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 devided 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 diferences 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-

Key Words : Culture medium, ECS, FCS, bovine blastocyst.

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 slaugther 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 (Nunclon, Denmark) containing 400 µl modified TCM + 20% ECS, 20 µl FSH (Foltropin), under 400 µl parafin oil. Oocytes were cultured for 20 – 24 h at 39°C under 5% CO_2 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 in adequate information about the requirements of bovine embryos for development in culture and of oocytes for achieving normal maturation (Bavister and Hellekant, 1992; Sirad, Coenan and Bilodeau, 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 reseachers (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 begining of the culture period in medium supplemented with FCS. Replacement of ECS with FCS after two or three days of culture 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 mechanims and nutrition requirement of bo-

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

AKNOWLEDGEMENT

This work was done as part of first author study programme and was funded by Austrian Academic Exchange Services (OAD).

REFERNCES

Aoyogi, Y., Y. Fukui, Y. Iwazumi, M. Urakawa, Y. Minegishi and H. Ono. 1989. Effects of culture system on development of in vitro-fertilized bovine ova into blastocysts. *Theriogenology* **31** : 168.

Bavister, B.D. and T.A.R. Hallekant. 1992. Development of in vitro matured/in vitro fertilized bovine embryos into morula and blastocyst in defined culture media. *Theriogenology* **37 (1)** : 127–143.

Blakewood, E.G. and L. Zhang. 1993. The use of chick embryo amniotic fluids for the in vitro culture of early stage mammalian embryos. *Theriogenology* **39** : 189.

Bracket, B.G. and K.A. Zuelke. 1993. Analysis of factors involved in the in vitro production of bovine embryos. *Theriogenology* **39** : 43–64.

Chung, Y.G. and G.E. Seidel Jr. 1993. Development of in vitro-derived bovine embryos after maturation of oocytes in TCM – 199 supplemented with EDTA and pyruvate. *Theriogenology* **39** : 202.

Del Compo, M.R., M.X. Donoso, A.T. Palasz, A. Garcia and R.J. Mapletoft. 1993. The effect of days in coculture on survival of deep frozen bovine ivf blastocysts. *Theriogenology* **39** : 208.

Fukui, Y. 1989. Effects of sera and steroid hormones on development of bovine oocytes matured and fertilized in vitro and co-cultured with bovine oviduct epitelial cells. *J. Anim. Sci.* **67** : 1318–1323.

Fukui, Y., M. Urakwa, C. Sasaki, N. Chikamatsu and H. Ono. 1989. Development to the late morula or blastocyst stage following in vitro maturation and fertilization of bovine oocytes. *Anim. Reprod. Sci.* **18** : 139–148.

Fukui, Y., L.T. Gowan, R.W. James, P.A. Pugh and H.R. Tervit. 1991. Factors affecting the in-vitro development to blastocysts of bovine oocytes matured and fertilized in vitro. *J. Reprod. Fert.* **92** : 125–131.

Keefer, C.L. 1992. Development of in vitro produced bovine embryos cultured individually in simple medium : Effects of EGF and TGFB. *Theriogenology* **37 (1)** : 236.

Kim, C.I., J.E. Elington and R.H. Foote. 1990. Maturation, fertilization and development of bovine oocytes in vitro using TCM 199 and a simple defined medium with coculture. *Theriogenology* **33 (2)** : 433–439.

Leibo, S.P. and N.M. Loskutoff. 1993. Cryobiology of in vitro-derived bovine embryos. *Theriogenology* **39** : 81–94.

Parrish, S.S., J.L. Susho-Parrish, M.L. Libfried-Rutledge, E.S. Crister, W.H. Eyestone and N.L. First. 1986. Bovine in vitro fertilization with frozen-thawed semen. *Theriogenology* **25** : 591.

Petters, R.M. 1992. Embryo development in vitro to the blastocyst stage in cattle, pigs and sheep. *Anim. Reprod. Sci.* **28** : 415–421.

Polland. J.W., K.P. Xu, R. Rorie, W.A. King and K.J. Betteridge. 1989. Influence of various oviductal epitelial cell culture system on the development of early stage bovine embryos in vitro. *Theriogenology* **31** : 239.

Sanbuissho, A and W.R. Threlfall. 1985. The effects of estrus cow serum on the maturation and fertilization of the bovine follicular oocyte in vitro. *Theriogenology* **23 (1)** : 226.

Schellander, K., F. Fuhrer, B.G. Brackett, H. Korb and W. Schleger. 1990. In vitro fertilization and cleavage of bovine oocytes matured in medium supplemented with estrus cow serum. *Theriogenology* **33 (2)** : 477–485.

Sirad, M.A., K. Coenen and S. Bilodeau. 1992. Effect of fresh or cultured follicular fractions on meiotic resumption in bovine oocytes. *Theriogenology* **37 (1)** : 39–55.

Takagi, Y., K. Mori, M. Tomizawa, T. Takahashi, S. Sugawara and J. Maraki. 1991. Development of bovine oocytes matured, fertilized and cultured in a serum – free, chemically defined medium. *Theriogenology* **35 (6)** : 1197–1204.

Takashi, Y. and N.L. First. 1992. In vitro development of bovine one-cell empbryos : influence of glucose, lactate, pyruvate, amino acids and vitamins. *Theriogenology* **37** : 963–978.

Trounson, A. 1992. The production of ruminant embryos in vitro. *Anim. Reprod. Sci.* **28** : 125–137.

Van Inzen, W.G., Th. A.M. Kruipp and S.M. Weima. 1993. Use of conditioned medium for ivm-ivf bovine embryos in vitro culture systems. *Theriogenology* **39** : 236.

Xu, K.P., R. Hoier and T. Greve. 1988. Dynamic changes of estradiol and progesterone concentrations during in vitro oocytes maturation on cattle. *Theriogenology* **30** : 245–255.

Younis, A.I., B.G. Brackett and R.A. Fayrer-Hosken. 1989. Influence of serum and hormones on bovine oocytes maturation and fertilization in vitro. *Gemete Research* **13** : 189–201.