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The morphological measurement of immature oocyte obtained from follicle different size in Indonesian local goat

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Abstract

The purpose of this study is to determine the diameter variations of immature oocytes and cumulus oocyte complex quality from different size of Indonesian local goat follicle. The ovaries obtained from slaughterhouse. Follicle diameter classified into 3 groups: small (<3 mm), medium (3-5 mm), and large (>5 mm). Oocytes was aspirated from follicles and it was divided into 3 groups based on the oocyte diameter: group 1 (<160.5 µm), group 2 (160.5-170 µm), and group 3 (>170 µm) and observe the quality of cumulus oocyte complex. The results indicate that size of follicles significantly affect to oocyte diameter ($p<0.05$). Large size follicle produces oocytes with a large diameter. The diameter of oocytes significantly correlated to the quality of oocyte cumulus complex ($p<0.05$). It was concluded that the follicle size significantly affect to the oocyte diameter and oocyte cumulus complex quality.

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Introduction

The quality of the immature oocyte is determined by the quality of cumulus oocyte complexes (COCs) and oocyte diameter (Arlotto *et al.*, 1996), but the result of aspiration of ovarian follicles are known have varying quality of cells (Lucas *et al.*, 2002). The specific cause of the variation oocyte quality is unknown, but may be caused by a variety of follicle size and oocyte diameter were used, which relates to the process of folliculogenesis.

Folliculogenesis is a process of follicular development in the ovary. During the folliculogenesis process, follicle diameter will be increases caused by an increase of oocyte diameter and increasing the number layers of granulose cells. During oocyte growth, oocyte diameter will change affect by physiological changes in the cytoplasm and nucleus. That changes will lead oocyte to the final developmental competence. Physiological changes in the cytoplasm include protein synthesis (Kastrop *et al.*, 1990) and the distribution of organelles (Szollosi, 1993 *sit* Ledda *et al*, 1999) which is needed in resuming germinal vesicle (GV) to the metaphase II (M-II). Blondin *et al* (1995) showed the development competence of meiotic in cattle, associated with the size of the follicle, where oocytes derived from small follicles showed low capacity to resume meiosis and to develop in the embryonic stage. Pujol *et al* (2004) states that only the specified size of oocytes have the ability to develop after processing in vitro. Research in sheep also showed oocytes from large follicles have a greater opportunity to reach metaphase II (Cognie *et al*, 1998; Lonergan *et al.*, 1994). In cattle and pigs are known, there is a relationship between oocyte diameter with capability development (Lucas *et al.*, 2002; Otoi *et al.*, 1997). In cattle (Lequarre *et al*, 2005; Lonergan *et al.*, 1994) and camels (Khatir *et al*, 2007) are known there is the influence of follicle diameter to the oocyte developmental capability.
However, we have a little information about the relationship between the size of follicles, immature oocytes diameter, and the oocyte quality of Indonesian local goat. So we need to study about the relationship between the quality of immature oocytes from follicles and oocytes of different sizes.

Materials and methods

Ovary Collection

Goat Ovaries were collected from Slaughterhouse Malang City, East Java, Indonesia. The ovary was washed with 0.9% physiological saline and were transported to the laboratory in a container kept at 37^{o}C in physiological saline with antibiotic (0.006 gram penicillin (Meiji, Japan) and 0.01 gram streptomycin (Meiji, Japan).

Diameter follicle and oocyte measurement

Research using a completely randomized design where this research used 359 follicles isolated from 148 ovarian. Research was conducted at the Central Laboratory of Biological Sciences Brawijaya University. Research is divided into two experiment. In the first experiment follicles isolated with surgical blade no. 11 then divided into 3 groups based on diameter: small (<3 mm), medium (3-5 mm) and large (> 5 mm) (Crozet *et al.*, 1995). In the second experiment oocytes aspirated from the all ovarian follicles group (group 1, group 2, and group 3) then grouped based on diameter: group 1 (<160.5 µm), group 2 (160.5-170 µm), and group 3 (> 170 µm). The diameter of follicle size and oocyte were measured with a micrometer eyepiece (Griffin *et al.*, 2006) previously calibrated on a stereo microscope (Olympus, Japan). Quality of cumulus oocyte complexes were analyzed based on layers of granulose cells. There are three categories of cumulus oocyte complexes: category A (good quality), oocytes surrounded by cumulus cells with large quantities (more than 3 layers) and compact with a homogeneous ooplasm; category B (medium quality) cumulus cells surrounding the oocytes or oocytes partially surrounded by less than 3 layers of cumulus cells with homogeneous ooplasm; category C (poor quality) the oocytes not covered by cumulus cells or oocytes surrounded by cumulus cells slightly (Pawshe *et al.*,1994 and Wang *et al.*, 2007).

Data analysis

The data were analyzed using one-way variance

(ANOVA) and continue with Duncan test using SPSS 20 for windows ($p<0.05$).

Results

Correlation between size of follicles with a diameter of oocytes

The diameter of oocyte were measure including zona pellucida (Lucas *et al.,* 2002) (Figure 1). The result show that there is a significant correlation between size of the follicles with a diameter of oocytes ($P<0.05$, Table 1). Oocyte with diameter <160.5µm (group 1) was found in small follicles (<3 mm), medium follicles (3-5 mm) and large follicles (>5 mm) are 52.78%, 18.99%, and 16.13 % respectively. In group 2, the oocytes (160.5-170 µm) were found in medium follicles, small follicles and large follicles are 47.47%, 28.7% and 15.05% respectively. In group 3, the oocytes (>170 µm) were found in large follicles, medium follicles and small follicles are 68.82%, 33.54% and 18.52% respectively.

Table 1. Correlation between size of follicles with a diameter of oocytes.

Follicle Size	Number Oocyte	Oocyte Diameter		
		Group 3 (>170µm)	Group 2 (160.5-170µm)	Group 1 (<160.5µm)
Small (<3 mm)	108	20 (18.52%)[a]	31 (28.70%)[b]	57 (52.78%)[c]
Medium (3-5 mm)	158	53 (33.54%)[b]	75 (47.47%)[c]	30 (18.99%)[b]
Large (>5 mm)	93	64 (68.82%)[c]	14 (15.05%)[a]	15 (16.13%)[a]

Percentages in the same column with different letters, were significantly different ($P< 0.05$).

Correlation between diameter of oocytes with COCs quality

In experiment 2 it is known that there is a significant relationship between the diameter of immature oocytes with COCs quality ($P<0.05$). In Table 2, it can be seen that good quality COCs found in oocyte diameter group 3 (>170 µm) and group 2 (160.5-170 µm) is 60.75% and 61.02% compared to the oocyte diameter group 1 (<160.5µm) is 29.31%. Meanwhile, medium and poor quality COCs are found in oocyte diameter group 1 is 34.48% and 36.21%.

Tabel 2. Correlation between diameter of oocytes with COCs quality.

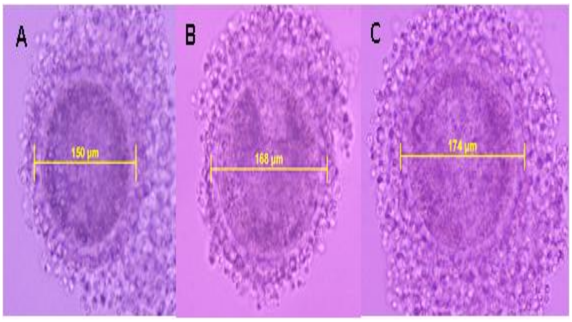
Oocyte Diameter	Number Oocyte	Quality of cumulus oocyte complex (%)		
		Good	Medium	Poor
Group 1 (<160.5µm)	58	17 (29.31%)[a]	20 (34.48%)[a]	21 (36.21%)[b]
Group 2 (160.5-170µm)	177	108 (61.02%)[c]	49 (27.68%)[b]	20 (11.30%)[ab]
Group 3 (>170µm)	107	65 (60.75%)[b]	30 (28.04%)[a]	12 (11.21%)[a]

Percentages in the same column with different letters, were significantly different ($P< 0.05$).

Discussion

The results showed a significant correlation between follicle size and oocyte diameter and between diameter of immature oocytes with COCs quality ($p<0.05$). In Table 1 shows the size of large follicles (>5 mm) produces more oocytes group 3 (>170 µm) when compared with medium (3-5 mm) and small follicles (<3 mm) are respectively 68.82%, 33.54%, and 18.52%. In Table 2 can be seen that in oocytes group 3 (>170 µm), a lot of good quality COCs found when compared with group 2 (160.5-170 µm) and group 1 (<160.5 µm) which is 60.75%, 61.02% and

29.31 %. These results show an association between follicles size and oocyte diameter and correlation between oocyte diameter with COCs quality. This situation because during the process of folliculogenesis, size of follicle will increase as a result of increase of diameter oocytes, increasing the number of layers of granulose cells and the result of the accumulation of follicular fluid.



Oocytes with different diameter

Fig. 1. Oocyte diameter measurement including zona pellucida. A) oocyte group 1 (<160.5 µm), B) oocyte group 2 (160.5-170 µm), C) oocyte group 3 (>170 µm).

In the process of folliculogenesis oocyte diameter growth and will continue to grow after antrum formation until to a certain diameter. According to Diaz *et al* (2007) the growth of the oocyte depend and concurrent with the development of follicular granulose cell layer. During oocyte growth, intracellular physiological changes in the nucleus and cytoplasm cause the oocyte have developmental competence.

This intracellular physiological changes may have a role in resuming the GV to the metaphase II. It is known that during follicular growth, oocyte diameter increases as a result of the accumulation of ions, carbohydrates, lipids and proteins (Heleil & Fayed, 2010; Fair *et al.*, 1997), where this component required in proses resuming meiosis from GV to MII. Research conducted Lucas *et al* (2002) and Blondin (1995) showed that the meiotic competence is closely related to the size of the follicle, where the oocytes derived from small follicles had low ability in the developmental competence after in vitro maturation.
Positive relationship between the size of follicles with meiotic and developmental competence was also reported by Cognie *et al* (1998) who showed that genotypic differences may affect the ability of oocytes to resuming meiosis on in vitro maturation. Research conducted De Smedt *et al* (1994) also showed that only oocytes from follicles larger than 3 mm, which have competence development and can reach metaphase II. The results of these studies, in line with research conducted by Sayuti *et al* (2007) and Griffin *et al.* (2006) who found large follicle will produce large-diameter oocytes. Oocytes with a large diameter, generally have a better quality of COCs and will give good results on oocyte maturation in vitro. The results of this study indicate that the diameter of immature oocytes can be used as selection criteria in the IVM.

Research conducted in pigs (Lucas *et al.,* 2002) and in cattle (Otoi *et al.,* 1997) shows that, oocytes with a large diameter resulting in a higher maturation rate when compared to oocytes with a small diameter. In other studies Haque *et al* (2012) and Da costa *et al* (2013) showed that oocytes with good quality COCs have a higher success rate, be it on cumulus expansion, core maturation and embryo development after fertilization. Results of research conducted showed a close relationship between follicle diameter, and diameter of oocytes and its relationship with oocyte quality on the ability of oocytes to develop.
The conclusion of this research are follicle size significantly affect to the oocyte diameter and oocyte diameter affecting on the quality of the oocyte cumulus complexes.

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