Comparative Superovulatory Response, Embryo Recovery, and Embryo Quality in Holstein-Friesian and Limousin Donor Cattle in Indonesia

Anggita Dian Pramudhita[1], Detry Al Bukhary[2], Putri Utami[1], Habib Asshidiq Syah[1], Anny Rosmayanti[3], Putri Indah Ningtias[3], Fahrudin Darlian[3], Trinil Susilawati[1], and Aulia Puspita Anugra Yekti[1]*

[1]Department of Animal Reproduction and Breeding, Faculty of Animal Science and Technology, Universitas Brawijaya, Malang, Indonesia
[2]PT. Bintang Ternak Abadi, Tegal Regency, Indonesia
[3]Cipelang Livestock Embryo Center, Bogor, Indonesia
***Corresponding author email: auliapay@ub.ac.id**

Abstract. Embryo transfer (ET) is a reproductive biotechnology used to accelerate the dissemination of superior genetics. However, its success under Indonesian tropical conditions remains variable and may be influenced by physiological and adaptive differences between dairy and beef cattle used as embryo donors. This study compared in vivo embryo production between Holstein-Friesian (HF) and Limousin donor cattle. The study was designed as a comparative observational study using selected donor cattle, comprising 11 HF and 25 Limousin cows maintained under standardized ET management. The observed parameters included superovulatory response, represented by the number of corpora lutea (CL), total ova/embryos collected, fertile embryos, transferable embryos, embryo quality, response rate, and recovery rate. Data were analyzed using descriptive statistics, and breed differences were analyzed using Welch's independent t-test when variance heterogeneity was detected, and Cohen's d was used to estimate effect size. Limousin cows showed higher CL counts than HF cows (12.72 ± 6.98 vs. 6.55 ± 2.88; $P < 0.001$), produced more total ova/embryos (11.44 ± 6.45 vs. 6.00 ± 3.00; $P < 0.001$), fertile embryos (9.16 ± 5.17 vs. 3.45 ± 2.81; $P < 0.001$), and transferable embryos (5.76 ± 4.29 vs. 1.91 ± 2.07; $P < 0.001$). Response rate between HF and Limousin cows was 94.12% vs. 92.86%, as was the recovery rate (89.92% vs. 90.12%). Overall, these findings indicate that under the conditions of this study, Limousin cattle exhibited higher superovulatory responses and embryo production than HF cattle, although breed-specific protocol optimization remains necessary.

Keywords: embryo transfer, embryo production, embryo quality, Holstein-Friesian, Limousin

Abstrak. Transfer embrio (TE) merupakan bioteknologi reproduksi yang digunakan untuk mempercepat penyebaran genetik unggul. Namun, keberhasilannya dalam kondisi tropis Indonesia masih bervariasi dan dapat dipengaruhi oleh perbedaan fisiologis serta kemampuan adaptasi antara sapi perah dan sapi pedaging yang digunakan sebagai donor embrio. Penelitian ini membandingkan produksi embrio in vivo antara sapi donor Holstein-Friesian (HF) dan Limousin. Penelitian dirancang sebagai studi observasional komparatif menggunakan sapi donor terpilih, yang terdiri atas 11 ekor HF dan 25 ekor Limousin yang dipelihara di bawah manajemen TE yang terstandar. Parameter yang diamati meliputi respons superovulasi, yang direpresentasikan oleh jumlah corpus luteum (CL), total ovum/embrio yang terkoleksi, embrio fertil, embrio layak transfer, kualitas embrio, response rate, dan recovery rate. Data dianalisis secara deskriptif dan perbedaan antarbangsa dianalisis menggunakan Welch's independent t-test. Jika heterogenitas varians terdeteksi, serta Cohen's d untuk mengestimasi ukuran efek. Sapi Limousin menunjukkan jumlah CL yang lebih tinggi dibandingkan sapi FH (12,72 ± 6,98 vs. 6,55 ± 2,88; P < 0,001), menghasilkan total ovum/embrio yang lebih banyak (11,44 ± 6,45 vs. 6,00 ± 3,00; P < 0,001), embrio fertil (9,16 ± 5,17 vs. 3,45 ± 2,81; P < 0,001), dan embrio layak transfer (5,76 ± 4,29 vs. 1,91 ± 2,07; P < 0,001). Response rate antara sapi FH dan Limousin adalah 94,12% vs. 92,86%, demikian pula recovery rate sebesar 89,92% vs. 90,12%. Secara keseluruhan, temuan ini menunjukkan bahwa, dalam kondisi penelitian ini, sapi Limousin memiliki respons superovulasi dan produksi embrio yang lebih tinggi dibandingkan sapi HF, meskipun optimasi protokol masih diperlukan.

Kata kunci: transfer embrio, produksi embrio, kualitas embrio, *Friesian-Holstein, Limousin*

Introduction

The limited genetic quality of local dairy and beef cattle remains a major constraint on livestock productivity in Indonesia, preventing domestic beef and milk production from meeting national demand. Beef demand has exceeded 700,000 tons per year, while domestic milk production supplies only approximately 20–30% of national needs (BPS, 2025). This dependence on imported superior livestock contributes to the livestock trade deficit, highlighting the need to accelerate genetic improvement through superior genetic resources.

Embryo transfer (ET) technology is an important reproductive biotechnology for accelerating the dissemination of superior genetics by increasing the number of offspring from genetically valuable donor females and superior sires, shortening generation intervals, and improving reproductive efficiency in dairy and beef cattle breeding programs (Hansen, 2024). Compared with conventional breeding, ET enables faster multiplication of superior genotypes, particularly when elite donors are limited. However, the effectiveness of ET under Indonesian tropical conditions remains variable due to heat stress, reproductive management, donor physiological status, and responses to in vivo hormonal protocols, with a success rate of only around 30-40% (Supriatna, 2018). Therefore, successful in vivo embryo production requires the integration of appropriate donor selection, estrus synchronization, superovulation response, artificial insemination success, embryo collection techniques, and operator competency (Baharun et al., 2025).

The donor cow's condition is a critical factor, making donor selection the most decisive initial stage. Producing high-quality offspring through ET must begin with genetically superior donor cows that have optimal body performance and are in good physiological condition. This is followed by superovulation induction to increase the number of oocytes available for fertilization (Say et al., 2021). Superovulation is an effort to increase the number of ova by suppressing follicular atresia, enhancing gonadotropin stimulation of the ovaries, and supporting estrous-cycle regulation (Khan et al., 2022). Superovulation uses various hormonal protocols, such as PMSG–hCG, FSH–LH, and GnRH. Responses to these protocols vary depending on the physiological condition and breed of cattle (Khodadadi et al., 2022). The physiological condition of the donor cow, including health status, endocrine balance, and body condition score (BCS), greatly influences the response to superovulation treatment and the quality of resulting embryos.

Previous studies have shown that donor-related internal factors, including age, follicular status, health status, nutritional condition, endocrine profile, and breed, can affect ovarian response, embryo yield, and embryo quality (Garza et al., 2023; Salek et al., 2025). In addition, external factors such as environmental conditions, ambient heat load, nutritional management, semen quality, hormonal protocol, insemination timing, and embryo recovery technique can further modify embryo production capacity (Gómez-Guzmán et al., 2024).

Technically, the stages of in vivo embryo production include donor selection, multiple-ovulation induction, artificial insemination (AI), embryo collection, and embryo evaluation and classification (Wahjuningsih et al. 2019). Failure at the donor selection and early reproductive management stages is likely to result in a low superovulation response and inferior quality embryos, thereby reducing the overall efficiency and success of the embryo production program. Differences in cattle breeds are known to influence superovulation responses, oocyte quality, and the success rate of embryo production (Muttaqin et al., 2023). Therefore, breed-specific physiological characteristics should be considered when evaluating embryo production efficiency, particularly under environmental conditions that may challenge reproductive performance. Recent evidence also indicates that hormonal stimulation and donor phenotype can influence the number and quality of oocytes or embryos produced, supporting the need for breed-adapted reproductive protocols (Salek et al., 2025).

In practice, embryo production programs in Indonesia generally use exotic cattle breeds, especially HF and Limousin, due to their high genetic potential. HF cattle represent the main dairy cattle breed with the potential for high milk production (Pramudhita et al., 2025), while Limousin cattle, as beef cattle, are known to have a fast growth rate (Yekti et al., 2023), relatively better environmental adaptability, and a high carcass percentage (Vlasova et al., 2020). However, each cattle breed has different physiological characteristics and different capacities for adaptation to hormonal treatments and tropical environmental conditions.

Differences in metabolism, thermoregulatory ability, and endocrine response between breeds contribute to variations in reproductive responses, especially under conditions of heat stress and high humidity, which can reduce follicular development, oocyte competence, and embryo viability at early stages of development (Dovolou et al., 2023; Lang et al., 2024). Heat stress can impair reproductive performance through cellular and molecular alterations in oocytes, granulosa cells, and early embryos, including oxidative stress, mitochondrial dysfunction, and altered developmental competence (Gómez-Guzmán et al., 2024).

Several studies have reported variations in embryo production success among cattle breeds, with Limousin cattle showing a relatively high percentage of viable embryos for transfer. In contrast, HF cattle show a more variable response depending on the superovulation protocol used. Setiawan et al. (2017) reported that Limousin cows produced 57.7% viable embryos for transfer, while HF cows yielded an average of 54.03% (Damaris et al., 2023). Previous studies at the Livestock Embryo Center have evaluated embryo production in relation to seasonal variation (Robbaani et al., 2024), superovulation methods (Darlian et al., 2021), and multiple cattle breeds, often involving one dairy breed and several beef breeds. However, focused comparisons between HF dairy donors and Limousin beef donors under standardized ET management in the Indonesian tropical highlands remain limited. Therefore, this study aimed to compare in vivo embryo production between HF and Limousin donor cattle based on super-ovulatory response, embryo recovery, total embryo yield, and transferable embryo production under type B tropical highland conditions at 800–1400 m above sea level, with temperatures of 18–22 °C and relative humidity of 70–80%.

Materials and Methods

Study Location and Design

The research was carried out at the Cipelang Livestock Embryo Center (CLEC), Cipelang Village, Bogor, West Java (6°43′12″ S, 106°46′12″ E), at an altitude of 800–1400 m above sea level with tropical climate type B (temperature 18–22 °C and humidity 70–80%). We applied a quantitative comparative observational design, in which we treated cow breed as a natural biological factor without manipulation. Donor cattle were selected purposively based on biological and reproductive criteria. Random allocation was not applied because each parent represented one breed. To reduce systematic bias, all donors were treated uniformly in management, hormonal protocols, insemination schedules, and embryo collection procedures; all in vivo embryo production procedures followed applicable SOPs and reproductive management practices.

Donor Cow Selection and Experimental Materials

This study involved donor cattle from two breeds: 11 HF and 25 Limousin, all maintained at CLEC. The donor cows were clinically healthy, showed no abnormalities in the reproductive organs, and had good reproductive histories. The donor cows had a body condition score (BCS) of 3.0–3.5 on a scale of 1–5 and were 2–8 years old, which falls within the reproductive period when cows are generally productive and physiologically stable. All donor cows had previously been inseminated as a prerequisite for in vivo embryo production.

The materials used in this study included progesterone preparations (Cue-Mate®) as an intravaginal progesterone device, follicle-stimulating hormone (Folltropin®) as a gonadotropin for follicular stimulation, flushing medium consisting of Ringer's lactate enriched with 1% calf serum and 0.1% penicillin, prostaglandin $F_2\alpha$ ($PGF_2\alpha$/Lutalyse®) to induce corpus luteum regression, and supporting equipment including a stereomicroscope, Foley catheter, cervical expander, 50cc, 20cc, 10cc, 5cc syringe, gun spool, and Pasteur pipettes.

Superovulation

Superovulation was initiated by inserting an intravaginal progesterone device (Cue-Mate®) on day 0 to support the luteal phase (Darlian et al., 2021). From day 9, FSH was administered intramuscularly twice daily: 4 mL morning and evening. On day 10, injections continued at 3 mL twice daily. On the morning of day 11, 2 mL FSH was given with prostaglandin F2α (PGF2α) to induce luteolysis; the same treatments were repeated in the afternoon, and the Cue-Mate® device was removed. The final FSH dose (1 mL twice daily) was given on day 12. All injections were performed by trained personnel following standard procedures (Figure 1).

Non-surgical In Vivo Embryo Collection

In vivo embryo collection was performed non-surgically via transcervical uterine flushing, as commonly used in bovine embryo transfer procedures (Darlian et al., 2021). The procedure began with the examination of the number of corpora lutea (CL) in post-superovulation donor cows to confirm ovarian response. The vulvar area was then cleaned and disinfected. The cervix was manipulated using a cervical expander, and a Foley catheter was inserted and positioned in the anterior third of the left or right uterine horn.

The catheter balloon was inflated using a 20-mL syringe with approximately 10–15 mL of air or fluid to secure its position. After the stylet was removed, the flushing device was connected to the catheter. The uterus was flushed repeatedly with a flushing medium of 10–60 mL per flush, depending on the capacity of the uterine horn, until all flushing medium had been used. The recovered flushing fluid was collected and stored for subsequent embryo searching and evaluation under a stereomicroscope. After embryo collection was completed, the donor cow received an intrauterine infusion of an antibiotic or antiseptic solution (10–15 mL) and was injected with prostaglandin $F_2\alpha$ ($PGF_2\alpha$) to induce luteolysis and promote the return of the estrous cycle.

Morning	Intravaginal progesterone (Cue-Mate)	FSH 4 ml	FSH 3 ml	FSH 2 ml + PGF 2α	FSH 1 ml	Artificial insemination	Artificial insemination	Embryo collection & Evaluation
Day-	0	9	10	11	12	13	14	20
Afternoon		FSH 4 ml	FSH 3 ml	FSH 2 ml + PGF 2α + CIDR removal	FSH 1 ml	Artificial insemination		

Figure 1. Timeline of superovulation, artificial insemination, and embryo collection protocol in donor cows

Embryo Evaluation and Classification

The obtained embryos were transferred into Petri dishes containing handling medium and examined under a stereomicroscope. Embryo evaluation was performed as a qualitative assessment of developmental stage and morphological quality in accordance with the standards of Shivaani & Madan (2024). Developmental stage was assessed during embryo evaluation using a 1–9 scoring system and was used as part of the criteria for determining embryo transferability. However, the developmental stage was not analyzed as an independent observational parameter because the main outcomes of this study focused on embryo yield, recovery rate, embryo quality, and transferable embryo proportion.

Embryo quality was evaluated using a 1–4 grading system (Helen et al., 2022): grade 1, excellent or good; grade 2, fair; grade 3, poor; and grade 4, dead or degenerated. Embryos classified as transferable were those that had reached at least stage 4, or morula, with quality grade 1 or 2. In contrast, degenerated embryos and unfertilized oocytes were categorized as non-transferable. Degenerated embryos were defined as embryos showing cellular damage or blastomere degeneration affecting more than 75% of the embryo.

Parameters Observed

Number of CL. The number of CL was calculated from the flushing results by adding up the total CL in each ovary previously identified through rectal palpation (Zampini et al., 2020).

Number of embryos/ova collected. The number of embryos/ova collected is the total of fertilized embryos and ova collected from the flushing results (Stádník et al., 2022).

Number of fertile embryos. Fertile embryos are created when spermatozoa successfully penetrate the zona pellucida (Occhio et al., 2019). Observations were made using a stereo microscope, focusing on embryo condition, number, intact cells, and shape.

Embryo quality. Embryo quality assessment is grouped into three groups, namely embryos suitable for transfer (LT), namely embryos that have grades 1 (Excellent), 2 (Fair), and 3 (Poor), as well as degenerated embryos (DG) and unfertilized oocytes (UF), with embryo quality assessment standards based on the (Shivaani & Madan, 2024).

Recovery rate. Recovery rate was defined as the ratio of the total number of embryos recovered to the total number of corpora lutea present in the ovaries (Darlian et al., 2021), calculated as follows:

$$\text{Recovery Rate} = \frac{\text{Total number of embryos recovered}}{\text{Total number of corpus luteum}} \times 100\%$$

Data Analysis

Data were collected and coded for analytical purposes. Descriptive statistics were used to present means ± standard deviations and percentages. Differences in superovulation response and success in in vivo embryo production between HF and Limousine cattle were analyzed using an independent t-test. The assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk test, respectively. The magnitude of differences between groups was measured using Cohen's d. All statistical analyses were performed in RStudio version 4.4.3, with a significance level of $P < 0.05$.

Results and Discussion

Superovulatory Response and Embryo Production

All parameters met the assumption of normality ($P > 0.05$). However, because the group variances were heterogeneous, comparisons between breeds were conducted using the Welch independent t-test (Table 1).

Table 1. Superovulatory response and in vivo embryo production outcomes in HF and Limousin donor cattle

Parameters	Treatment for different types of cattle		P-Value	Effect size
	HF (n = 11)	Limousin (n = 25)		
Total of CL	6.55 ± 2.88	12.72 ± 6.98**	<0.001	2.08
Total ova/embryos collected (embryos)	6.00 ± 3.00	11.44 ± 6.45**	<0.001	1.80
Embryos transferable (embryos)	1.91 ± 2.07	5.76 ± 4.29**	<0.001	2.02
Embryos fertile (embryos)	3.45 ± 2.81	9.16 ± 5.17**	<0.001	2.10

Note: Values are presented as mean ± standard deviation; ** indicates a highly significant difference (p < 0.01); HF = Holstein-Friesian; CL = Corpus Luteum; n = total number of donor cows.

The results demonstrated that bovine breed was a major determinant of the in vivo superovulatory response. Specifically, Limousin cattle produced significantly higher CL counts than HF (P < 0.001; Table 1 In line with this finding, the number of embryos collected from Limousin cattle was also higher (P < 0.01). Similarly, Limousin cattle had increased numbers of fertile embryos (P < 0.001) and transferable embryos (P < 0.01), indicating that their superiority was not only quantitative but also biologically meaningful in terms of oocyte efficiency and embryo transfer potential (Table 1).

The results of this research confirmed that bovine breed was a major biological determinant of superovulation efficiency, fertilization rate, and in vivo embryo quality, with direct implications for embryo production programs under tropical conditions. The CL is the main indicator of donor response to hormonal stimulation (Zampini et al., 2020). Donor cows are categorized as responsive if they produce more than one to three CL, depending on the criteria used (Stádník et al., 2022). A CL number exceeding the normal physiological value may result from FSH administration in accordance with follicular wave dynamics, thereby preventing the atresia of subordinate follicles and promoting multiple ovulation (Khodadadi et al., 2022). Thus, variations in CL number reflect not only the success of hormonal induction but also the quality of the ovarian response to the applied superovulation protocol, particularly to FSH. Differences in CL numbers between breeds indicate that the super-ovulatory response is influenced by ovarian efficiency in producing multiple ovulations. The higher number of CL in Limousin cattle may be related to genetic differences between breeds, including follicular pool size, FSH receptor sensitivity in granulosa cells, and patterns of follicular dominance and competition (Robbaani et al., 2024). This finding is in line with Helen et al. (2022), who reported that cattle breed significantly affects total CL formation. However, hormonal protocols, donor physiological status, and rearing environmental conditions can also influence variation.

The low number of CL in HF cows may have been associated with accelerated metabolism of gonadotropins and steroids, high rates of follicular atresia, or early luteinization, all of which can inhibit multiple ovulation (Damaris et al., 2023). Dairy cattle, including HF, have been intensively selected for metabolic efficiency and milk production, which are associated with higher basal metabolic rates (Walsh et al., 2024). This condition may cause reproductive hormones, including FSH, to be metabolized more rapidly, resulting in a shorter duration of follicular stimulation than in beef cattle (Stádník et al., 2022). As a result, follicular growth and super-ovulatory response in dairy cows tend to be lower, even when they are not in the lactation phase. In contrast, beef cattle, including Limousin cattle, tend to exhibit a more stable FSH response, resulting in higher CL numbers under the same protocol. Apart from genetic factors, the success of superovulation is also influenced by the route of administration, biopotency and half-life of the hormone, hormone concentration, and reproductive history of the donor animal (Mikkola et al., 2020).

The number of CL does not predict embryo yield linearly, as embryo recovery depends on multiple technical and biological factors. Technical variables, including semen quality, flushing technique, media volume, thoroughness of endometrial flushing, catheter placement, and aspiration speed, critically determine collection efficiency, and a fault in any single element can reduce embryo yield despite a strong ovarian response (Setiawan et al., 2017).

Breed-specific physiology further modulates these outcomes. HF dairy cows may experience a high metabolic load associated with milk production, which can alter gonadotropin pharmacokinetics, steroid balance, and uterine blood flow, thereby compromising early embryo survival and increasing the risk of embryonic loss (Gutierrez-Reinoso et al., 2025). By contrast, beef breeds such as Limousin typically exhibit a more stable metabolic status, which better supports fertilization and early embryonic development (Damaris et al., 2023). Thus, both meticulous technique and breed-related physiology must be considered when interpreting CL–embryo relationships.

In addition, heat stress exposure can modulate uterine perfusion and progesterone secretion. Heat stress activates the hypothalamic–pituitary–adrenal (HPA) axis, increases cortisol secretion, and suppresses gonadotropin-releasing hormone (GnRH) release, thereby reducing LH and FSH secretion and disrupting follicular growth, oocyte quality, and super-ovulatory response (Stefanska et al., 2024).

The basis for linking heat stress with ovarian dysfunction is that elevated thermal load induces cellular heat-shock responses and oxidative stress in ovarian tissues, particularly in granulosa and luteal cells. These responses can alter heat-shock protein expression, increase reactive oxygen species formation, impair steroidogenic activity, and reduce cellular responsiveness to gonadotropins (Gómez-Guzmán et al., 2024; Lang et al., 2024). At the ovarian level, heat stress can therefore reduce granulosa cell sensitivity to gonadotropins, inhibit luteinization, and impair corpus luteum function (Melo-sterza, 2021). Simultaneously, the thermoregulatory response redistributes blood flow and reduces uterine perfusion, resulting in decreased progesterone secretion and a less prepared uterine environment for supporting early embryonic development.

Progesterone deficiency causes asynchrony between embryo and uterine development, thereby increasing embryonic vulnerability to premature loss (Susilawati et al., 2022). At the cellular level, heat stress increases embryonic oxidative stress by increasing reactive oxygen species accumulation (Ibayashi et al., 2021), disrupting mitochondrial function and the regulation of developmental gene expression, particularly during the early embryonic stages. This mechanism is closely related to the heat-shock response because embryos and reproductive tissues exposed to elevated temperature activate heat-shock proteins as a protective response against protein denaturation, oxidative injury, and mitochondrial dysfunction (Lang et al., 2024). However, prolonged or severe heat stress may exceed this protective capacity and compromise embryo viability. This vulnerability is more pronounced in Bos taurus cattle than in Bos indicus cattle due to differences in thermoregulatory ability and hormonal stability, which may explain variations in the success of in vivo embryo production between breeds under tropical conditions (Okamoto et al., 2025).

Environmental stress, especially heat stress, reduces uterine blood flow, progesterone concentration, and early embryonic viability, ultimately reducing embryo production efficiency (Susilawati et al., 2023). This condition is particularly relevant in HF cattle, which originated from temperate regions and tend to be more sensitive to tropical environments than Limousin cattle. This interpretation is consistent with Vieira et al. (2014), who reported that seasonal variation significantly influenced the production of fertile embryos in HF cattle, with the number of fertile embryos being higher in winter than in summer (5.1 ± 0.3 vs. 3.7 ± 0.4), confirming the important role of environmental factors in fertilization success and in vivo embryo production.

Meanwhile, embryos suitable for transfer indicate the final success of in vivo embryo production, as only embryos with specific morphological qualities are eligible for transfer, and only higher-quality embryos are suitable for freezing (Madureira et al., 2020). Several mechanisms may explain why Limousin cows produced more embryos suitable for transfer, including superior oocyte quality or larger follicular reserves, allowing more high-quality oocytes to be available for fertilization. Breed differences in ovarian sensitivity to FSH protocols may also result in more uniform oocyte maturation and reduced follicular aging. Favorable metabolic conditions, including nutritional profile, uterine blood flow, and steroid balance, can minimize embryo fragmentation and degeneration (Zampini et al., 2020). Meanwhile, cytoplasmic lipid composition and embryo capacity to tolerate technical stress, such as flushing and temperature changes, influence the proportion of embryos that remain morphologically normal (Supriatna, 2018). In accordance with Mikkola et al. (2020), embryo quality is influenced by complex interactions among oocyte quality, hormonal balance, uterine condition, nutrition, and environmental management.

Embryo Quantity and Quality

Descriptively, HF cows showed a marginally higher response rate. In contrast, Limousin cows produced substantially more CL (Table 2), indicating similar initial responsiveness to superovulation but greater capacity for multiple ovulation. Embryo yield correlated closely with recovery rate, which integrates ovulation success, fertilization, and flushing efficiency (Khan et al., 2022). Despite differences in CL number and total embryos among breeds, embryos harvested per ovulation were comparable, suggesting that the conversion of ovulations into recoverable embryos depended more on procedural and physiological success than on breed. Comparable recovery rates between breeds indicated equivalent effectiveness of flushing and handling in this study. Zago et al. (2023) reported an embryo recovery rate of 60.0% in HF cattle, whereas Damaris et al. (2023) reported up to 87.81% in Limousin cattle. This variability confirms that the recovery rate is influenced not only by breed but also by differences in hormonal protocols, reproductive management, and characteristics of the livestock population used.

Table 2. Descriptive profile of in vivo embryo recovery and embryo quality in HF and Limousin donor cattle

Parameters	Treatment for different types of cattle	
	HF (n = 11)	Limousin (n = 25)
Response rate (%)	94.12	92.86
Recovery rate (%)	89.92	90. 12
UF oocyte (oocytes)	28	57
Percentages of UF oocytes (%)	41.43	19.93
DG embryo (embryos)	17	85
Percentages of DG embryo (%)	25.76	29.02
Fertility rate (%)	57.24	83.66

Note: HF = Holstein-Friesian; n = total number of donor cows; UF = unfertilized oocytes (oocytes not fertilized); DG = degenerate embryos (degenerated embryos); response rate = percentage of donor cows responding to superovulation treatment; recovery rate = percentage of oocytes/embryos successfully recovered from total ovulations.

Salzano et al. (2018) added that several factors contribute to high or low recovery rates, including excessive ovarian size, ineffective oocyte capture by the uterine tube, steroid-level changes that trigger oocyte loss in the reproductive tract, formation of the corpus luteum without complete ovulation, and operator accuracy during the flushing process.

Although harvest efficiency was relatively similar between breeds, differences in embryo quality were still observed. The proportion of DG in Limousin cows was higher than that in HF cows (Table 2), indicating that the high number of ovulations and embryos formed was not always accompanied by optimal embryo quality. Baharun et al. (2025) reported that degenerated embryos are damaged embryos that are not suitable for transfer. The higher proportion of DG in beef cattle, such as Limousin, compared with dairy cattle, such as HF, may be influenced by several factors, including nutritional status, BCS, oocyte cytoplasmic lipid composition, and heat or oxidative stress (Ruebel et al., 2022). Beef cattle are generally maintained at relatively high BCS, which is associated with impaired oocyte maturation, increased cytoplasmic fragmentation, and reduced embryo development. This over-conditioning can trigger metabolic and hormonal imbalances that negatively affect oocyte quality before ovulation. In addition, embryos from beef cattle tend to have higher cytoplasmic lipid content, particularly in the form of triacylglycerol droplets (Sudano et al., 2016). Although lipids function as energy sources and cell-signaling mediators during early embryonic development (Li et al., 2023), excessive lipid accumulation increases susceptibility to oxidative stress and membrane damage during fertilization, embryo transport, and technical manipulations, such as flushing and in vitro evaluation (Stefanska et al., 2024). This condition can accelerate embryonic degeneration, especially when combined with temperature fluctuations and environmental stress.

Differences in embryo quality were also reflected in fertilization status. The fertilization rate of Limousin cows in this study was higher than that of HF cows, indicating that Limousin superiority was related not only to the number of ovulations but also to oocyte quality and fertilization success. However, the number of UF in Limousin cows was slightly higher than that in HF cows, although the difference was relatively small (Table 2). Differences in the proportion of UF between breeds were also reported by Khodadadi et al. (2022), who described UF as ova that are not fertilized by spermatozoa. Several studies have reported that estradiol supplementation in superovulation protocols increases fertilization rates compared with protocols without estradiol, confirming that the hormonal protocol composition influences fertilization outcomes. Damaris et al. (2023) reported an average number of unfertilized ova of 2 ± 2.76 in HF and 1.83 ± 2.08 in Limousin. In contrast, this study found that Limousin cows produced more UF, although the difference was relatively small. This difference may have been caused by variations in protocol, including hormone type and dose, timing of progesterone treatment, semen quality, timing of insemination relative to ovulation, nutritional status or BCS, donor age, and sample size.

Conclusions

Based on the findings of this study, Limousin cattle demonstrated better in vivo embryo production performance than Holstein-Friesian cattle across the evaluated parameters, particularly in superovulatory response, total embryo yield, fertile embryos, and transferable embryos. However, these findings should be interpreted within the specific scope and conditions of this study, as Holstein-Friesian and Limousin cattle represent different production types and may differ in their reproductive physiology and management requirements. Therefore, the results should not be regarded as evidence of absolute breed superiority. Further optimization of reproductive management, donor selection, and breed-specific superovulation protocols is needed to improve embryo quality and donor efficiency. Overall, this study provides useful comparative information to support the development of breed-adapted in vivo embryo production and embryo transfer programs under tropical conditions in Indonesia.

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