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The Respond of Immature Oocytes from Prepubertal Indonesian Goat After Estrus Goat Serum (EGS) Supplementation On *In-vitro* Maturation Rate.

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ABSTRACT

The aims of this research were to evaluate in vitro maturation of oocytes from prepubertal goats obtained from a local slaughterhouse. In this work oocytes were matured in vitro to determine the effect supplementation of Estrus Goat Serum (EGS) in TCM 199. The immature goat oocytes were aspirated from follicles of 2.0 to 6.0 mm in diameter using 10 ml syringe and 18G needle. Oocytes were matured for 26 hours at 39^{o}C and 5% CO_2 with maximum humidity. Results showed that the percentage of cumulus expansion were 32,7% (TCM 199), 80,1% (TCM+10% EGS), 81,8% (TCM+15% EGS) and 83,1% (TCM+20% EGS) respectively. Meanwhile, the percentage of nuclear maturation of M-II were 26,5%, 61,2%, 80,8%, and 81,3%. The percentage of nuclear maturation (M-II) was also significantly different ($p<0.05$). The level of EGS supplementation resulted in significantly different maturation rate. As well as the EGS concentration increased, the higher maturation rate was also observed. The highest maturation were obtained with 15% and 20 % EGS, but the difference is not significant. It was concluded that immature oocytes from prepubertal goat supplemented with EGS were a significant effect to their maturation rate, both base on cumulus expansion and nuclear maturation.

Keyword: Serum, oocyte, maturation, local goat, prepubertal.

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INTRODUCTION

The assisted reproductive technologies (ART) such as in vitro production (IVP) of embryos is widely used to solve various problems in the field of animal reproduction and fertility. The IVP comprises, at least, tree important protocols of in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) of embryos.

In vitro fertilization (IVF) was performed to produce embryos by using oocytes isolated from the ovaries of live animals or selected oocytes from slaughtering houses. IVF process begins with the process of in vitro maturation (IVM). The development of oocytes to reach maturation is influenced by many factors such as oocyte quality and maturation medium [1,2]. This condition causes the medium to be used in IVM, as much as possible to have a condition similar to in vivo conditions so that the gap junctions between oocytes and surrounding cumulus cells remain intact.

The oocyte quality is very important and involved in the development competence of embryos. In its development to reach maturation, oocyte need protein, hormones and growth factors. This causes the necessary improvement of the quality culture media that can be done with the addition of proteins and hormones [3]. Besides the addition of proteins and hormones, can also be done with the addition of serum. The serum is known to contain a variety of essential components such as hormones, growth factors, and other components, which can increase the success of oocyte maturation. Various types of serum that had been used as supplementing the maturation media, among others, fetal bovine serum (FBS) [4], estrus sheep serum (ESS) [5], bovine serum albumin (BSA) and fetal calf serum (FCS) [6,7]. The use of estrus goat serum (EGS) as the IVM medium supplemented on various studies show positive results, but the results are different between one and the other researchers. This condition can be caused by different methods, skills, and other external factors. This study was conducted to determine the effect of supplementation EGS in IVM medium on the oocyte maturation of prepubertal goat namely Peranakan Ettawa (PE) as one on the most important local Indonesian goat.

MATERIAL AND METHODS

Oocyte collection

Ovaries were collected from Slaughter House, a Malang city. Briefly, ovaries were collected in bottles containing 0.9% sterile saline (200 ml) added with penicillin 0.06 gr/200 ml and streptomycin 0.1 gr/200 ml and were taken using a box with a 38^{o}C of temperature. Aspiration and selection of oocytes using a modified phosphate buffered saline (mPBS). Aspiration performed on follicles with 3-6 mm of diameter using 10 ml syringe with needle 18 G. Follicles fluid which contain oocytes selected under a stereo microscope using microhematocrit. Oocytes were chosen for culture is a good quality oocytes surrounded by more than 2 layers of cumulus cells and compact [7].

Maturation media

Maturation medium using Tissue Culture Medium 199 (TCM-199) that supplementation with serum, i.e. estrus goat serum (EGS). The concentration of EGS supplemented in TCM-199 was 0%, 10%, 15% and 20% **respectively.**

Oocyte maturation

Oocytes which have been selected was cultured in the medium drop of 100 µl. Medium drop then covered with paraffin oil and put in a 5% CO_2 incubator at a 39^{o}C of temperature and matured for 26 hours. Variables observed were the development of cumulus cells observed through a cumulus cell expansion and nuclear maturation of M-II. The nuclear maturation was observed by aceto-orcein staining base on maturation phase which are categorized as the germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase 1 (MI) and metaphase 2 (M-II).

Data analysis

The research data obtained were tabulated with Microsoft Excel 2003 and analyzed using SPSS 2.0 by ANOVA followed by Duncan different test.

RESULT AND DISCUSSION

Effect of supplementation EGS on cumulus cell expansion

The supplementation of EGS in medium TCM showed the significant effect (p<0,05) against expansion level of cumulus cells (Table 1). Results showed that the percentage of oocytes that reached the expansion level 3, tend to higher in medium supplemented EGS, rather than medium without supplementation of EGS. The percentage of oocytes that reached the expansion level 3 in each treatment is 32,7% (TCM), 80,1% (TCM+10% EGS), 81,8% (TCM+15% EGS), and 83,1% (TCM+20% EGS). This condition indicates that the medium with supplementation EGS, it can support the process of oocyte maturation, which can be seen with the expansion of cumulus cells. Meanwhile, in a medium with EGS supplementation, supplementation with 20% EGS has the highest percentage of the results is 83.1%, but statistically, the percentage value of the expansion 3 on medium supplemented EGS (10%, 15%, 20%) did not differ significantly. Meanwhile, the cumulus cell expansion level 1 and level 2 are found at most in the medium TCM treatment, respectively 24% and 43.3%.

Cumulus cell expansion is still used as a reference in determining oocyte maturation. This condition is due to the expansion of cumulus cells in the process of in vitro oocyte maturation, development can be seen directly. According to (8) the rate of expansion of cumulus cells can be used as an indicator of oocytes reaching M-II stage. Cumulus cells during oocyte maturation process serve as gap junctions, which plays a role in the transfer of nutrients that support the nuclear maturation. In addition, the cumulus cells play an important role in assisting the development of oocyte maturation [9,10], as a mediator transport energy, and micronutrients which play a role in the oocyte cytoplasmic maturation during the process of oocyte maturation. Cumulus cells also play a role in the complex mechanism that involves intracellular communication oocyte and somatic cells during the process of maturation [11].

Table 1: Effect of supplementation EGS to expansion level of cumulus cell

Treatment	Number of oocytes	Expansion level of cumulus cell		
		Expansion 1	Expansion 2	Expansion 3
TCM-199	150	36 (24%)^{b}	65 (43,3%)^{b}	49 (32,7%)^{a}
TCM-199+10%EGS	161	14 (8,7%)^{a}	18 (11,2%)^{a}	129 (80,1%)^{b}
TCM-199+15%EGS	159	15 (9,4%)^{a}	14 (8,8%)^{a}	130 (81,8%)^{b}
TCM-199+20%EGS	154	11 (7,1%)^{a}	15 (9,7%)^{a}	128 (83,1%)^{b}

The notation letters (a, b, c) are different on the same column, showed significantly different (p <0.05)

Effect of supplementation EGS on nuclear maturation

The results indicate that supplementation of EGS in medium TCM has a significant effect (p <0.05) on the development of nuclear maturation (M-II stage) in prepubertal goat oocytes. In Table 2 presented the percentage of oocytes that reach each stage of maturation of the nucleus. The total percentage of oocytes that are at GV stage, GVBD, and MI most are found in the treatment with TCM medium, with a percentage respectively 6.1%, 24.5%, and 42.9%. Meanwhile, the percentage of the value of M-II stage are respectively 26.5% (TCM), 61.2% (TCM + 10% EGS), 80.8% (TCM + 15% EGS), and 81.3% (TCM + 20% EGS). The percentage of oocytes reaching M-II on TCM treatment significantly different with TCM + 10% EGS, and significantly different to TCM + 15% EGS and TCM + 20% EGS. Meanwhile, the percentage of oocytes reaching M-II between TCM + 15% EGS, and TCM + 20% EGS were not significantly different. This condition shows that supplementation EGS after supplementation 15% gave no significant results (not significant) to increase the number of oocytes reaching M-II stage.

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Table 2: Effect of different EGS supplementation on maturation response rate of oocytes in vitro

Treatment	Number of Oocyte	Maturation rate (nucleus)			
		GV	GVBD	M-I	M-II
TCM-199	49	3 (6,1%)[a]	12 (24,5%)[ab]	21 (42,9%)[a]	13 (26,5%)[a]
TCM-199 +10%EGS	129	6 (4,7%)[a]	15 (11,6%)[b]	30 (23,3%)[b]	79 (61,2%)[b]
TCM-199 +15%EGS	130	3 (2,3%)[a]	8 (6,2%)[ab]	14 (10,8%)[a]	105 (80,8%)[c]
TCM-199 +20%EGS	128	4 (3,1%)[a]	5 (3,9%)[a]	15 (11,7%)[a]	104 (81,3%)[c]

- The notation letters (a, b, c) are different on the same column, showed significantly different ($p < 0.05$)
- GV (germinal vesicle), GVBD (germinal vesicle breakdown), M-I (metaphase 1), M-II (metaphase 2)

Supplementation of EGS on maturation culture medium intended to create environmental conditions as closely as possible to the conditions in vivo in order to support the process of oocyte maturation. This condition is caused during the maturation process of the nucleus, oocyte requires hormones, proteins, and growth factors to support metabolism and in vitro development [12]. The results obtained in this study indicate that supplementation EGS in medium TCM is proven to increase goat oocyte maturation. This condition indicates that the hormones, proteins and other components contained in EGS can support the development of oocytes reaching M-II stage. According to [13], serum has a wide variety of content, such as proteins, fatty acids, vitamins and growth factors. In addition, the serum also serves in transport proteins, carrying hormones, minerals, trace elements, spreading factors, stabilizing and detoxifying factors [14]. Increased oocyte maturation in medium TCM with EGS supplementation in this study are consistent with the results [15] which showed increased rates of oocyte maturation by supplementing estrus buffalo serum (EGS) in medium TCM. Results of research conducted by [16] in oocytes of cattle and the sheep oocytes (5) showed that the serum on the maturation medium increases the rate of oocyte maturation. Increased oocyte maturation in medium with serum EGF supplementation may be caused by the hormones and other growth factors it contains. This condition is caused by EGS is a serum which taken from animal estrus, where according to [17] that the serum collected from estrus animal gave a good response to the level of maturation. According to [14] also states that serum plays a role in providing hormonal factors that play a role in supporting the growth and proliferation of cells. But the effectiveness of serum in the IVM can vary due to the variation of existing components such as hormones, growth factors, proteins, amino acids and other factors [18].

CONCLUSION

Supplementation of estrus goat serum (EGS) in medium TCM-199 showed a significant effect on the increase in oocyte maturation of prepubertal oocytes of local Indonesia goat. The highest percentage of oocytes reaching the stage of M-II present in 20% EGS supplementation, even though it is not significantly different with the percentage at 15% EGS supplementation. Prepubertal oocytes of local Indonesian goats were competence to reach complete maturation in vitro.

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