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Relationship between the Number and Size of the Follicle on the Oocyte Diameter of the Pig Ovarium

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ABSTRACT

Background: The availability of productive sows slaughtered in commercial and traditional pig slaughterhouses makes it possible to use their ovaries as a source of oocytes, which can be used as raw materials in the *in vitro* Maturation (IVM) process. Oocyte diameter is one indicator that determines the success rate of oocyte nuclear maturation *in vitro*. Scientific information on the relationship between the number and size of the follicles on the diameter of porcine oocytes is still very limited. Therefore, this research was conducted to add data that can later be used for *in vitro* Maturation and *in vitro* Fertilization.

Methods: This research was carried on in 2021; samples were collected from sow ovaries from commercial and traditional pig slaughterhouses in Badung and Tabanan Regency. The aspiration oocyte collection method was carried out at the Embryology Laboratory of the Faculty of Veterinary Medicine, Udayana University. This research was conducted by grouping ovaries into three groups: group 1 is ovaries with small follicles that have a diameter <2 mm, group 2 were ovaries with medium follicles which have a diameter of 3.1-5 mm and group 3 were ovaries with large follicles that have diameter >5 mm.

Result: The results showed that the larger the size of the follicle, the larger the diameter of the oocyte. There is a closer relationship between oocyte diameter and follicle diameter with a correlation value of 1 and has a very strong relationship with the number of follicles (negative relationship, correlation value = -0.703) and with oocyte quality (positive relationship with a correlation value 0.703). Oocyte quality correlated perfectly with follicle diameter and number of follicles (correlation value 1) and quite strongly correlated with oocyte diameter (correlation value 0.703). The close relationship between follicle diameter and the number of follicles on oocyte diameter is shown by the regression equation $Y= 0.848 + 6.359\times1 - 3.797\times2$ and the close relationship between follicle diameter and oocyte diameter on oocyte quality is shown by the regression equation $Y= 0.900+ 0.310\times1 + 0.005\times2$.

Key words: Follicles, Oocytes, Ovaries, Pigs, Pig slaughterhouse.

INTRODUCTION

The pig is a meat-producing livestock commodity with great potential to be developed because it has beneficial properties and abilities, including a fast growth rate, high number of piglets per birth (litter size) and good ration efficiency (70-80)%) and high carcass percentage (65-80%) (Ardana and Putra, 2008) can be developed to meet the needs of meat and animal protein, especially in areas where people consume pork.

Pigs in Bali are in great demand and this can be seen from the population, which by 2021 will reach 409.960 pigs (Directorate General of PKH, 2021). Observing the opportunities and challenges of developing bali pigs rests on integrating five main pillars: breeders, traditional villages, local governments, entrepreneurs and academics. The synergy of the five pillars resulted in a strategy for developing bali pigs to accelerate pig growth faster so that the productivity of raising bali pigs can be increased (Suarna and Suryani, 2015). The availability of productive sows slaughtered in commercial and traditional pig slaughterhouses makes it possible to utilize their ovaries, which are usually discarded but still have oocytes that can be used as raw material in the *in Vitro* Maturation (IVM) process.

Genetic factors are influenced by parents and males (Sumantri and Anggraeini, 1999). The main factor in terms of the environment that affects embryo development is the quality of the oocytes used. The factors that determine the quality of these oocytes are their diameter and morphology (Arlotto *et al.,* 1996). Oocytes in the ovaries of female pigs have different diameters and oocyte diameter is one indicator that determines the oocyte nuclear maturation success rate *in vitro*. Scientific information on the relationship between the number and size of the follicles on the diameter of porcine oocytes is still very limited. Therefore, this research was conducted to add data that can later be used for *in vitro* maturation and *in vitro* fertilization.

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MATERIALS AND METHODS

The oocytes used in this study were sourced from pig ovaries from commercial and traditional pig slaughterhouses in Badung and Tabanan districts. Follicular fluid aspiration was carried out in the Laboratory of Anatomy and

Embryology of the Faculty of Veterinary Medicine, Udayana University. In this study, data in the form of oocytes were obtained from selected follicles and divided into three groups based on their diameter size. Small diameter measuring <2 mm, medium 3-5 mm and large >5 mm. The ovaries are divided into three groups based on the number of follicles. Group 1 had 5 follicles, group 2 had 6-9 and group 3 had 10.

The aspiration procedure is as follows by Sayuti *et al*. (2007). Insert a small amount of sterile physiological NaCl into a disposable syringe, then insert the needle around the follicle and aspirate/aspirate; insert it into a petri dish with a diameter of 60 mm × 15 mm. The liquid looks yellow. Avoid follicles that contain blood (black follicles) because they will contaminate all the liquid in the petri dish. Add a new physiological NaCl solution to the aspirated follicular fluid, then wash it slowly. After washing, the oocytes were put back into a 60 mm × 15 mm petri dish and added physiological NaCl, then observed under a binocular microscope to see the morphology of the oocytes.

Oocyte morphology (quality of oocyte) was observed under a binocular microscope based on the classification of Lonergan (1992), namely: Complete there are cumulus oophorus cells, consisting of more than three thick layers (5 layers), the oocytes look compact, Expanded there are cumulus oophorus cells, consisting of 3 (3-5) thick layers, with one part not intact, Partial there are only two layers of cumulus oophorus cells, Nude there are no cells surrounding the oocyte, the oocyte is only surrounded by the zona pellucida evenly. This study used a completely randomized design. The effect of follicle size on oocyte diameter was analyzed using simple regression and correlation analysis. In contrast, the effect of follicle size and number on oocyte morphology was analyzed using a one-way analysis of variance. The measurement of oocyte diameter was carried out using the Image J application (https://imagej.nih.gov/ij/download.html.). Data analysis was carried out with SPSS version 23 software.

RESULTS AND DISCUSSION

The data of relationship and the closeness between variables used a partial correlation was a perfect relationship (Table 1). There was a perfect relationship between oocyte diameter and follicle diameter (correlation value 1) and a very strong relationship between the number of follicles (negative relationship, correlation value -0.703) and oocyte quality (positive relationship, correlation value 0.703) (Table 1). This study is in accordance with the results of the study of Sayuti *et al*. (2007), which stated that oocyte quality (morphology) was perfectly correlated with follicle diameter and the number of follicles (correlation value 1) and quite strongly correlated with oocyte diameter (correlation value 0.503). Factors that can affect the number of oocytes obtained are temperature and storage time of the ovaries, as well as quality, follicle size and oocyte diameter (Arlotto *et al*., 1996; Gordon, 2003). Oocytes with a larger diameter will have a greater ability to reach meiosis I and oocytes from larger follicles have a greater chance of reaching metaphase II than oocytes from smaller-diameter follicles. This result can be seen in studies using oocytes from different animals, such as sheep oocytes (Lonergan *et al*., 1992) and bovine oocytes derived from small antrum follicles that have a low ability to undergo germinal vesicle breakdown (GVBD) and metaphase I (Pavlok *et al*., 1992). Lonergan *et al*. (1992) stated that the diameter of the oocyte has a strong relationship with the quality of the oocyte produced. This study found that the size of the follicle is directly proportional to the diameter of the oocyte, where the larger the size of the follicle, the larger the diameter of the oocyte. Gordon (2003) reported that several factors could affect the number of oocytes obtained, namely the temperature and storage time of the ovaries and the quality and size of the follicles. Studies in sheep have shown that the meiotic ability of oocytes occurs progressively during follicular growth.

The close relationship between follicle diameter and the number of follicles on oocyte diameter is shown by the regression equation Y= 0.848 + 6.359X1 – 3.797X2 (Table 2). The close relationship (r) between follicle diameter and the number of follicles and oocyte diameter was 0.848 (close to 1). The direction of the positive relationship between the diameter of the follicle and the diameter of the oocyte indicates that the larger the diameter of the follicle, the larger the diameter of the oocyte tends to be. Meanwhile, the number of follicles on the oocyte diameter has a negative relationship, which indicates that the more follicles, the smaller the oocyte. According to Gordon (2003), the size of the ovary does not affect the number of oocytes produced. However, the number and quality of oocytes are influenced by the number of follicles in the ovary. According to Martino *et al*. (1994), 3-6 mm medium-sized follicles produced good oocyte quality, which was higher than medium-sized follicles. This was due to the increase in oocyte quality in large follicles caused by the intrafollicular environment, which could improve oocyte quality. Changes in intracellular conditions that occur in growing oocytes also affect oocyte diameter. Changes in intracellular conditions that occur in oocytes can be in the form of accumulation of ions, carbohydrates and an increase in the number of organelles such as ribosomes and mitochondria, which also affect the increase in oocyte volume and diameter (Fair *et al*., 1997; Hyttel *et al*., 1997;

Table 1: Relationship between variables of oocyte diameter, follicle diameter, number of follicles, and oocyte quality.

	Variable		Diameter follicles	Number of follicles	Quality of oocyte
Diameter of oocytes	Diameter of follicles	Correlation	1.000	-0.703	0.703
	Number of follicles	Correlation	-0.703	1.000	-1.000
	Quality of Oocyte	Correlation	0.703	-1.000	1.000

Johnson and Everitt, 2007). Some oocytes are able to continue meiosis, or germinal vesicle break down (GVBD) during antral follicles with a diameter of 0.5-0.8 mm and can reach metaphase I in follicles reaching a diameter of 1.0-1.8 mm (Crozet *et al*., 1995) and progresses to metaphase II in follicles larger than 3 mm (Mermillod *et al*., 2000). The proportion of oocyte complete nuclear maturation increases markedly as the follicle enlarges from 2-5 mm. The oocyte reaches full development in follicles larger than 5 mm (Crozet *et al*., 1995). Arlotto *et al*. (1996) have examined the relationship between oocyte diameter and follicular growth. The oocyte continues to grow after the formation of the antrum, which involves oocytes derived from follicles measuring 10-15 mm in diameter. The difference in the diameter of oocytes from large and small follicles is about 5%. Differences in oocyte diameter will affect the development of oocytes from early development to blastocyst. At the maturity level of Metaphase-II (MII), oocytes with a diameter >120 m had the highest percentage and the lowest was <110 m. This is because oocytes with a diameter of <110 m have a low percentage of reaching the MII stage and have not been able to reach the MII stage optimally and it takes a long time for oocyte development. Oocytes with a diameter of >120 m are more competent to reach the stage of meiosis II and are ready to be fertilized, have a higher ability to reach the M-II stage and can be collected from follicles measuring 3-4 and > 4 mm (Ferreira, 2009). Oocytes with a diameter of <110 m cannot completely synthesize maternal RNA 23 and some essential proteins, so they cannot reach the M-II stage (Otoi *et al*., 1997). Ledda *et al*. (1999) also stated that the ability of oocytes to develop to reach metaphase II is influenced by oocyte diameter. The results of other studies regarding the ability of oocyte development in pigs (Lucas *et al*., 2002), cattle (Lequarre *et al*., 2005) and camels (Khatir *et al*., 2007) showed that there was an effect of oocyte diameter on the ability of oocyte development. These conditions indicate the criteria for oocyte selection for IVM purposes, in addition to looking at cumulus cells. It is also necessary to consider the size of the oocyte diameter (Arlotto *et al*., 1996; Lucas *et al*., 2002).

The close relationship between follicle diameter and oocyte diameter on oocyte quality is shown by the regression equation Y= 0.900+ 0.310×1 + 0.005×2 (Table 3). The close relationship (r) between follicle diameter and the number of follicles and oocyte diameter is 0.900. This shows a very close relationship (close to 1), where the larger the follicle's diameter and the oocyte's diameter, the better the quality of the oocyte. The diameter of the oocyte affects the expansion ability of the cumulus cells. This condition can be seen from the number of oocytes that reach level 1 expansion, which increases with the increase in oocyte diameter. These conditions indicate that between groups of oocyte diameters have different developmental competencies, which indicates the differences in intracellular physiological conditions. It is stated that during the oocyte growth process, it undergoes intracellular modification (Ledda *et al*., 1999; Harris *et al*., 2015). Oocytes with a larger diameter will have a greater ability to reach meiosis I and oocytes from larger follicles have a greater chance of reaching metaphase II than oocytes from smaller-diameter follicles. Pavlok *et al*. (1992) also stated that oocytes obtained from small-diameter follicles resulted in significantly lower maturation capacity when compared to oocytes obtained from larger follicles. collected. Follicular size and oocyte diameter have a close relationship and as both increase, the developmental ability of the oocyte also improves (Gandolfi *et al*., 2005). Increased oocyte quality in large follicles is also caused by the follicular intrafollicular environment, which can improve oocyte quality. The follicular intrafollicular environment refers to steroid and peptide hormones, growth factors, cytokines and other molecules that may act singly or combine with one or more other factors that influence oocyte and follicular development. The intrafollicular environment of the dominant follicle is

Table 2: The relationship between the number of follicles and the diameter of the follicle on the diameter of the oocyte.

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Constanta	0.848	12.619		8.343	0.000
Diameter of follicle	6.359	1.448	0.701	4.391	0.000
Number of follicles	-3.797	3.771	-0.161	-1.007	0.318

Dependent variable: Diameter of oocyte.

Table 3: Close relationship between follicle diameter and oocyte diameter on oocyte quality.

Model	Unstandardized Coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
Constanta	0.900	0.436		0.714	0.478
Diameter of follicle	0.310	0.041	0.807	7.472	0.000
Diameter ofoocyte	0.005	0.005	0.109	1.007	0.318

Dependent variable: Quality of oocyte.

dominated by estrogen, the compact cumulus cells associated with the oocyte (Lonergan *et al*., 1992; Assey *et al*., 1994). Meanwhile, subordinate follicles are characterized by the dominance of progesterone and degenerated cumulus cells. Oocytes will develop rapidly *in vivo* in pigs if the theca cell layer and granulosa cell layer are more complex and this will only be obtained from larger follicles (King *et al*., 1991).

CONCLUSION

The results showed that the size of the follicle and the diameter of the oocyte have correlated. There is a perfect relationship between oocyte diameter and follicle diameter with a correlation value of 1 and it has a very strong relationship with the number of follicles (negative relationship, correlation value = -0.703) and with oocyte quality (positive relationship, correlation value 0.703). Oocyte quality correlated perfectly with follicle diameter and number of follicles (correlation value 1). The close relationship between follicle diameter and the number of follicles on oocyte diameter is shown by the regression equation Y= 0.848 + 6.359X1 -3.797X2 and the close relationship between follicle diameter and oocyte diameter on oocyte quality is shown by the regression equation Y= 0.900 + 0.310X1 + 0.005X2.

Conflict of Interest: None.

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