

**EVALUATION OF SUPEROVULATION RESULTS ON *IN VIVO* EMBRYO PRODUCTION IN THREE DIFFERENT COWS BREEDS AT THE LIVESTOCK EMBRYO CENTER**

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**Volume 12 Issue 1  
(April 2026)**  
e-ISSN: 2550-0740

**doi:**  
10.30997/jpn.v12i1.18415

**ABSTRACT**

The purpose of this study was to compare the superovulation response in several breeds, namely Angus, Friesian Holstein (FH), and Ongole crossbreed (OC). A total of 152 cows were used in this study, namely 39 Angus cows, 62 Friesian Holstein cows, and 51 Ongole crossbreed cows. The parameters of this study were the number of CL, response rate, recovery rate, number of embryos collected (1, 2, 3, 4 (DG/UFO)), proportion of transferable embryos, proportion of DG, and proportion of UFO. The collected data were tested using the Kruskal-Wallis test, if there was a difference, it was continued using the Mann-Whitney test. The results of this study included the number of CL, recovery rate, total embryos collected, proportion of DG, and proportion of UFO of Angus, FH, and OC cows showed significantly different results ( $P < 0.05$ ), the response rate showed very significantly different results ( $P < 0.01$ ), but the proportion of transferable embryos was not significantly different ( $P > 0.05$ ). This study concludes that CO cattle have the highest average of CL, response rate, recovery rate, and total embryos collected. Meanwhile, Angus cattle produce the lowest percentage of DG and UFO embryos

**ARTICLE INFO**

**Article history:**

Received: 29 Mei 2025  
 Revised version received: 29 mei 2025  
 Accepted: 15 April 2026  
 Available online: 28 April 2026

**Keywords:**

Angus; FH; OC; embryo; superovulation.

**How to Cite:**

Rismasari, Wijayanti D, Darlian F, ningtias PI, Kusmayadi A. (2026). Evaluation of superovulation results on *in vivo* embryo production in three different cows breeds at the livestock embryo center. *Jurnal Peternakan Nusantara*. Vol 12 (1). 1-18.

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## INTRODUCTION

The average amount and per capita consumption of meat worldwide are rising due to population growth and average individual income. The growth rate in each region varies, with low-income countries' average meat consumption being low and stable, middle-income countries tending to increase gradually to high levels, and high-income countries being static or declining (Godfray *et al.*, 2018; Arifin *et al.*, 2019). Over the course of the next five decades, it is projected that the global population will reach nine billion (Brameld and Parr., 2016). Simultaneously, the economic expansion in developing nations will amplify the need for meat, milk, and other animal-derived proteins. Therefore, it is necessary to increase the population with superior genetic quality in individual livestock on a large scale to help fulfill the demand for meat and milk.

The application of reproductive biotechnology to the superovulation program can be used as a way to increase the population of superior genetic quality in a relatively short time. The aim of superovulation treatment is to obtain the maximum number of transferable embryo before carrying out the embryo transfer program so that maximum results are obtained, embryos can be used for embryo transfer programs with a large percentage of producing pregnancies in large numbers (Bó & Mapletoft, 2020) (Kamath *et al.*, 2020) (Köse *et al.*, 2022). If we look at the percentage of transfer embryo (TE) success, the percentage of TE using fresh embryos is 59.90% and TE using frozen-thawed embryos is 52.49% (Damaris *et al.*, 2023). In contrast to the reported efficiency percentage of 86.4% achieved with IB, the current range for TE from the superovulation program is 48.1% (Tadesse *et al.*, 2022), Consequently, it is premature to declare TE a viable solution at this time. Nevertheless, through the implementation of the superovulation program on female livestock possessing exceptional genetic quality, it is expected that artificial insemination (AI) improves the genetic quality of females and males resulting from. These calves will subsequently serve as sires of superior genetic quality, while males will produce semen for utilisation in the AI program, thereby facilitating the production of seeds of superior genetic quality.

This research was conducted to determine the success rate of superovulation in production to produce genetic quality Angus (*Bos taurus*), Friesian Holstein (*Bos taurus*), and Ongole crossbreed cattle (*Bos indicus*) which was held at the Cipelang Livestock Embryo Center (CLEC). In order to address the national need for meat and milk, it is necessary to discover a solution that eliminates the need for ongoing product imports once the demand for cattle has been satisfied. The aim of this research is to determine the level of superovulation responses in the embryo production process of three breed which are included in the types of beef and dairy cow studied, because the higher the number and quality of embryos produced can help increase the livestock population with superior genetic quality.

## MATERIALS AND METHODS

### Research Location and Materials

This research was carried out at the Cipelang Livestock Embryo Center (CLEC) located in Cipelang Village, Cijeruk District, Bogor Regency, West Java, Indonesia. This research was conducted using data on the quality and number of embryos from donors Angus, Friesian Holstein, and Ongole crossbreed cows obtained from the CLEC during the implementation of the superovulation program from February 2020 to October 2023. There were 152 cows used in this research, namely 39 Angus cows, 62 Friesian Holstein cows, and 51 Ongole crossbreed cows with an average age of 5 years.

### Research procedures

The procedures for implementing embryo production at CLEC from the start of donor cow selection to storage of the resulting embryos are as follows (Figure 1).

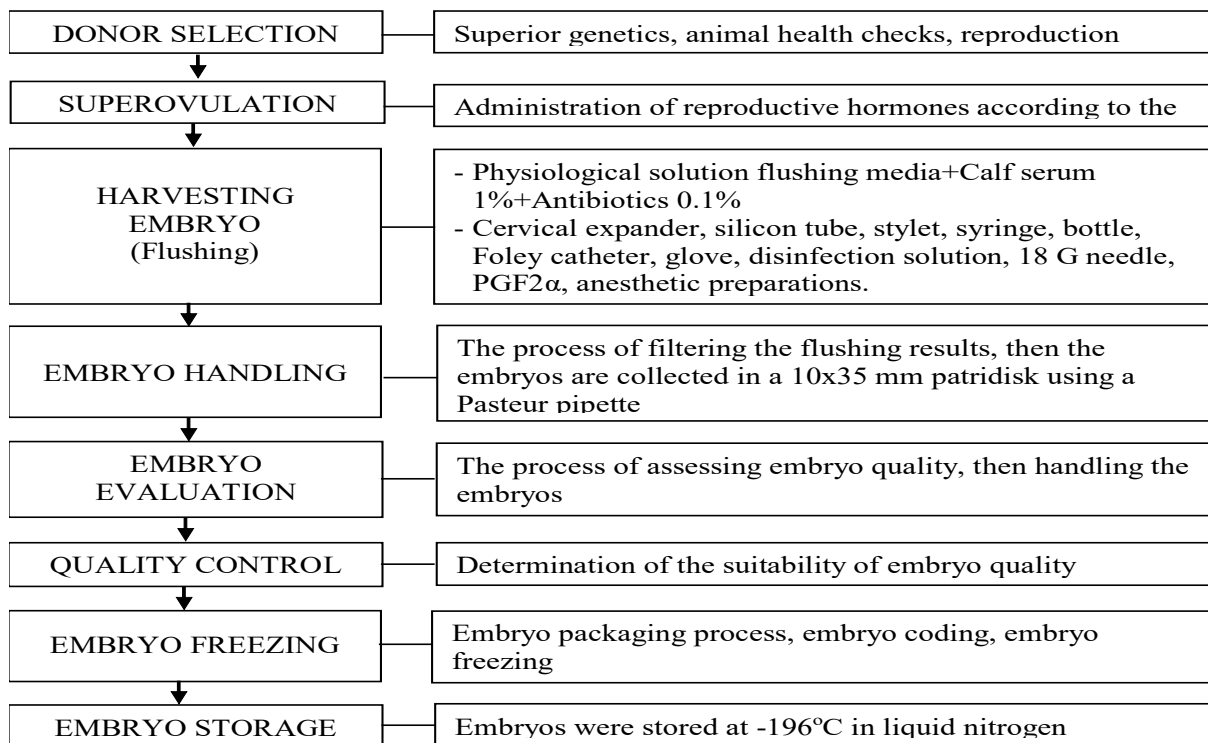


Figure 1. Steps in the superovulation program and in vivo embryo production, starting from donor selection to embryo storage.

The procedure for performing superovulation in CLEC from the initial selection of donor cows to the storage of embryos produced is as follows (Figure 2).

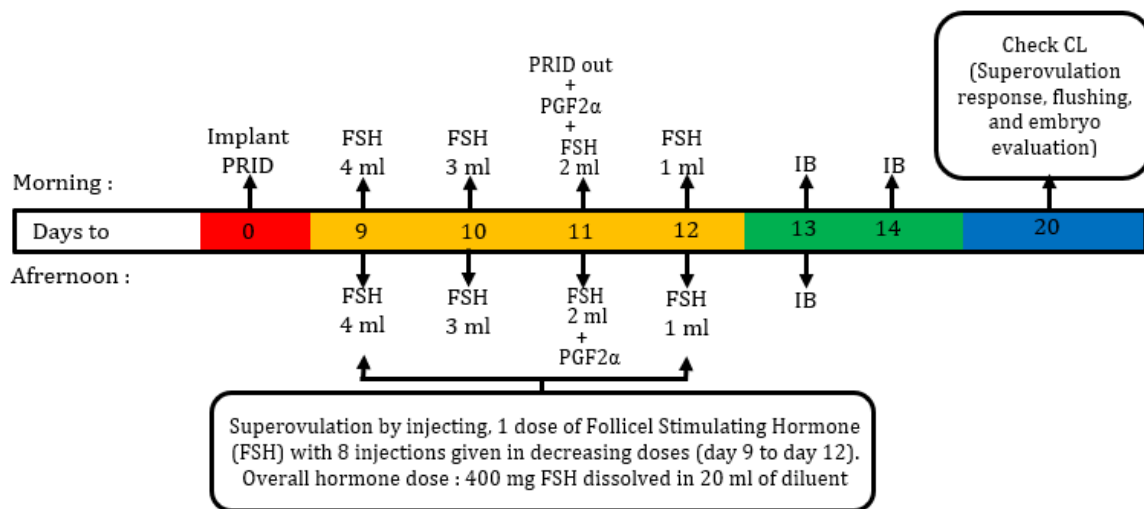


Figure 2. Superovulation Procedure. Day 0, installation of a progesterone device for follicular wave synchronization. Day 9 to day 12 of the superovulation program with FSH injection using a reduced dose. Day 11, synchronize lust by releasing the progesterone device and injecting the PGF2 $\alpha$  hormone. Day 13 and 14 artificial insemination 3 times. Day 20 of rinsing.

**Synchronization of Follicular Waves and Superovulation**

The implementation of follicular wave synchronization is achieved by implanting a progesterone device (Cue - mate®), the cow is declared on the day of the estrous donor cow in the cycle, the progesterone device implant (Cue - mate®) is in the vagina from day 0 to day 11. The

superovulation program is carried out by injecting FSH on days 9 to 12 with decreasing dosage (4 ml, 3 ml, 2 ml, 1 ml in the morning and evening) intramuscularly.

### **Synchronization of Oestrus and Artificial Insemination (AI)**

To synchronize oestrus, PGF<sub>2α</sub> hormone was administered via injection on day 11, concurrent with the release of the progesterone device (Cue-mate®). Following this, artificial insemination (AI) is carried out 3 times at 12-hour intervals after showing signs of heat.

### **Embryo Flushing**

Embryo flushing is carried out on the 7<sup>th</sup> day after the first AI, either non-surgically or by flushing is done by rinsing the right left uterus and body, which is carried out repeatedly using 500 ml of lactic solution riger, supplemented with 1% bovine serum, and antibiotics 0,1%. So, from each cattle will get 1500 ml which is divided into three bottles. The flushing process is carried out using a Folley Catheter. The flushing fluid that has been collected into bottles will be evaluated for embryos in the laboratory. Meanwhile, after the embryo flushing process, the livestock will be given 50 ml of 2% iodine povidone intravaginally to prevent infection, after that the livestock will be injected with PGF<sub>2α</sub> to shed CL to prevent pregnancy and speed up reproductive recovery (Damaris *et al.*, 2023; Darlian *et al.*, 2021)

### **Embryo Evaluation**

The evaluation of embryos can commence with the filtration of the flushing media whit embryo through a 75µm embryo filter (Damaris *et al.*, 2023). The embryos search is conducted using embryo filtering media in the presence of a 40x magnification microscope (Table 1).

Table 1 Embryo Quality Assessmen

<b>Embryo Quality</b>	<b>Description</b>
Quality 1 (A): Excellent or Good	<ol style="list-style-type: none"> <li>1. The shape of the embryo is symmetrical and round (spherical) with blastomeres that are uniform in size, color, and density.</li> <li>2. The embryo must have a shape consistent with the estimated developmental phase of the embryo itself. Relatively minor irregular shape.</li> <li>3. Have a minimum of 85% intact cellular material and live embryo mass.</li> <li>4. The zona pellucida should be round, smooth, not sticking to the petri dish or pipette.</li> </ol>
Quality 2 (B): Fair	<ol style="list-style-type: none"> <li>1. In general, it has an irregular shape in the medium category in terms of embryo mass, size, color and density of individual cells.</li> <li>2. Have intact cells and live embryo mass of at least 50%</li> </ol>
Quality 3 (C): Poor	<ol style="list-style-type: none"> <li>1. Embryos are predominantly irregular in shape, size, color and density of individual cells.</li> <li>2. Have intact cells and a live embryo mass of at least 25%.</li> </ol>
<i>Degenerative</i>	Degenerative embryos are embryos that were once alive but died or did not develop according to their phase
<i>Unfertilized</i>	Unfertilized embryos are embryos that are not fertilized because when fertilization occurs the egg cells have not yet matured.

### **Research Parameters**

1. Number of Corpus Luteum (CL)

$$2. \text{ Response Rate} = \frac{\text{Number of responding donor cows}}{\text{Number of donor cows in superovulation}} \times 100\%$$

$$3. \text{ Recovery Rate} = \frac{\text{Total number of embryos collected}}{\text{The number of CL formed in the ovaries}} \times 100\%$$

#### 4. Embryo Collection

$$5. \text{ Transferable Embryos} = \frac{\text{Number of transferable embryos}}{\text{Number of embryos collected}} \times 100\%$$

$$6. \%DG = \frac{\text{Number of DG embryos}}{\text{Number of embryos}} \times 100\%$$

$$7. \%UFO = \frac{\text{Number of UFO}}{\text{Number of embryos collected}} \times 100\%$$

#### Statistical Analyses

The number of corpus luteum (CL), response rate, recovery rate, total embryos collected, proportion of transferable embryos, proportion of degenerative embryos (DG), proportion of unfertilized ova (UFO), will be analyzed using the Kruskal-Wallis test, if there are differences real will be tested further using the Mann-Whitney U Test with a significance level of ( $P < 0.05$ ) and ( $P < 0.01$ ) using IBM SPSS 23 software.

## RESULTS AND DISCUSSION

### Number of Corpus Luteum (CL)

The number of CL produced indirectly indicates the number of ova ovulated as a response to the cattle's ovaries to the superovulation program. Using the Kruskal-Wallis test, the amounts of CL made by Angus, Friesian Holstein, and Ongole crossbred cow were found to be significantly different ( $P < 0.05$ ) (Figure 3).

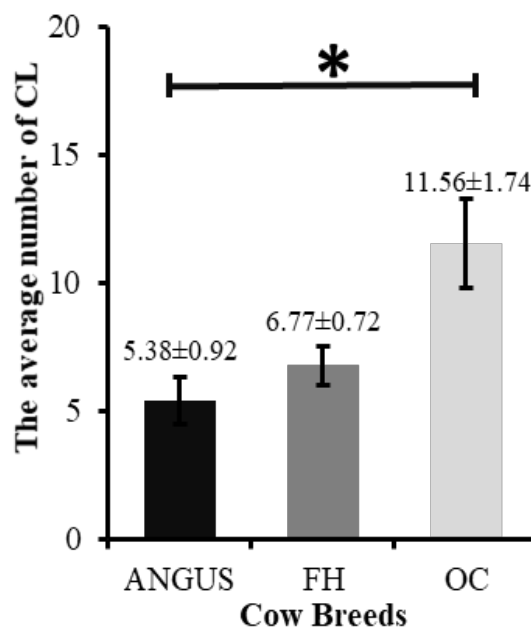


Figure 3. Number of corpus luteum in three cattle \* $P < 0.05$ . Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbreed).

Table 2 Comparison of the number of CL in different cow breeds using the Mann-Whitney test

Comparison of the number of CL in different cow breeds					
Angus X FH		OC X Angus		FH X OC	
5.38±0.92	6.77±0.74	11.56±1.74 <sup>A</sup>	5.38±0.92 <sup>B</sup>	6.77±0.74	11.56±1.74

Note : values with different superscripts in the same column (A, B) are highly significant;  $P < 0.01$ ; FH (friesian holstein); OC (ongole crossbreed).

Based on the Mann-Whitney U test results, the research results showed that there were differences in the number of CL between different cow breeds (Table 2). The Angus cow breed showed results that were not significantly different from the Friesian Holstein cow ( $P > 0.05$ ). The Angus cow and Ongole crossbreeds exhibited highly significant differences in their results ( $P < 0.01$ ). Friesian Holstein cattle and Ongole crossbreeds showed results that were not significantly different ( $P > 0.05$ ).

In this study (Table 2), there was no significant difference in the CL numbers of Angus and Friesian Holstein cows because both came from the same breed, namely *Bos taurus*. Therefore, it is highly probable that the two breeds possess similar reproductive characteristics. According to Sartori *et al.* (2016), *Bos taurus* cattle have a larger CL volume and ovulation follicle diameter, but the steroid hormones circulating in the body are low. The absence of significant distinctions between Angus and Friesian Holstein cows can be attributed to their shared ancestry in the *Bos taurus* family, which ensures consistency in the quantity and dimensions of corpus luteum, and follicles produced. The number of CL Angus cattle and Ongole crossbreed cows show very real differences. According to Degefa *et al.* (2016), *Bos indicus* cow have higher circulating steroid hormones and have a smaller dominant follicle size compared to *Bos taurus* cow. *Bos taurus* cattle and the other group of cattle have an inverse relationship, resulting in significant differences between them. Meanwhile, Friesian Holsteincow and Ongole crossbreed cows showed results that were not significantly different. In fact, according to Maqhashu *et al.* (2021), dairy cow will produce low CL numbers because lactating cattle are stressed due to negative energy balance.

It was found that Ongole crossbred cows from the *Bos indicus* cattle grove had a higher than Angus and Friesian Holstein cows from the *Bos taurus* cattle families (Figure 3). This is due to the ability of each animal to handle stress differently, especially with the superovulation method which is carried out by injecting FSH twice in one day which can cause stress which can affect the decrease in the superovulation response (Maqhashu et al., 2021)

**Response Rate**

The results of the research showed that the response rates produced by Angus, Friesian Holstein and Ongole cross breed cow showed very significantly different results ( $P < 0.01$ ) (Figure 4).

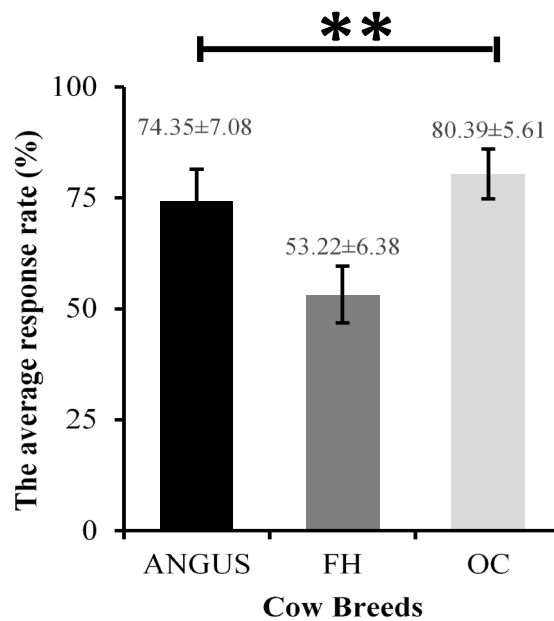


Figure 4 The response rate for three cattle  $**P < 0.01$ . Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbred).

Table 3 Comparison of response rates in different cow breeds using the Mann-Whitney test

Comparison of Response Rates in Different Cow Breeds					
Angus X FH		Angus X OC		OC X FH	
74.35±7.08 <sup>a</sup>	53.22±6.38 <sup>b</sup>	74.35±7.08	80.39±5.61	80.39±5.61 <sup>A</sup>	53.22±6.38 <sup>B</sup>

Note : values with different superscripts in the same column (A, B) are highly significant;  $P < 0.01$ ; (a, b) means significant  $P < 0.05$ ; FH (friesian holstein); OC (ongole crossbred).

Based on the results of the Mann-Whitney U test, the research results show that there are differences in response rates between different cow breeds (Table 3). Angus and Friesian Holstein cow showed significantly different results ( $P < 0.05$ ). Angus cow and Ongole crossbred cow showed results that were not significantly different ( $P > 0.05$ ). Friesian Holstein cow and Ongole crossbreeds show very different results ( $P < 0.01$ ).

In this research, Angus cow and Friesian Holstein cow showed significantly different results. Based on the statement of Damaris et al. (2023), High milk production will cause low circulation of progesterone due to high levels of steroids, negative energy balance in lactating cows at the beginning of lactation, as well as decreased blood calcium and glucose in dairy cow related to the level of embryo growth which can be said to influence the superovulation response. Angus cow and Ongole

crossbreed cow showed results that were not significantly different. Based on research by Damaris *et al.* (2023), the superovulation responses of *Bos taurus* and *Bos indicus* showed no difference. Friesian Holstein cow and Ongole crossbreeds show very different results. According to Sartori *et al.* (2016), progesterone metabolism in dairy cattle is higher than in beef cattle, this is a factor causing the low number of CL produced as a sign of the superovulation response.

According to Figure 4, Ongole crossbred cows (*Bos indicus*) have the highest average response rate, at 80.39%. The average response rate for Ongole crossbreed cows is smaller compared to research by Damaris *et al.* (2023), which states that the average response rate for Ongole crossbreed cows is 100%. Meanwhile, the Friesian Holstein cow (*Bos taurus*) as a type of cattle representing the dairy cow breed showed the lowest average response rate. According to Bó and Mapletoft (2020), The low superovulatory response in Friesian Holstein cows is probably due to FSH absorption that rapidly or an excessive percentage of atresia caused by follicular luteinization or follicular atresia that appears in the *Bos taurus* breed of dairy cow. The decreased response is possibly related to the acceleration of gonadotrophin metabolism in dairy cows.

### Recovery Rate

The results of the study showed that the recovery rates for Angus, Friesian Holstein, and Ongole breed cows showed significantly different results ( $P < 0.05$ ) (Figure 5).

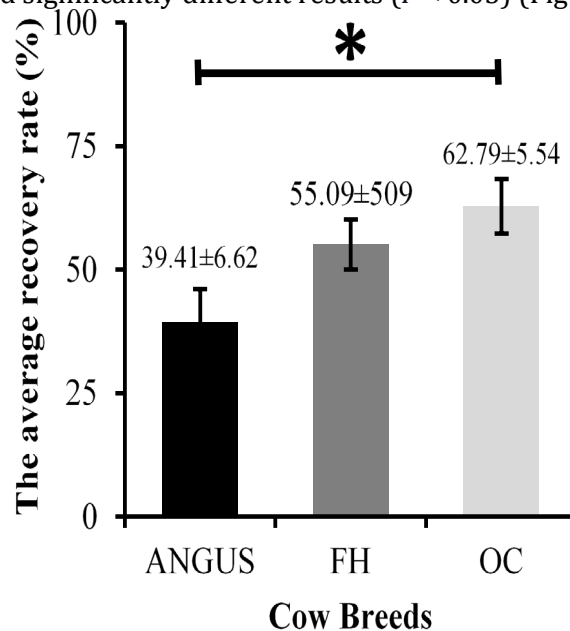


Figure 5 Recovery rate of three cattle  $*P < 0.05$ . Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbreed).

Table 4 Comparison of recovery rates in different cattle breeds using the Mann-Whitney test

Comparison of Recovery Rates in Different Cow Breeds					
Angus X FH		OC X Angus		FH X OC	
39.41±6.62	55.09±5.09	62.79±5.54 <sup>A</sup>	39.41±6.62 <sup>B</sup>	55.09±5.09	62.79±5.54

Note: values with different superscripts in the same column (A, B) are highly significant;  $P < 0.01$ ; FH (friesian holstein); OC (ongole crossbreed).

According to the Mann-Whitney U test results, there are differences in recovery rates between different breeds of cattle (Table 4). Angus and Friesian Holstein cows showed results that were not significantly different ( $P > 0.05$ ). The Angus cattle and Ongole crossbreeds exhibited highly significant variations in their outcomes ( $P < 0.01$ ). Friesian Holstein cow and Ongole crossbreeds showed results that were not significantly different ( $P > 0.05$ ).

In the research (Tabel 4), Angus and Friesian Holstein cows showed results that were not significantly different. Factors that influence the absence of real differences between Angus and Friesian Holstein cows are gonadotropin hormones, donor cows, nutrition, BCS, and dominant follicles in the ovaries (Damaris et al., 2023)(Horn et al., 2022). The Angus cow and Ongole crossbreeds exhibited highly significant differences in their results. According to Redhead et al. (2018), The response to superovulation can be influenced by the number of healthy follicles in the follicle collection. There was a very significant difference in recovery rates between Angus cow and Ongole crossbreeds, indicating that these two cows may have different physiological conditions, resulting in different levels of healthy follicles that are ovulated and thus giving very significantly different results. Friesian Holstein cow and Ongole crossbreeds showed results that were not significantly different. Based on research by Sartori et al. (2016), Bos taurus cows have a higher ovulation rate than Bos indicus.

Based on Figure 5, Angus cows have the lowest recovery rate. This may be influenced by the reproductive ability of the livestock itself, or it may also be influenced by the AI process carried out on the livestock, where these two things can be supported to continue to play a role and show results by providing optimal nutrition. According to Kruse et al. (2017), nutritional restrictions can affect the early development of the embryo. Bos taurus would be more susceptible to embryo loss when exposed to negative nutrition, this could affect the cow recovery rate Fontes et al. (2019). The Friesian Holstein cow in this study had a greater average recovery rate than the Angus cow. This value is still stated to be smaller when compared to the research results (Damaris et al., 2023), Friesian Holstein cows have a recovery rate of 55.09%. However, in this study, it was not proven because the recovery rate value for Angus cows was smaller than for Friesian Holstein cows as dairy cows, this was caused by several factors such as the flushing technique, and the physiological condition of the cow. Meanwhile, Ongole crossbreed cows have the highest recovery rate. This value is still considered small when compared to research by Damaris et al. (2023) namely, Ongole crossbreed cows have a recovery rate of 62.79%.

**Total Embryos Collected**

The statistical analysis revealed significant differences in the total number of embryos produced by Angus, Friesian Holstein, and Ongole crossbreed cows ( $P < 0.05$ ) (Figure 6).

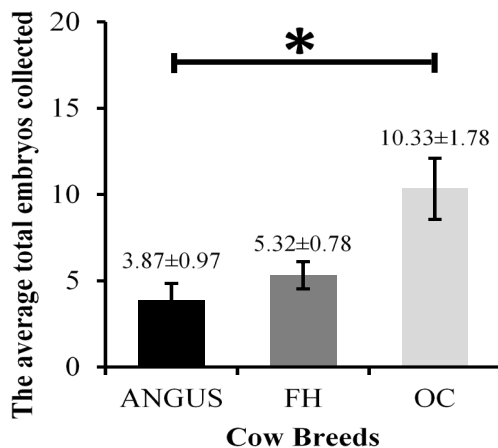


Figure 6 Total embryos collected from three cattle breeds \* $P < 0.05$ . Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbreed).

Table 5 Comparison of total embryos collected in different cattle breeds using the Mann-Whitney test

Comparison of Total Embryos Collected in Different Cattle Breeds					
Angus X FH		OC X Angus		FH X OC	
3.87±0.97	5.32±0.78	10.33±1.78 <sup>A</sup>	3.87±0.97 <sup>B</sup>	5.32±0.78	10.33±1.78

Note: values with different superscripts in the same column (A, B) are highly significant;  $P < 0.01$ ; FH (friesian holstein); OC (ongole crossbred).

There were differences in the number of embryos collected between different breeds of cow (Table 5). Total embryos collected from Angus and Friesian Holstein cows were not significantly different ( $P > 0.05$ ). Angus cows and Ongole crossbred cows produce very different results ( $P < 0.01$ ). Friesian Holstein cow and Ongole crossbreeds showed results that were not significantly different ( $P > 0.05$ ).

Based on the data in Table 5, the number of embryos collected from Angus and Friesian Holstein cattle in this study showed results that were not significantly different. This can be caused by the uniformity of livestock age, in the research the average age of the livestock used was 5 years. According to Jatón *et al.* (2016), the optimum age for cattle to induce superovulation is before 5 years, because cows that are more than 8 years old will produce fewer embryos (González *et al.*, 2017). The results of Angus cow and Ongole crossbred cattle show very real differences. This could be because, the body condition score (BCS) can also serve as a distinguishing factor between the two breeds of cow, namely *Bos taurus* and *Bos indicus*. These breeds exhibit different BCS ratios due to the positive correlation between superovulation response and BCS (Smuts *et al.*, 2019). The superovulation response can be observed through the total number of collected embryos produced (Mondragón *et al.*, 2016). Friesian Holstein cow and Ongole crossbreeds showed results that were not significantly different. *Bos Indicus* cow and dairy cow have a higher progesterone metabolism than *Bos taurus* beef cow, which results in a lower level of CL gain (Damaris *et al.*, 2023). Cow embryos consist of nine phases and are grouped into four according to the quality of the embryos produced by each of the three breeds of cow (Figure 7).

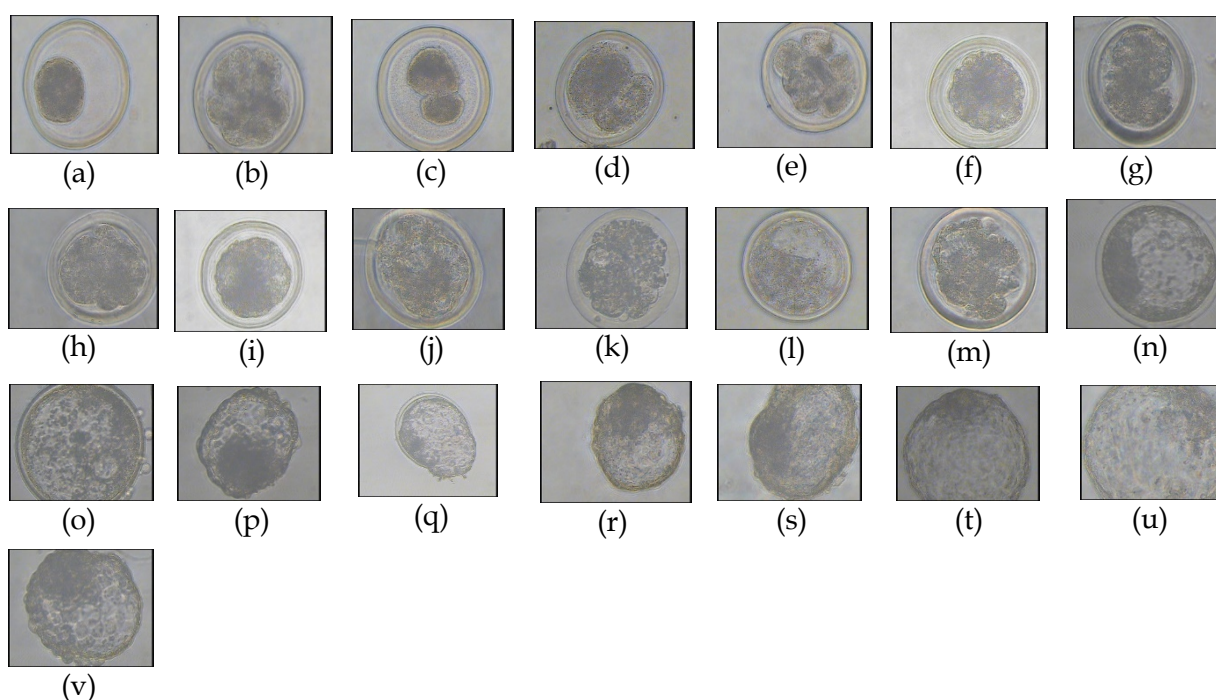


Figure 7 Embryo levels based on phase and quality. (a) Unfertilized, (b) Degenerative, (c) Degenerative (2 Cell), (d) Degenerative (3 Cell), (e) Degenerative (4 Cell), (f) Morulla (Quality 1), (g) Morulla (Quality 2), (h) Morulla (Quality 3), (i) Early Blastocyst (Quality 1), (j) Early Blastocyst (Quality 2), (k) Early Blastocyst (Quality 3), (l) Blastocyst (Quality 1), (m) Blastocyst (Quality 3), (n) Expanded Blastocyst (Quality 1), (o) Expanded Blastocyst (Quality 2), (p) Hatched Blastocyst (Quality 1), (q) Hatched Blastocyst (Quality 2), (r) Hatched Blastocyst (Quality 3), (s) Hatched Blastocyst (Quality 4), (t) Expanded Hatched Blastocyst (Quality 1), (u) Expanded Hatched Blastocyst (Quality 2), (v) Expanded Hatched Blastocyst (Quality 3).

### Proportion of Transferable Embryos

The proportion of transferable embryos produced by Angus, Friesian Holstein, and Ongole crossbred cows showed results that were not significantly different ( $P > 0.05$ ) (Figure 8).

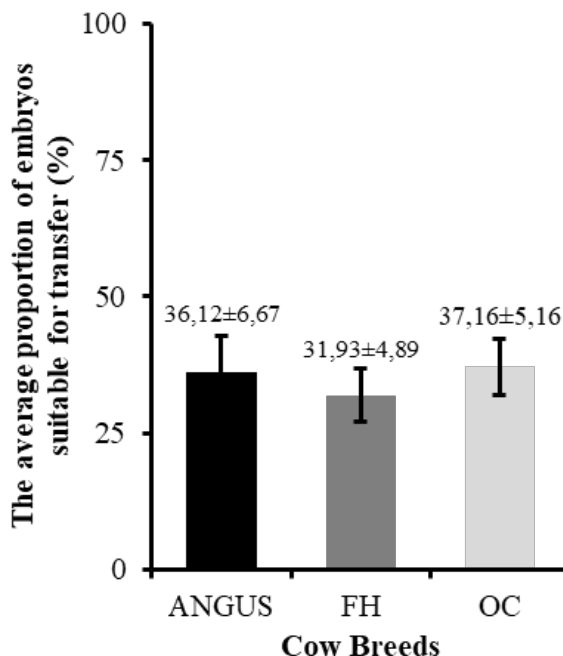


Figure 8 Proportion of embryos viable for transfer in three cow breeds. Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbreed).

According to the research findings, Friesian Holstein cows have the lowest percentage of transferable embryos among the three cow breeds studied, at 31.91%. Furthermore, Angus cows have a proportion of transferable embryos that is not significantly different from Friesian Holstein cows, namely 36.12%. Ongole crossbred cows exhibited the highest proportion of transferable embryos compared to Angus and Friesian Holstein cows, albeit with a marginal difference of 37.16%.

In terms of the number of CL, response rate, recovery rate, and total embryos collected, Ongole crossbred cows consistently exhibit the highest average among all *Bos indicus* beef cattle varieties. However, *Bos taurus* cows produced more embryos than *Bos indicus* transferable embryos, in the same literature it was explained that the low number of transferable embryos was influenced by the frequency of superovulation of each individual (Damaris et al., 2023). According to Jatón et al. (2016), the frequency of flushing performed on each influences the number of transferable embryos.

If you look at the research results (Figure 8), of the three breeds of cow studied, namely Angus cow, Friesian Holstein, and Ongole crossbreeds, it shows that the Friesian Holstein cow has the lowest percentage of transferable embryos, namely 31.93%. Research result Damaris et al. (2023), also stated that the lowest number of transferable embryos produced by Friesian Holstein is because of a type of dairy cow that has a high level of progesterone metabolism when compared to beef cow. According to Kasimanickam et al. (2020), energy status plays a role in metabolic homeostasis and reproductive performance of dairy cows.

### Proportion of Degenerative Embryos

The proportion of degenerative embryos produced by Angus, Friesian Holstein, and Ongole crossbred cows showed significantly different results ( $P < 0.05$ ) (Figure 9). There are differences in the proportion of degenerative embryos between various cow breeds (Table 6). Angus and Friesian

Holstein cows did not show any notable variations ( $P > 0.05$ ). Angus cow and Ongole crossbreeds showed strongly significant differences ( $P < 0.01$ ). Friesian Holstein cow and Ongole crossbred cow showed no significant differences ( $P > 0.05$ ).

Based on research data (Table 6). Angus and Friesian Holstein cows in this study did not show any significant differences. This could be because the two breeds have equally good physical or physiological conditions, this can be seen from the cow's BCS (Bezdiček *et al.*, 2020), cow that experience a superovulation response are cows that have an intermediate BCS. The differences between Angus cow and Ongole crossbreeds are very significant. Differences in types of cow herds cause differences which influenced by the genetics of each herd. The very significant differences in Angus cow and Ongole crossbreeds also be caused by abnormalities in oocyte maturation or asymmetry between oocyte and follicle maturation (Peralta-Torres *et al.*, 2017) in one of the cattle clumps. Friesian Holstein cow and Ongole crossbred cow did not show significant differences. Based on the opinion of Sartori *et al.* (2016), *Bos indicus* cow and *Bos taurus* dairy cows have high progesterone metabolism, and have a rapid FSH absorption time or have an excessive percentage of anesthesia caused by luteinization of the follicles.

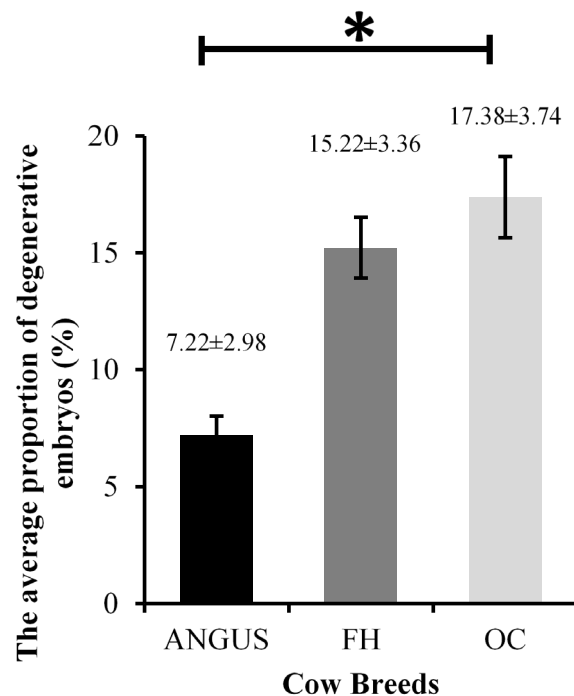


Figure 9 Proportion of degenerative embryos  $*P < 0.05$ . Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbred).

Table 6 Proportion of degenerative embryos in different cow breeds using the Mann-Whitney Test

Comparison of the Proportion of Degenerative in Different Cow Breeds					
Angus X FH		OC X Angus		FH X OC	
7.22±2.89	15.22±3.36	17.38±3.74 <sup>A</sup>	7.22±2.89 <sup>B</sup>	15.22±3.36	17.38±3.74

Note: values with different superscripts in the same column (A, B) are highly significant;  $P < 0.01$ ; FH (friesian holstein); OC (ongole crossbred).

According to Figure 9, Angus cows have the lowest average percentage of degenerative embryos compared to the other two breeds, which is 7.22%. This is to by research by Damaris *et al.*

(2023), *Bos taurus* cows produce the lowest percentage of degenerative embryos among other cow breeds. Cow with great genetics can also influence the low proportion of degenerative embryos produced because genetic selection to enhance the superovulation response is possible (Jaton et al., 2016), there is some genetic component to some of the traits associated with superovulation and selection is possible (Parker Gaddis et al., 2017; Wijayanti et al., 2022). Meanwhile, two other breeds of cow, the Friesian Holstein cow, and Ongole crossbreed cow, produced a higher percentage of degenerative embryos, namely 15.22% and 17.38%, respectively. This is by research by Damaris et al. (2023), if the quality of degenerative embryos in *Bos taurus* dairy cow and *Bos indicus* beef cow is higher than in *Bos taurus* beef cow. According to Naranjo-Chacón et al. (2019), high temperatures can have an impact on oocyte quality and embryo development, increasing embryo mortality and lowering embryo quality. So the location of the Cipelang Livestock Embryo Center (CLEC) which is in the highlands as a place for embryo production can be declared appropriate to minimize the space for the embryos produced

### Proportion of Unfertilized Ova

The proportion of unfertilized ova produced by Angus, Friesian Holstein, and Ongole crossbred cows when tested using the Kruskal Wallis test showed significantly different results ( $P < 0.05$ ) (Figure 10).

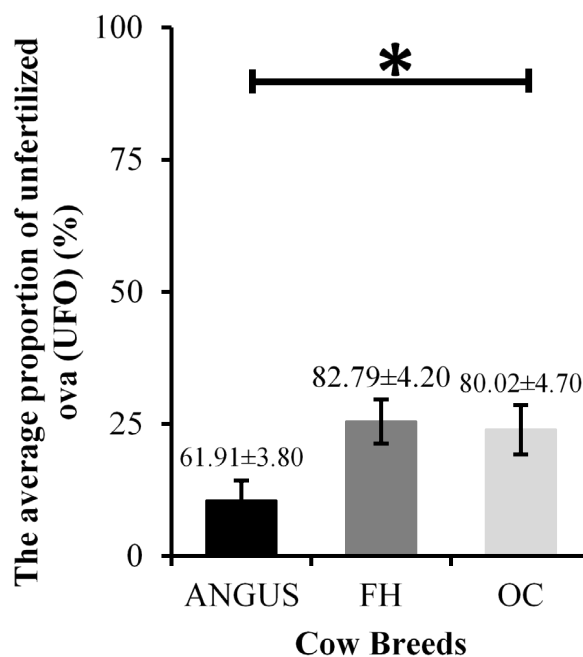


Figure 10 Proportion of unfertilized ova in three cow breeds  $*P < 0.05$ . Each bar indicates the average level of expression. Error bars are the standard error of the mean value. FH (friesian holstein); OC (ongole crossbreed)

Table 7 Comparison of the proportion of unfertilized ova in different cow breeds using the Mann-Whitney test

Comparison of the Proportion of Unfertilized ova in Different Cattle Breeds					
FH X Angus		OC X Angus		FH X OC	
25.42±4.20 <sup>a</sup>	10.49±3.80 <sup>b</sup>	23.88±4.70 <sup>a</sup>	10.49±3.80 <sup>b</sup>	25.42±4.20	23.88±4.70

Note: values with different superscripts in the same column (A, B) are highly significant;  $P < 0.01$ ; FH (friesian holstein); OC (ongole crossbreed).

The proportion of unfertilized ova produced by Angus, Friesian Holstein, and Ongole crossbred cows showed significantly different results. The obtained results were inconsistent with the research findings, as the proportion of unfertilized ova in the various cattle groups was not significantly different (Damaris *et al.*, 2023). According to Bó & Mapletoft (2020), increasing the dose of crude pituitary extract containing FSH and LH resulted in low levels of egg fertilization. The Mann-Whitney U test results revealed differences in the proportion of unfertilized ova among different breeds of cow (Table 8). Angus and Friesian Holstein cows showed significantly different results ( $P < 0.05$ ). Angus cow and Ongole crossbreeds showed significantly different results ( $P < 0.05$ ). Friesian Holstein and Ongole crossbreed cows showed no significant differences ( $P > 0.05$ ).

Based on the data in Table 7, Angus and Friesian Holstein cattle showed significantly different results. The real difference between the two breeds is probably caused by differences in the fertilization process because the fertilization process is an indicator of successful superovulation which can influence embryo quality (Damaris *et al.*, 2023). Angus cow and Ongole crossbreeds showed significantly different results. The difference between the dominant follicles of *Bos taurus* and *Bos indicus* is one factor, where the diameter of *Bos taurus* follicles is 8.5 mm while that of *Bos indicus* is 6.2 mm. This difference can affect the performance of FSH on follicle growth which is related to the process of ovulation of the dominant follicle and can trigger the egg not being fertilized (Damaris *et al.*, 2023). Friesian Holstein and Ongole crossbreed cows showed no significant differences. According to Sartori *et al.* (2016), *Bos indicus* cows have ovaries containing fewer antral follicles, larger ovulation follicle sizes, and a greater metabolic rate than dairy *Bos taurus* cows. In addition, *Bos taurus* has lower circulating peak concentrations of estradiol and progesterone than *Bos indicus*.

According to Figure 10, the average proportion of unfertilized ova in Angus cows is lower, at 10.49%. This average value can be stated if Angus cows have a fairly high fertility level. Meanwhile, two other breeds of cow, namely Friesian Holstein cow and Ongole crossbreeds, had a higher proportion of unfertilized ova, namely 25.42% and 23.88%, respectively. According to Damaris *et al.* (2023), this is influenced by the differences in follicular waves in *Bos taurus* and *Bos indicus* cow, where *Bos indicus* cow have 4 follicular waves and have high progesterone metabolism. The average unfertilized ova should be in the range of 5 – 20%. Meanwhile, from the research results, only Angus cattle produced an average of less than 20% unfertilized embryos.

## CONCLUSION AND IMPLICATIONS

This study concluded that CO cow had the highest average response for the number of CL, response rate, recovery rate, and total embryos collected, even though they produced a high number of DG and UFO. However, Angus cow produce the best quality embryos because they have a percentage of transferable embryos that is not much different from the other two breeds of cow and produce the lowest percentages of DG and UFO. Thus, of the three breeds of cow studied, Angus cow had the best superovulation response

## ACKNOWLEDGEMENT

Thank you to the leadership and staff of the Cipelang Livestock Embryo Center, Bogor, who helped provide the three herds of cattle used in this research. And has helped the process of implementing and collecting research data.

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