Original Research

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Comparison of the quantity and quality of cow ovarian oocytes extracted using aspiration, slicing, and flushing medium techniques

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ABSTRACT

High-quality oocyte basis materials are essential for *in-vitro* embryo formation in animal reproductive biotechnology. Researchers revealed that cow oocytes produced *in-vitro* can grow and develop. Selection of oocytes is crucial to *in-vitro* fertilization success and the production of excellent embryos. The aim of study was about the comparison of the number and morphology of oocytes in bovine ovaries obtained through aspiration, slicing and flushing medium techniques. The study used 300 ovaries derived from the remaining slaughtered female cattle in the Surya Slaughterhouse in Pegirian Surabaya. Data obtained included ovarian weight, number of follicles, number and morphology of oocytes, and were then analysed with the SPSS Mann-Whitney test. The results obtained from the study showed that there were differences in the number and morphology of oocytes between aspiration, slicing and flushing medium techniques with a p-value (0.000) < α (0.05). Therefore, H0 was rejected, leading to the conclusion that there were significant differences in the number and morphology of oocytes extracted using between these techniques. The number of oocytes was found to be higher in the slicing method. Morphology of grade A and B oocytes was found to be more prevalent in the aspiration method.

Introduction

Reproductive biotechnology is used to improve the genetic quality of livestock. The implementation of reproductive biotechnology is anticipated to enhance livestock quality, potentially resulting in increased economic value (Menchaca, 2023). Reproductive biotechnology has had an impact on various fields, as some materials help preserve the quality of oocytes (Shabira *et al*., 2025). In the field of livestock reproduction, biotechnology is carried out, among others, for the production of *in-vitro* embryos, which begins with the isolation of oocytes from the ovaries, oocyte maturation and *in-vitro* fertilization (Aguila *et al*., 2020).

Embryo production can be done in vivo and *in vitro*. In vivo oocyte collection comes from live cattle donors while *in-vitro* embryo production can be done using oocytes from the remaining ovaries of female cattle slaughtered at slaughterhouses (Landeo *et al*., 2022). In vivo, embryo production begins with superovulation using gonadotrophin hormones (Berliana *et al*., 2023). This technology is limited by the skill of the operator during ovum pick up, the high cost of hormones and the varying responses of donor cattle (Surjus *et al*., 2014). *In-vitro* generation of high-quality embryos at a reasonable cost is feasible done by isolating oocytes from the remaining ovaries of female cattle slaughtered at slaughterhouses (Wilson *et al*., 2005; Nourin *et al*., 2024). Also, some researchers found that the oocytes of cows cultured in an *in-vitro* will still be able to grow and develop to the next stage (Ríos *et al*., 2015; Telfer *et al*., 2019).

Utilization of leftover ovarian organ pieces from slaughterhouses is a way to obtain oocytes in large quantities and does not require expensive costs (Duncan *et al*., 2016). Collection of cow oocytes can be done using several methods including aspiration and slicing. According to Aguila *et al*. (2020) the method of collecting oocytes in the ovaries is carried out depending on the type of livestock. In goats, an effective method to use in producing a good number and quality of oocytes is to use the slicing method, while in cattle the effective method used is the aspiration method (Pawshe *et al*., 1994). The quantity of oocytes acquired with the slicing technique surpasses that obtained via aspiration. On the other hand, Cuervo-Arango *et al*. (2019) highlighted the critical role of carefully selecting flushing media components to enhance the efficiency of oocyte collection.

The aspiration method is a method that is carried out by utilizing the appearance of the follicles, so that the oocytes obtained are intact, but the number of oocytes obtained is not much (Georgiou *et al*., 2018). The slicing method is carried out by cutting the oocytes so that it produces a greater number of oocytes because all the follicles are chopped, but the quality of the oocytes is not good. Both methods can be combined to get maximum results (Akter *et al*., 2022).

The aspiration technique is the most often employed procedure in bovine ovaries. This method has a disadvantage, namely that the oocytes collected from a single needle puncture are only 30–60% (Chandramohan *et al*., 2024). The faster the process of collecting oocytes in the laboratory, the better the quality of the oocytes obtained. In the slicing method, the number of oocytes produced is 2 times (Akter *et al*., 2022). While Dawaymeh *et al*. (2023) found that flushing medium techniques improved oocyte recovery (5–15%), yielding up to 2.3 complexes per flush versus 0.8–1.3 by conventional methods.

Assessment of oocyte morphology is an effort to select oocytes and greatly affects the success of the *in-vitro* fertilization process that will produce quality embryos (Ahmad *et al*., 2024). The prevalent method for

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oocyte selection relies on the shape of the surrounding cumulus cells. Typically, oocytes surrounded by multilayer cumulus are utilized in *in-vitro* embryo formation. The criteria for identifying high-quality oocytes include a uniform ooplasm and compact cumulus cells encircling the zona pellucida (Turathum *et al*., 2021).

In general, and based on the literature review (Robin *et al*., 2021), only a limited number of studies have investigated the quantity of oocytes retrieved using different methods. The efficacy of *in-vitro* fertilization (IVF) is significantly contingent upon the quality, specifically the morphology of oocytes; thus, optimal oocyte quality is crucial. The purpose of this study was to compare the number and morphology of oocytes from female cattle slaughtered at the RPH using the aspiration, slicing collection and flushing medium techniques.

Materials and methods

Research Design

This research has been conducted at the Obstetrics Laboratory of the Veterinary Reproduction Division, Faculty of Veterinary Medicine, Airlangga University, Surabaya. The ovaries were taken from female cows slaughtered at the slaughterhouse. This research was conducted in February-April 2024. This study used 200 ovaries obtained from 100 pairs of female cow ovaries slaughtered at the Surya Pegirian slaughterhouse in Surabaya.

Collection of Ovary

The ovaries were inserted into plastic containing PBS and then added with antibiotics Penicillin 100 IU/ml and Streptomycin 0.1 mg/ml. The ovaries were stored at a cold temperature (4°C) to be taken to the laboratory. The ovaries were then washed with 0.9% physiological NaCl solution.

Collection of Oocytes

Oocytes were collected using either the slicing approach or the aspiration method or flushing medium techniques. Oocytes collected by the slicing method used a scalpel and tweezers. The ovaries were placed in a Petri dish and held using tweezers, then the follicles that appear on the surface of the ovary were cut with a scalpel. The chopped ovarian tissue was filtered, and the filtrate were observed under a microscope, to count the number of oocytes obtained and classify them.

Oocyte collection by the aspiration method is carried out by utilizing the appearance of the follicles. The follicles in the ovaries were punctured and the fluid was positioned in a Petri plate and then observed under a microscope, according to the research conducted.

The flushing media procedure was performed by repeatedly puncturing the ovarian surface. Subsequently, the ovaries were rinsed with D-PBS medium gradually through punctures distributed uniformly throughout the entire surface, utilizing a syringe filled with 1.0–1.5 milliliters of medium and equipped using a 21 G needle (Wongtra-ngan *et al*., 2010).

Oocyte Quality Examination

Oocyte quality is grouped into 4 grades (Rahma *et al*., 2020): Grade A oocytes were distinguished by their encasement in over five layers of compact and uniform cumulus cells, along with a homogeneous cytoplasm; High-quality oocytes (B) were distinguished by their uniformity, possessing fewer than five layers of cumulus cells and exhibited black cytoplasm; Poor-grade oocytes (C) were distinguished by their surrounding uneven and non-compact cumulus cells, together with a more translucent and irregularly colored cytoplasm; The lowest quality oocytes (D) were distinguished by the lack of cumulus cells and the presence of clear cytoplasm.

Data Analysis

The research was executed via an experimental methodology. The research strategy employed to distinguish strategies based on the quantity and quality of oocytes was a completely randomized design (CRD), after which the transformed data were analyzed using the Mann Whitney test. The collected data were statistically tested using Statistical Analysis Software program (SPSS).

Results

The aspiration procedure yielded a minimum of 0 oocytes, a maximum of 9 oocytes, and an average of 2.7 oocytes. The slicing approach yielded a minimum of 1 oocyte, a maximum of 15 oocytes, and an average of 5.11 oocytes. The flushing medium techniques procedure yielded a minimum of 0 oocytes, a maximum of 6 oocytes, and an average of 1.41 oocytes.

Based on the results of the research that has been done (Figures 1 and 2) in grade A oocytes, the oocytes were surrounded by more than 5 layers of compact and uniform cumulus cells, and homogeneous cytoplasm. In grade B oocytes, uniform oocytes were seen and have less than

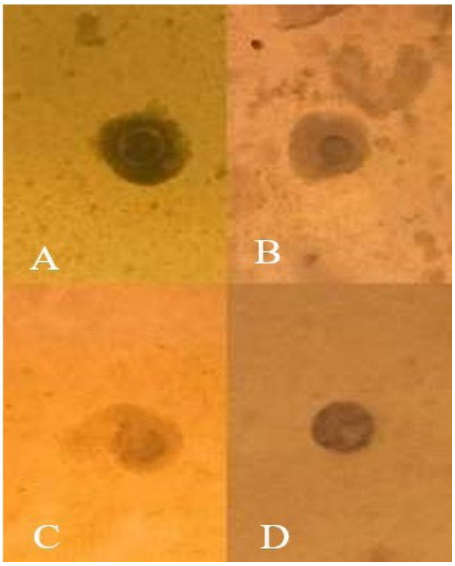

Figure 1. Oocyte collection by aspiration method. (A) Best category cow oocytes/grade A; (B) Good category cow oocytes/grade B; (C) Less good category cow oocytes/grade C; (D) Bad category cow oocytes/grade D.

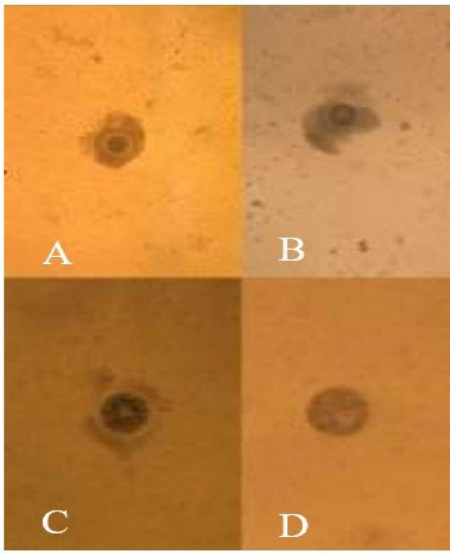

Figure 2. Oocyte collection using the slicing method. (A) The best cow oocytes/grade A; (B) Good cow oocytes/grade B; (C) Less good cow oocytes/grade C; (D) Bad cow oocytes/grade D.

five layers of cumulus cells and have dark cytoplasm. In grade C oocytes, the oocytes were surrounded by uneven and non-compact cumulus cells and the color of the cytoplasm is more transparent and uneven. In grade D oocytes, there are no cumulus cells, and the cytoplasm is transparent.

According to the findings of the conducted research (Table 1), the aspiration procedure yielded a total of 270 oocytes, comprising 115 morphological grade A oocytes, 120 grade B oocytes, 20 grade C oocytes, and 15 grade D oocytes. Utilizing the slicing procedure, 511 oocytes were acquired, comprising 7 morphological grade A oocytes, 12 grade B oocytes, 181 grade C oocytes, and 311 grade D oocytes. The flushing medium techniques procedure yielded a total of 141 oocytes, comprising 60 morphological grade A oocytes, 34 grade B oocytes, 26 grade C oocytes, and 21 grade D oocytes.

Table 1. Results of the oocyte number variable test based on the method

Method	N	Mean rank	P-value
Aspiration	100	74.71	0
Slicing	100	126.29	
Flushing medium	100	25.72	

Based on the data analyzed using the SPSS program, the slicing method, aspiration approach and flushing medium techniques differed significantly from one another. In the slicing method group, the average value was greater, namely 126.29 compared to the average value of the aspiration method, namely 74.71 and flushing medium techniques namely 50.5 (Table 1).

When the p-value (0.000) is less than the α (0.05), H0 is rejected so that with a real level of 5% it can be concluded that there is a significant difference in the number of oocytes using the aspiration method and the slicing method so that it can be said that there is an influence of the method about the quantity of oocytes.

Based on the data analyzed using the SPSS program, there were significant distinctions between the slicing approach, aspiration method and flushing medium techniques. In the aspiration method group, the average oocyte morphology values for grades A, B, C, and D were 133.56, 129.40, 67.02, and 53.77, respectively. While in the slicing method group, the appearance of oocytes was classified as A, B, C, or D., that had an average value of 67.44; 71.60; 133.98; and 147.23, respectively. The flushing medium techniques group, the appearance of oocytes was classified as A, B, C, or D., that had an average value of 27.81, 25.72, 26.39, and 27.96, respectively (Table 2).

Table 2. Results of oocyte morphology test for each grade based on method

Grade	Method	N	Mean rank	P-value
A	Aspiration	100	133.56	0
	Slicing	100	67.44	
	Flushing medium	100	27.81	
B	Aspiration	100	129.4	0
	Slicing	100	71.6	
	Flushing medium	100	25.72	
C	Aspiration	100	67.02	0
	Slicing	100	133.98	
	Flushing medium	100	26.39	
D	Aspiration	100	53.77	0
	Slicing	100	147.23	
	Flushing medium	100	27.96	

Based on the calculation of the p-value (0.000) less than α (0.05), H0 is rejected so that with a real level of 5% it can be concluded that there is a significant difference in the morphology of oocytes grades A, B, C, and D using the aspiration method, the slicing method and flushing medium techniques so that it can be said that there is an influence of the method on the morphology of oocytes of each grade.

Discussion

This study yielded 270 oocytes via the aspiration method, 511 oocytes with the slicing approach and 141 oocytes with the flushing medium techniques. This demonstrates that the quantity of oocytes is superior using the slicing technique compared to the aspiration technique and flushing medium techniques. This is supported by research by Jaffe *et al*. (2009) who reported that the slicing method can cut to the inside of the ovary so that it is possible to remove not only large follicles but also other follicles underneath. This possibility is supported by the number of oocytes obtained with moderate and poor quality (Wang *et al*., 2007).

The number of oocytes obtained by the slicing method is greater than the aspiration method and flushing medium techniques because the oocytes collected by these methods one needle puncture are only 30-60% so that not all oocytes can be taken as a whole (Wani *et al*., 2000). The number of oocytes is one of the factors that affects the *in-vitro* fertilization. The number of quality oocytes affect the percentage of success of the fertilization process. The greater the number of quality oocytes, the greater the percentage of the fertilization results (Guimarães *et al*., 2021).

In this study, differences in oocyte morphology were found between aspiration, slicing and flushing medium techniques. Oocyte morphology is a critical factor in the efficacy of *in-vitro* fertilization (Aguila *et al*., 2020). The oocyte collection method greatly influences the morphology of the oocyte, this is important for the next process including maturation, fertilization, and embryo development (Evecen *et al*., 2009). Oocyte collection by aspiration is done by aspirating the visible follicles to obtain their number and level. Oocyte collection by slicing is done by counting the follicles that appear on the surface of the ovary so that there is a possibility of more oocytes in the follicles obtained (Georgiou *et al*., 2018). The variation in the number of oocytes observed in this study is attributed to the oocyte collection method used.

Based on the results of the study, it was shown that good oocyte morphological quality (A, B) was more often obtained by aspiration than by slicing or flushing medium techniques. Good oocyte morphological quality for *in-vitro* fertilization is grade A and B oocytes, this is supported by the statement of Sianturi *et al*. (2002) that oocytes with grade A, B have a much better maturation rate than bald oocytes (D) or partially bald (C).

Grade A oocytes are encased by many layers of compact cumulus cells and possess homogeneous cytoplasm, whilst grade B oocytes are enveloped by two to three layers of cumulus cells, also exhibit homogeneous cytoplasm (Rahma *et al*., 2020). The zona pellucida is connected to cumulus cells play a role in maintaining intracellular communication and regulating oocyte growth and maturation by facilitating the process of hormonal metabolism and nutrient transformation. Cumulus cells are a specific tool in the transduction mechanism for transferring gonadotropin signals to oocytes through the gap junction system. This is supported by Handarini *et al*. (2014), that the gap junction is a nutrient pathway for oocytes.

Good oocyte morphology is essential for *in-vitro* fertilization and early embryogenesis (Hasbi *et al*., 2022). Good quality oocytes are not only reach successfully to the nuclear maturation stage but also be able to pass through various stages of cytoplasmic maturation required for the fertilization process. Oocyte quality affects oocyte maturation (Handarini *et al*., 2014). Acquiring additional grades, A and B oocytes is anticipated to enhance the *in-vitro* fertilization program.

In the results of this study, there were differences in the morphology of grade C and D oocytes between the aspiration, slicing and flushing medium techniques. The number of grade C and D oocytes was found to be greater through the slicing method and less in the flushing medium techniques and in the aspiration method. This is in accordance with the research of Febretrisiana and Pamungkas (2017), that incisions in the slicing method must be considered so as not to hit blood vessels and cause

bleeding which can cause poor oocyte morphology because cumulus cells are uneven and compact.

Oocytes with grade C morphology have a cumulus layer that is not too dense with an irregular ooplasm shape and has a dark layer, while grade D oocyte morphology does not have a cumulus layer. Determination of oocyte quality can be done by conducting several evaluations of the oocytes that will be used in the FIV process. The most widely used oocyte selection is the selection of oocytes based on the morphology of the cumulus cells around the oocytes (Aguila *et al*., 2020).

Good oocyte maturation is obtained in oocytes with morphological quality grade A and B because oocyte maturation requires a cumulus cell layer to encourage normal *in-vitro* fertilization oocyte nucleus maturation. Oocytes with morphological grade C, D are oocytes that experience degeneration (Sianturi *et al*., 2002).

Oocytes with grade C and D morphology cannot be used for *in-vitro* embryo production because they do not have cumulus cells surrounding the oocytes so that oocyte maturation cannot be carried out. The *in-vitro* oocyte maturation process can be seen by the presence of cumulus oophorus which acts as a mediator providing energy transport, micronutrients and carrier molecules (barriers) for oocyte development and mediates the influence of hormones on the cumulus complex around the oocyte (Zhang *et al*., 2023).

Oocyte morphology is determined by the presence of cumulus cells. The role of these cumulus cells is related to the gap junction mechanism, regulating signal regulation related to hormones and the metabolic ability of oocytes (Del Bianco *et al*., 2024). Cumulus cells surrounding oocytes play a very important role in oocyte maturation *in-vitro* (Turathum *et al*., 2021).

Conclusion

The number of oocytes obtained by the slicing method is greater than the aspiration method or flushing medium techniques. The morphology of grade A and B oocytes is greater through the aspiration method compared to the slicing method or flushing medium techniques.

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Conflict of interest

The authors have no conflict of interest to declare.

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