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Effect of a Simple Serum-Free Medium, CR1, on the Development of IVM/IVF Bovine Embryos

Se Pill Park[1], Seun Eui Kim[1], Sang Jun Uhm[1], Eun Young Kim[1], Teoan Kim[2], San Hyun Yoon[3] Kil Saeng Chung[4] and Jin Ho Lim[3]

Maria Infertility Medical Institute, Seoul[1] School of Medicine, Taegu Hyosung Catholic University[2]
Maria OB/GYN, Seoul[3] College of Animal Husbandry, Kon-Kuk University[4]

체외생산된 소수정란의 체외발생에 미치는 혈청무첨가 단순배양액인 CR1의 효과

서울마리아 기초의학 연구소[1], 대구효성카톨릭대학교 의과대학[2],
서울마리아 산부인과[3], 건국대학교 축산대학[4]

박세필[1] · 김선의[1] · 엄상준[1] · 김은영[1] · 김태완[2] · 윤산현[3] · 정길생[4] · 임진호[3]

=국문초록=

본 연구는 혈청 무첨가 단순배양액인 CR1이 체외에서 생산된 소 수정란의 체외 배발생에 미치는 영향을 검토하고자 실시하였다. 본 연구에서 얻어진 결과를 요약해보면, 1) 총 1,250개의 체외성숙 난자로 부터 체외 수정결과 본 실험의 목적상 이용될 수 있는 1,025개(82.0%)의 분할란 (>1세포기)을 얻을 수 있었으며, 체외배양 결과 배반포기와 부화율은 각각 27.1%와 20.2%였다. 2) CR1 배양액은 소난포란 (>1세포기)의 체외발생시 난관상피세포, 난구세포, 영양배엽세포 등의 체세포와 공동배양을 유도하지 않고서도 높은 배발생율을 얻을 수 있었으며, 이러한 결과로 미루어 볼때 CR1은 난자의 체외배양시 난자성장촉진인자를 연구하는데 효과적으로 사용될 수 있다는 것을 시사한다.

INTRODUCTION

A major obstacle to the application of bovine embryos manipulating technique, such as embryo transfer, cloning and gene transfer, has been cleavage block which often occurs *in vitro* at the 8-to 16-cell stage(Camous et al., 1984; Eyestone & First, 1986).

In addition, the energy requirements of bovine embryos during cleavage stages have not been fully defined (Rexroad, 1989).

However, the use of trophoblastic vesicle (Camous et al., 1984), cumulus cell (Goto et al., 1989), and oviductal cell (Eyestone & First, 1989) for embryo co-culture has been beneficial for the development of bovine embryos. Only recently has there been success with culture in cell-free media. These successes include use of a medium based on the composition of oviduct fluid (Synthetic oviduct fluid; SOF; Takahashi & First, 1993), conditioned medium produced from the co-culture systems (Eyestone & First, 1989) and a simple medium based on NaCl, KCl, $NaHCO_3$, lactate and amino acids, called CR1 (Rosenkrans et al., 1993). Especially, CR1 medium has been reported to be as effective as co-culture in supporting preimplantation bovine embryo development to the blastocyst

Table 1. The chemical composition of CR1 medium

Component	Concentration (mM)
Sodium Chloride	114.7
Potassium Chloride	3.1
Sodium bicarbonate	26.2
L-Glutamine	1.0
Lactic acid	5.0
Pyruvate	0.4

stage (Rosenkrans et al., 1993).

The objective of this experiment was to evaluate the effect of a simple serum-free medium, CR1, on the development of IVM/IVF bovine embryos.

MATERIALS & METHODS

1. Oocytes maturation

Bovine ovaries were transported from a slaughterhouse to the laboratory suspended in saline (0.9% NaCl; 32±2℃) in container. Cumulus oocytes complexes (COCs) were collected from visible follicles (2-6mm) of ovaries. The COCs were washed three times with TALP-HEPES medium containing BSA (0.1%). The basic medium for maturation was tissue culture medium (TCM-199). The additives were FCS (10%), sodium pyruvate (0.2mM), FSH (1μg/ml), estradiol-17β (1μg/ml), and gentamycin (25μg/ml). The COCs were placed in each maturation plate (10 COCs/50μl drop), and then cultured at 39℃, 5% CO_2 incubator in air with high humidity.

2. *In vitro* fertilization

Frozen bovine semen ($1x10^8$ cells/ml) was used for fertilization. Highly motile sperm were recovered from frozen-thawed semen separated on a discontinuous percoll gradient previously used by Rosenkrans et al. (1993). Briefly, frozen bovine semen was layered on the top of bilayered -discontinuous percoll column (45% and 90% of 2ml each) and centrifuged at 700xg for 15min. at room temperature. The pelleted sperm were resuspended with Sp-TALP at a concentration of $2.5x10^7$ cells/ml.

In vitro fertilization was carried out by a method of Sirard et al. (1988) with some modifications. After 24hr *in vitro* maturation, COCs were washed three times with Sp-TALP, and transferred to fertilization plates.

Fertilization plates consisted of sixteen 44μl drops of Fert-TALP without glucose in 60mm petri dishes covered with 10ml of paraffin oil. Ten COCs were moved to each fertilization drop, followed by 2μl of motile sperm ($5x10^4$ cells/50μl drop), 2μl of heparin (2μg/ml), and 2μl of PHE stock (Rosenkrans et al., 1993).

3. Embryo development

After 44±2 coincubation with the sperm, the cumulus cells were removed by repeated pipetting through a fine-bore pipette of 200μm (I.D.), and the cleavage rate (>1-cell) was assessed. The basic medium for embryo

Table 2. The effect of CR1 medium on *in vitro* development of IVM/IVF bovine embryos

Replication	No. of oocytes examined	No. of embryos with >1-cell (%)	No. of embryos developed to (%)	
			Blastocyst	Hatched
I	312	250	34	42
II	318	264	69	50
III	285	234	57	48
IV	335	277	78	67
Total (%)	1,250	1,025 (82.0)	278(27.1)	207(20.2)

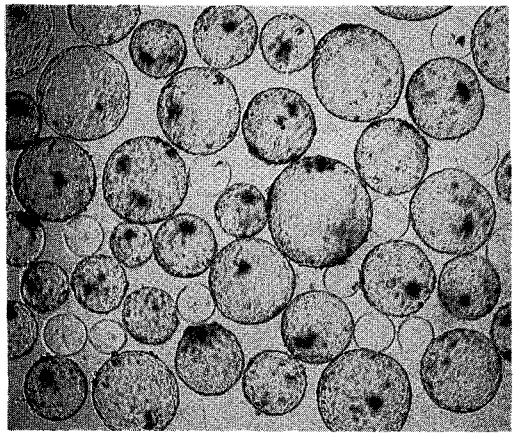

Fig. 1. Eight-day bovine hatched blastocyst embryos.

development was CR1 (Charles Rosenkrans, 1993). The components are described in Table 1. In addition, the additives were Minimum Essential Medium (MEM), Basal Medium Eagle's (BME), Fatty acid-free BSA (FAF-BSA, 3mg/ml), and Gentamycin (25μg/ml). The cleaved embryos were placed in 50μl drop of CR, medium for 7 days (9 days post insemination).

RESULTS AND DISCUSSION

As shown in Table 2, this experiment was replicated four times using a total of 1,612 oocytes. The average cleavage rate was 82.0%, resulting in 1,025 embryos used for experimentation. The average of embryos reaching the blastocyst and hatched blastocyst stage was 27.1% and 20.2%, respectively (Fig. 1).

The energy requirements of murine embryos have been studied extensively (Chatot et al., 1989; Lawitts & Biggers, 1991; Quinn & Wales, 1973; Cross & Brinster, 1973). However, few investigations have been published on energy metabolism by embryos from domestic animals. Recently, CR1 medium based on the composition of bovine oviductal and uterine fluids has been using for the *in vitro* culture of bovine 1-cell embryos (Rosenkrans et al., 1993). This medium, a simple serum-free medium, mainly consists of NaCl, KCl, $NaHCO_3$, lactate, and pyruvate. Also, additives are 20 amino acids (12 BME essential amino acids, 7 MEM non-essential amino acids, and 1.0mM glutamine). As shown in Table 2, *in vitro* development of cleaved embryos (>1-cell) to the blastocyst stage appeared to be similar with data from co-culture (Camous et al., 1984; Takahashi & First, 1993) and resulted in a higher frequency of hatched blastocyst stage. Bovine oviductal and uterine fluids contain 20 and 25 amino acids, respectively (Fahning et al., 1967; Stanke et al., 1974). Especially, glutamine has been reported to be effective in promoting the development of early embryos in hamster (Bavister et al., 1983) and pigs (Rosenkrans et al., 1989). and can be utilized as an energy source by bovine blastocysts (Rieger & Guay, 1988). These results suggest that *in vitro* development of cleaved embryos (>1-cell) to the blastocyst stage promotes by addition of 19 amino acids and glutamine (1mM).

SUMMARY

This study was to evaluate the effect of a simple serum-free medium, CR1, on the development of bovine embryos *in vitro*.

The results obtained in these experiments were summarized as follows:

1. Of total 1,250 oocytes, 1,025 (82.0%) were cleaved (>1-cell) and the average of embryos reaching the blastocyst and hatched blastocyst stage was 27.1% and 20.2%, respectively.

2. CR1 medium promotes the *in vitro* development of early bovine embryos in the absence of somatic cells, such as oviductal cell, cumulus cell and trophoblastic cell. The use of a simple serum-free medium might be the medium of choice studying the role of embryotrophic factors in culture.

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