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Spontaneous Parthenogenesis of Porcine Oocyte Induced by Prolonged Culture in Various Media

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다양한 배지에서 장시간 배양에 의한 돼지 난자의 단위발생 유도

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SUMMARY

본 연구에서는 TCM-199, mSOF, NCSU-23 세 종류의 배지의 자연 단위 발생 유도로 형성된 돼지 배아의 초기 발육에 대한 효과를 조사하였다. 실험 1에서는 세 종류의 배지에서 체외 성숙된 도축장 유래 돼지 난자의 성숙율을 성숙 48 시간째 핵상 관찰로 조사하였다. 각 배지에서의 핵 성숙율은 mSOF군에서 83.1±2%, NCSU-23 군에서 78.0±3%, TCM-199 군에서 83.5±2%로 세 배지의 난자 성숙율에 대한 유의적 차이는 없었다(P(0.05). 실험 2에서는 돼지 난자의 전핵 형성률, 단위 발생으로 형성된 배아의 분할율, 6 - 8세포기까지의 발육율 등이 각각 체외 성숙 55-58 시간, 96시간 그리고 168 시간 후에 관찰되었다. 전핵 형성율 (5.4±2 % in mSOF and 3.7±1% in NCSU-23 vs 1.7±3% in TCM-199) 그리고 6 - 8 cell까지의 발육율은 (3.2±3% in mSOF and 4.0±1% in NCSU-23 vs 1.4±3% in TCM-199) mSOF군과 NCSU-23군에서 유의적으로 높았다(P<0.05).

이상의 결과를 볼 때 mSOF 배지와 NCSU-23 배지에서 장시간 배양에 의한 난자의 단위 발생 유도율이 유의적으로 높았다.

(Key words : spontaneous parthenogenesis, porcine oocytes, early development)

INTRODUCTION

Activation is termed by the initiation a cascade of events in the oocyte by fusion of the male and female pronuclus in normal fertilization and is also is a crucial step in the cloning procedure (Hyun et al., 2002). Activation includes the cortical reaction, resumption of meiosis (transition from meiotic metaphase to mitotic interphase), increased metabolic activity and cytoskeletal remodeling (Li et al.,

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1997). Parthenogenetic activation of mammalian oocytes can be induced by various methods, such as exposure to ethanol, calcium ionophore and electric pulse (Joliff et al., 1997). The LT mouse MI arrested oocytes exhibit a high incidence of spontaneous parthenogenetic activation (Eppig et al., 2000). Eppig et al. demonstrated that cumulus cells participate in the incidence of parthenogenetic activation of LT-related mouse. Rat oocytes are spontaneously activated after being released from the oviduct, but this spontaneous activation is incomplete, terminating at a metaphase III-like stage without pronuclear formation (Keefer et al., 1982). Spontaneous activation does not preclude preimplantation development because aged mouse oocytes spontaneously activated without special treatment, can develop to blastocysts *in vitro* (Otaegui et al., 1999). In pigs, Kikuchi et al. and Kim et al. reported that prolonged culture of pig oocytes in Waymouth's media and TCM-199 resulted in spontaneous parthenogenetic activation, respectively (Kikuchi et al.,1995; Kim et al.,1994).

The aim of this study was to determine the optimal IVM medium leading to completion of nuclear and cytoplasmic maturation for pig oocyte activation by prolonged culture.

MATERIALS AND METHODS

1. Preparation of Oocytes

Ovaries were obtained from prepubertal gilts at a local abattoir and transported to the laboratory in physiological saline at 30 to 35℃. Antral follicles 3 to 6 mm in diameter were aspirated using an 18-gauge needle attached to a 5-ml disposable syringe. Cumulus-oocyte complexes (COCs) with compact cumulus cells were collected from the aspirate and washed several times in Hepes-buffered tissue culture medium (TCM)-199 (Life Technologies, Rockville, MD). The COCs were placed in three different IVM media (TCM-199, NCSU-23 or mSOF) supplemented with 10% (v/v) porcine follicular fluid (pFF), 10 ng/ml EGF, 10 IU/ml eCG and hCG. The pFF was aspirated from superficial antral follicles 8 to 10 mm in diameter from prepubertal gilts. After centrifugation at 1,600 ×g for 30 min, supernatant was collected and filtered through 1.2 μm and 0.45 μm syringe filters (Gelman Sciences, Ann Arbor, MI). Prepared pFF was then stored at −20℃ until use. A group of 50 COCs was cultured in 500-μl IVM medium at 39℃ in a humidified atmosphere of 5% CO_2 and 95% air. After culturing for 22 hr, COCs were transferred to eCG- and hCG-free IVM medium and cultured further for 24 hr.

2. Media

Three maturation media were used in this study: TCM-199 (Life Technologies, Rockaville, MD), NCSU-23 and mSOF. The TCM-199 was supplemented with 0.57 mM cysteine, 3.05 mM D-glucose and 0.91 mM sodium pyruvate. The mSOF were supplemented with 4 mg/ml BSA, ITS 10 μg/ml and 0.57 mM cysteine.

3. Spontaneous Activation Induced by Prolonged Culture

After culturing for 46 hr, COCs were cultured in eCG- and hCG-free IVM medium further for 24 hr in three maturation media.

4. Assessment of Activation

1) Pronuclei (PN) Formation

In vitro matured oocytes in three different media were analyzed for chromosomal stage by aceto-orcein staining. Oocytes were mounted and fixed for 24 h in 25% acetic acid in ethanol at room temperature. The fixed oocytes were then stained with 1% (w:v) orcein in 45% (v:v) acetic acid and examined at (Leitz) with phase-contrast optics under ×200 or ×400 magnification.

2) Statistical Analysis

Data were assessed by analysis of variance using a SAS program. Percentage data were arcsined transformed. Differences between the means were determined by means of Duncan's multiple range test. Data were represented as the mean±SEM and the level of statistical significance was taken as $P<0.05$.

RESULTS

1. Nuclear Maturation in Three Different IVM Media

As shown in Table 1, maturation of oocytes was evaluated by examining the nuclear status of oocytes (GV, M-I, and M-II). When assessed by each status after IVM, IVM media did not appear to make significant difference in maturation rate of metaphase II stage among three groups. The 83.1±2, 78.0±3 and 83.5±2% of maturated oocytes in three media were obtained at 48h after IVM, respectively. However, cumulus expansion was prominent in TCM-199 or NCSU-23 than in mSOF.

2. Pronuclear Formation and Early Development of Spontaneously Activated Oocytes

As seen in Table 2, a significantly higher ($P<0.05$) pronuclear formation rate cleavage rate and early developmental rate of embryos were observed in mSOF (5.4±2%) and NCSU-23 (3.7±1%) after spontaneous activation compared to that observed in TCM-199 (1.7±3%). The cleavage rate and early developmental rate of embryos were higher ($P<0.05$) in mSOF (3.2±3%) and NCSU-23 (4.0±1%), compared to that observed in TCM-199 (1.4±3%) (Table 2).

DISCUSSION

In this study, we examined if *in vitro* maturation media affect the early development of porcine oocyte after spontaneous activation induced by prolonged culture. Demonstration of normal fertilization and early development is a proper way of assessing the developmental ability of IVM oocyte.

Table 1. Effect of maturation media on nuclear status of oocytes

Maturation medium	Oocytes examined (%)	Nuclear status of oocytes (%)		
		GV	M-I	M-II
mSOF	89±2	5.6±3	11.2±2	83.1±3
TCM199	91±3	9.8±1	12.1±2	78.0±1
NCSU-23	85±2	8.2±3	8.2±2	83.5±2

Table 2. Effects of maturation media on the spontaneous activation of pig oocytes

Maturation medium	[1]Pronucleus formation (%)	No. of embryos developed (%)	
	1PN	[2]Cleavage rate (%)	[3]6~8cell
mSOF	5.4±2[a]	7.1±2[a]	3.2±3[a]
TCM199	1.7±1[b]	3.9±2[b]	1.4±2[b]
NCSU-23	3.7±3[a]	6.7±1[a]	4.0±2[a]

Values with different supercripts within each column are significantly different ($P < 0.05$).

[1] Pronucleus formation were assessed at 55-58 h after IVM.

[2] The cleavage rate were assessed at 96 h after IVM.

[3] 6~8 cell embryos were assessed at 168h after IVM.

However, involvement of various factors derived from spermatozoa and parthenogenetic activation obtained after combined treatment of pig oocytes with electrical stimulation and DMAP may cause complex interpretation (Kim et al.,1994). Therefore, we employed spontaneous activation induced by prolonged culture of pig oocyte in three different media (mSOF, TCM-199 and NCSU-23) as a method of assessing the developmental ability of IVM oocytes. Kikuchi et al. reported that prolonged culture of pig oocytes in Waymouth's media (72 hr) resulted in high spontaneous parthenogenetic activation (24%) (Kikuchi et al., 1995). Suzuki et al. reported no evidence of spontaneous activation after culture up to 72 hr in NCSU-23 medium without EGF. In this study, we observed low spontaneous parthenogenetic activation (1.7% to 5.4%) in each medium. Although these results cannot be compared directly due to different culture systems used, the present study showed higher rates of pronuclear formation and 6~8 cell development of parthenogenotes in oocytes matured in mSOF and NCSU-23. Activated with electrical stimulation alone, porcine parthenogenotes did not develop beyond the two-cell stage when the diploidization step was omitted (Jiang et al., 2002). However, we examined that porcine spontaneous activated parthenogenotes developed to 8 cell stage without any treatment for diplodization. In our study, pig oocytes underwent spontaneous activation without treatment and exhibited pronuclear formation (1PN) and 8cell development. Our results indicate that mSOF and NCSU 23 were more effective media for development after spontaneous activated pig oocytes. In conclusion, we assessed embryos derived from various maturation media using spontaneous activation induced by prolonged culture. These results will further enlighten the research for pig oocyte activation.

SUMMARY

This study was undertaken to investigate the effect of three different porcine IVM media, TCM-199, mSOF and NCSU-23 on early development of porcine spontaneous parthenogenotes. Spontaneous parthenogenotes were induced by a prolonged culture of porcine oocytes in each medium. In Experiment 1, oocytes derived from gilts were matured in three IVM media and maturation of oocytes was evaluated by the status of chromatin configuration. Oocytes matured in mSOF, NCSU-23 and TCM-199 showed no significant difference ($P>0.05$) in maturation. Maturation rates at 48h after IVM were $83.1\pm2\%$ (mSOF), $78.0\pm3\%$ (NCSU-23) and $83.5\pm2\%$ (TCM-199). In Experiment 2, pronucleus formation and development to 6~8 cell stage of pig oocytes activated spontaneously. Pronucleus formation, cleavage rate and development to 6~8 cell embryos of porcine spontaneous parthenogenotes were assessed at 55~58 h, 96 h and 168h after IVM, respectively. Pronucleus formation ($5.4\pm2\%$ and $3.7\pm1\%$ vs $1.7\pm3\%$) and development to the 6~8 cell (3.2 ± 3 % and $4.0\pm1\%$ vs $1.4\pm3\%$) was significantly ($P<0.05$) higher in mSOF or NCSU-23 than TCM-199. In conclusion, the present study showed that oocytes matured in mSOF and NCSU-23 were spontaneously activated with higher frequency.

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