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CAPABILITY OF DIFFERENT BREEDS OF DONOR CATTLE TO PRODUCE EMBRYOS AT THE CIPELANG LIVESTOCK EMBRYO CENTER, BOGOR, WEST JAVA

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ABSTRACT

The objective of this research was to investigate the capability of the donor cattle to produce embryos by comparing three cattle breeds, namely *Bos taurus* beef cattle (Limousin), *Bos taurus* dairy cattle (Friesian Holstein/FH), and *Bos indicus* beef cattle (Peranakan Ongole/PO). This research was observational method with *in vivo* embryo production method and a nonsurgical embryo collection method (flushing). The data collected were then processed using the one way ANOVA method. The results showed that the number of CL, response rate, total number of embryos, and recovery rate in PO cattle, FH cattle, and Limousin cattle did not differ significantly (P>0.05). The appropriate number of embryos to be transferred and degenerative embryo were significantly different among PO cattle, FH cattle, and Limousin cattle (P<0.05), while the unfertil ova, fertilization rate, and transferable embryo did not differ among cattle breed (P>0.05). This research concluded that each breed of the donor cattle produced embryos with varies quality and the best quality of embryo production is found in Limousin cattle.

Key words: *Bos indicus*, *Bos taurus*, embryo production, superovulation treatment

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui kemampuan produksi embrio sapi donor dengan membandingkan 3 bangsa sapi yaitu Bos taurus sapi potong (Limousin), Bos taurus sapi perah (FH) dan Bos indicus sapi potong (PO). Penelitian ini menggunakan metode observasi dengan metode produksi embrio secara in vivo dan metode pemanenan secara non bedah (flushing). Data yang diperoleh dianalisis dengan one way ANOVA. Hasil analisis data menunjukkan bahwa jumlah CL, response rate, total perolehan embrio dan recovery rate pada sapi PO, sapi FH, dan sapi limousin tidak berbeda nyata (P>0,05). Jumlah embrio layak transfer dan embrio degenaratif menunjukkan perbedaan nyata (P<0,05) diantara sapi PO, sapi FH, dan sapi Limousin, sedangkan unfertil ova, fertilization rate, dan transferable embryo tidak berbeda nyata (P>0,05) diantara bangsa sapi (P>0,05). Kesimpulan penelitian bahwa setiap bangsa sapi donor memberikan produksi embrio yang bervariasi dengan total perolehan embrio terbanyak yang diperoleh sapi PO dan produksi embrio kualitas terbaik pada sapi Limousin.

Kata kunci: Bos indicus, Bos taurus, produksi embrio, perlakuan superovulasi

INTRODUCTION

The low genetic quality of local dairy and beef cattle causes low productivity. This further leads to insufficient milk and meat production to meet domestic needs that continue to increase every year. The availability of high-quality breeds as genetic agents for superior dairy cattle and beef cattle is also limited. The government has attempted to overcome this shortage by importing livestock products and superior breeds from overseas. This causes the livestock export-import balance in 2019-2020 to remain at a deficit (when the import value is greater than the export) (Ditjen Peternakan dan Kesehatan Hewan 2020). The main objectives of cattle breed selection are to produce offspring that inherit superior traits and high productivity and to use the production input efficiently. Thus, in the selection of dairy cattle, the progeny test is used to compare the average milk production of their female offspring (Anggraeni 2012). Meanwhile, the selection of beef cattle is done using a performance test. A careful selection process is crucial if the genetics of the offspring are to be improved. It is also necessary to examine the possibility of the interaction between the genetics and environmental factors to produce superior bulls. The government has attempted to produce superior bulls from various domestic breeds to improve the domestic bulls' genetic quality so that the number of imported bulls can be decreased. The latest effort made by the government is Multiple Ovulation and Embryo Transfer (MOET) biotechnology expected to be able to produce excellent breeds to fulfil national demands.

MOET is the second generation of reproductive biotechnology after AI. It has several processes, namely livestock donor selection, estrous synchronization, superovulation, AI, embryo collection, embryo handling and evaluation, embryo transfer to the recipient up to the pregnancy examination, and the delivery process. MOET has several benefits, such as allowing the offspring to have the best characteristics of its parents, shortening the generation interval, accelerating genetic quality improvement and helping to achieve reproductional efficiency (Parlindungan 2021). The government has established a center that facilitates the use of MOET including for the production of embryos which will later be distributed throughout Indonesia to improve the genetic quality of livestock. The Center for Livestock Embryo (BET) in Cipelang, Bogor, West Java, is the only center that focuses on the MOET. The main programs of BET Cipelang include the independent program to produce high-quality breeds/self-sufficiency in bulls, the "Goes to Farm" program to select local breeds in the area, and the germplasm development program/local genetic resources.

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One of the determining factors for the success of MOET is embryo production. Strategies for the success of *in-vivo* embryo production in cattle are still being developed, and the results vary from one bull to another in different environments. This is due to the different characteristics of each breed in producing an embryo; thus, these different characteristics of the donor cattle need to be investigated beforehand to obtain optimal embryos. Jodiansyah *et al*. (2013) mentioned that the factors affecting embryo production in donor cattle are the donor responses to superovulation treatment. Meanwhile, according to Hardiyanto *et al*. (2016), the factors that may affect the cattle are the environmental factor within the conservation, the treatment period, reproduction and health status, stress level, food distribution management, and the cattle's responses after receiving gonadotropin. In the same research, it was also stated that variations in individual cattle and breeds of bulls can cause differences in embryo production. The characteristics of each breed in responding to superovulation treatment as well as external factors that can affect embryo production in each donor cattle breed need to be considered.

Cattle species with potential and genetic advantages are *Bos taurus* and *Bos indicus*. These breeds were selected for the study because they have rapid growth potential (Sodiq and Yuwono 2016). *Bos indicus* can endure high temperatures, low-quality feeds, and parasites (Kuswati *et al*. 2020). One type of the *Bos taurus* breed in Indonesia is Limousin cattle that possesses some excellent traits, such as rapid growth with daily weight gain of 1.0-1.4 kg, 800-900 kg of a two-year-old cattle, and 1,000-1,100 kg on the adult cattle, and good meat quality favourable by livestock farmers (Muada *et al*. 2017). The livestock breed in Indonesia that produces the most milk is *Bos taurus*. One of the dairy cattle from the *Bos taurus* breed which has been nurtured is Friesian Holstein (FH). The average FH dairy cattle production is 4,185.89± 990.43 kg per cattle and per lactation, with an average lactation duration of 317.97±26.15 days (Makin and Suharwanto 2012). However, the cattle production from *Bos taurus* breed in Indonesia is not as high as that of its origin. Several factors cause this condition, such as weather, food quality, and the genetic quality of the exported cattle from their origin country, which are not as excellent as they are in the origin country (Setiawan, 2019). These factors, related to the genetic improvement of both domestic and imported dairy and beef cattle, must be considered to make the cattle more adaptive and have superior characteristics.

Therefore, this research needs to be conducted to generate embryo production information in donor cattle in various breeds. This research was conducted after considering the lack of information on embryo production in various breeds. This research was expected to fill the gap by providing information about the topic.

MATERIALS AND METHODS

This research was carried out at Cipelang Livestock Embryo Center at KH. Halimi Street No. 09, Pasir Pogor, Cipelang, Cijeruk Subdistrict, Bogor, West Java. A total of 15 donor cattle from 3 different breeds, namely FH, Limousin, and Peranakan Ongole (PO) cattle (five of each) at the age of 3 years old were used in this study. The body condition score (BCS) for beef cattle and dairy cattle were 3.0-3.5 and 2.75-3.25, respectively. The cattle were selected based on the donor cattle's criteria.

This research used an observational method. The samples were selected through the purposive sampling technique. The donor cattle was reared in a free-stall cage and fed with elephant grass (*Pennisetum purpureum*) of 30-40 kg/cattle/day for food. They were also supplemented with special concentrate for the donor with the amount of 5 kg/cattle/day. The concentrate contained 17.6% crude protein, 14.6% fiber, 70.2% total digestible nutrient, and water in *ad libitum*. The *in vivo* embryo production with superovulation used progesterone releasing intravaginal device (PRID).

Pre-Treatment Synchronization

The purpose of the synchronization process was to align the follicle wave growth prior to superovulation. The synchronization used PRID (Cue-Mate containing 1.56 mg of progesterone in two silicon pods, Bioniche Animal Health (A/Asia) Pty.Ltd., Australia). The progesterone preparation was conducted for 11 days using applicators with a lubricant gel.

Superovulation

Superovulation on the whole livestock was performed using Follicle-Stimulating Hormone (FSH) preparation (Folltropin V containing 400 mg of NIH-FSH-P1 and 20 mL of Bacteriostatic sodium chloride solvent, Vetoquinol USA, Inc., USA), with a total dose of 400 mg/20 mL. The FSH injection started on the 9th day after the progesterone preparation was inserted. The FSH injection was performed twice a day at morning and afternoon with a declining dose (4, 4; 3, 3; 2, 2; and 1, 1 mL) delivered intramuscularly.

The prostaglandin F2α (Prostate C, 5 mg etiproston per 2 mL; Virbac Animal Health, France) hormone injection was conducted on the eleventh day along with the progesterone preparation release. The injection was done intramuscularly in the morning and afternoon with a dose of 2 mL. The AI was carried out three times, with 12-hour intervals using imported frozen semen depending on the cattle's breed. The AI used the rectovaginal method with rectal palpation in manipulating the cervix on the 13th and 14th days. It was then followed by embryo collection seven days after the first AI. The superovulation treatment can be seen in Figure 1.

Embryo Collection

The embryos were collected seven days after the first AI or on the 20th day of the first embryo

production program cycle. This process used a non-surgical method through trans-cervical. The first step was rinsing the uterus three times, right, left, and body uterus, using 500 mL of rinse media for each of them. The media was a lactated ringer that had been added 1% of bovine serum (Sigma-Aldrich, USA) and 0.1% of penicillin and streptomycin (100 IU/mL) (Sigma-Aldrich, USA), and also by using an 18FR two-way foley catheter (FHK Fujihira, Japan). The rinse media was inserted into the uterus and rinsed. Then, it was flushed out to a particular bottle. Next, the embryos were searched in the rinsed result media and evaluated in the laboratory. After the rinsing process, 2% of iodine povidone was given with a total amount of 50 mL via intra vaginal to avoid post-rinsing infection. Finally, 2 mL of prostaglandin F2α was injected into the donor cattle to exuviate the corpus luteum (CL) to prevent the cattle from getting pregnant and to restart the cycle.

Embryo Evaluation

The uterus rinsing result was then filtrated by an embryo filter with a diameter of 75 mm (Agtech inc., USA), and left at 10 mL. After that, the filtration result was separated and placed in a striped petri dish with a size of 100x100x10 mm (Falcon, USA). Its surface was then burned to remove the air bubble and the search for the embryos using a stereomicroscope started (Olympus SZ61, Japan) with 50 times magnification. The collected embryos were then moved into a circle petri dish with the size of 35x10 mm (Falcon, USA) to be evaluated by using a 200-time inverted microscope. The embryo evaluation was conducted based on the embryo growth phase and embryo quality, based on the International Embryo Transfer Society (2010), namely grade 1 (excellent or good), grade 2 (fair), grade 3 (poor), and grade 4 (dead or degeneration). In determining embryo quality, the embryo growth phase was considered. The embryo growth phase consists of nine phases according to the IETS (2010), namely unfertilized embryos, 2-16 embryo cells, early morula, morula, early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst and expanded hatched blastocyst. The transferrable embryos are embryos from the 4th (morula) to the 7th growth phase (expanded blastocyst phase) with a quality of 123. Meanwhile, the untransferable ones are unfertilized ova, an ovum that does not have a division mark, and degenerative embryo or an embryo with wrecked blastomere cells (with more than 75% of damage) (Darlian *et al*. 2021).

Observed Parameters

The total of CL was counted by rectal palpation and considered as the superovulation response rate indicator. Cattle are considered to give a good response when the CL number is ≥3 (Jodiansyah *et al*. 2013). A total embryo obtained was the total of embryos and ovum obtained after the flushing process consist of the transferable and non-transferable embryos. While embryo quality consists of the transferrable embryos (classified into 1, 2, and 3 quality), degenerative embryos, and unfertile ova.

Response rate, recovery rate, fertilization rate, and transferrable embryos were calculated as follows:

$$Response\ Rate = \frac{\sum flushed\ donor\ cattle}{\sum superovulated\ donor\ cattle}\ x\ 100\%$$

$$Recovery\ Rate = \frac{\sum obtained\ embryo\ and\ ovum}{\sum CL\ on\ the\ ovary}\ x\ 100\%$$

$$Fertilization\ Rate = \frac{(\sum transferable\ embryo + degenerative\ embryo)}{\sum obtained\ embryo}\ x\ 100\%$$

$$Transferable\ Embryo = \frac{\sum embryo\ with\ 123\ quality}{\sum obtained\ embryo}\ x\ 100\%$$

Data Analysis

The data were analyzed using statistically with one-way analysis of variance.

RESULTS AND DISCUSSION

Total of Corpus Luteum (CL) in the Different Donor Cattle Breeds

CL was formulated from follicle de Graaf after the ovulation occurred (Noakes *et al*. 2016). The formed CL was then used as the superovulation response

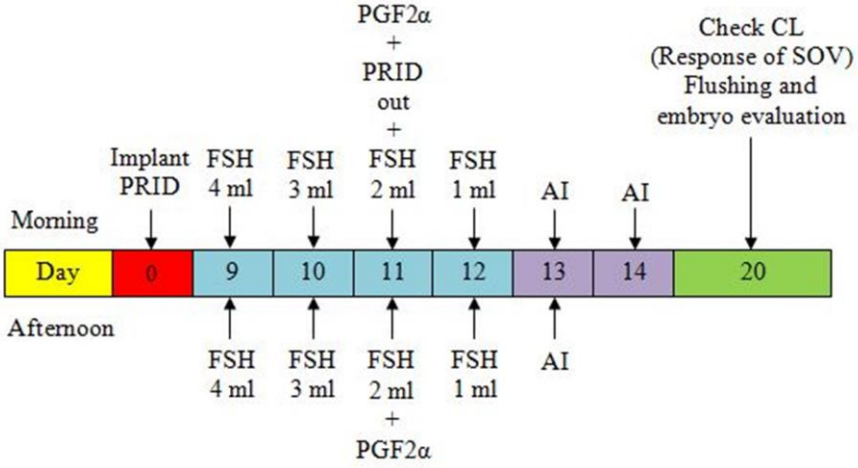

Figure 1. The superovulation treatment using FSH hormone and a declining dose through intramuscular injection

indicator in the livestock because the total CL can represent the total ovulated ovum. The CL checking process was performed using the rectal palpation method. The cattle used for this research were FH representing *Bos taurus* breeds for dairy cattle, Limousin representing *Bos taurus* breeds for beef cattle, and Ongole or PO cattle representing *Bos indicus* breeds for beef cattle. For the first variable, the total CL collected was presented in Table 1 with the highest average coming from the PO cattle with a total of 12.29±5.44, while statistically, there were no significantly different results between breeds (P>0.05). All cattle responded well to the superovulation, indicated by their average CL of ≥3, which is in line with the statement of Jodiansyah *et al*. (2013) that superovulation employing FSH is effective when the total CL reaches more than 3.

According to Sartori *et al*. (2016), the progesterone concentration in *Bos indicus* was higher than that of *Bos taurus*. The progesterone metabolism in dairy cattle is also higher than that in beef cattle. These are the factors leading to lower progesterone concentration and shorter half-life in dairy cattle. In the FH cattle, including dairy cattle, the CL total was the lowest, which seemed to correlate with the lower progesterone concentration inside the body and faster half-life.

The differences in the average CL collected from each of the cattle breeds were affected by the individual responses to the superovulation, which were related to the genetic factors of each breed. Cattle generally have three follicle waves which appear on the second, ninth, and sixteenth day. However, the follicle waves on *Bos indicus* cattle usually appear four times due to the delayed luteolysis and interestrus interval. Individual cattle with two follicle waves per cycle tend to have shorter interestrus interval and ovulate larger and less-fertile follicles (Noakes *et al*. 2016). In the same literature, it was mentioned that lactation status also affects folliculogenesis, ovulation, and CL formation. Thus, milk production hormones affect the amount of CL in dairy cattle, but not in beef cattle.

The calculation of total CL using the palpation method allows uncounted CL due to being stacked or skipped. According to Noakes *et al*. (2016), size and protrusion facilitate the finding of CL through the rectal.

The Response Rate of Donor Cattle in Different Breeds

The superovulation response rate in the livestock was marked by the CL formation. The success of the superovulation process can be seen from the total of CL formed after the FSH hormone injection (Pranata *et al*. 2016). It can be calculated by comparing donor cattle flushed to the superovulated donor cattle. From Table 1, it was clear that all of the cattle breeds gave a high response to the superovulation treatment. All breeds gave the highest responses (100%), even though statistically, the results between breeds were not significantly different (P>0.05). Both responded well since the value reached over 70%. According to Supriatna (2018), an acceptable response rate is higher than 70%. In the research done by Supriatna (2018), the superovulation response by FSH resulted in a higher value in beef cattle rather than in dairy cattle. FSH stimulation in this research started to be carried out on day 9 after the PRID implant, in which the second follicle wave entered the diestrus phase. In the diestrus phase, natural progesterone is the highest and most stable; natural FSH is also basal. In this phase, the development of primary and secondary follicles begins. The superovulation response in this study gave high results due to the FSH injection given in the diestrus phase of the recruitment step along with the presence of FSH receptors on the cell surface that prepare these early follicles for maturation to the Graafian stage, thereby increasing viability through the selection step. According to Permadi (2012), in the recruitment phase, pre-antral follicles have receptors for FSH but do not have Luteinizing Hormone (LH) receptors, which are assumed to prepare early-stage follicles for maturation, enter the Graafian follicle stage, and make granulosa cells respond well to FSH.

In addition to the donor breeds used, other factors also affected the response rate, such as the donors' age, BCS, and the total of livestock used as donors (Liamanu *et al*. 2018). Previously Jodiansyah *et al*. (2013) stated that there are two factors leading to different response rates in superovulation, namely internal and external factors. Internal factors consist of the livestock's genetic condition (breed and its sensitivity to the hormone), physiological characteristics (age, ovaries condition, dominant follicle availability), nutrition (BCS and nutrition fulfilment), and the condition of reproductive organs. Meanwhile, the external factors include the FSH preparation used (the equality of FSH and LH), FSH dosage, weather, and the execution process. According to Bo and Mapletoft (2020), the FH superovulation response may be low because of over-rapid absorption of FSH or an excessive percentage of atresia caused by the luteinization of the follicle and/or follicle atresia. It also appears in the *Bos taurus* breed for dairy cattle; the reduction of the responses may be related to the accelerated metabolism of gonadotropin in dairy cattle, as is already reported for steroid hormones.

Table 1. The average of superovulation responses in different breeds

Variables	Cattle breeds			P
	Friesian Holstein n= 11	Limousin n= 12	Peranakan Ongole n= 7	
Amount of corpus luteum (CL)	9.18±4.77	12.08±3.87	12.29±5.44	0.25
Response rate (%)	100.00±0.00	100.00±0.00	100.00±0.00	-
Total of obtained embryos	8.09±4.91	10.83±4.04	11.430±5.03	0.25
Recovery rate (%)	82.90±19.24	87.81±8.46	93.38±9.99	0.32

All cattle used in this research were three years old. They were considered young, had high productivity, and were able to give good superovulation responses. These criteria were in accordance with those set by Hardiyanto *et al*. (2016) that 3-4 years old donor cattle would give optimal results in embryo production.

Total Embryos Obtained in the Different Donor Cattle Breeds

The obtained embryos were the embryos that had undergone the flushing and searching process. Table 1 shows that the highest total embryos obtained were from PO cattle (11.43±5.03), while statistically, there are no significantly different results between breeds (P>0.05). According to Bo and Mapletoft (2020), the factors affecting the production of an embryo of one of the donor cattle are the population of antral follicles, ovarian physiology, and gonadotropin biochemistry. Jodiansyah *et al*. (2013) explained that the different responses of each individual might be caused by several factors, such as the differences in genetic condition, FSH preparation types used, and the quality and clarity of the FSH used. In the same research, it is also mentioned that the embryo and ovum obtained were especially determined by the success of superovulation treatment using FSH hormone to the follicles on ovaries, which was the superovulation target. Before the superovulation treatment, the synchronization process and progesterone preparation using PRID were done. The progesterone level in the follicular phase was 2-3 ng/mL. Meanwhile, the ideal progesterone level for FSH injection is around 4-6 ng/mL (Jodiansyah *et al*. 2013). Therefore, the progesterone level in the individuals' body was also affected the number of obtained embryos because the progesterone preparation dose used on all livestock was identical.

The breed's characteristics also affected their tolerance towards stress from the surrounding. Heat stress would affect the reproductive system of female cattle, such as reducing the blood flows in the uterus, reducing progesterone circulation, and affecting the embryo loss rate in *in vivo* embryo production (Silva *et al*. 2013). FH cattle from *Bos taurus* obtained the lowest total embryos. Generally, dairy cattle produced fewer obtainable embryos compared to beef cattle due to the effect of milk production hormone. Supriatna (2018) mentioned that beef cattle produce a higher number of obtainable embryos compared to dairy cattle. In the same literature, he also explained that the average number of embryos obtained per flushing during embryo collection is 5-6. Among the three cattle breeds observed in this research, the average number of obtained embryos was more than 5 per flushing, which was higher than what has been mentioned in the literature. It might be caused by the high individual response rate to the superovulation, and also the compatibility of hormone type and superovulation method used on the donor cattle.

The average number of total embryos on all cattle breeds was lower than the number of CL since the fertilized ova were spread throughout the reproductive tract; thus, they might be skipped when the flushing process was carried out. According to Supriatna (2013), the ovum was spread within the fallopian tube; 10% of the ovum was inside the fallopian tube; 70% of which was spread throughout the 10 cm uterine horns (cornua uterine), and the rest was spread near the uterine horns section. Using the non-surgical rinsing method, the result would show up to 90% ovulated ovum. Primarily, around 70% of ovulated ovum would be obtained.

Recovery Rate Percentage on the Different Breeds of Donor Cattle

The recovery rate percentage is the comparison between the total embryos obtained to the total CL. Table 1 shows the highest recovery rate came from PO cattle, with a value of 93.38%±9.99%, while statistically, there were no significantly different results between breeds (P>0.05). Setiawan *et al*. (2017) mentioned that the average recovery rate on cattle with the non-surgical embryo collecting method is 68.2-74%, while according to Supriatna (2018), a good value of recovery rate ranges from 40% to 80%. The research results from the *Bos taurus* dairy cattle and *Bos indicus* beef cattle were lower than those mentioned in the literature. In the same literature, it is also mentioned that a low recovery rate might be caused by embryos falling into the abdomen due to a high amount of rinsing fluid inserted, causing the embryos to remain within the uterus. It became too large and hanging which made the catheter balloon unable to completely close, and the fluid leaked from the other side. That is one of the drawbacks of the non-surgical flushing process because, during the flushing process, the catheter placed inside the female cattle reproductive organ cannot be seen directly and can only be felt. Besides, the variety of reproductive organ forms in each individual also affected the flushing process. Darlian *et al*. (2012) also issued a similar statement that recovery rate is affected by the flushing technique and the accuracy of folley catheter placement during the flushing process.

Quality of the Different Donor Cattle Breeds
Transferrable embryos grade of 1, 2, and 3

A successful indicator during the superovulation treatment is the highest percentage of transferrable embryos. A transferable embryo is an embryo in the 4^{th}-7^{th} development phase, namely the Morula phase up to the Expanded Blastocysts phase at the grade of 1, 2, and 3. The embryo flushing was done seven days after the first AI, which corresponded to the embryo development phase on the 7^{th} day; the embryos had developed to the Early Blastocyst phase. After the research was finished, the total transferrable embryos in each cattle breed are shown in Table 2. The highest transferable embryos average was from Limousin cattle with 7.08±4.17, while statistically, there were no significantly different results between breeds (P<0.05). According to Cizmeci and Guller (2018), the average transferrable embryos can be classified as 'economic'

when it is ≥5. From the three different cattle breeds used in this research, only Limousin cattle as the representative of *Bos taurus* beef cattle met the criteria of economic transferrable embryos. Meanwhile, the FH and PO cattle embryo production was still under the average production to meet the economic value. The transferrable embryos obtained were affected by the genetic factors of each individual, which can be seen from the research variables that have been discussed previously, where the Limousin cattle provided the highest embryo production besides its type as beef cattle and coming from the *Bos taurus* breed.

A low number of transferrable embryos obtained might be due to the superovulation frequency of each individual. According to Jaton *et al*. (2016), the flushing frequency done to each individual affects the number of transferrable embryos. A low number of transferrable embryos obtained was also related to the fertilization rate, and based on Table 2, the highest fertilization rate came from Limousin cattle. Another factor that affects the transferrable embryos obtained is the physiological factor of the livestock during the flushing process. During this process, the donor cattle is in the luteal phase with a high level of CL, but without an un-ovulated follicle, causing the progesterone level to be high and able to keep the embryos alive (Jodiansyah *et al*. 2013). The FH cattle showed the lowest number of transferrable embryos obtained, despite belonging to *Bos taurus*. FH cattle are dairy cattle with higher progesterone metabolism compared to beef cattle. Thus, their progesterone concentration is lower than that of beef cattle, lowering the hormone that can support an embryo's life.

Degenerative embryos

The non-transferrable embryos can be divided into two types: degenerative (damaged) embryos and unfertile ovum (unfertilized). The difference in embryo quality produced by each individual might be caused by the different oocyte maturity rates (Jodiansyah *et al*. 2013). Superovulation treatment in the livestock was done to accelerate the growth rate of follicles inside the body; hence, the obtained embryo quality would have various maturity rates which would produce varied embryo quality. Based on the data in Table 2, Limousin cattle have the lowest average of degenerative embryos with a total of 11.92±2.02, or 17.69%. Meanwhile, statistically, there are no significantly different results between breeds (P<0.05). The lower number of non-transferrable embryos obtained the better embryo production from the donor cattle which accept superovulation treatment. The degenerative embryo percentage of *Bos taurus* breeds in this research was still considered acceptable. The average of damaged (degenerative) embryos is around 15-20% (Supriatna, 2013). However, the other two cattle from dairy cattle under *Bos indicus* and *Bos taurus* breeds resulted in high percentages of degenerative embryos, which were 33.75% and 38.20%, respectively. Even though classified under the same breeds, the donor cattle were still varied, such as having different BCS although still qualified under the donor qualification. The higher the BCS in the individual donor, the more fat inside the cattle's body, including in the cattle's reproductive organs. According to Kadokawa *et al*. (2008), the embryo from a high BCS donor might have slower or earlier growth, which may damage the embryo or delay its growth. The cattle's genetic factors also influence the formation of non-transferrable embryos. Meanwhile, according to Prihatno *et al.* (2021) early embryonic death is caused by complex factor, factors from the mother related to abnormalities in the mother, influence of parental age, genetic factors, uterine infection, return of the estrous cycle, anatomical abnormalities in the female reproductive tract, hormonal dysfunction, poor follicular development, and the influence of nutrition. The hormone that plays a role in maintaining the viability of the embryo is progesterone. A low level of progesterone can lead to degenerative embryos. Panjaitan *et al*. (2019) found one of the main causes of embryo death is insufficient luteal function as indicated by low concentrations of progesterone. The mechanism of embryo death due to low progesterone is related to the secretion of interferon tau. Interferon tau is a pregnancy signal produced by trophoblast tissues that prevent the release of PGF2 alpha. The decrease in progesterone secretion on the 7th day after IB causes disruption of endometrial protein secretion, disturbance to the embryo's living environment, inhibition of embryonic development, and early embryonic death (McNeill *et al*. 2006).

The breakdown of *tight junctions* can disrupt communication between cells and result in a less compact bond between blastomeres, as in the discovery of degenerated morula by Zayani *et al*. (2016). Meanwhile, Yasyri *et al*. (2017) found that failure to penetrate and decondensate spermatozoa into oocytes is assumed to cause embryo degeneration, and there are

Table 2. Average embryo quality in different breeds

Variables	Cattle breeds			P
	FH n = 11	Limousin n = 12	PO n = 7	
Embryo quality:				
– Transferrable embryo	3±2.83^{a}	7.08±4.17^{b}	4.14±4.14^{ab}	0.04
– Degenerative embryo	3.09±4.32^{a}	1.92±2.02^{a}	3.86±2.97^{b}	0.004
(%)	38.20	17.69	33.75	
– Unfertile ova	2±2.76	1.83±2.08	3.43±4.08	0.57
(%)	24.72	16.92	30	
Fertilization rate (%)	80.06±28.92	79.91±28.57	74.69±25.65	0.90
Transferable embryo (%)	54.03±40.65	62.81±26.49	42.98±44.06	0.53

$^{a, b, ab}$Different superscripts within the same row indicate significant differences (P<0.05)

more than one spermatozoa that can penetrate the vitelline membrane of the oocyte called polysperm. This embryo abnormality likely inhibits further embryonic development.

Unfertile ova

Unfertile ova (unfertilized ovum) are also classified into non-transferrable embryos. Table 2 shows that the best unfertile ova percentage came from Limousin cattle (16.92%), while statistically, there are no significantly different results between breeds (P>0.05). According to Supriatna (2013), the average of unfertilized (unfertile) embryos is around 15-20%. Among the three cattle used in the study, only Limousin cattle had good unfertile ovum production with approximately 15-20%. Meanwhile, FH and PO cattle had a low unfertile ova percentage as they exceeded the acceptable unfertile ova percentage. The non-transferrable embryos might also be caused by the failure in the ovulation process due to the low LH level produced by the individual (Jodiansyah *et al*. 2013). Therefore, the ovulation process is delayed, causing the fertilization process to stop after the AI and the ova to become infertile. According to Bo and Mapletoft (2020), increasing the dose of crude pituitary extract containing FSH and LH resulted in a low rate of fertilized ova. The LH levels that are too high in the animal's body can affect the quality of the ovum/embryo produced. According to Mapletoft and Bo (2012), the sizes of the dominant follicles of *Bos taurus* and *Bos indicus* are different. In *Bos taurus*, the diameter of the dominant follicle is 8.5 mm while in *Bos indicus*, it is 6.2 mm. The differences between the two breeds affect the performance of the FSH hormone on follicular growth which is related to the speed of ovulation of the dominant follicle; it can trigger an unfertilized ovum.

A high or low number of unfertile ova obtained were affected by the fertilization level. The higher the fertilization level, the lower the unfertile ova was obtained. In Table 2, it was shown that Limousin cattle representing beef cattle from *Bos taurus* breed had the highest fertilization level. According to Liamanu *et al*. (2018), the differences in unfertile embryos obtained may be the result of a low fertilization level and the internal factors of the livestock, including genetic factors. According to Mapletoft *et al*. (2015), the 4-day superovulation treatment caused some follicles to have insufficient time to mature, regulate appropriate gene expression, and acquire the capacity to ovulate. This is related to the differences in the number of follicle waves in *Bos taurus* and *Bos indicus*.

The environmental factor also played a role in the number of unfertile ova obtained. Heat stress can damage oocytes and decrease their fertilization ability as well as ovum growth. Heat pressure can also delay embryo growth. *Bos taurus* is more sensitive to heat pressure compared to *Bos indicus*. The dairy cattle performance in oocyte growth is still lower compared to beef cattle (Silva *et al*. 2013). However, the research showed that the best result came from the Limousin cattle of the *Bos taurus* breed. Therefore, not only is the breed superior, the cattle's genetics is excellent as well because it has a high tolerance to heat. According to the previous research, beef cattle had better embryo performance than dairy cattle; hence, the unfertile ova obtained from the beef cattle in the research were lower than those of the dairy cattle.

Fertilization Rate on the Donor Cattle from Different Breeds

The fertilization rate is the comparison between the fertile embryos (transferrable and degenerative embryos) and the total embryo obtained. The fertilization rate is one of the successful indicators of superovulation treatment for the donor cattle since it affects the embryo quality produced. From Table 2, it is known that the highest fertilization rate came from FH cattle with 80.06% ± 28.92% from the dairy cattle of *Bos taurus* breed, while statistically, there were no not significantly different results between breeds (P>0.05). The fertilization rate result from the research showed that the fertilization rate from *Bos taurus* was higher than that of *Bos indicus*. According to Liamanu *et al*. (2018), a low fertilization rate might be due to low semen quality. In the research done by Vieira *et al*. (2014), a bad fertilization result was likely caused by the disturbance during the transportation process of spermatozoa or ovum. Spermatozoa quality that decreases due to storage or handling before use may reduce its ability to capacitate and produce enzymes that are useful in the fertilization process. According to Pratiwi *et al.* (2019), fertilization occurs when the spermatozoa have undergone capacitation. The fertilization process requires three critical steps: 1) the spermatozoa must penetrate the cumulus; 2) the spermatozoa must touch, penetrate and interact with the layer of the zona pellucida; 3) the fusion of the spermatozoa with the oocyte plasma membrane. In all three processes, spermatozoa secrete various enzymes to facilitate fertilization. Failure of fertilization may stem from the number of UF oocytes. Although intracytoplasmic sperm injection has been carried out, it can also cause the failure of oocyte activation. Ningtias *et al*. (2022) stated Ca concentration is associated with oocyte activation in embryo production.

Besides, the estrous synchronization treatment using progesterone preparation can make the ovulation similar, which can increase the fertilization rate (Darlian *et al*. 2021). Every breed produces different reproduction results. Superovulation in this research was carried out by using the same method and hormonal type for all breeds. Every individual gave distinct responses to the treatment, including the fertilization rate in all individuals. According to Barros and Nogueira (2001), the steroidogenic profile on the follicle changes during the pra-ovulation period can cause a disturbance in oocyte maturation and sperm transportation. It makes the fertilization rate of each breed different.

Transferrable Embryo Percentage on the Donor Cattle from Different Breeds

Transferrable embryo is the percentage of embryos that can be transferred or the comparison between the

transferrable obtained embryos and the total number of embryos obtained. Based on the research data in Table 2, the highest transferable embryo rate was in Limousin beef cattle of the *Bos taurus* breed (62.81±26.49%), while statistically, there were no significant results between breeds (P>0.05). The transferrable embryo percentage corresponds to the transferrable embryos obtained from each individual. According to Supriatna (2013), the transferrable embryo percentage is affected by physiological characteristics (ovaries condition and the availability of dominant follicles). Meanwhile, Mikolla and Taponen (2017) explained that high variability between cattle can give varied responses to gonadotropin stimulation. It results in responsive follicle variation towards gonadotropin which affects the embryo produced. This is in accordance with the finding of Bo and Mapletoft (2020) that the use of the same superovulation protocol in different breeds (*Bos taurus* and *Bos indicus*) can produce different transferrable embryo gains between breeds used in the research.

The superovulation treatment in the research was carried out using FSH hormone and declining dose injection, which means that the injection was recurrent in the morning and afternoon for four days. The recurring injection can cause stress in the donor cattle. The individual donor with low-stress tolerance can affect embryo production and decrease embryo quality; as a consequence, the transferrable embryo percentage will be low. This was supported by the statement of Amiruddin *et al*. (2013) that recurrent FSH injection can stress the embryo and lower the embryo quality.

CONCLUSION

Based on the research findings, it can be concluded three cattle from two different breeds, namely Limousin and FH cattle from *Bos taurus* breed, and PO cattle from *Bos indicus* gave varied responses to embryo production with the highest number of embryos being obtained from PO cattle and the best quality embryo production belonging to Limousin cattle. Meanwhile, FH cattle gave different embryo production for each variable. The three breeds of cattle used in this study have the potential to produce embryos when viewed from the response level of each breed. However, the superovulation protocol must be adapted to the characteristics of each breed to achieve optimal embryo production.

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