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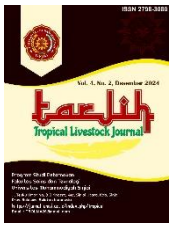

Comparison of Embryo Production between Simmental and Limousin Cattle in Different Seasons at Cipelang Cattle Embryo Center

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ABSTRACT

Indonesia is a tropical country that has two different seasons, namely the rainy season and the dry season. Embryo production can be influenced by several factors, one of which is the season factor. Seasonal factors can affect the quality and quantity of embryos produced. The purpose of this study was to determine the comparison of embryo production of Simmental cattle with Limousin cattle in the rainy season and the dry season. The material used was 40 donor cattle divided into 20 Simmental cattle and 20 Limousin cattle aged 3 to 6 years with a body weight of 450-700 kg. The research procedure starts from superovulation using reproductive hormone injection, artificial insemination, embryo collection using flushing technique and embryo evaluation. The research method used was a case study. Data processing and analysis in this study used the independent t-test with research variables of the number of embryos collected, the quality of embryos produced, the number of corpus luteum produced, the level of response, and the level of recovery. The results of this study were no significant difference (P> 0.05) in the production of Limousin and Simmental cattle embryos in the rainy season. In contrast to the dry season, the results were (P <0.05) which means there is a difference in the production of Limousin and Simmental cattle embryos in the dry season. On the other hand, Limousin cattle showed that Limousin cattle were slightly superior to all variables in the dry season.

INTRODUCTION

Beef is a livestock product that has high protein to meet the nutritional needs of the Indonesian people, but based on the facts in the field, public consumption of beef is still very low. Based on the report of the Central Statistics Agency (BPS) 2023, beef consumption by the Indonesian population in a week was 0.01 kilograms in 2023. The solution is to provide investment funds to the directorate of livestock breeding and production to improve the genetic quality of beef cattle in order to accelerate breeding with embryo production.

Reproductive technology that can be used to accelerate increased productivity and improve genetic quality is embryo transfer. Embryo transfer has many benefits, including producing offspring that inherit traits from both parents directly and can shorten the distance between generations, so that improvements in the genetic quality of livestock can be achieved more quickly. The application of embryo transfer also increases reproductive efficiency as measured by several variables such as the number of corpus luteum, response rate, number of embryos collected, embryo quality, and donor-parent recovery rate. One factor that can affect the success of conception during embryo transfer is the environment (Wieczorkiewicz *et al*., 2021).

As a tropical country, Indonesia has two seasons, namely the dry season and the rainy season, each of which has different temperatures and humidity that can affect the amount of embryo production and cause stress. According to Vieira *et al*. (2014), the dry season has an overall detrimental impact on embryo production and quality, with a more pronounced negative effect on donor cattle than heifers. Martínez *et al*. (2021) also stated that seasonal effects were more pronounced in donor and calf performance than in recipients. High ambient temperature and temperature-humidity index can have a negative impact on cow conception rates (Nishisozu *et al*., 2023). Cattle exposed to high temperatures result in overheating of the reproductive organs, thus disrupting the processes of oogenesis and embryogenesis (Miętkiewska *et al*., 2022). In the early stages of cow embryo cleavage (before the 8-16 cell stage), the genome is not yet active and the embryo is sensitive to many stressors, including high temperatures (Satrapa *et al*., 2013).

Feed is one of the external factors that is greatly influenced by the season. In the dry season, the availability of green fodder tends to decrease, while in the rainy season, the availability of green fodder is abundant. This is supported by the statement of Bira *et al*., (2024) that there is an imbalance in the availability of feed between the rainy season and the dry season, so livestock productivity fluctuates according to seasonal changes. Of course, this problem can affect the production of embryos produced in different seasons, because feed is closely related to livestock productivity.

Simmental and Limousin cattle have the potential to be developed in Indonesia because they have quite high body weight performance. The body weight of Limousin cattle reaches 650 kg in females and 850 kg in males, while Simmental cattle can reach a body weight of 1150 kg in males and 800 kg in females (Hermawansyah *et al*., 2023). The purpose of this study was to determine the comparison of embryo production of Simmental cattle with Limousin cattle in the rainy season and the dry season.

MATERIALS AND METHODS

Research Materials

This study used 40 donor cows divided into 20 Simmental and 20 Limousin donor cattle, each aged 3 to 6 years with a body weight of 450-700 kg at the Cipelang Embryo Transfer Center, Bogor Regency. The selected donor cows had met the criteria as donor cows. In this study, the cows were fed elephant grass (*Pennisetum purpureum*) of 30 to 40 kg per cow per day, supplemented with additional concentrate feed of 5 kg per cow per day, and drinking water provided ad libitum.

Superovulation

Naturally, female cows only release one egg during estrus. To obtain more than one egg at the same time, a superovulation program is carried out on selected donor cows. Superovulation is carried out by injecting reproductive hormones, the hormones used include FSH (Follicle Stimulating Hormone), PMSG (Pregnant Mare Serum Gonadotropin), or a combination of PMSG (Pregnant Mare Serum Gonadotropin) with hCG (Human

Chorionic Gonadotropin) (Wenda *et al*., 2013). These hormones play a role in stimulating the growth and development of follicles, regulating the ovulation process, forming the corpus luteum, and influencing the concentration of endogenous hormones in the body (Bó & Mapletoft, 2014). Superovulation uses a 20-day period starting from the implant of progesterone preparations on day 0 in the morning, then FSH hormone injections are carried out in the morning and evening 3 times with decreasing doses of 5 ml, 3 ml, and 2 ml. On the 11th day or the 3rd day of FSH injection, in the morning the progesterone preparation implant is removed along with PGF2a injection. In the afternoon, FSH + PGF2a injections are given.

Artificial Insemination

Two days after the progesterone preparation was removed, artificial insemination was performed 3 times with interval of 12 hours starting from the first estrus in the superovulation program. If the first estrus is in the morning, then artificial insemination is carried out in the afternoon or 12 hours after the estrus up to three times. This aims to increase the number of ova fertilized by sperm (Subchan & Handarini, 2016).

Embryo Collection

After 7 days from the first artificial insemination, a rectal palpation examination was performed to evaluate the superovulation response by examining the condition of the ovaries (Ponsania *et al*., 2023). At this stage, the number of corpus luteum (CL) formed was recorded. After recording, embryo collection was carried out using the flushing method. Cows that were going to undergo the flushing process were put into a clamp cage, then given epidural anesthesia. After the anesthesia worked, a cervix expander was inserted into the vagina and placed in the cervical lumen to manipulate the cervix. This was done so that the balloon path could be lifted properly so that the flushing medium could flow out completely (Subchan & Handarini, 2016). Flushing was carried out in three different places, namely on the right and left cornua and on the corpus uteri, each place required 400-500 ml of lactated ringer as a flushing medium (Jodiansyah *et al*., 2013). The goal is to ensure that all embryos are collected in the flushing medium. The flushing media containing the embryos is then collected in a media bottle and immediately taken to the laboratory for further evaluation of the embryos (Irma *et al*., 2023).

Embryo Evaluation

The quality assessment of embryo evaluation is based on the International Embryo Transfer Society (IETS). According to Liamanu *et al.* (2018) stated that embryo quality has five groups consisting of three transferable (grade 1, 2, 3) (TF), degenerative (DG), and unfertile (UF). The embryo quality is evaluated using a light microscope and the percentage of embryos that are suitable for transfer and not suitable for transfer is calculated based on morphological characteristics (Ponsania *et al*., 2023). The evaluation process begins with filtering or filtration which aims to separate embryos from foreign objects using a 70 µ filter. After the embryos are obtained, embryo classification is carried out using a stereo microscope with a magnification of 100 times.

Research Variables

Number of Corpus Luteum (CL)

The number of corpus luteum (CL) can be calculated by rectal palpation or viewed using ultrasound.

Total Embryos Collected

The number of embryos collected from flushing results, including embryos that are transfer-worthy, degenerative, and unfertile.

Embryo Quality

The IETS classification is based on the assessment of embryo morphology, which includes shape, color, cytoplasmic density, and

degenerated parts. The observed embryos are collected in storage media to be evaluated based on their morphological development stage to determine the quality of the embryo. The quality of embryos that are transfer-worthy is quality 1 to 3, while for the embryo phase from phase 4 to 8. In addition, there are also qualifications of degenerative and unfertile embryos.

$$\frac{\sum \text{embrio grade (TF or DG or UF)}}{\sum \text{embrio collected}} \; x\; 100$$

Response Rate

Response rate can be stated as a response donor cow is a donor cow that produces more than or equal to 3 numbers of corpus luteum (CL) from the administration of follicle stimulating hormone (FSH) (Jodiansyah *et al*., 2013).

$$\frac{\sum \text{donor cow responded (CL} \geq 3)}{\sum \text{superovulated donor cow}} \; x\; 100$$

Recovery Rate

The recovery rate can be calculated using the following formula (Jodiansyah *et al*., 2013):

$$\frac{\sum \text{collected embryos}}{\sum \text{CL is formed in the ovary}} \; x\; 100$$

Data Analysis

The data used in this study is embryo production data obtained from the Animal Embryo Center in 2022. Data relevant to the objectives of this study will be collected, processed, and analyzed using non-parametric tests with the SPSS version 29 application. Before the Independent T-test analysis, normality and homogeneity tests must be carried out, if the results are not normal and not homogeneous, a non-parametric test will be carried out. The non-parametric test used in this study is the Mann-Whitney test. Data obtained from the research object will be interpreted appropriately to find facts systematically, factually, and accurately.

RESULTS AND DISCUSSION

Number of Corpus Luteum

Examination of the number of corpus luteum (CL) functions as an indicator of the number of embryos produced during flushing and as a determinant of the success rate of the superovulation program. This is in line with Singh *et al*. (2020) who stated that if the CL formed has a large number, then the number of ovum ovulated will also be large. The results of observations of the number of CL from Limousin and Simmental cows in different seasons can be seen in the Figure 1.

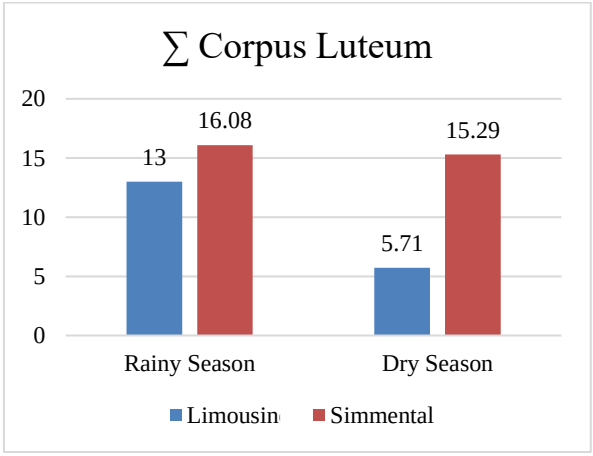

Figure 1. Diagram of number of corpus luteum in different season

Based on the Figure 1, it is known that in the comparison of the number of CL of Limousin and Simmental cattle in the rainy season, the results obtained showed that there is no difference (P>0.05). This result is different from previous research that the number of CL in Simmental cattle was higher (15.96) when compared to Limousin cattle (12.28) (Liamanu *et al*., 2018). In previous research located at Cipelang Embryo Transfer Center by Pranata *et al*. (2016) reported that the number of CL of Simmental cattle in the rainy season obtained an average result of 13.4

while in this study the average number of CL was 16.08±9.56. Meanwhile, Pangestu *et al*. (2024) reported an average CL of Simmental cattle of 8.43. This shows that the results of this study are better compared to previous studies.

While in the dry season, the results of the comparison of the number of CL of Limousin and Simmental cattle showed that there is a difference (P<0.05). The average number of CL of Limousin cattle in the dry season was 5.71 ± 2.81 lower than the CL of Simmental cattle namely 15.29 ± 7.54. In a previous study located at Cipelang Embryo Transfer Center by Jodiansyah *et al.* (2013) reported that the number of CL of Simmental cattle in the dry season was 7.7 while in this study the number of CL was 15.29 ± 7.54. This shows that the results of this study are better compared to previous studies.

The amount of CL is seen to be higher in the rainy season compared to the dry season. Differences in corpus luteum can be caused by environmental factors that are less suitable for the body condition of the donor cow. Setiawan *et al*. (2019) stated that heat stress in cows causes a decrease in appetite, this can affect the concentration of hormones in the body. If the concentration of hormones in the body is disturbed, it will affect the amount of corpus luteum produced by the donor cow. The process of CL formation can be influenced by two factors, namely intrinsic and extrinsic factors. Nurcholis & Muchlis (2023) stated that intrinsic factors include genetics, hormones, age, and reproductive conditions. While extrinsic factors include the environment, feed, and breeders.

Number of Embryo Collected

The embryo harvesting method used is flushing. Flushing is done three times, this is done to maximize the number of embryos collected. According to Irma *et al.* (2023) uterine flushing is carried out in three parts of the uterus, namely the right uterine horn, the left uterine horn, and the uterine body. The purpose of flushing in these three parts is to determine the results of the superovulation program in the livestock. The results of the embryo collection comparison can be seen in the Figure 2.

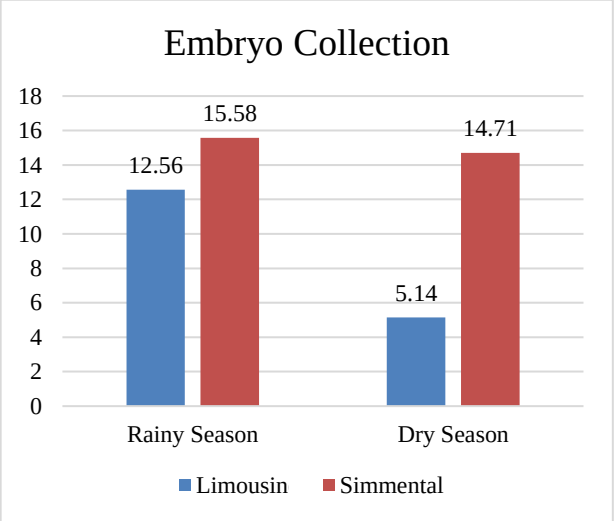

Figure 2. Diagram of the number of embryo collected in different season

The results of the data analysis that have been carried out show that the comparison of the total collection of Limousin and Simmental cattle embryos in the rainy season shows no difference (P>0.05). The average total of Limousin cattle embryos collected in the rainy season is 12.56 ± 8.11 while the average total of Simmental cattle embryos collected in the rainy season is 15.58 ± 9.41.

While in the dry season, the results show that the comparison of the number of Limousin and Simmental cattle embryo collections has a difference (P<0.05). The average total of Simmental cattle embryos collected in the dry season is higher (14.71 ± 8.12) than Limousin cattle embryos (5.14 ± 3.58). In previous research located in Cipelang Embryo Transfer Center by Jodiansyah *et al*., (2013) reported that the average total Simmental cattle embryos collected in the dry season obtained results of 6.7. Meanwhile, Pangestu *et al*. (2024) reported that the average number of embryos collected in

Simmental cattle was 6.2. This shows that this study is better than previous studies.

The number of embryos collected in this study tended to be higher in the rainy season, especially in Limousin cattle. Differences in collected embryos can be caused by genetics, breed, reproductive health, feed consumption, environment, cage temperature, age of the cow, and the embryo production capacity of the donor cow (Irma *et al*., 2022).

Embryo Quality

Embryo evaluation is carried out after flushing to determine the quality of the collected embryos. This evaluation is important to ensure that the resulting embryos meet the standards required for the next process, either for embryo transfer to recipient cows or for further research purposes. The results of observations of embryo quality are categorized into three classifications, namely Transferable (TF), Degenerate (DG), and Unfertile (UF) (Subchan and Handarini, 2016).

The collected embryos are then evaluated and categorized into grades 1, 2, and 3 (transferable) and grade 4 (not transferable). Embryos with grade 1 and 2 can be frozen directly, while grade 3 embryos can be directly transferred to the recipient (fresh TE). Grade 4 embryos that are considered unfit for transfer will be discarded (Djunaedi *et al*., 2018). Embryos of quality 1 and 2 tend to produce high pregnancy rates (Hasler, 2014).

Based on the data above, it is known that the comparison of the quality of Limousin and Simmental cattle embryos in the rainy season. The quality of Limousin cattle embryos in the rainy season that are transferable (TF) is 15%, degenerate (DG) is 52%, and unfertile (UF) is 33%. While the quality of Simmental cattle embryos in the rainy season that are transferable is 52%, degenerate is 19%, and unfertile is 29%. Overall, in the dry season simmental cattle have more embryos that are Transferable (52%), compared to Limousin cattle.

Based on the data, it is known that the comparison of the quality of Limousin and Simmental cattle embryos in the dry season. The quality of Limousin cattle embryos in the dry season that are transferable is 39%, degenerate 25%, and unfertile 36%. While the quality of Simmental cattle embryos in the dry season that are transferable is 30%, degenerate 37%, and unfertile 33%. Overall, in the dry season Limousin cattle have more embryos that are transferable (39%), compared to Simmental cattle.

The number of degenerate embryos tends to be higher in Simmental cattle than Limousin cattle in the dry season, this is in line with the study of Liamanu *et al.* (2018) that Simmental donor cattle produce more degenerate embryos than Limousin cattle. However, the number of transferable embryos in this study was lower than the previous report, namely from 18 Simmental cattle embryos collected in Turkey, 88.9% were transferred embryos and 11.1% were degenerate embryos (Köse *et al*., 2022).

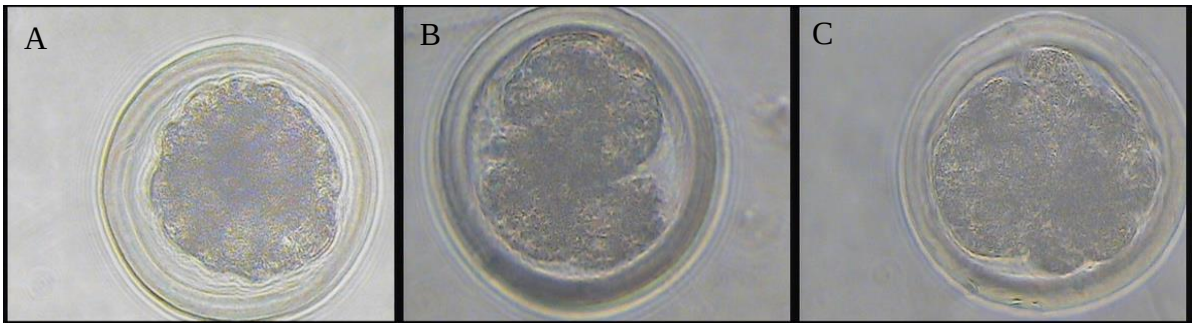

Figure 3. Transferable embryos. A: Grade 1, B: Grade 2, C: Grade 3

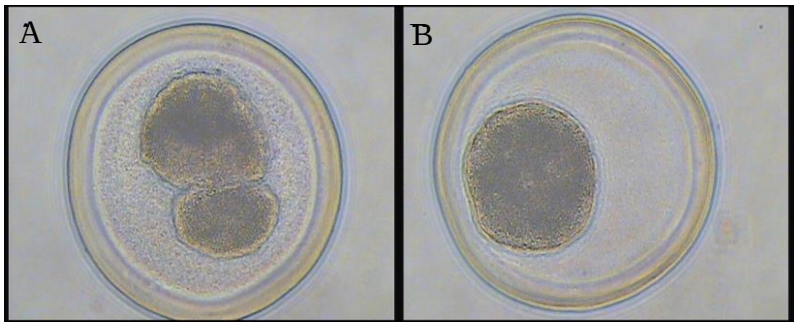

Figure 4. Degenerate and unfertile embryos. A: Degenerate, B: Unfertile

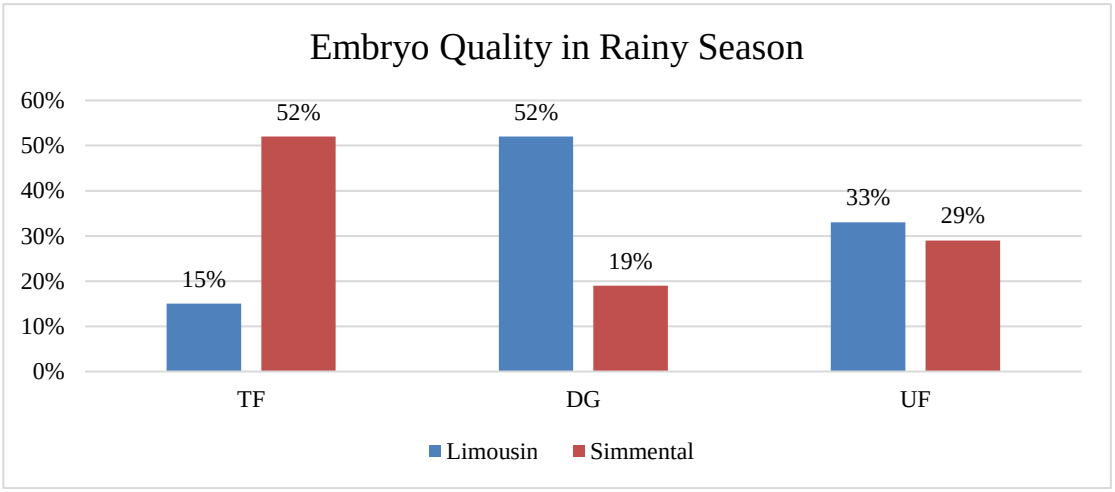

Figure 5. Embryo quality diagram in rainy season. TF: Transferable, DG: Degenerate, and UF: Unfertile

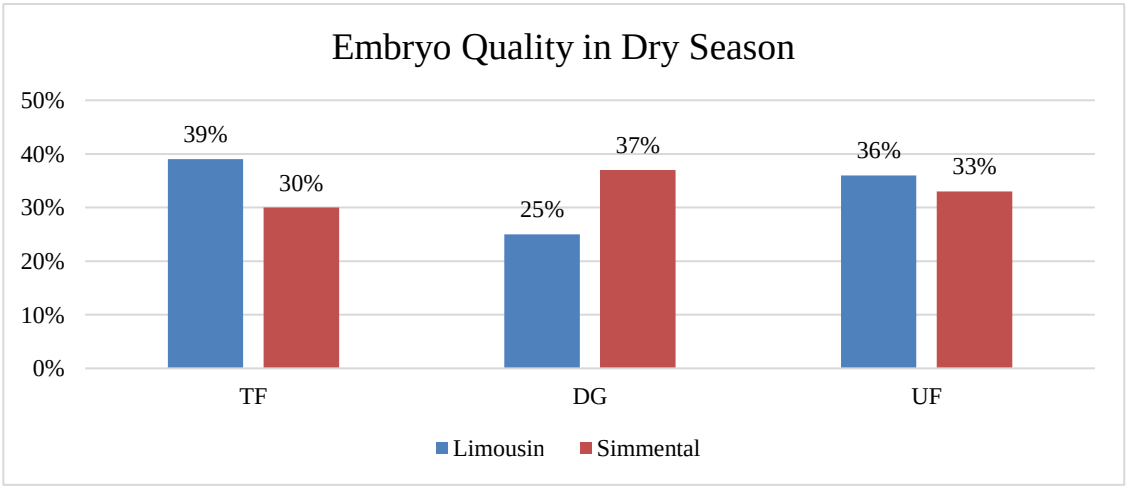

Figure 6. Embryo quality diagram in dry season. TF: Transferable, DG: Degenerate, and UF: Unfertile

From the data it can be seen that each breed of cow has different embryo quality, this is caused by variations in semen used during artificial insemination because each individual cow has a different response during the fertilization process. Hardiyanto *et al*. (2016) stated that variations in individual cows and breeds of bulls have different body resistance to the environment.

In a previous study conducted by Chrenek *et al*. (2014) the results of Limousin cattle embryo production in three seasons had the following quality, in the summer transferable 22.2% and unfertile 14.8%. In spring, transferable was 34.8%, degenerate 24.8% and unfertile 24.8%. While in autumn, transferable was 38.1%, degenerate 10.2% and unfertile 10.2%. From previous research, it can be seen that the percentage of transferable embryos of Limousin cattle in the dry season in Indonesia is better than in the summer in Slovakia.

The results showed that embryos collected in the dry season had more severe cell damage; in contrast, embryos cryopreserved in the rainy season appeared morphologically more capable of producing pregnancy after transfer (Marquez *et al*., 2003). This is supported by the study of Martínez *et al*. (2021) that the number of good quality embryos suitable for transfer and unfertilized ones increased in the rainy season. According to Köse *et al*. (2022), heat stress causes an increase in ovarian temperature which has a negative impact on oocyte quality, changing the intrauterine environmental conditions because blood flow decreases and uterine temperature increases. These changes inhibit embryo development, increasing the rate of early embryo mortality.

Response Rate

Response rate is a parameter of success of cows programmed for superovulation by looking at the comparison between the number of cattle that respond to the total number of cattle that are superovulated. It is said to respond if the number of CL formed is ≥ 3. This is in accordance with Irma *et al*. (2023) who stated that superovulated cows can be categorized as response cows if they produce more than 3 CL. The formation of CL during superovulation is caused by various factors, one of which is environmental factors. The environment, especially the season, can provide peace if the surrounding temperature is ideal. Likewise, if the temperature is too high it will cause stress and affect livestock reproduction (Setiawan *et al*., 2019).

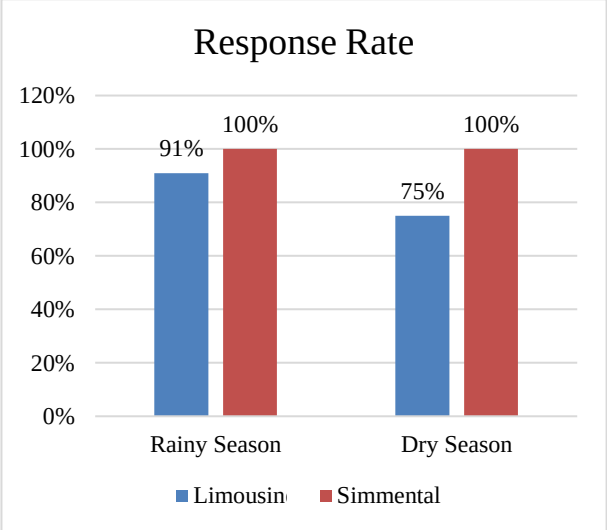

Figure 7. Response rate diagram in different season

Based on the data known above, the success of Simmental cattle superovulation carried out during the rainy season was stated to have responded completely (100%), in other words, all Simmental cattle superovulated during the rainy season produced CL ≥ 3. The 100% superovulation response rate indicates that the gonadotropins used are effective in stimulating the development of subordinate follicles into dominant follicles and then into de Graaf follicles, follicles that are ready to be ovulated (Liamanu *et al*., 2018). While the success of Limousin cattle superovulation during the rainy season only got 91% results. During the dry season, the results showed that Simmental cattle responded completely

(100%), in other words, all Simmental cattle superovulated during the dry season produced CL ≥ 3. While the success of Limousin cattle superovulation during the dry season only got 75% results. From the two data above, it shows that Simmental cattle are more responsive when superovulated during the rainy and dry seasons compared to Limousin cattle. This result is quite different from the study of Liamanu *et al*. (2018) that the response rate of 25 Limousin and Simmental cattle showed the same figure, namely 100%. In a previous study conducted by Afriani *et al*. (2019) reported that the response rate results of 15 Pesisir cows in the dry season got 100% results or all cows responded (producing CL ≥ 3). Similar to this study, it obtained 100% results in the response of Simmental cows that were superovulated in the dry season.

The difference in response values is caused by several factors such as cage temperature, age, application of artificial insemination, determination of the estrus cycle, and the accuracy of FSH administration. Hardiyanto *et al*. (2016) stated that the effect of age on superovulation response and embryo production is very high, the older the donor cow, the lower the superovulation response and embryo production of the cow. Setiadi *et al*. (2005) stated that administration of gonadotropin hormone during the follicular wave emergence phase has the potential to significantly increase the superovulation response. This effect is most optimal when the hormone application is carried out when the follicular wave is taking place, but without the presence of a dominant follicle in the ovary. This condition allows the developing follicles to respond more uniformly to hormones, thereby increasing the efficiency and success of the superovulation process.

Recovery Rate

Recovery rate is the percentage of the number of embryos successfully obtained from superovulation after flushing. This is in accordance with Pranata *et al*. (2016) who stated that the recovery rate is the retrieval of successfully collected ovum and embryos produced from superovulation, as well as the percentage of embryo or ovum recovery after ovulation. Factors that affect the recovery rate include the quality of sperm and egg cells, the condition of the reproductive organs, the health of the livestock, and the technical skills and methods used in the procedure (Efendy & Luthfi, 2019).

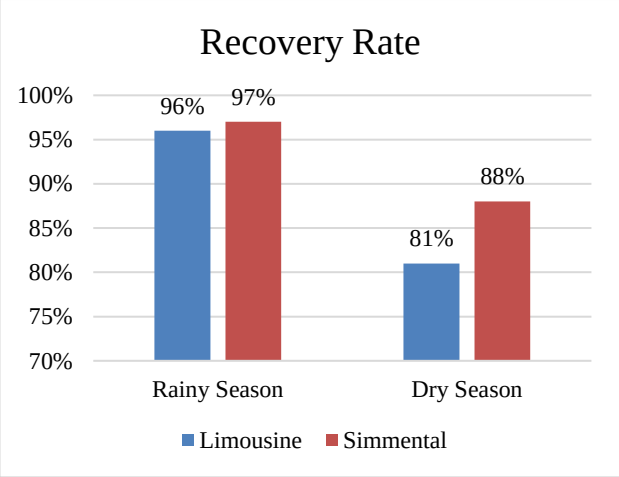

Figure 8. Recovery rate diagram in different season

Based on the data above, it is known that the comparison of the recovery rate of Limousin and Simmental cattle in the rainy season obtained results that showed that (P>0.05) which means there is no difference between the recovery rate of Limousin and Simmental cattle in the rainy season. The average recovery rate in Limousin cattle in the rainy season is 96±6% while the average recovery rate in Simmental cattle in the rainy season is 97±4%. In the dry season, the results showed that the comparison of the recovery rate of Limousin and Simmental cattle did not have a difference (P>0.05). The average recovery rate in Limousin cattle in the dry season is 81±38% while the average recovery rate in Simmental cattle in the dry season is 88±25%.

From the two data above, it shows that the recovery rate of Simmental and Limousin cattle in the rainy and dry seasons have the same results in each season, namely there is no

difference. In previous research conducted by Imron *et al*., (2016) reported that the average recovery rate in Ongole crossbreed cattle during the dry season was 84.81 %. In another study conducted by Jodiansyah *et al.* (2013) reported that the average recovery rate of Simmental cattle during the dry season was 45 %. This shows that the average recovery rate of Simmental cows in this study was better than previous studies.

Differences in recovery rate data can occur due to several factors, one of which is stress in livestock. If livestock are stressed, it will affect the hormonal conditions of the livestock. The response rate greatly affects hormonal conditions. Irma *et al.* (2022) stated that decreased appetite is often associated with stress or disease that can affect the overall physiological condition of the cow. Stress can also be caused by increased cage temperatures and cause heat stress.

CONCLUSION

The results of this study indicate that there is no significant difference in embryo production between Simmental and Limousin cattle in the dry season, but in the rainy season there is a significant difference. Simmental cattle have more transferable embryos than Limousin cattle in the rainy season, while Limousin cattle have more transferable embryos than Simmental cattle in the dry season. Simmental cattle are superior in terms of superovulation response (response rate) and recovery rate compared to Limousin cattle. This indicates that seasonal variations can significantly affect embryo production in both cattle breeds.

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