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Development of Pig IVM/IVF Produced Embryos to Hatching Blastocysts *In Vitro* as Affected by Amino Acids and Serum

Uhm, S. J., E. Y. Kim, M. K. Kim, S. H. Yoon*, S. P. Park, K. S. Chung and J. H. Lim***
Maria Infertility Medical Institute

아미노산과 혈청이 돼지 체외수정란의 부화에 미치는 영향

엄상준 · 김은영 · 김묘경 · 윤산현* · 박세필 · 정길생 · 임진호***
마리아 기초의학연구소

요 약

체외성숙과 수정된 돼지 난자의 체외발달능이 체외배발생 배양액인 NCSU 배양액에 0.4% BSA, 10% 혈청 혹은 아미노산 (2% BME 아미노산 용액과 1% MEM 아미노산 용액)을 첨가함으로써 조사되었다. 본 실험에 공시된 난자는 체외수정 후 30 시간 (2-세포기) 혹은 48 시간 (2~4-세포기)에 회수하였다. 실험 I 에서 0.4% BSA가 첨가된 NCSU 배양액에서 2-세포기 난자들의 배양경과시간에 따른 발달능을 조사한 결과, 배양 후 72 시간 (체외수정 후 102 시간)에 상실배와 배반포기가 나타났으며, 배양 후 120 시간째 (체외수정 후 150 시간)에도 팽창된 배반포까지만 발달하였다. 실험 II 는 체외수정 후 48 시간의 분할된 (2~8-세포기) 난자들의 핵과 외관적 분할구와의 수적 차이를 조사한 결과, 2~4-세포기보다는 5-세포기 이상에서 핵과 분할구의 조화에 차이가 많았다. 실험 III 에서는 BSA, 혈청 혹은 아미노산이 첨가 혹은 무첨가된 배양액내에서 2~4-세포기 난자들의 배반포 후 부화능력을 조사한 결과, 모든 군에 있는 난자들은 팽창된 배반포까지 발달할 수 있었던 반면, 난자의 부화는 아미노산 혹은 혈청이 포함된 배양액에서만 일어났다. 더우기 상실배와 배반포시기에 혈청의 첨가는 부화 배반포기 배의 발달을 현저히 증가시켰다. 이상의 결과로 미루어 볼 때, 배양액내에 대한 아미노산과 혈청의 첨가는 돼지 배반포의 부화를 유도할 수 있다고 본다.

I. INTRODUCTION

Many researchers, in pig, have been investigated for *in vitro* culture system of immature oocytes (Mattioli et al., 1989; Funahashi et al., 1993; Yoshida et al., 1993; Niwa, 1993; Nagai, 1994). However, development from immature oocytes to blastocysts *in vitro* a little limmited in pig. Therefore, in pig, many reports have been more examined by *in vivo* than *in vitro* embryos compared with other species (Archibong et al., 1989; Krisher et al., 1989ab; Prather et al., 1991; Rieger et al., 1992).

* 마리아 산부인과 (Maria Obs. & Gyn., Seoul)
** 건국대학교 축산대학 (College of Animal Husbandry, Kon-Kuk University)

Many factors (BSA, serum, amino acids, growth factors, etc.) for improvement of culture system have used as the additive in culture medium in pig as well as others (Stone et al., 1984; Ding and Foxcroft, 1994; Harper and Brackett, 1993; Rosenkrans and First, 1994). Rosenkrans et al. (1994) reported that the blastocyst formation of bovine embryos was affected by the culture medium supplemented with amino acids. The presence of amino acids in the culture medium increases blastocyst formation, hatching, and cell number on the development of embryos (Stone et al., 1984; Meyen et al., 1989; Gardner and Lane, 1993; Rosenkrans and First, 1994). In pig, the development of zygotes in simple medium can develop to blastocyst, but the development of blastocysts ceases *in vitro* after blastocyst expansion (Wright, 1977; Menino and Wright, 1982; Petters et al., 1990; Hagen et al., 1991; Beckmann and Day, 1993). Hatching and trophoblast expansion after pig blastocyst formation were stimulated by including serum and amino acids in the culture medium (Robl and Davis, 1981; Stone et al., 1984; Rosenkrans et al., 1989; Pollard et al., 1995). Pollard et al. (1995) suggested that the development of *in vivo* pig zygotes to blastocyst can be supported by simple culture media, but the development of morulae to hatching was superior to complex medium contained amino acids and serum. These results showed that development of embryos necessarily requires to nutrients and energy sources. These factors more affect at the continued development and hatching of late embryo stage after morula and blastocyst formation than cleavage of early embryo stage. Therefore, this report examined to effects by amino acids and fetal bovine serum (FBS) on the blastocyst development as well as hatching after the blastocyst formation of pig IVM/IVF produced embryos.

Ⅱ. MATERIALS AND METHODS

1. Recovery of immature oocytes

Ovaries were collected from prepubertal gilts at a local slaughterhouse and transported to the laboratory in saline (35 to 39℃) within 1 hr. The oocyte-cumulus complexes (OCCs) were recovered by aspiration from the follicles (2~6 mm in diameter) using a 10 ml disposable syringe fitted with an 18-gauge needle. The OCCs were washed three times with TL-HEPES (1 mg/ml BSA, low carbonate TALP; Parrish et al., 1988) and the maturation medium, respectively.

2. *In vitro* maturation (IVM)

The oocytes (about 50 oocytes) were transferred into a 0.5 ml of maturation medium equilibrated for 2 hr in 5% CO_2 and 95% O_2 incubator under warm mineral oil in a four well culture dish. The maturation medium consisted of TCM-199 (with Earle's salts: Gibco, USA) supplemented with 25 mM $NaHCO_3$, 10% FBS (fetal bovine serum, Gibco), 0.2 mM pyruvate, 0.6 mM L-cysteine, 10 μg/ml p-FSH, and 25 μg/ml gentamycin. Culture was carried out at 39℃ in 5% CO_2 in air for 42-44hr.

3. Sperm capacitation and *in vitro* fertilization (IVF)

Semen were collected from cauda of epididymis obtained from a local slaughterhouse. Semen (1 ml) were diluted into 5 ml Sp-TALP (Rosenkrans et al., 1994), and the extender was removed by washing two times (1,000 rpm) for 5 min. Then the sperm pellet was resuspended with Sp-TALP, and motile sperm were collected by swimup separation after incubation for 10 min.

Highly motile sperm were added into the fertilization medium containing about 50 oocy-

velopment and hatching of pig blastocysts.

V. SUMMARY

In vitro development potential of pig IVM/IVF produced embryos was investigated in culture medium (NCSU) supplemented with BSA (0.4%), serum (10%), or amino acids (2% BME amino acids solution and 1% MEM non essential amino acid solution). Embryos collected at 30 hr (2-cell) or 48 hr (2- to 4-cells) after IVF. In the first experiment, the development ability of 2-cell embryos in the culture medium (NCSU+0.4% BSA) following time course was examined. Morulae and early blastocysts revealed at 72 hr (102 hr after IVF) after culture, and expanded blastocysts showed at 120 hr (150 hr after IVF). In the second experiment, the cleaved (2- to 8-cell) embryos at 48 hr after IVF compared number of nuclei status with morphological blastomeres. The harmony of the number between nuclei and blastomeres more differed from over 4-cell than 2- to 4-cell embryos. In the third experiment, the ability of hatching after blastocyst formation of 2- to 4-cell embryos in the culture medium supplemented with/without BSA, serum, or amino acids was examined. Embryos in all groups can develop to expanded blastocyst, but hatching of embryos can be developed by the culture medium containing amino acids or serum. In addition, the addition of serum at morula and early blastocyst stages was significantly enhanced the hatching rate of blastocysts. Therefore, This result suggests that amino acids and serum in the culture medium can support to the continued development and hatching of pig blastocysts.

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