; Murdoch *et al.*, 1986; Sogn *et al.*, 1987).

TC199 hCG 가

PGF<sub>2</sub> , HCS 가

PGF<sub>2</sub> PGF<sub>2</sub>

PGE Schuetz (1992) E<sub>2</sub>, T

가 hCG 가 24

P<sub>4</sub> 가 (p=0.048)

P<sub>4</sub> Khoury (1994),

Vanderhyden (1993)

TC199

Ham's F-10

TC199

, TC199 hCG 가 -

P<sub>4</sub> 가

TC199 -

, HCS LH P<sub>4</sub>

가



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#### FIGURE LEGENDS

- Fig. 1. OCCs (prophase ) with a germinal vesicle and compact cumulus cells before culture ( $\times 200$ ).
- Fig. 2. OCCs cultured in TC199 supplemented with 0.4% BSA for 24 hrs showing no cumulus expansion ( $\times 200$ ).
- Fig. 3. OCCs cultured in TC199 supplemented with 10 IU hCG for 8 hrs showing partial cumulus expansion ( $\times 200$ ).
- Fig. 4. OCCs cultured in TC199 supplemented with 10 IU hCG for 24 hrs showing marked expansion of cumulus ( $\times 200$ ).
- Fig. 5. OCCs cultured in TC199 supplemented with 10% HCS for 8 hrs showing partial cumulus expansion ( $\times 200$ ).

Fig. 6. OCCs cultured in TC199 supplemented with 10% HCS for 24 hrs showing marked expansion of cumulus ( $\times 200$ ).

Fig. 7. OCCs cultured in TC199 supplemented with 10% HCS plus 10 IU hCG for 8 hrs showing partial cumulus expansion ( $\times 200$ ).

Fig. 8. OCCs cultured in TC199 supplemented with 10% HCS plus 10 IU hCG for 24 hrs showing complete cumulus expansion ( $\times 200$ ).

**Table 1. Concentrations of sex steroids after 4 hr and 24 hr culture in the three different media containing BSA, HCS, and HCG**

Culture time	culture media $\pm$ OCC <sup>a</sup>	No. of cultures	E (pg/M $\ell$ ) <sup>2</sup>	P (pg/M $\ell$ ) <sup>4</sup>	T (pg/
4 hr	TC199 + 0.4 % BSA	6	-	-	-
	TC199 + 0.4 % BSA + OCC		-	-	-
	TC199 + 10 % HCS	6	429.3 $\pm$ 170.4 <sup>b</sup>	2315.4 $\pm$ 98.8	46.5 $\pm$
	TC199 + 10 % HCS + OCC		479.8 $\pm$ 173.5	2798.5 $\pm$ 370.6	59.7 $\pm$
	TC199 + 10 IU HCG	4	4.0 $\pm$ 8.0	6.2 $\pm$ 3.9	-
	TC199 + 10 IU HCG + OCC		7.7 $\pm$ 8.9	12.4 $\pm$ 11.4	-
24 hr	TC199 + 0.4 % BSA	6	-	-	-
	TC199 + 0.4 % BSA + OCC		-	-	-
	TC199 + 10 % HCS	6	439.6 $\pm$ 176.9	2488.4 $\pm$ 476.5	71.2 $\pm$
	TC199 + 10 % HCS + OCC		434.3 $\pm$ 147.0	2531.9 $\pm$ 385.3	81.1 $\pm$
	TC199 + 10 IU HCG	4	7.0 $\pm$ 11.2	11.8 $\pm$ 4.2 <sup>*</sup>	-
	TC199 + 10 IU HCG + OCC		7.8 $\pm$ 12.6	33.7 $\pm$ 7.0 <sup>*</sup>	-

<sup>a</sup> With or without 25 oocyte cumulus complexes were cultured in media.

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

<sup>\*</sup> p=0.048

**Table 2. Concentrations of PGF<sub>2</sub> after 4 hr and 24 hr culture in the three different media containing BSA, HCS, and HCG**

Culture time	culture media $\pm$ OCC <sup>a</sup>	No. of cultures	PGF <sub>2</sub> (pg/M $\ell$ )
4 hr	TC199 + 0.4 % BSA	4	-
	TC199 + 0.4 % BSA + OCC		-
	TC199 + 10 % HCS	6	322.3 $\pm$ 125.0 <sup>b</sup>
	TC199 + 10 % HCS + OCC		376.4 $\pm$ 139.5
	TC199 + 10 IU HCG	4	368.5 $\pm$ 267.9
	TC199 + 10 IU HCG + OCC		661.0 $\pm$ 19.7
24 hr	TC199 + 0.4 % BSA	4	-
	TC199 + 0.4 % BSA + OCC		-
	TC199 + 10 % HCS	6	181.0 $\pm$ 115.9
	TC199 + 10 % HCS + OCC		443.7 $\pm$ 145.3
	TC199 + 10 IU HCG	4	509.5 $\pm$ 60.1
	TC199 + 10 IU HCG + OCC		653.7 $\pm$ 182.3

<sup>a</sup> With or without 25 oocyte cumulus complexes were cultured in media.

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

Table 3. Cumulus expansion and oocyte maturation of OCC in 4 different media

culture media	No. of cultures	culture time							
		4 hr				8 hr			
		expansion(%)				expansion(%)			
		-	+	++	%M <sup>b</sup>	-	+	++	%M
TC199 + 0.4 % BSA	10	100	0	0	100	100	0	0	100
TC199 + 10 % HCS	10	98 $\pm$ 1.3 <sup>a</sup>	2.0 $\pm$ 1.3	0	100	45.8 $\pm$ 9.5	53.9 $\pm$ 9.6	0	100
TC199 + 10 IU HCG	10	100	0	0	100	0	100	0	100
TC199 + 10 % HCS + 10 IU HCG	10	97 $\pm$ 1.5	3.0 $\pm$ 1.5	0	100	8.0 $\pm$ 4.4	92.0 $\pm$ 4.4	0	100

<sup>a</sup> Values are means  $\pm$  SEM.

<sup>b</sup> Percentage of metaphase oocyte.

<sup>c</sup> Percentage of metaphase oocyte.