



Superovulation Response in Friesian Holstein and Ongole Grade Cows by Intramuscular Injection of Follicle Stimulating Hormone

Respon Superovulasi Sapi Friesian Holstein dan Peranakan Ongole terhadap Penyuntikan Follicle Stimulating Hormone secara Intramuskular

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ABSTRAK

Ketersediaan benih unggul di Indonesia yang masih terbatas sehingga penggunaan metode superovulasi yang melibatkan hormon perangsang folikel untuk mempercepat perkembangan folikel dan meningkatkan ovulasi dapat dijadikan sebagai solusi dalam proses transfer embrio. Penelitian ini bertujuan untuk menganalisis respon superovulasi pada sapi Friesian Holstein dan Peranakan Ongole dilihat dari jumlah corpus luteum (CL), embrio yang dikumpulkan, embrio layak transfer (ELT), embrio tidak layak transfer (ETLT), dan proporsi ELT dan ETLT, masing-masing dikelompokkan berdasarkan usia. Penelitian ini dilakukan di Balai Embrio Ternak Cipelang, menggunakan 49 ekor pada masing-masing sapi FH dan PO, dan melibatkan sinkronisasi pra-perlakuan, superovulasi, pemanenan embrio, serta evaluasi embrio, dengan analisis data menggunakan uji-T tidak berpasangan pada IBM SPSS Statistics. Hasil penelitian menunjukkan tidak adanya perbedaan antara sapi PO dan sapi FH dalam hal jumlah corpus luteum (CL), embrio yang terkumpul, dan kualitas embrio. Sapi PO memiliki jumlah CL dan embrio terkumpul yang lebih tinggi, tetapi proporsi embrio yang tidak layak transfer juga lebih besar, menunjukkan signifikansi dalam kualitas reproduksi antara kedua jenis sapi ini. Sehingga penelitian ini menekankan pentingnya memahami dinamika reproduktif berbasis genetik dan umur dalam strategi pengembangan reproduksi ternak. Studi ini memberikan wawasan baru dalam teknik pemuliaan dan dapat meningkatkan efisiensi produksi bibit unggul di Indonesia.

ABSTRACT

Considering the limited availability of superior livestock seeds in Indonesia, using superovulation methods, which involve follicle-stimulating hormones to accelerate follicle development and increase ovulation, can solve embryo transfer processes. This study aims to analyze the superovulation response in Friesian Holstein (FH) and Ongole Grade (OG) cattle based on the number of corpus luteum (CL), collected embryos, viable embryos (VEs), non-viable embryos (NVEs), and the proportions of VEs and NVEs, categorized by age. The research was conducted at the Cipelang Embryo Center,

KEYWORDS:

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involving 49 FH and OG cows, with synchronization, superovulation, embryo collection, and evaluation. Data were analyzed using an independent T-test in IBM SPSS Statistics. The results showed no differences between OG and FH cattle regarding CL count, embryo collection, and embryo quality. OG cattle had a higher CL count and embryo collection but also a larger proportion of degenerate embryos, indicating reproductive quality differences between these cattle breeds. This study highlights the importance of understanding genetic and age-based reproductive dynamics in livestock reproduction strategies, offering new insights into breeding techniques to improve the efficiency of superior livestock production in Indonesia.

1. Introduction

The availability of superior livestock breeds is a measure of the quality of the livestock sector in Indonesia. However, the availability of superior cattle breeds remains low (Mutenje et al. 2020; Widyas et al. 2022). Enhancing superior breeds can be achieved through genetic quality improvement, but this process is time-consuming if conducted naturally (Fathoni et al. 2022). To address this issue, the application of reproductive biotechnology methods, such as embryo transfer, is necessary (Ferré et al. 2020). Embryo transfer is the process of extracting pre-implantation embryos from genetically superior female donor animals and transferring them to the uterus of female recipient animals, resulting in pregnancy (Mazzoni et al. 2020). The concept of embryo transfer involves engineering the reproductive functions of female livestock through superovulation, which allows for the ovulation of many ova that are then fertilized by superior spermatozoa from artificial insemination (Menkir and Ebrahim 2021).

Superovulation is used to grow, develop, and mature egg follicles, as well as to increase the number of eggs ovulated within the same estrous cycle (Darlian et al. 2021). An additional hormone, Follicle Stimulating Hormone (FSH), is administered to donor livestock to achieve superovulation. FSH is a hormone that stimulates follicular development in the ovaries, and the number of corpus luteum (CL) formed can be observed through rectal palpation of the ovaries (Afriani et al. 2020). The corpus luteum (CL) is formed from a ruptured follicle that has ovulated an egg. Therefore, the number of CLs formed indicates the number of eggs ovulated, which is the effect of superovulation (Darlian et al. 2021). Intramuscular injections of FSH are given for 3-4 days to reach the maximum number of follicles (Maciel et al. 2019), as FSH has a short half-life and requires repeated administration (Casarini et al. 2020).

Several factors, including the age and breed of the donor cattle, can influence the superovulation response in donor cattle. Studies show that older donor cattle have lower superovulation responses (Hirayama et al. 2019; Pohler et al. 2021; Simenta et al. 2021). Additionally, the breed of the donor cattle also affects the superovulation response. For example, Limousin cattle have the highest average of transferable embryos compared to Simmental and Angus, while Simmental cattle have the highest average of non-transferable and unfertilized embryos (Lubis et al. 2021). Madureira et al. (2020) also noted differences in ovulation response between *Bos indicus* and *Bos taurus* breeds.

This research aligns with previous studies on using FSH to evaluate superovulation response. Earlier studies, such as Karl et al. (2021), investigated the effects of high doses of FSH on ovulatory follicle function and estradiol production in young dairy cattle with small ovarian reserves. Reinoso et al. (2022) used FSH for superovulation in red Angus cattle, focusing on ovarian response and embryo quality. Afriani et al. (2022) evaluated the superovulation response in coastal cattle using GnRH, with parameters such as the number of corpus lutea and embryos produced.

However, this study is unique in evaluating the superovulation response in two different types of cattle, Friesian Holstein (FH) and Ongole Grade (OG), focusing on the comparison of the number of corpus lutea, collected embryos, and the proportion of transferable and non-transferable embryos by age group. Thus, this study differs from previous studies that emphasized the dose of FSH, source of FSH, or use of GnRH, as the aim of this study is to analyze the superovulation response in FH and OG cattle based on the number of corpus lutea, collected embryos, transferable embryos, non-transferable embryos, and the proportion of transferable and non-transferable embryos, each grouped by age. The hope is to obtain a large number and quality of ova and embryos suitable for embryo transfer in recipient cattle. Thus, this study contributes new insights into how age factors influence the outcomes of superovulation in two different types of cattle in Indonesia, providing important insights for enhancing the production of superior breeds through biotechnological reproductive technology.

2. Materials and Methods

This research was conducted at the Livestock Embryo Center (BET) Cipelang in Cijeruk District, Bogor, West Java, from December 2023 to January 2024. The study

utilized samples of FH and OG cattle, each comprising 49 individuals ranging from young (27 months) to old ages (up to 114 months). The donor cattle used in this study met specific criteria: possessing superior genetics, high reproductive capabilities, a normal oestrous cycle ranging from 18 to 21 days, high fertility, and a Body Condition Score (BCS) between 2.8 and 3.2.

2.1. Pre-Treatment Synchronization

Pre-treatment synchronization aimed to synchronize the growth of follicular waves before the superovulation process. This synchronization used a progesterone device (Cue-Mate®, containing 1.56 mg of progesterone in two silicone pods), implanted in the vulva for 11 days, with the implantation day considered as day 0 in the superovulation process sequence.

2.2. Superovulation

Superovulation in this study was induced using an FSH preparation (Folltropin-V®, containing 400 mg NIH-FSH-P1) at a total 400mg/20ml dose. Intramuscular FSH injections began on the ninth day after the insertion of the progesterone device. The injections were administered twice daily (morning and evening) for three consecutive days with decreasing doses (5, 5, 3, 3, and 2, 2 ml). This procedure was adapted from the study by Darlian et al. (2021) conducted at the Cipelang Livestock Embryo Center, Bogor, and was by the superovulation SOP at BET Cipelang. In that study, injections were given intramuscularly in the morning and evening for three consecutive days with decreasing doses (5:5 ml, 3:3 ml, 2:2 ml). The total dose was 400 mg of FSH dissolved in 20 ml of solvent. Prostaglandin F2 α injections (Prostavet-C®, 5 mg etiproston per 2 ml, Virbac Lab) were also administered twice a day, on the 11th day after the insertion of the progesterone device and in the morning of the day the device was removed. Artificial insemination was performed three times over 12 hours, starting on the 13th day (morning and evening) and the morning of the 14th day. The sequence of the superovulation process is illustrated in **Figure 1**.

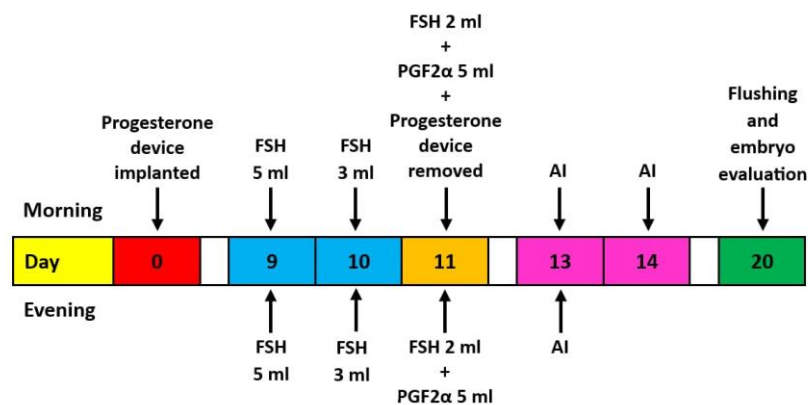


Figure 1. Series of superovulation processes

Based on the image of the superovulation process sequence above, it complies with the standard operating procedures (SOP) implemented at BET Cipelang, as referenced in the research by Darlian et al. (2021).

2.3. Embryo Collection

Embryo collection was conducted on day 20 or 7 days after the first artificial insemination using a non-surgical method via trans-cervical access. The procedure involved rinsing the uterus three times (right, left, and body of the uterus) with a lactated Ringer's solution. Initially, the cattle received epidural anaesthesia between the last sacrum and first coccygeal bones. A cervix expander was inserted to open the cervical cavity, followed by the placement of a Foley catheter in the right/left uterus and rinsing until the lactated Ringer's solution was exhausted. After rinsing, 50 ml of 2% iodine povidone solution was administered intra-vaginally, and prostaglandin hormone was injected to accelerate the reproductive system's recovery.

2.4. Embryo Evaluation

The flushing solution from each 500 ml bottle was filtered using an embryo filter. The search for embryos in the filtered flushing media was performed using a stereo microscope with 50x magnification. Observed embryos were collected in an embryo storage media and evaluated based on their morphological and developmental stages to determine the embryo quality. The number of collected embryos was then classified based on their grades according to the guidelines from the International Embryo Transfer Society (IETS 2010), as follows: grades 1, 2, and 3 (suitable for transfer), grade 4

(unsuitable for transfer), and UF. Embryos of grade 1 and 2 could be immediately frozen, and those of grade 3 could be transferred directly to the recipient, while grade 4 and UF embryos were discarded.

2.5. Observed Parameters

The parameters observed included:

- a. Number of CLs formed: CL (Corpus Luteum) indicates ovulation and follicular development in the ovaries. The number of CLs is counted through rectal palpation one day before embryo collection. Palpation is performed to evaluate the superovulation response by observing the number of CLs formed on the ovarian surface. The more CLs formed, the greater the possibility of ovulated ova.
- b. Number of embryos collected: Embryos are collected on day 20, or seven days after the first artificial insemination, using flushing. Before embryo collection, rectal palpation is performed to determine the number of CLs. The embryo collection process involves gradually flushing the uterine horns with lactated Ringer's solution. The collected embryos are then counted, and their quality is evaluated.
- c. Number of Viable Embryos (VE) and Non-Viable Embryos (NVE):

Viable Embryo (VE): These are embryos suitable for transfer, specifically those of good quality (grades 1, 2, and 3), based on the guidelines of the International Embryo Transfer Society (IETS). Evaluation is conducted using a stereo microscope to determine the stage of morphological development. Embryo Quality Based on Each Criterion is presented in **Table 1**.

Non-Viable Embryo (NVE): These are embryos unsuitable for transfer, categorized as grade 4 and unfertilized. These embryos show signs of degradation or failure in fertilization between the ovum and sperm cells.

Table 1. Embryo quality based on each criteria (International Embryo Transfer Society (IETS) (2010))

Embryo Quality		Standard	Criteria	Embryo Group
Freezable (Freezing)	1	Excellent	Very good, symmetrical, and round embryo shape with uniform blastomeres in terms of size, color, and density, with no defects, and	Suitable for Transfer

Embryo Quality	Standard	Criteria	Embryo Group
Fresh Transfer	2	Good	having a near-perfect viable embryo mass.
		Fair	The embryo surface is not perfectly smooth with cell degeneration of around 0-10%.
	3	Poor	Irregular shape (moderately irregular) in terms of embryo mass, size, color, and individual cell density, with at least 50% viable embryo mass.
			The embryo is predominantly irregular in terms of embryo mass shape, size, color, and individual cell density, with at least 25% viable embryo mass.
Discard	4	Dead or Degenerate	Embrio degradasi, massa embrio viable 0% (non viable)
	UF	Unfertilized	Cells are damaged and unfertilized, with the presence of oocytes.
			Not Suitable for Transfer

The observation procedure began with the injection of FSH, which was administered intramuscularly on the 9th day after progesterone implantation. This injection was performed for three consecutive days with a decreasing daily dose. PGF2 α hormone injection was carried out on the 11th day to stimulate ovulation. After this injection, the progesterone device was removed. Artificial Insemination (AI) was performed on the 13th and 14th days to ensure fertilization. On the 20th day, embryo harvesting or flushing was conducted. Before the flushing procedure, rectal palpation was done to count the corpus luteum (CL) number. Flushing was carried out using a lactated ringer solution to collect the embryos. The collected embryos were then evaluated in the laboratory using a stereo microscope. Based on their morphology, the embryos were classified as viable embryos (VE) and non-viable embryos (NVE).

- d. Recovery rate (RR), the proportion of VE (PVE), and the proportion of NVE (PNVE), formulated as:

$$\begin{aligned}
 RR &= \frac{\sum \text{collected embryos}}{\sum \text{formed CL}} \times 100\% \\
 PVE &= \frac{\sum \text{VE}}{\sum \text{collected embryos}} \times 100\% \\
 PNVE &= \frac{\sum \text{NVE}}{\sum \text{collected embryos}} \times 100\%
 \end{aligned}$$

- e. The number of each CL, collected embryos, VE, and NVE were grouped by various ages according to tooth wear (FSIS 2021; Karisch 2022) presented in **Table 2**.

Table 2. Tooth eruption with age range

Tooth Eruption	Age (months)
Eruption 1 (I ₁)	18-24
Eruption 2 (I ₂)	25-36
Eruption 3 (I ₃)	37-42
Eruption 4 (I ₄)	43-48
> Eruption 4 (> I ₄)	> 48

Considering the limited availability of donor cows, age was classified based on tooth eruption, ranging from 18 months to over 48 months. We also compared the superovulation response between FH and OG cattle within these age groups. This analysis aimed to evaluate whether the age of the cows influences the number of corpus luteum, collected embryos, and the quality of the embryos produced in both cattle breeds (**Table 4**).

2.6. Data Analysis

The data obtained are secondary data, which are subsequently calculated and expressed as mean, standard deviation, and percentage. Data collection was conducted by grouping the number of individuals based on different categories and further classified by various age groups (I₁, I₂, I₃, I₄, and > I₄). The data were then compared based on different categories, age groups, and the comparison between different age groups. The data were analyzed and processed using a Kolmogorov-Smirnov for the abnormality of the data (caused less of data were analyzed afterwards), then using independent T-test in the IBM SPSS Statistics 25 application, with the following formula (Nurmalasari 2018):

Homogeneity of Variances Test, with H₀: $\sigma_1 = \sigma_2$; H₁: $\sigma_1 \neq \sigma_2$

F-statistic:

$$F = \frac{s^a(\text{variance of the larger value})}{s^b(\text{variance of the smaller value})}$$

Homogeneous Variance ($\sigma_1 = \sigma_2$) :

$$t = \frac{\bar{x}_a - \bar{x}_b}{sp \sqrt{\frac{n_a + n_b}{n_a \cdot n_b}}} \quad \text{where } sp = \sqrt{\frac{(n_a - 1)S_a^2 + (n_b - 1)S_b^2}{n_a + n_b - 2}}$$

Heterogeneous Variance ($\sigma_1 \neq \sigma_2$) :

$$t = \frac{\bar{x}_a - \bar{x}_b}{\sqrt{\frac{S_a^2}{n_a} + \frac{S_b^2}{n_b}}}$$

3. Results and Discussion

3.1. Total Number of Corpus Luteum Formed

The success of superovulation can be observed from the number of corpora lutea (CL) formed. The number of CL can estimate the number of ovulated eggs; therefore, the more CL that form, the more eggs are ovulated. The number of CL formed in FH and OG cattle is presented in **Table 3**. In this study, the average total CL in OG cattle was higher than in FH cattle, but the statistical results showed no significant difference ($P>0.05$). However, this difference (number of CL) may be due to the type of cattle, *Bos taurus* (FH) versus *Bos indicus* (OG), which exhibit different responses to heat stress. Sajjanar et al. (2024) stated that *Bos indicus* breeds are more resistant to heat stress compared to *Bos taurus* breeds. Kasimanickam and Kasimanickam (2021) noted that heat stress in *Bos taurus* causes abnormal development of preovulatory follicles, followed by disruptions in the formation and function of CL, leading to suboptimal progesterone production in the uterus. Average total CL, collected embryos, embryo quality, and Recovery Rate is presented in **Table 3**.

Table 3. Average total CL, collected embryos, embryo quality, and recovery rate

Variable	FH (n=24)	OG (n=29)
CL formed	11.71±5.39 ^a	12.41±7.03 ^a
Collected Embryos (CE)	10.67±4.54 ^a	11.83±6.83 ^a
Embryo quality:		
- Viable Embryo (VE)	7.40±4.72 ^a	6.18±3.66 ^a
- Non-Viable Embryo (NVE)	6.62±4.29 ^a	6.96±4.77 ^a
Recovery Rate (RR) (%)	91.12	95.32

Notes: ^aSame superscript letters on the same line indicate a non significant difference ($P>0.05$)

The variation in the number of CLs formed can also be attributed to the differences in the number of small follicles on the ovaries of FH and OG cattle formed before FSH

hormone stimulation. Mossa and Ireland (2019) stated that examining the number of small follicles can depict the level of fertility in the breeders, which can be linked to superovulation, indicating that the more small follicles detected, the higher the fertility rate, which then becomes embryos. The number of small follicles increases with age, as explained by Lopes et al. (2020), showing an increase in the number of small follicles as parity increases. The number of CLs in different age groups is presented in **Table 3**.

In this study, the average number of CL in OG cattle was higher compared to FH cattle across all age ranges, with a trend of increasing CL count as the cattle aged. These results suggest that OG cattle have higher fertility levels than FH cattle in terms of superovulation. The number of CLs formed in OG cattle (12.41 ± 7.03) was higher than in FH cattle (11.71 ± 5.39), but statistically, there is no significant difference ($P > 0.05$). Additionally, the embryo recovery rate in OG cattle was higher (95.32%) compared to FH cattle (91.12%). The number of collected embryos (CE) in OG cattle (11.83 ± 6.83) was greater than in FH cattle (10.67 ± 4.54). This variation may be due to differences in heat stress response between *Bos indicus* (OG) and *Bos taurus* (FH) cattle, as noted by Sajjanar et al. (2024) and Kasimanickam and Kasimanickam (2021).

However, statistically, there was no significant difference ($P > 0.05$) in the number of CLs based on specific age groups. Furthermore, no significant difference ($P > 0.05$) was found in the total samples of FH and OG cattle when compared by different age groups. The increase in corpus luteum (CL) with age is closely related to the number of collected embryos. This can be attributed to the reduced ability of follicles to respond to exogenous gonadotropins in older livestock, which limits follicle growth and development. Specifically, in older age of animals, their follicles become less responsive to stimuli from hormones like FSH, resulting in fewer follicles maturing and ovulating, ultimately leading to fewer oocytes being ovulated, as reflected by fewer CLs formed. This phenomenon has been documented in several studies, which indicate that although CL formation may increase with age (in line with ability of follicular sensitivity), there is a specific limits of it and it will tends to decrease afterwards (Moorey et al. 2022).

3.2. Total Number of Collected Embryos and Recovery Rate

An embryo is the result of fertilization between an ovum and a sperm cell, either through natural mating or artificial insemination (AI). The number of CE can be seen in

Table 3. In this study, the average number of CE in OG cattle was higher compared to FH cattle, but statistical results showing no significant difference ($P>0.05$). However, this difference (number of CE) could be due to higher levels of progesterone in *Bos indicus* compared to *Bos taurus* (Batista et al. 2020), especially since the metabolism of progesterone in dairy cattle is higher than in beef cattle (Damaris et al. 2023). However, Mapletoft et al. (2002) and Mapletoft et al. (2015) reported that *Bos indicus* had a lower number of collected embryos (12.1 embryos) compared to *Bos taurus* (13.2 embryos). The RR values in **Table 3** indicate that OG cattle had higher rates than FH cattle. The differences in RR values may be due to technical issues, such as rectal palpation for counting CLs being less accurate than ultrasonography (USG) (Samir et al. 2023). Assessments by different experts may also influence the variance in estimated CL numbers (Fordham et al. 2022). The number of collected embryos by age group is presented in **Table 4**.

In this study, the average number of CE from OG (*Bos indicus*) cattle was higher than FH (*Bos taurus*) cattle. For example, in the age group I₄, OG cattle had an average of 10.67 ± 5.03 embryos collected, while FH cattle had an average of 7.71 ± 3.64 embryos. This supports the finding that *Bos indicus* cattle may have higher progesterone levels, which may contribute to the increased number of embryos collected. Previous studies have explained that progesterone levels are higher in *Bos indicus* than in *Bos taurus* (2.3 ± 0.2 vs 2.0 ± 0.2 ng/mL under HDMI (High Dry Matter Intake) and 3.4 ± 0.2 vs 2.3 ± 0.2 ng/mL under LDMI (Low Dry Matter Intake)) (Batista et al. 2020), mainly due to higher progesterone metabolism in dairy cattle than in beef cattle (Damaris et al. 2023). However, Mapletoft et al. (2002) dan Mapletoft et al. (2015) reported that *Bos indicus* had fewer embryos collected (12.1 embryos) compared to *Bos taurus* (13.2 embryos). The RR values in **Table 3** indicate that OG cattle have a higher ratio than FH cattle. The difference in RR values may be due to technical issues, such as rectal palpation being less accurate for counting CL than ultrasonography (USG) (Samir et al. 2023). The assessment by different experts may also contribute to variance in estimating the number of CL (Fordham et al. 2022). The number of collected embryos by age group is presented in **Table 4**.

Table 4. Average on CL, collected embryos, VE, and NVE by age group.

Variable	Age	FH cattle		OG cattle		$\bar{x}$ (FH+OG)
		n	$\bar{x}$	n	$\bar{x}$	
CL	I ₂	5	8.20±2.86 ^a	4	5.75±3.20 ^a	8.29±2.36 ^c
	I ₃	4	7.50±1.92 ^a	3	11.67±5.51 ^a	8.00±2.53 ^c
	I ₄	7	7.86±3.81 ^a	3	11.00±5.00 ^a	9.14±3.13 ^c
	> I ₄	14	13.57±7.63 ^a	3	12.60±7.69 ^a	12.85±6.28 ^c
CE	I ₂	5	7.80±2.39 ^a	4	10.00±8.66 ^a	7.71±1.98 ^c
	I ₃	4	6.75±2.75 ^a	3	11.00±4.58 ^a	10.43±4.50 ^c
	I ₄	7	7.71±3.64 ^a	3	10.67±5.03 ^a	7.00±2.50 ^c
	> I ₄	13	12.54±6.74 ^a	20	12.00±7.41 ^a	10.55±3.97 ^c
VE	I ₂	5	5.20±2.28 ^a	3	4.00±3.54 ^a	5.67±2.34 ^c
	I ₃	4	3.75±3.10 ^a	3	2.67±2.08 ^a	2.67±2.08 ^c
	I ₄	5	5.00±3.46 ^a	3	4.33±4.04 ^a	3.75±2.36 ^c
	> I ₄	12	8.67±5.12 ^a	12	6.92±3.20 ^a	6.80±2.67 ^c
NVE	I ₂	4	3.25±1.71 ^a	3	9.33±5.13 ^a	5.40±2.07 ^c
	I ₃	6	4.83±3.87 ^a	3	9.00±5.29 ^a	5.60±2.07 ^c
	I ₄	4	3.25±1.89 ^a	3	6.67±6.43 ^a	3.80±2.05 ^c
	> I ₄	4	12.75±3.86 ^a	9	11.11±4.54 ^a	6.90±2.56 ^c

^a Values with the same superscript letter on the same line indicate no significant difference (P>0.05);

^c Values with the same superscript letter in the same column indicate no significant difference (P>0.05).

The highest average of CE was found in FH cattle aged > I₄, while the lowest average was found in FH cattle aged I₃. Statistical results showed that the difference in the average number of collected embryos was not significant (P>0.05) at certain ages when comparing FH and OG cattle. The average number of collected embryos across the total sample (FH and OG) at each age had the highest average at age >I₄ and the lowest at age I₄, but statistical results indicated no significant difference (P>0.05) in age differences. Mikkola et al. (2019) state that age is a very minor factor that can influence the response to superovulation and embryo collection in both dairy and beef cattle.

3.3. Number of Viable Embryos

The success of the superovulation process can be observed from the proportion of viable embryos (VE) collected (Damaris et al. 2023). The number of VEs is presented in **Table 3**. In this study, the average number of VEs in OG cattle was higher than in FH cattle, but statistical results showed no significant difference (P>0.05). This could be due to the breed differences between FH cattle (*Bos taurus*) and OG cattle (*Bos indicus*), with *Bos indicus* having higher progesterone levels than *Bos taurus*, as shown by the difference in the number of corpus lutea formed (**Table 3**). Nagy et al. (2021) explained that progesterone regulates molecular, biochemical, and physiological interactions in the

uterus, affecting embryo growth, development, and survival. In dairy cattle, progesterone levels tend to be lower than in beef cattle due to high progesterone metabolism (Damaris et al. 2023), which could be associated with the different numbers of VEs in each breed.

The number of VEs by age group is presented in **Table 4**. This study found the highest average VEs in OG cattle aged $>I_4$ and the lowest in those aged I_2 . Statistical results showed that the average VEs were insignificant ($P>0.05$) at specific ages compared to FH and OG cattle. The average VEs for the total sample (FH and OG) at each age had the highest average at age $>I_4$ and the lowest at age I_3 . However, statistical results showed no significant age differences ($P>0.05$). The differences in the number of VEs at young (I_2) and old ($>I_4$) ages in this study, as explained by Tutt et al. (2023), are due to insufficient glucose transporter activity, suboptimal protein translation size, and low metabolism and cytoplasmic maturation of oocytes in young cattle.

3.4. Number of Non-Viable Embryos

In addition to the viable embryos (VE) collected, there were also embryos of non-viable embryos (NVE), which included degenerative embryos (DG) and unfertilized embryos (UF) (International Embryo Transfer Society (IETS) 2010). The number of NVE is presented in **Table 3**. In this study, the average number of NVE in OG cattle was higher compared to FH cattle, but the statistical results showing no significant difference ($P>0.05$). However, this difference (number of NVE) can be linked to variations in estrogen levels between the two cattle breeds, which influence progesterone levels in the uterus. Previous research indicated that OG cattle exhibited much higher estrogen concentrations, particularly during the estrus phase, with a peak value of 122.38 ng/mL (Widyaningrum et al. 2020). In contrast, FH cattle showed lower estrogen concentrations, averaging around 9.81 ng/L to 11.18 ng/L, depending on the dietary supplementation provided (Zulfarniansyah and Safitri 2022). One of the functions of progesterone is to stimulate the secretion of interferon tau (IFN-T) (Melia et al. 2021). IFN-T serves as a pregnancy signal produced by trophoblast tissues to prevent the secretion of PGF2 α (Damaris et al. 2023). It is known that the secretion of PGF2 α can lyse the CL, which can damage and inhibit embryo growth. With high estrogen levels, progesterone levels decrease, which may result in suboptimal IFN-T secretion, leading to the secretion of PGF2 α . The number of NVE by age group is presented in **Table 4**.

In this study, the highest average NVE on different age was found in FH cattle aged $>I_4$, and the lowest average was found in FH cattle aged I_2 . However, the statistical results showed no significant difference ($P>0.05$) for specific ages. The total average of NVE (FH and OG) for each age had the highest average at age $>I_4$ and the lowest at age I_4 , but the statistical results showed no significant difference ($P>0.05$) for age differences. These are aligns with Chacón et al. (2019), who reported similar findings. As shown in **Table 4**, the highest number of NVE is 6.90 embryos at age $>I_4$, which may be caused by abnormalities in oocyte maturation or asynchrony between oocyte and follicle maturation (Chacón et al. 2019).

3.5. Proportion of VEs and DEs

The success rate in performing superovulation can be assessed by having a high proportion of viable embryos (VEs) and as minimal a proportion of non-viable embryos (NVEs) as possible. The proportion of VEs and NVEs in the age group of FH and OG cattle that presented in **Table 5** shows that the highest proportion of VEs was found in FH cattle (55.16%). In comparison, the highest percentage of NVEs was found in OG cattle (59.11%). In the age group, the highest proportion of VEs in FH cattle was found at age I_2 (66.67%) and the lowest at age I_3 (28.26%), while the highest proportion of VEs in OG cattle was found at age $>I_4$ (45.28%) and the lowest at age I_3 (15.15%). This may explain that although OG cattle have a high number of CL and CEs, it does not necessarily correlate with a high number of collected VEs, as stated by Damaris et al. (2023). Proportion of collected VEs and NVEs by age group is presented in **Table 5**.

Table 5. Proportion of collected VEs and NVEs by age group

Age Group	FH		OG	
	VE	NVE	VE	NVE
I_2	66.67%	33.33%	21.05%	78.95%
I_3	28.26%	71.74%	15.15%	84.85%
I_4	49.09%	50.91%	34.37%	65.63%
$>I_4$	61.76%	38.24%	45.28%	52.72%
Total	55.16%	44.84%	40.89%	59.11%

4. Conclusion

This study found a non significant difference in the superovulation response between FH and OG cattle, from variable of the number of corpus luteum (CL), collected

embryos (CE), viable embryos (VE), and non-viable embryos (NVE). OG cattle had a higher average CL and number of collected embryos than FH cattle, but the embryo quality was lower, with a higher number of NVE. Statistically, the number of VE between OG and FH cattle did not significantly differ. Based on age, both cattle types showed an increase in the number of CL and collected embryos with age, without a significant difference in the quality of VE. This study has limitations in sample size and variation in CL counting techniques, which could affect the results. Therefore, future research should increase the sample size, use more consistent CL counting techniques, such as ultrasonography (USG), and consider environmental and livestock management variables for a more comprehensive analysis.

Furthermore, although FSH has been widely used in the standard operating procedures for superovulation in embryo transfer, this study provides novelty from a comparative perspective between two cattle types, Friesian Holstein and Ongole Grade. The focus of this study is not only on the dosage or application of FSH itself but also on how the superovulation response, corpus luteum (CL) count, and embryo quality are influenced by genetic and age factors in these two cattle types, which have not been thoroughly explored in previous literature.

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