The Analysis of Ca^{+2} Intensity Profile of M-II Oocyte Natural Activated by Crude Sperm Extract

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ABSTRACT

Sperm factors or cytoplasmic factor in the spermatozoa have been used to activate mammalian M-II oocytes artificially. Several chemicals activator have been reported for their capability in parthenogenetic activation of M-II oocyte, but still resulted in improper, lower and partially achievement, especially for reconstructed oocytes. The aims of these research are to study the potential natural activator Crude Sperm Extract (CSE) for improving activation of the M-II oocytes. The isolate of CSE and the 100 kD protein of CSE were supplemented to culture medium of TCM 199. The intentity of calsium during activation was observed by flou-3 stainning with Concocal Laser Scanning Microscope (CLSM). Result showed image analysis of Calcium intensity, demonstrate the variations among activated M-II oocyte. Profile of Ca+2 intensity of M-II oocyte diffrent compare to M-II oocyte non activated. Result from In vitro culture of these oocyte observed that activation using CSE of 2.5 ug/ml resulted in about 36.33 % oocyte was cleavaged compare to activation using 100 kDa protein of CSE of 2,00 % of M-II oocyte was cleavaged . The CSE is relatively better than spesific protein of GSE of 100 kDa for their role as natural agen of activation to oocytes. It was suggested to additional researh in both the intracelluler calcium concentration and identification of spesific protein or sperm factors of CSE.

Key words: *Calcium Intensity, Crude Sperm Extract, Activation, Oocyte*.

Introduction

Two theories have been proposed to explain the induction of Ca^{+2} release in fertilizes M-II oocyte following fertilization by sperms: (1). the sperm receptor theory and (2) the sperms factor theory. Sperm factor, postulated as cytoplasmic factor in the spermatozoa, has been used to partenogenetically activate mammalian oocytes. Spermatozoa release cytosolic substances into oocyte at fertilization and its induces Ca^{+2} oscillations (Fissore et al, 1998, Oda 2006, Wu et al, 1997). The parthenogenetic activation of oocytes is an interesting and valid tool to investigate the comparative roles of paternal and maternal genomes in controlling early embryo development.

Repetitive Ca^{+2} rises at fertilization are necessary to accomplish degradation of MPF. Calcium oscillation in oocytes is responsible for pronuclear formation. Egg activation is caused by dramatic increase in intracellular Ca^{+2} concentrations common to every animal species including mammalian (Miyazaki and Ito, 2006). Mammalian oocytes at the time of fertilization occurred exhibit a series of transient increase in intracellular calcium ion (Ca^{+2}) concentration which are prerequisite and sufficient for oocyte activation. Immediately after the sperm attaches to the oocyte, increase in intracellular Ca^{+2} in the oocytes are firstly seen near the site of sperm-oocyte fusion.

Parthenogenetic activation of mammalian oocytes was commonly artificially induced by chemical agents, but these agents did not effectively activate pig oocyte (Okada, 2005) or reconstructed goat oocytes (Ciptadi, 2005). Crude Sperm Extract (CSE) that was injected into egg showed the repetitive Ca^{+2} *oscillation* as well as like normal fertilization by sperm (Han et al., 2003). this research was focused on study the effect of supplementation of CSE transfected in the conventional chemical medium activation. on activation rate base on both parameters of Ca^{+2} intensity and the cleavage rate of M-II oocyte,

Experimental Details

In vitro Maturation (IVM) of Oocyte

The oocytes-cumulus cell complexes were isolated from goat anthral follicles of 2 – 6 mm, cultured in bicarbonate buffered medium 199 supplemented with 10 % FBS, 0.1 mg sodium pyruvate (Tanaka, 2001). The culture was carried out in 5 % CO_2 in humidified air at 38 ^{o}C for 48 hours. Oocytes were completely denuded by pipetting after treated with 0.01 % hyaluronidase (Sigma).Only those with a compact, non-atretic cumulus

and a homogeneous cytoplasm which were previously classified as Grades A and B (Neglia et al, 2000, Tanaka , 1981), were selected. Oocytes emitting the 1st polar body were selected in this experiment.

Preparation of CSE

The CSE was prepared from local goat sperm as describe by Okada et al (2004) and Swan (1990). Briefly, the sperms were distrupted by ultra sonification and fractionated by ultracentrifugation. The sperm suspension containing approximately 10 x 10 ^{8} cells/ml was sonificated for 15 minute at 4 ^{o}C. Homogenated sperm suspension was centrifuged twice at 10.000 g for 45 minute at 4 ^{o}C and the supernatant was collected as the crude sperm extract (CSE), and then stored at – 80 ^{o}C before used.

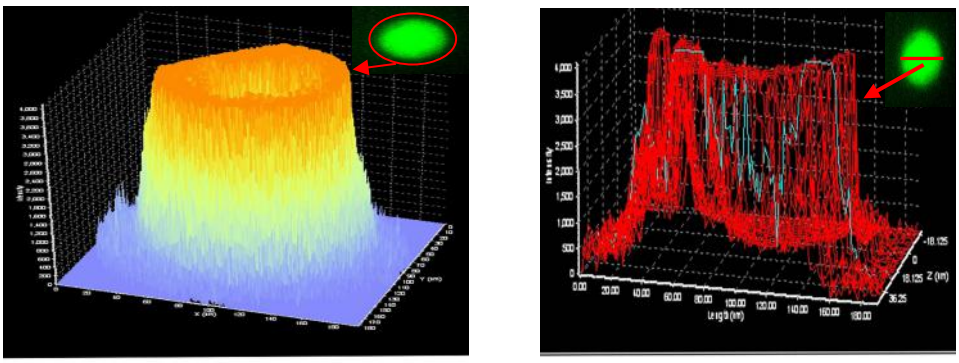
The Experiments

Treatment of this experiment were different CSE supplementation of protein concentration based on the early concentration of sperm 10 x 10 ^{8} spermatozoa/ml isolation medium. GCSE was supplemented into chemical activation medium, with the experiment treatments of M-II oocyte parthenogenecally refer to Ciptadi (2005), Ongeri et al (2001) are : (1) Control (M-II) oocyte, (2). 7 % ethanol + CSE 2.5 ug/ml, and (3). 7 % ethanol + CSE of 100 kDa. In this research, the selected M-II oocytes were exposed in these medium activation treatment after a slit of zona pellucida was performed (transfection) by micromanipulator .

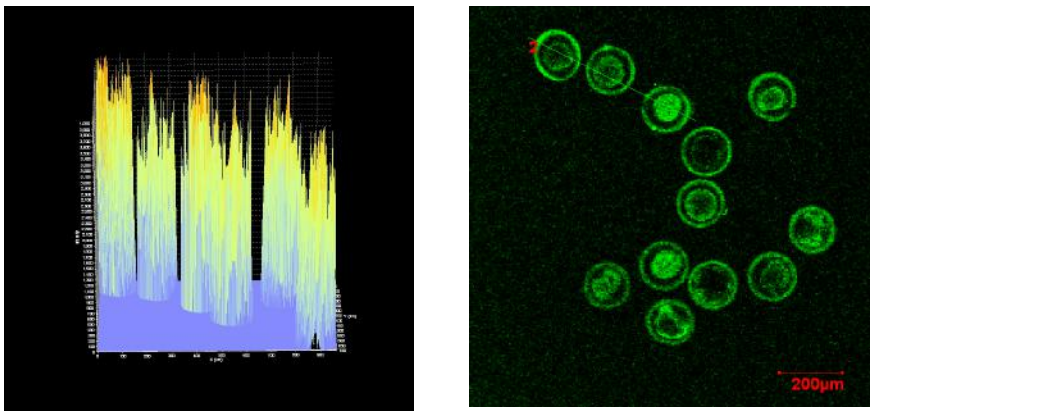
Result and Discussion

Calsium (Ca+2) Intensity Profile

The CSE used in this research was characterised for their protein profile and its indicate the 11 kinds of protein weight molecular included the 100 kD (Wahyuni, et al.. 2009).) The protein of 100 kDa in this extract sperm might be potential as activation agent. Matsuura et all.(2006) showed that sprem extract with molecular weight 100 kDa contain effective materials for porcine oocyte activation.



A. B.



C. D.

Figure.1. A. Histogram profile of oocyte non activated (control),48 hour of culture. Calsium intensity expressed mainly zona pellucida. B. Calcium intensity profile of oocyte activated by CSE, intensity calsium is well expressed spread out in cytoplasm C,D. Variation of oocyte respons on different oocyte treated with CSE, 48 hour after treatment and cultured. Analysis was done by CLSM-fluo -3.

In this research, the intensity of Ca^{+2} showed different profile of each treatment. M-II oocyte with no treatment showed strong enough intensity on the area of zonna pellucida. Meanwhile, the oocyte exposed with CSE, Ca^{+2} is well expressed spread out on cytoplasm. However, the responses on CSE supplementation are varied after 24 to 48 hour of culture (Figure 1). It was assumed that if Calcium $^{+2}$ was expressed in the cytoplasm, it mean that its exhibits increase in Calcium intracellular. Oda (2006) and Miyazaki and Oda (2006) mentioned that egg exhibits increase in Ca^{+2} is indications of egg activation. Several recent research results suggest that mammalian sperm factor would be introduced in to the oocyte cytoplasm through sperm-egg cytoplasmic connection, initially distributing evenly throughout the cytoplasm (Ogunuki et al., 2001).

Some experiments have attempted to study the molecular identity of sperm factor protein. Phospholipase C (PLC) zeta was identified as a strong candidate for the mammalian sperm factor (Oda, 2006). The molecular weight of PLC zeta is approximately 74 kDa (Matsuura and Maeda, 2006). Fujinomoto et al (1994) confirmed that the most convincing evidence that PLC(z) is the mammalian sperm factor. Further work is required to clarify the component of sperm factor in this goat CSE.

Oocytes activation

Activation of mammalian oocytes could be induced by a wide variety of chemical (Kline and Kline, 1994) and physical (Ozil, 1990) stimuli that mimic the fertilization process by providing calcium signals to the MII arrested eggs by different mechanisms. The transfection method used in this research might be cause the lower result because of improper method of activation Result of cleavage rate especially using GCSE alone (2 %) was lower than achieved by Okada et al (2003) which resulted in approximately 30 % of oocyte cleaved using microinjection method of CSE..(table 1.). CSE may be representation of the sperm factor complex that having better influence in activation compare to CSE with 100 kD proteins specific. It is necessary that a complex of unknown composition of the active factor in CSE to be determine in next research.

Table1. Cleavage rate of different treatments of parthenogenetic using GCSE supplementation.

Activation method	Selected M-II Oocytes	Cleavage rate (%)
(1). Control (M-II) oocyte	40	(0.00)
(2). CSE 2.5 ug/ml	251	(36. 33)
(3). CSE of 100 kDa protein	240	(2.00)

The confirmation of Ca^{+2} intensity using fluo-3 through Confocal Laser Scann Microscope showed the different profile of M-II oocyte activated using GCSE. After 48 hours activation of the oocyte, it was observed an increase in Ca^{++} intensity only in outer region of M-II activated by CSE alone) in contrary the increase of Ca^{+2} intracellular intensity of the oocytes was observed at all sites of M-II activated using chemical agent and CSE. The increase of Ca^{+2} intensity. that equal to Ca^{+2} concentration, might lead to egg activation. Oocytes activation was induced by chemical agent supplemented with CSE, with the evidence of rise Ca^{+2} intensity. This was considered to be sufficient factor for activation. Matsura and Maeda (2006) suggested that if a large quantity of protein with high ability for oocyte activation was extracted from spermatozoa, it might improve the rate of oocyte activation. A qualitative image analysis of Ca^{+2} intensity done by histogram and line series was presented in Fig. 1 B,.

Conclusion

The increase of Ca^{+2} intensity was considered to be sufficient indicator that showed activation of M-II oocyt is occurred.The CSE effecct on culture medium is relatively better than spesific protein of GSE of 100 kDa for their role as activation effect to goat oocytes. The supplementation of CSE in conventional chemical medium activation could improve the induction of M-II oocyte activation. It was suggested to additional research in both intracellular calcium concentration and identification of specific protein or sperm factor component of CSE.

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