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Effect of Insulin Transferin Selenium (ITS) on oocyte maturation *in vitro* in Indonesian goats

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Abstract
The aim of the present study was to evaluate the effects of Insulin-Transferin-Selenium (ITS) on maturation of oocytes *in vitro* in Indonesian Goats. Goat ovaries were collected at a local slaughterhouse and washed twice in sterilized NaCl solution. The follicular content including cumulus-oocyte complexes (COCs), were collected by aspirating the visible small antral follicles (around 2-6 mm diameter) with a 10 ml syringe equipped with an 8-gauge needle. The oocytes with more two layers were cultured for 28 hours at 38.5^{o}C and 5%CO_2. After maturation the cumulus cells were removed and the denuded oocytes were stained with 2% aceto-orcein to determine the nuclear oocyte status. The result showed that the rate of nuclear maturation in ITS-treated groups was significantly higher ($P<0.05$) than in the ITS-untreated group, were 38.14%^{a}; 61,40%^{b} and 71,55%^{b} , respectively for medium without ITS, 5 ng/ml ITS and 10 ng/ml ITS. This observation indicated that supplementation of ITS into culture medium was effectively enhance oocyte nuclear maturation *in vitro*.
Keywords: oocyte, nuclear maturation, Insulin Transferin Selenium, in vitro maturation

Introduction

Availability of large quantities of mature oocytes is needed for in vitro fertilization (IVF) purposes as a basis of embryo transfer program. Immature oocytes could be obtained either from the abatoir ovaries by aspirating the visible follicle (de Smedt *et al*., 1992) or from life animal by guidance of laparoscopy or ultra sonography equipments (Wikland *et al.*, 2007). The ovaries from a slaughterhouse is the cheapest and abundant source of immature oocytes. This immature oocytes then cultured in strelizied incubator at temperature 39^{o}C with 5% CO2 and 95% humidity.

Some researches have reported culture media to produce high quality of mature oocyte following in vitro maturation process. Mature oocytes at metaphase II (M-II), are ready to use for in vitro fertilization, the metabolism are optimal and enough energy to perform division (Hyttel, *et al*., 2000). Oocytes cultured in vitro system must be in similar condition with the *in vivo* environment (Djati, 2006).

Raghu, *et al*. (2002) showed that supplementation of Follicle Stimulating Hormone (FSH) and Epidermal Growth Factor (EGF) to the culture medium increased the number of maturated oocytes. But the higher blastocyst stage harvested in media containing EGF and ITS. By The addition of FSH and Luteinizing Hormone or insulin to the culture medium resulted cultured oocytes from secondary follicles reached 90% M-II more less same as at the ovulation (Djati, 2006). The supplementation of Gonadotropin hormone, insulin transferrin selenium (ITS) and epidermal growth factor (EGF) to the culture medium have successfully increase the rate of maturation mouse oocytes from preantral follicles collected from the prepubertal mice by 92.2% (Gao, *et al*., 2007). Insulin Transferrin Selenium (ITS) was used as a supplement for the culture of mouse oocytes (De La Fuente *et al.,* 1999), goats (Herrick et al., 2004) and pigs (Jeong, *et al.,* 2008). The transferrin and selenium are essential for catalytic activity of glutathione peroxidase could be role as the antioxidant defense system in the oocyte (Cerri, *et al.,* 2009). The aim of this research was to evaluate the effect of ITS supplementation to the cultured medium on maturation in vitro in the Indonesia goats.

Materials and Methods

Oocyte collection

Goats ovaries (n= 172) were collected from local slaughtered house. The ovaries were transported in distilled water containing 0.9 %NaCl and 600 IU pennicilinn and 100 mg/l streptomycin at 37 °C (Kochhar, *et al*., 2002) to the laboratory LSIH, Brawijaya University, Malang City, Eat Java, Indonesia.
The distance from the slaughter house to the laboratory was about 15 km. Before aspiration, the overies were washed 3 times using 0.9% NaCl solution plus antibiotics penicillin and streptomycin. The Aspiration were done for the follicles with diameter between 2-6 mm (De Smedt, *et al.*, 1992) using a 10 G needle connected to a 10 ml syringe containing a solution of handling medium (Djuwita, *et al*., 1998). Only the immature oocyte of A and B quality used in this study (Kelly, *et al.,* 2007). The immature oocytes were selected in handling media, washed three times in handling media and once in washing media (Catt, *et al*., 2009), washed again in medium

treatment before it planted.

In Vitro Maturation

The compotition of 10 ml of basic culture medium was αMEM (Sigma, USA), 10% FBS (Sigma), penicillin 600 IU/ml (Meiji, Seika), Streptomycin 100 mg/ml (Meiji, Seika), FSH 100 IU/ml (Sigma), LH 100 IU/ml (Sigma). The treatments were the supplemention of 0. 5 ng/ml and 10 ng/ml ITS (Sigma) in medium cultured.

Each petri dish filled with the 3 drops of 50µl maturation medium coated with paraffin oil, then incubated over night (in 5% CO2, 95% humidity and temperature 38,5 ^{o}C) before planted. Approximately 10 oocytes would be included in each drop (Catt, *et al*., 2009). Furthermore oocytes IVM medium were cultured for about 28 hours (Pamungkas, *et al*., 2012), and then evaluated for cumulus expansion (Wahyuningsih, 2005) and the maturity status of the oocyte nucleus by aceto orcein staining (Wattimena and Saija, 2005).

Data obtained in the form of cumulus expansion rate, the percentage status of the oocyte nuclei were analyzed using MINITAB Versi 14.

RESULTS AND DISCUSSION

Cumulus Expansion

In vitro oocyte maturation process is characterized by the expansion of cumulus cells surrounding the oocyte (Goto and Iritani, 1992) and is also characterized by cytoplasmic maturation and enhanced maturation of the nucleus (Mulyawan, 1995). The oocytes cultured in vitro for 28 hours showed cumulus expansion was not different between the treatments (P >0.5), respectively 90.24%, 96.77% and 99.22% cumulus expansion rate of 3 for without ITS, 5 ng/ml ITS and 10 ng/ml ITS (Figure 1.).

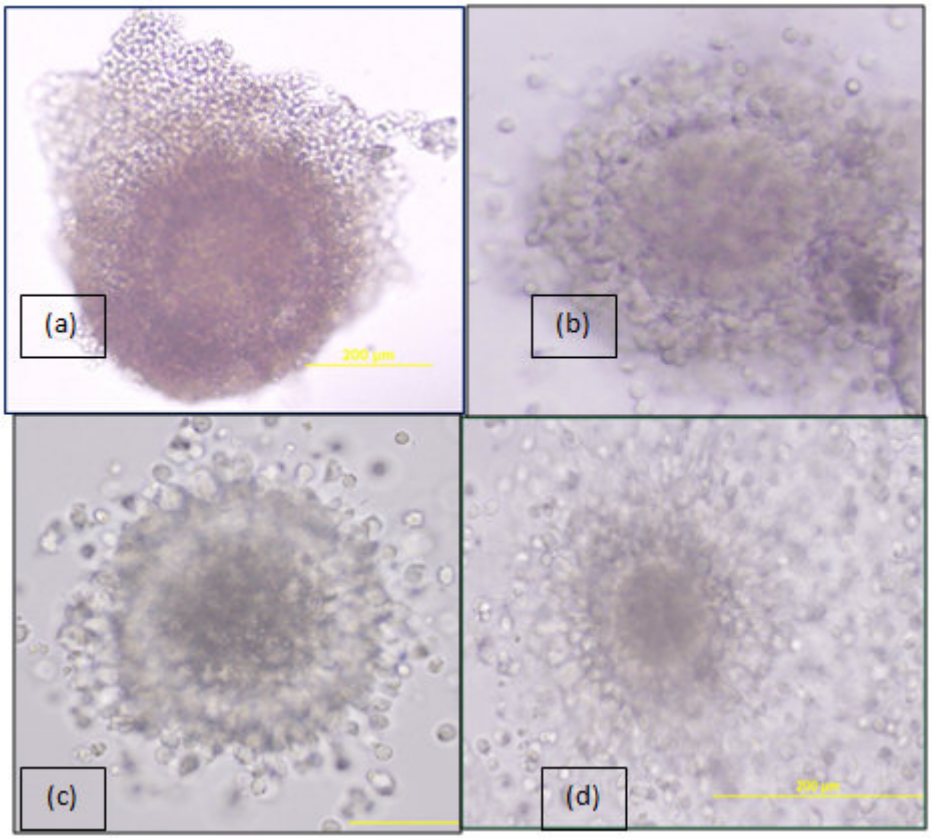

Figure 1. The view grade of expansion cumulus of oocyte culture in vitro for 28 hour. (a) Oocyte before in vitro maturation; (b) Grade 1 was no expansion or only some cumulus cells remain attack to the cumulus; (c) Grade 2 was partly expanded (sligty expanded cumulus with scare intercellular matrix); (d) Grade 3 was fully expanded (widely expanded cumulus with loads of elastic intercellular matrix.

All of the oocytes in this research showed expansion. This was possible because the culture medium of the treatments were αMEM basic medium contained a number of amino acids and water-soluble vitamins such as vitamin B group, choline, folic acid, inositol, and glucose. These vitamins play an important role in energy metabolism, helping intermediate amino acid which is very useful for cell growth and energy metabolism, especially when entering the TCA cycle that produces energy ready for growth, and to maintain other energy cell

(Djati, 2006). The amino acids, vitamins, nucleic acid precursors, are the important ions corresponding to the growth of oocytes and embryos in vitro (Gardner and Lane, 2000). Cumulus cells, included of granulosa cells (GCs) surrounding oocytes, supply the nutrition for the growth of the oocytes. The cumulus will be expanded as the oocytes have grown (Chian *et al*., 1995), and is affected by gonadotrophin hormone. The expansion of the GCs could increased the succesfull of fertilization and embryo development. FSH and LH have a great influence on the oocyte maturation (Squires, 2003; Chian *et al.*, 2004; Assidi *et al*., 2013). After evaluation of cumulus expansion, the oocytes were examined of nuclear maturation.

Nuclear maturation

In this study the three treatments showed perfect cumulus expansion(> 90%.). There was a positive correlation (r = 0.58) between cumulus expansion and the emergence of PB I (Ciptadi, 2012). In mammals, PB I can only be observed on average 50% with M-II configuration, but it does not mean that there are no mature oocytes (Gordon, 1994). The nuclear maturation process classified Germinal Vesicle (GV), Germinal Vesicle Break Down (GVBD), Mataphase-I and Metaphase II (M-II) (Ciptadi, 2009). The effect addition of ITS on nuclear maturation of goat oocyte could be seen in Table 1.

Table 1. Effect addition of ITS on nuclear maturation of goat oocyte

Treatment ITS	n	Stage of nuclear maturation (%)			
		GV	GVBD	M-I	M-II
0 ng/ml	118	11,02[a]	21,19[b]	29,66[b]	38,14 [a]
5 ng/ml	114	5,26[a]	7,89[a]	25,44[ab]	61,40[b**]
10 ng/ml	116	4,31[a]	5,17[a]	18,97[a]	71,55[b**]

[a,b,c,d] values with different superscripts within the same column are significantly different (P<0.05*) and (P<0.01**)

The experiment result showed that ITS supplementation in the medium gave higher oocytes maturation. ITS could increased glutathione (GSH) concentration in oocytes in pigs.(Jeong, *et al*., 2008). Glutathione is a thiol tripeptide component in all cell types, and has a important role in the ransportation of amino acid, the synthesis of the protein and DNA , and the reduction of disulfide bonds (Meister and Anderson, 1983). So, insulin is a polypeptide hormone which increases glucose and amino acid up take and has a mitogenic effect (Spicer and Echternkamp, 1995). And Transferrin (Tf) and selenium (Se) are trace elements and antioxidants system (Wu, *et al*., 1973) dan Gutteridge (1986). In culture cell system, sodium selenite protect cell from oxidative damage, free radicals and obstructed lipid perioxide products (Ebert, *et al*., 2006; Tatemoto, *et al*. 2004). Tf and Se could have a role in the antioxidant defense system in the oocyte, which is essential for catalytic activity of glutathione peroxidase (Cerri, *et al.*, 2009). Hasbi (2010) studied the supplemented of 1 mM GSH in maturation sheep oocytes medium and the result showed that it increased the nuclear maturation of M-II stage. Yaday, *et al.(* 1997) and Shamsuddin, *et al*. (1993) reported that the stage M-II of nuclear maturation is the optimal time for undergoing fertilization and development as an embryo (Hananel, *et al*., 2007). Therefore, ITS can be used as a supplement for medium cultured mouse (De La Fuente, *et al.*, 1999), goat (Herrick, *et al.*, 2004), and pig oocytes (Jeong, *et al.*, 2008) because ITS enhanced the oocytes development (Eppig, *et al*., 1992).

Conclusion

This observation indicated that supplementation of ITS into medium of culture of goat oocytes *in vitro* was effectively enhances oocyte nuclear maturation.

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