

## EFFECTS OF SUBSTITUTION ESTRUS COW SERUM WITH FETAL CALF SERUM ON CULTURE BOVINE EMBRYOS PRODUCED IN VITRO

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### RINGKASAN

Kapasitas perkembangan sel telur sapi yang dimatangkan secara in vitro telah diteliti dengan mengubah komposisi culture medium dari serum sapi yang sedang estrus (ECS) dengan Fetal Calf Serum (FCS). Penelitian dibagi dalam 4 grup : Grup (A) kultur dilakukan pada ECS dan Grup (B), (C), (D) masing-masing dikultur 1, 2 dan 3 hari pada ECS dan seterusnya dilakukan kultur yang mengandung FCS. Hasil penelitian menunjukkan tidak ada perbedaan nyata Cleavage rate yang terjadi, tetapi perkembangan blastocyst rate tertinggi diperoleh pada Grup D.

### ABSTRACT

The development capacity of bovine oocytes matured in vitro was investigated by replacement culture medium composition from Estrus Cow Serum (ECS) to Fetal Calf Serum (FCS). The experiment was divided into four groups : group (A) cultured in Medium with ECS and groups (B), (C) and (D) cultured 1, 2 and 3 days in ECS, respectively and the rest of the culture period in medium containing FCS. The results of the experiment show that no significant differences on cleavage rates were observed within the experimental groups, but blastocyst development showed significant differences with the highest rates in group D.

### INTRODUCTION

The culture of mammalian embryos in vitro requires a suitable environment, so that the early embryos can undergo cleavage with formation of blastocyst

stage embryos (Petter, 1992). Many researchers showed that development of early bovine embryos in vitro is generally arrested at the 8 to 16 cell stage (Fukui, 1989; Kim Elington and Foote, 1980; Takagi *et al.*, 1991; Keefer, 1992). Se-

veral culture systems were used to overcome the block and to increase full blastocyst development such as bovine Oviductal Epithelial Tissue (BOET) (Fukui, 1989; Nagao, Saeki, Hoshi and Kaimuna, 1990; Delcompo *et al.*, 1993). Synthetic Oviduct Fluid (SOF) (Takasi and First, 1992), Buffalo Rat Liver (BRL) (Inzen, Kruip and Wiemna, 1993), Chick Amniotic Fluid (CAF) (Black Wood and Zhang, 1993) and Esture Cow Serum (ECS) (Fukui, 1989, Schellander *et al.*, 1990). Actually it is difficult to determine whether the problem is due to sub optimal culture conditons or is a result of reduced developmental competence of oocytes matured and fertilized in vitro (Trounson, 1992).

The objective of this study was to investigate the effect of serum supplementation of the culture medium on the development of embryos produced in vitro.

## MATERIAL AND METHODS

### In Vitro Maturation

Oocytes were collected from small antral follicles (1–6 mm in diameter) of ovaries from slaughter house. Only oocytes with intact cumulus cell were used for this experiment. The cumulus-oocytes complexes were washed twice in modified TCM 199 (Sigma) supplemented with 20% ECS (Younis *et al.*, 1989). The ECS was heat inactivated at 56°C, 30 min. Using this medium supplemented with FSH. oocytes were transfered into a plastic multidish (Nun-

clon, Denmark) containing 400 µl modified TCM + 20% ECS, 20 µl FSH (Folotropin), under 400 µl parafin oil. Oocytes were cultured for 20 – 24 h at 39°C under 5% CO<sub>2</sub> in air.

### In Vitro Fertilization

Frozen semen packaged in 0.25 ml straw was thawed in water bath 39°C for 8 seconds. A motile sperm fraction was isolated by swim up medium and centrifuged at 1200 rpm for 10 min. The sperm pellets were resuspended and equilibrated for 5 min into the incubator. Following the steps, then resuspended and equilibrated for 5 min into the incubator. Following the steps, the resuspended sperm pellet was centrifuged at 1200 rpm for 10 min for the second time. For in vitro fertilization, the pellet from the second centrifugation was introduced into TALP medium supplemented with BSA, heparis and PHE (Parrish *et al.*, 1986).

## RESULT

Results of the experiment show that there were no significant differences on cleavage rate between control gorup and experimental group. The cleavage rates range between 29.2% and 40.9%. However it seems that alteration of the culture medium composition affected further development, particularly that replacement medium one day after culture had a significant effect. Development to morula and blastocyst stages was severely retarded (Table 1).

**Table 1. Development of embryo in different culture medium**

Group	No. Oocyt	Cleavage rate (%)	6-8 cell (%)	Morula (%)	Blastocyst (%)
A	110	40,9	55,6	35,6	28,8
B	70	30,0	14,3	4,8	4,8
C	72	29,2	61,9	28,6	19,0
D	70	38,6	66,7	48,1	33,0

### DISCUSSION

Efficiency blastocyst production *in vitro* is unsatisfactory, due to inadequate information about the requirements of bovine embryos for development in culture and of oocytes for achieving normal maturation (Bavister and Hellkant, 1992; Sirad, Coenan and Bildeau, 1992).

Results of our experiment show that cleavage rates in the control experimental groups were similarly high due to the use of the same medium supplemented with ECS and FSH. Whereas the beneficial effect of ECS has been proven by several researchers (Sanbuissho and Threlfall, 1985; Schellander *et al.*, 1990; Younis *et al.*, 1989; Fukui, 1989). The ability of fertilized oocytes to reach 2-cell stage is not an adequate indicator for full embryonic developmental competence (Xu, Hoier and Greve, 1988; Bavister and Hellkant, 1992).

Culture for one day in ECS and the rest in FCS (group B) showed lower blastocyst development as compared with the other groups. The replacement of ECS with FCS did significantly influence blastocyst development only. When zygotes were cultured from the beginning of the culture period in medium supplemented with FCS. Replacement of ECS with FCS after two or three days of cul-

ture did not influence further development. This indicates, that acquisition of developmental competence takes place at very early cleavage stages. This acquisition is somehow associated with content of the estrus cow serum.

Blastocyst rate ranged in this experiment from 4.8% to 33%. Culture in ECS gives better result than FCS. This result is correspond with results observed by several author groups (Fukui, 1989; Schellander *et al.*, 1990; Durnford and Stubbing, 1992). Although, bovine IVM/IVF embryos can develop *in vitro* in the absence of serum, other proteins and without using somatic cell co-culture.

Standard culture medium employs serum supplement. Our data indicated that factors present in the serum are required at specific developmental stages. One of the stages is the change from cleavage round to other shape which is useful for activity of the embryonic genome. Preliminary data (not shown) suggest also that serum supplementation during culture through the 8 cell block seem to be also critical for further development.

In conclusion ECS is not required for further development of IVM-IVF embryos after 3 days of culture. Further investigation on regulatory mechanisms and nutrition requirement of bo-

vine early embryonic development should be done to obtain optimum culture result.

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