The Correlation between the Weight of Ovary, Number of Follicles, and Quality of Oocytes of Culling Female Bovine in Slaughterhouse

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ABSTRACT

The purpose of this research was to determine the correlation between the weight of the ovary, the number of follicles, and the quality of the oocytes from the culling female bovine obtained from the slaughterhouse. The variation may affect the potential of the ovary to produce follicles. Paired ovaries were obtained from female bovines. Follicle numbers were recorded and divided into three size categories (small: 3 mm, medium: < 3 mm-8 mm, and large: > 8 mm). Oocytes were aspirated and the number of oocytes was recorded and graded into four categories (grades A, B, C, and D). There was a positive correlation between the weight of the ovary and the number of follicles, which is 0.560 with the regression equation y = 3.52 + 0.501 x. There was a positive correlation between the number of follicles and the number of oocytes, which is 0.546 with the regression equation y =2.48 + 1.204 x. There was a positive correlation between the number of oocytes and the grade of oocytes, which is 0.520, with the regression equation y = 0.93 + 0.800 x. There was no correlation between the weight of the ovary and oocyte grade A, which is 0.013.

Keywords: Bovine, Correlation, Follicle, Oocyte, Ovary.

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INTRODUCTION

Reproduction is a biological process to produce new individuals or offspring. Reproduction is the basic way to produce the next generation. However, the ability of the new generation of individuals to survive in nature is determined by the qualities of the oocytes and uterine environment during prenatal growth. Therefore, improving the quality of oocytes and the environment during gestation can lead to the production of quality offspring. Oocytes themselves are important components in various aspects of female reproduction, including the development of follicles and early embryogenesis (Moussa et al., 2015).

Reproductive biotechnology is an important tool for grading the genetic quality of livestock. The development of this technology has economic benefits that include a potential increase in the quantity of offspring or the protection of the gene pool of infertile livestock. There is an impact on livestock productivity and diseases due to various factors (Kappes et al., 2023). The use of biotechnology in reproduction has provided a wide range of research, treatments, and phenomena both in vitro and in vivo. The most common reproductive technologies are artificial insemination (AI), embryo transfer (ET), preservation of semen, and in vitro maturation (IVM). This technology involves the IVM of oocytes, which is further fertilized by a superior sperm (Britt, 2008).

In the bovine, the estrus cycle consists of two phases, the follicular phase and the luteal phase. At the same age, bovines have different size variations of ovary in both the follicular phase and the luteal phase. The size of the ovary, which contains follicles, should be optimal in order to get good fertility (Mossa et al., 2012). The follicle surrounding the oocyte is the fundamental reproductive unit of the ovary. The cortex of young female bovines consists mainly of follicles, while in mature female bovines most of these follicles are replaced by fibers as a consequence of ovulation or atresia. As the follicle grows in size, oocytes travel deeper into the follicle as the time of ovulation approaches (Mohr-Sasson et al., 2020).

It is important to understand the meaning of oocyte quality. From this perspective, oocyte quality may be consistent with their developmental ability. There are five levels of oocyte development. i.e., the ability to: resume meiosis; divide after fertilization; develop the blastocyte stage; gestation; and develop a healthy period (Sirard et al., 2006). The intrinsic quality of the oocyte is one of the major factors affecting early embryogenesis (Aguila et al., 2020), and embryo culture conditions play an important role in determining blastocyst quality (Rizos et al., 2002). The oocyte grading technique is an easier and more objective way to evaluate complex oocyte cumulus cells. The presence of cumulus cells supports the maturation of the oocyte to the metaphase II stage and the maturation of the cytoplasm, which is necessary for the ability to develop after fertilization (Abeydeera, 2002).

Successful in vitro fertilization (IVF) is highly dependent on oocyte quality. It is important to provide good-quality oocytes.

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The oocytes are derived from follicles. Based on this background, it is necessary to conduct research on the relationship between the weight of the ovary, the number of follicles, and the quality of the oocytes of female bovines. Therefore, through this research, it is expected to know the correlation between the weight of the ovary, the number of follicles, and the quality of the oocytes of the culling female bovine in the slaughterhouse.

MATERIALS AND METHODS

Research Design

This research involves 100 ovaries from 50 culling female bovines that had been slaughtered at Slaughterhouse Surabaya. Parameters were the weight of the ovary, the number of follicles, the number of oocytes, and the quality of the oocyte. This experiment was conducted at the Laboratory of In-Vitro Fertilization, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. The ovaries had been collected directly after slaughtering at Slaughterhouse Surabaya. This experiment was conducted in April 2023.

Collection of Ovary

The left and right ovaries were excised. The ovary was placed into plastic containing phosphate-buffered saline (PBS) supplemented with 1 ml of penicillin/streptomycin (10 000 IU/ml/10 mg/ml) (Yu et al., 2009). Then the ovary was placed in a thermos flask. This was done so that the temperature of the ovaries remained stable during the trip (Wang et al., 2007). In the laboratory, the ovary was taken out of the thermos. The excessive tissue was trimmed out and the ovary was cleaned with physiological NaCL. The clean ovary was placed in a water bath at 37°C (Mytton et al., 2017).

Collection of Oocytes

Excessive tissues attached to the ovaries were carefully trimmed off and the ovaries were weighed using an electronic scale (Kouamo et al., 2014). The collection of oocytes was carried out using the follicle aspiration method with a diameter ranging from 2 to 5 mm (Georgiou et al., 2018). Aspire uses an 18-gauge needle connected to a 3 ml plastic syringe containing (1.0-1.5 ml) of PBS solution. The collected oocytes were then placed in a petri dish and observed under a light microscope at a low magnification of 10x to get oocytes in the desired category (Rao and Mahesh, 2012).

Determination of Follicular Number

For each ovary, visible follicles were counted and measured with electronic Vernier calipers. Follicular was classified into three categories (Acar et al., 2013):

Small	= (< 3 mm)
Medium	= (3 to 8 mm)
Large	= (> 8 mm)

Examination of Oocytes Quality

Oocytes that were collected were then divided into four groups, namely grade A, B, C, and D, based on the cumulus cell layer and its cytoplasmic representation, according to Bakri et al. (2016). Oocytes are graded into four groups:

Grade A, if covered by more than five layers of cumulus cells with cytoplasm that is homogeneous. The percentage of Grade A oocytes was obtained from a comparison of grade A oocytes to the total oocytes obtained multiplied by 100%.

Grade B, if it surround oocytes by less than five layers of cumulus cells with

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homogeneous cytoplasm. The percentage of Grade B oocytes was obtained from a comparison of Grade B oocytes to the total oocytes obtained multiplied by 100%.

Grade C is still visible with a little cumulus, zona pellucida is still visible, and the cytoplasm is not homogenous. The percentage of Grade C oocytes was obtained from a comparison of Grade C oocytes to the total oocytes obtained multiplied by 100%.

In Grade D, if the oocyte has transparent cytoplasm, the zona pellucida is half visible or absent, and the cumulus oophorous has almost disappeared. The percentage of Grade D oocytes was obtained from a comparison of Grade D oocytes to the total oocytes obtained multiplied by 100%.

Data Analysis

The data obtained from this research were processed using two formulas. A simple correlation coefficient was used to interpret the correlation between the weight of the ovary, the number of follicles, and the quality of the oocytes. A regression formula was used to interpret the correlation between the weight of the ovary, the number of follicles, and the quality of the oocytes. The results were compared to support the hypothesis. The collected data were tabulated and analyzed statistically with SPSS (Statistical Analysis Software Program

RESULTS AND DISCUSSION

The research that has been conducted is on the correlation between the weight of the ovary, the number of follicles, and the quality of the oocytes of culling female bovine from the slaughterhouse. In this study, 100 bovine ovaries from female bovines were collected from Slaughterhouse Surabaya. Bovine that are slaughtered around the age of 8 years and above and are non-cycling or less reproductive are called culling bovine.

The Weight of Ovary

This research involves 100 bovine ovaries that were collected from slaughterhouses and the weight of each ovary was observed using an electronic scale. The ovaries weight ranges from 2.060 g to 15.586 g, with a mean value of 5.62. The results of the weight of each ovary are presented in Table 1.

Table 1. The result of weight of ovary

Right				Left			
Sample code	**Weight of Ovary (g)**	**Sample code**	**Weight of Ovary (g)**	**Sample code**	**Weight of Ovary (g)**	**Sample code**	**Weight of Ovary (g)**
1	6.368	26	5.318	51	7.191	76	4.693
2	7.327	27	4.273	52	3.220	77	7.128
3	4.267	28	5.929	53	7.423	78	3.599
4	11.800	29	6.331	54	10.344	79	3.653
5	3.048	30	8.249	55	7.474	80	4.823
6	5.638	31	7.293	56	3.822	81	6.261
7	6.407	32	6.081	57	3.490	82	4.937

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Right				Left			
Sample code	Weight of Ovary (g)	Sample code	Weight of Ovary (g)	Sample code	Weight of Ovary (g)	Sample code	Weight of Ovary (g)
8	3.397	33	7.996	58	4.634	83	3.993
9	5.477	34	4.468	59	11.678	84	2.060
10	5.290	35	3.448	60	9.670	85	2.380
11	3.824	36	4.860	61	5.771	86	3.460
12	6.422	37	5.446	62	8.572	87	3.301
13	4.848	38	3.590	63	4.685	88	2.446
14	4.401	39	2.563	64	5.875	89	3.383
15	3.366	40	9.373	65	5.684	90	9.150
16	8.655	41	7.832	66	6.440	91	5.183
17	3.962	42	4.471	67	4.918	92	5.410
18	3.589	43	4.731	68	9.051	93	3.410
19	6.883	44	5.341	69	8.555	94	6.236
20	4.314	45	9.199	70	5.308	95	7.589
21	3.395	46	3.892	71	5.097	96	5.125
22	2.423	47	9.966	72	3.856	97	7.873
23	15.586	48	4.290	73	4.531	98	3.226
24	4.594	49	6.901	74	3.649	99	5.741
25	2.906	50	7.341	75	3.218	100	6.432

The results show that the mean weight of the right and left ovaries is 5.72 and 5.51, respectively, with a total mean of 5.62, which is similar to those obtained by Karamishabankareh et al. (2015). The difference may be due to the stage of the estrous cycle, physiological state, or breed. The ovary, with its corpus luteum and follicles state, will increase the size and weight of the ovary (Ginther et al., 2014). It states that the right ovary has a higher mean number than the left ovary. Follicle activity and ovulation are more regular in the right ovary than the left ovary, as supported by the research of Dorice et al. (2019).

Number of Follicles

The number of follicles was counted from each ovary that had a visible follicle. From the calculation, the lowest number of follicles obtained is one and the highest number of follicles obtained is 14, with a mean value of 4.18. The data also stated that 7% of the sample did not contain follicles due to certain factor. The results of the number of follicles are presented in Table 2. The visible follicles were counted based on the size of the follicles (Acar et al., 2013), as presented in Table 3. Ovaries that did not have follicles due to certain factors, as presented in Figures 1 and 2.

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Table 2. The results of number of follicles from each ovary

Right				Left			
Sample code	**Number of follicles**	**Sample code**	**Number of follicles**	**Sample code**	**Number of follicles**	**Sample code**	**Number of follicles**
1	7	26	5	51	6	76	6
2	12	27	3	52	0	77	14
3	4	28	4	53	5	78	4
4	12	29	3	54	13	79	5
5	3	30	4	55	0	80	3
6	5	31	0	56	2	81	0
7	6	32	7	57	3	82	3
8	2	33	8	58	3	83	3
9	5	34	3	59	12	84	0
10	5	35	2	60	10	85	2
11	2	36	4	61	4	86	4
12	5	37	3	62	3	87	0
13	5	38	2	63	1	88	2
14	7	39	2	64	3	89	3
15	3	40	9	65	6	90	12
16	4	41	4	66	4	91	3
17	2	42	6	67	2	92	3
18	5	43	3	68	6	93	3
19	2	44	0	69	3	94	0
20	3	45	4	70	3	95	5
21	5	46	3	71	3	96	7
22	1	47	6	72	3	97	5
23	10	48	4	73	5	98	2
24	3	49	5	74	2	99	3
25	2	50	6	75	4	100	5

Table 3. Measurement and visible follicular number of bovine ovaries

Size of follicles	Visible Follicles Number	Mean
< 3 mm	151	4,24
3mm-8mm	180	4,80

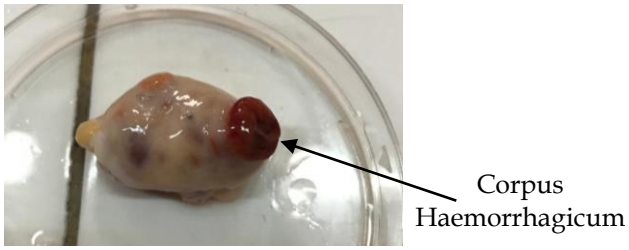

Figure 1. Presence of Corpus Haemorrhagicum

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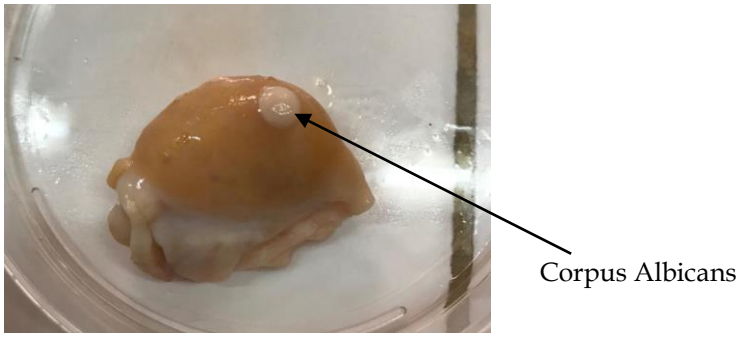

Figure 2. Ovaries that has no visible follicles and presence of Corpus Albicans

The lowest number of follicles per bovine is one and the highest number of follicles is 14, which is comparable to the study of López-Gatius et al. (2022). The difference may be due to the breed or follicular wave before being slaughtered. The hormone that plays a role in follicle development is the follicle stimulating hormone (FSH) (Ginther et al., 2014). At birth, the number of antral follicles is still low and their size is not over 1 mm in diameter. Based on Acar et al. (2013), the size of follicles is divided into three groups (Table 3), which states that follicles in the range of 3 mm to 8 mm have the highest mean number. Seven samples (Table 2) do not have follicle, which may be due to the absorption of follicles back when there is no follicular cycle

Oocyte Grading

Oocytes were aspirated from follicles. The aspirated oocytes were then placed in a petri dish (Figure 3). The oocytes were examined under a microscope with a magnification 100X. Oocytes were graded based on cumulus cell layers into grades A, B, C, and D (Figure 4), according to Bakri et al. (2016). From the calculation, the lowest number of oocytes was one and the highest number of oocytes was six. The percentage value of Grade A oocytes was 59%, while Grade B was 34%, Grade C was 25%, and Grade D was 20%. The results are presented in Table 4.

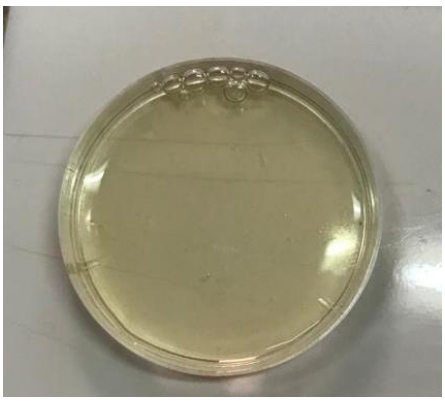

Figure 3. Follicle fluid that contains oocyte

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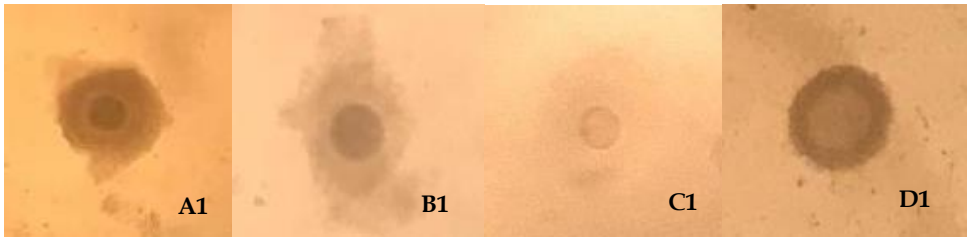

Figure 4. Oocytes grade based on cumulus cell layer ; A1: Grade A; B1: Grade B; C1: Grade C; D1; Grade D

Table 4. The results of number of oocytes and oocyte grade

Sample	Number of Oocyte	Oocyte grade				Sample	Number of Oocyte	Oocyte grade			
		A	B	C	D			A	B	C	D
1	2	1	1			51	1		1		
2	1		1			52	0				
3	0					53	0				
4	3	2		1		54	4	3		1	
5	0					55	0				
6	2		2			56	1			1	
7	2			1	1	57	1				1
8	0					58	0				
9	1			3		59	3	1	2		
10	1		2			60	2			2	
11	0					61	2		1	1	
12	6		3	1	2	62	1	1			
13	2			2		63	0				
14	0					64	1	1			
15	3	2	1			65	4				4
16	2			2		66	0				
17	1	1				67	0				
18	1	1				68	2			2	
19	3	3				69	2		1		1
20	3	3				70	4		1	1	2
21	2	1	1			71	2	1			1
22	1	1				72	1		1		
23	1		1			73	0				
24	1	1				74	0				
25	1	1				75	1	1			
26	3	2			1	76	1	1			
27	0					77	3	2		1	
28	3	2	1			78	0				
29	2		2			79	1	1			
30	0					80	0				
31	0					81	0				

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Sample	Number of Oocyte	Oocyte grade				Sample	Number of Oocyte	Oocyte grade			
		A	B	C	D			A	B	C	D
32	2			2		82	0				
33	1		1			83	0				
34	0					84	0				
35	1	1				85	1	1			
36	0					86	2	2			
37	1			1		87	0				
38	2		1	1		88	1		1		
39	1	1				89	1	1			
40	2	2				90	5	3	1		1
41	1		1			91	2		2		
42	4	3			1	92	1				1
43	2	2				93	1	1			
44	0					94	0				
45	2	1	1			95	2	1	1		
46	1	1				96	3	3			
47	4				4	97	1	1			
48	0					98	1			1	
49	2	1		1		99	0				
50	3		3			100	3	2		1	

Oocytes are aspirated from follicles to obtain the number and grade (Table 4). The number of oocytes that are suitable for IVF (Grades A and B) was higher than Grade C or Grade D. According to Moghadam et al. (2022), Grade A oocytes are characterized by being surrounded by multiple layers of compact cumulus cells and homogenous cytoplasm, whereas Grade B oocytes are surrounded by 2-3 layers of cumulus cells and homogeneous cytoplasm. Variation in the number of oocytes observed in this study is due to bovine noncyclic. Bovines with low reproduction are generally slaughtered. This is why slaughterhouses have noncyclic ovaries, which also contribute to a lower number of oocytes (Landoe et al., 2022).

Correlation between the Weight of Ovary and the Number of Follicles

Based on the data collected using the SPSS program, the Spearmen method, where the correlation coefficient was 0.560 with a significant level of 1%, it showed that there was a significant relationship because it was in the interval 0.40–<0.70 (Hauke and Kossowski, 2022). This showed there was a correlation with each other; the greater the weight of the ovary, the greater the number of follicles, and vice versa.

Based on the result, it was known that the significant value or Sig. (2-tailed) is 0.000; this value is less than <0.005, so it could be concluded that there is a significant relationship between the weight of the ovary and the number of follicles. The result of ANOVA regression shows that the

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two statements have a linear relationship, which can be described by the following equation: Y = 3.52 + 0.501 X. So, the Y intercept is 3.52, because when X = 0.501 and Y = 3.52.

In this study, there is a positive correlation between the weight of the ovary and the number of follicles. Ovary weights vary greatly between bovines due to the corpus luteum (CL) and the presence of preovulatory follicles. The result is strongly supported by previous results by Jerez et al. (2022) who reported that ovaries that are aspirated without CL have a higher number of follicles than those in the presence of CL. These results indicate that the weight of the ovary reflects the number of small and medium-sized follicles. The weight of the follicle itself affects the total weight of the ovary. Based on this finding, it is observed that the heavy ovaries have more follicles and that these follicles have an impact on ovary weight (Murasawa et al., 2005).

Correlation between the Number of Follicles and Number of Oocytes

Based on data collected using the SPSS program, the Spearmen method, where the correlation coefficient is 0.546 with a significant level of 1%, it shows that there is a significant relationship because it is in the interval 0.40–<0.70 (Hauke and Kossowski, 2022). This shows there is a correlation with each other: the more follicles, the more the number of oocytes, and vice versa.

Based on the result, it is known that the significant value or Sig. (2-tailed) is 0.000; this value is less than <0.005, so it can be concluded that there is a significant relationship between the number of follicles and the number of oocytes. The result of ANOVA regression shows that the two statements have a linear relationship, which can be described by the following equation: Y = 2.48 + 1.204 X. So, the Y intercept is 2.48 because X = 1.204 and Y = 2.48.

In this study, there is a positive correlation between the number of follicles and the number of oocytes. This indicates that the more follicles, the more oocytes are collected. The results of the analysis show that there is a very significant difference (P<0.01). This may have caused the number of follicles on the surface to have a high FSH hormone (Recchia et al., 2021).

Correlation between the Number of Oocytes and Grade A oocyte

Based on data collected using the SPSS program, the Spearmen method, where the correlation coefficient is 0.520 with a significant level of 1%, it shows that there is a significant relationship because it is in the interval 0.40–<0.70 (Hauke and Kossowski, 2022). This shows there is a correlation with each other; the higher the number of oocytes, the more oocytes in Grade A, and vice versa.

Based on the result, it is known that the significant value or Sig. (2-tailed) is 0.000; this value is less than <0.005, so it can be concluded that there is a significant relationship between the number of oocytes and oocytes with Grade A. The result of ANOVA regression shows that the two statements have a linear relationship, which can be described by the following equation: Y = 0.93 + 0.800 X. So, the Y intercept is 0.93 because X = 0.800 and Y = 0.93.

In this study, there is a correlation between the number of oocytes and oocytes in Grade A. This study was limited by the number of ovaries that contain follicles and oocytes. Good oocyte quality is important to

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the IVF process and early embryogenesis (Hasbi et al., 2022). Obtaining more oocytes with Grade A is expected and good to support the IVF program.

Correlation between the Weight of Ovary and Grade A oocyte

The result of the analysis with SPSS using the Spearman method, where the correlation coefficient is 0.013, which means that there is a relationship that can be excluded, is under the smallest provisions in the correlation coefficient of 0.200 (Hauke and Kossowski, 2022), with a significance value of 0.898, which is more than 1%.

The result of the correlation between the weight of the ovary and oocytes in Grade A does not show a relationship because the total number of follicles increased with ovary weight and size. However, oocyte yield and quality decreased according to the weight of the ovary (Kouamo et al., 2020).

CONCLUSION

There is a positive correlation between the weight of the ovary and the number of follicles, the number of follicles and the number of oocytes, the number of oocytes and Grade A oocytes, and the weight of the ovary and oocytes in Grade A, that is 0.013.

ETHICS APPROVAL

This research took ovaries from female cows that had been slaughtered at the slaughterhouse, so ethical clearance was not required.

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