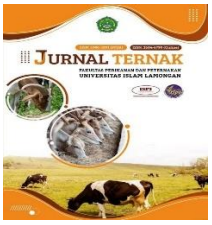
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Success Rate Of Artificial Insemination With Frozen Semen Sexing In Beef Cattle After Foot And Mouth Disease

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ABSTRACT

The purpose of this study was to analyze the success of sexing frozen semen AI in beef cattle of Limousin Cross breed after FMD. The material in the study used 30 Limousin cross cattle without a history of FMD and 30 Limousin Cross cattle with a history of FMD. This research method used an experimental field experiment with research treatments were P0: sexing semen AI acceptors without a history of FMD and P1: sexing semen AI acceptors with a history of FMD. Research variables included NonReturn Rate (NRR), Service Per Conception (S/C) and Conception Rate (CR). The data obtained were analyzed descriptively. The results showed NRR 1 in P0 AI acceptors without a history of FMD was 90.00%, in P1 NRR 1 was 76.67%, NRR 2 values were respectively P0 and P1 were 80.00% and 73.33%. S/C research results in P0 was 1.18 and in P1 1.44. CR at P0 was higher at 80% compared to P1 at 56.67%. The study concluded that the success rate of frozen semen sexing AI in AI acceptors without a history of FMD was higher than in AI acceptors with a history of FMD.

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1. Introduction

The fattening business is gaining increasing interest among livestock farmers, both on an industrial and household scale. According to Purwoko (2015), livestock farmers consider cattle fattening to be more economical than cattle breeding due to its faster capital turnover, lower business risks, and higher profit margins. Problems arise when breeding businesses are unable to supply local feeder cattle as raw material, forcing fatteners to import feeder cattle. This situation presents a challenge for local cattle breeding businesses to increase production.

Foot-and-Mouth Disease (FMD) is a disease caused by the Aphtae Epizooticae virus, belonging to the genus Aphthovirus and the family Picornaviridae, and affects even-toed ungulates, including beef cattle. Beef cattle are a staple food source, providing meat with economic value and contributing to public consumption. The foot-and-mouth disease outbreak that occurred in 2022 slowed livestock

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growth and production in Indonesia. According to Firman et al. (2022), the Indonesian government stated that foot-and-mouth disease (FMD) would spread from May 6, 2022, to May 20, 2022. This indicates that the spread of FMD is an extraordinary event that requires prompt and effective treatment.

Clinical signs of foot-and-mouth disease are used to establish a diagnosis. Adjid (2020) mentions several clinical signs, such as a body temperature above 40°C, emaciated animals, hypersalivation, and lesions on the nose, muzzle, cheeks, tongue, gums, and inside the lips. Both the livestock health industry and the livestock economy have suffered significant losses due to the spread of FMD in cattle. Many farmers have suffered financial losses, such as reduced productivity and difficulties selling livestock (Tawaf, 2016). The economic impacts of this disease include a 25% reduction in milk production, a 10%–20% reduction in the development rate of beef cattle to maturity, infertility, miscarriage, delayed pregnancy, and calf mortality (Naipospos, 2014).

Foot-and-mouth disease can cause long-term productivity and reproductive problems. One impact of substandard management, which leads to repeated breeding, is impaired reproductive performance due to foot-and-mouth disease (Nurul et al., 2022). This occurs because animals with FMD refuse to eat or drink, leading to nutritional deficits. The ovaries shrink and become less functional due to nutritional deficiencies, which also inhibit the anterior pituitary gland from secreting the GnRH hormone (Danus et al., 2020). Reproductive hormones play a crucial role in controlling the reproductive cycle of cattle when disrupted or inactive. This is consistent with the opinion of Wahyuni et al. (2018) found that stimulation of gonadotropin hormone release is significantly influenced by nutritional intake. Yudiani (2021) also added that nutrition influences the intensity of estrus for functional reproductive needs.

At the end of 2022, livestock farmers were impacted by an outbreak of foot-and-mouth disease (FMD) in cattle, which reduced the productivity of beef cattle. FMD causes long-term reproductive disorders in estrus in cattle and can lead to abortions in pregnant cows. Therefore, simultaneous replacement is necessary to support productivity and improve estrus to prevent further abortions. Artificial insemination (AI) is a reproductive technology aimed at improving the genetic quality of livestock, which is expected to increase the productivity of local livestock in the beef supply. Meeting meat demand must be accompanied by accelerating livestock population growth through the use of technology towards greater efficiency (Wahyudi, Susilawati, and Isnaini, 2014).

The separation or sexing of X- and Y-chromosome spermatozoa is a reproductive biotechnology that plays a crucial role in supporting AI programs to improve livestock efficiency. This sperm sexing is expected to produce crossbred livestock with the desired sex. AI using frozen semen from Y-chromosome spermatozoa (Y-sperm) will increase the male livestock population as raw material for fattening efforts in meat production. One of the methods used for sexing is Percoll density gradient centrifugation (SGDP) which is a simple and economical alternative to flow cytometry (Yekti et al., 2023; Safa et al., 2025). The Conception Rate (CR) in IB frozen semen non-sexing and sexing results was 44.44% and 25.92% (Wahyudi, et al., 2014) and 35.48% and 43.47% (Setiyani, Yekti, Kuswati and Susilawati, 2018). The method of using sex-selected spermatozoa can cause a decrease in sperm quality, so artificial insemination (AI) using sex-selected semen is carried out with a double dose (Fatahillah, Susilawati, and Isnaini, 2017). In small-scale livestock farming, beef cattle have been repeatedly mated with Limousine cattle, resulting in a low pregnancy success rate with a high number of matings per fertilization. Based on this, research is needed to determine the success rate of AI using frozen semen in beef cattle after FMD.

2. State of the Art

Previous studies have consistently shown that FMD affects cattle fertility beyond the acute phase of infection. A retrospective cohort study on a large dairy farm in Kenya reported that animals susceptible to fertility suppression at the time of the outbreak had a lower hazard of conception than animals served before the outbreak (Chaters et al., 2018). In Indonesia, Susilo et al. (2024) found reproductive disorders in 61.9% of FMD-infected dairy cows, dominated by ovarian hypofunction (53.5%), and reported S/C, CR, and pregnancy rate values of 2.02, 51%, and 85%, respectively, after GnRH administration. The estrus response of dairy cows that survived FMD has also become a target

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of intervention, for example through Superbooster and Immunobooster supplementation (Zulfanisa et al., 2024).

Studies on AI success after the 2022 outbreak in Indonesia have mostly used dairy cattle and unsexed semen. Syah et al. (2024) compared crossbred Friesian Holstein cows inseminated before and after FMD in Malang Regency and reported NRR-1 of 77.00% vs 86.96%, NRR-2 of 62.00% vs 73.04%, and CR of 49.00% vs 46.09%, with no significant differences (P>0.05). However, the marked decrease from NRR-2 to CR in post-FMD cows was suspected to result from early embryonic death associated with low progesterone levels, suggesting that NRR alone may overestimate pregnancy in cattle with a history of FMD.

Sex-sorting technology supports the efficiency of beef production but is generally associated with reduced fertility. A meta-analysis of 45 studies showed that sex-sorted sperm reduced NRR 24/60 by 13% and pregnancy rate by 23% compared with conventional sperm (Reese et al., 2021), although that analysis covered only flow cytometry-sorted sperm and excluded Percoll-based methods. For Percoll density gradient centrifugation, Yekti et al. (2023) reported that sexed frozen semen had lower motility, viability, concentration, and total motile spermatozoa than unsexed semen, while the separation accuracy of X and Y spermatozoa exceeded 80%. Therefore, FMD-related reproductive impairment and the reduced quality of sexed semen may act as two constraints on AI success at the same time, yet studies evaluating Percoll-sexed frozen semen AI in beef cattle with a history of FMD remain limited. This study evaluated NRR, S/C, and CR of sexed frozen semen AI in Limousin crossbred cattle with and without a history of FMD.

3. Method

Location and Materials

This research was conducted in February 2024-January 2025. Artificial Insemination (AI) was carried out within the service area of the Inseminator officers in Pakis District, Malang Regency. The research material was 60 Limousin cross cattle ready for AI, consisting of 30 cows with no history of FMD and 30 cows with a history of FMD. The research material was AI in double doses. The female cattle were AI using frozen semen sexing with the Percoll density gradient centrifugation separation method.

Research Method

The method used in this study was a field experiment. The sampling technique used purposive sampling, using the criteria of cows AI acceptors: sexually mature females aged 2-10 years and with a Body Condition Score (BCS) of more than 4. This study used 60 Limousin cross cattle ready for AI, divided into two groups:

P0: cows without a history of FMD

P1: fcows with a history of FMDData Analysis

The variables observed in this study include:

a. Non-Return Rate (NRR) is the percentage of acceptors who do not show signs of estrus again within 18-21 (NRR 1) and 40-42 (NRR 2) days after insemination (Wiranto et al., 2020). The NRR calculation formula adapted from Syah et al. (2024) is:
   NRR= (Total inseminated cattle - Total re-inseminated cattle) ÷ Total inseminated cattle x 100%
b. Service Per Conception (S/C) ratio was used to determine the success rate of artificial insemination (AI) through the percentage of AI services performed by the inseminator until pregnancy occurred. The mathematical formula for calculating the S/C percentage (Tadesse et al., 2022):
   S/C= (Total of AI services until pregnancy ÷ Total of pregnant acceptors)
c. Conception Rate (CR) was used in research to determine the success of AI by calculating the percentage of animals that become pregnant in the first AI. The following is the formula for determining the CR percentage (Syah et al., 2024):
   CR = (Total pregnancy cattle from the first insemination ÷ Total inseminated cattle) × 100%

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Data Analysis

The data obtained was analyzed using a descriptive method with a quantitative approach based on the results of observations and and the data on AI success rates were analyzed using the Pearson Chi-Square test to compare them against the 100% expectation.

2. Results and Discussion

Non-Return Rate (NRR)

Non-Return Rate (NRR) is one method of estimating pregnancy in IB acceptor cows after IB. The pregnancy estimation indicator is by observing IB acceptor cows that do not show signs of heat after IB. According to Varotto, Finocchiaro, Kaam, Marusi, and Cassandro (2016), the non-return rate (NRR) is one indicator of livestock fertility and an evaluation of reproductive performance without waiting for parturition. NRR observations are carried out twice, namely NRR 1 observed on days 18-21 after IB and NRR 2 observed on days 40-42 after IB (Syah et al., 2024). The NRR percentages from this study can be seen in Figure 1.

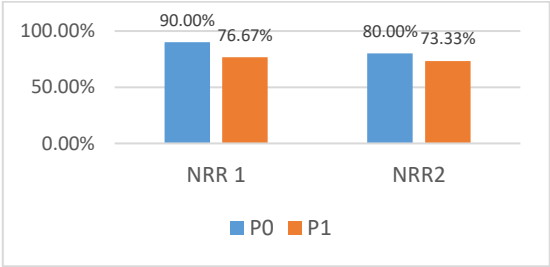


Figure 1. Non-Return Rate (NRR) from artificial insemination (AI) under treatments P0 and P1

Figure 1 shows that 90.00% of P0 AI acceptors without a history of FMD showed no signs of estrus, or 27. Meanwhile, 76.67% of P1 AI acceptors with a history of FMD showed no signs of estrus, or 23. Descriptively, the pregnancy rate in NRR 1 observation among P0 AI acceptors was higher than that among P1 AI acceptors, and statistically, the pregnancy rates for P0 and P1 AI acceptors were still below expectations. NRR 2 observation showed a decrease in pregnancy rate due to the increase in the number of estrus-inducing AI acceptors. The estimated pregnancy rates for P0 and P1 using the NRR method were 80.00% and 73.33%, respectively, with 24 and 22 cows, respectively.

The chi-square analysis for NRR 2 at P0 and P1, compared to 100% pregnancy, showed a highly significant difference ($P<0.01$). Descriptively, the pregnancy rate observed at P0 was higher than at P1, and statistically, the pregnancy rates for AI acceptors at P0 and P1 were still below expectations. This study compares favorably with the study by Setiyani, Yekti, Kuswati, and Susilawati (2018), which found that the pregnancy rate in Ongole crossbred cattle decreased from NRR 1 to NRR 2, from 93.54% to 83.87% after frozen semen AI. A similar decline from NRR 1 to NRR 2 was reported in crossbred Friesian Holstein cows before and after FMD (77.00% to 62.00% and 86.96% to 73.04%; Syah et al., 2024) and in Holstein Friesian cows inseminated with double-dose Percoll-sexed semen (80% to 65% and 85% to 75%; Utami et al., 2026), which suggests that some of the cows assumed to be pregnant at NRR 1 returned to estrus later. Sex-sorted semen is itself associated with lower non-return rates (Reese et al., 2021); since both groups in this study received sexed semen, the additional decline in P1 is more likely related to the history of FMD.

The emergence of post-AI re-estrus is indicated by symptoms such as high vocal intensity, swollen and slightly shiny vulva, mucus, restlessness of the mother and when approached will be silent and some mothers experience a decrease in appetite and drinking. One cause of post-AI re-estrus is the death of the embryo before implantation. In cattle with a history of FMD, this may be related to hormonal disturbance: FMD virus infection was shown to elevate estrogen and reduce progesterone levels in infected cows (Shaban et al., 2022), and low progesterone was suspected to cause the marked decrease from NRR 2 to CR in post-FMD cows (Syah et al., 2024). Cows in post-FMD AI programs may also have been vaccinated, as in Syah et al. (2024), and FMD vaccination was reported to increase pregnancy

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Nisa'us, et. al./Jurnal Ternak 17 (1) 2026 pp. 393-401

failure in beef cows, with 44.1% failure in cows vaccinated within 29 days after mating compared with 36.3% in unvaccinated cows (Garcia-Pintos et al., 2021). The vaccination status of the cows should therefore be taken into account when interpreting fertility after FMD.

Service Per Conception (S/C)

Assessing the success of artificial insemination (AI) based solely on the pregnancy rate does not fully reflect a livestock's reproductive efficiency; therefore, the "services per conception" (S/C) value is also evaluated. S/C serves as a benchmark for reproductive efficiency, measuring the number of AI services required for an animal to achieve a pregnancy confirmed via rectal palpation. Generally, pregnancy diagnosis via rectal palpation is conducted around 60 days post-AI (Syah et al., 2024; Utami et al., 2026). A higher S/C value indicates lower reproductive efficiency, whereas a lower S/C value indicates higher reproductive efficiency (Tadesse et al., 2022). The S/C values for this study are presented in Figure 2.

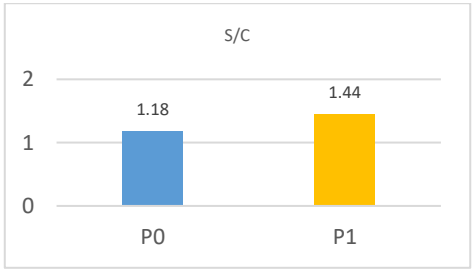


Figure 2. Service per Conception (S/C) from artificial insemination (AI) under treatments P0 and P1

The optimal S/C value is 1, meaning a single AI service results in pregnancy. Figure 2 shows S/C values of 1.18 for treatment P0 and 1.44 for treatment P1, indicating that, on average, more than one AI service was required to achieve a pregnancy confirmed by rectal palpation. Chi-square analysis comparing the number of AI services required to achieve pregnancy in P0 and P1 groups against the benchmark of a single service showed no significant difference ($P>0.05$). Descriptively, this indicates that the P0 group required fewer AI services than the P1 group; statistically, however, the number of services for both P0 and P1 exceeded the ideal benchmark of one AI service. The S/C of P0 (1.18) was close to the mean number of services per conception of 1.15 reported in a ten-year retrospective study of dairy cows (Tadesse et al., 2022), whereas the higher S/C of P1 (1.44) is consistent with the impaired fertility reported in FMD-affected cattle (Chaters et al., 2018; Susilo et al., 2024). Both values were lower than the S/C of 2.02 reported for FMD-infected dairy cows after GnRH treatment (Susilo et al., 2024), which may reflect differences in the animals and management. Failure to conceive at the first service extended the calving-to-conception interval by about 45 days in the study of Tadesse et al. (2022), so a higher S/C also has economic consequences for farmers.

The S/C value is influenced by the frequency of AI recipients returning to estrus after the initial insemination. In this study, factors influencing the S/C (Service per Conception) ratio include delays by farmers in detecting estrus and in contacting the inseminator for Artificial Insemination (AI). Tadesse et al. (2022) recommended that farmers be trained to identify cows in estrus and that AI technicians be trained to perform AI accurately in order to increase conception and reduce economic loss. The ability of farmers to recognize signs of estrus and the skill and experience of the inseminator have also been named as factors behind low non-return rates (Syah et al., 2024), and the success of AI with frozen sexed semen has been evaluated in relation to estrus characteristics (Utami et al., 2022). Detecting estrus may be even more difficult in cows that have been affected by FMD, as noted by Syah et al. (2024), which may delay reporting to the inseminator and increase the S/C in P1.

The success of Artificial Insemination (AI) can be assessed not only by pregnancy rates and Service per Conception (S/C) values but also by the proportion of AI recipients confirmed pregnant via rectal palpation following the first service—a metric known as the conception rate (CR). CR is crucial for determining the reproductive efficiency of female livestock and serves as a measure of the AI program's success. The CR percentage has not yet been determined, as rectal palpation for pregnancy diagnosis

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has not been performed. Typically, pregnancy diagnosis via rectal palpation is conducted around 60 days post-AI (Syah et al., 2024; Utami et al., 2026). The CR percentages are presented in Figure 3.

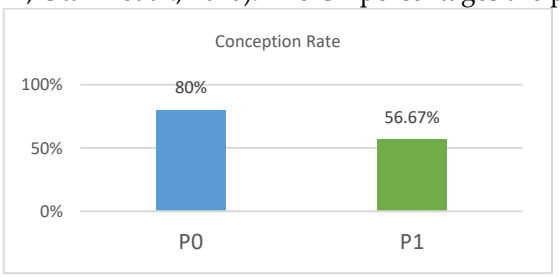


Figure 3. CR results of AI in treatments P0 and P1

Figure 3 indicates that the CR for AI using frozen semen in group P0 (80.00%) was superior to that in group P1 (56.67%). Chi-square analysis comparing the CR of AI recipients in groups P0 and P1 against a 100% pregnancy benchmark revealed a significant difference ($P<0.01$). Descriptively, the pregnancy rate among P0 recipients was higher than that of P1 recipients; however, statistically, the pregnancy rates for both groups remained below expectations. The CR of P0 was higher than the CR reported for AI after FMD in crossbred Friesian Holstein cows (46.09%; Syah et al., 2024) and in FMD-infected dairy cows after GnRH treatment (51%; Susilo et al., 2024), while the CR of P1 (56.67%) was within the range reported for sexed semen AI in Indonesian cattle, such as 50%–75% in Holstein Friesian cows inseminated with double-dose Percoll-sexed semen (Utami et al., 2026) and 63.15% with albumin-sedimentation sexed semen (Syah et al., 2023). The relatively high CR in P0 may be associated with the double dose used in this study, which compensates for the reduced number of viable sperm after sexing (Utami et al., 2026), considering that sex-sorting is generally associated with lower pregnancy rates (Reese et al., 2021). The lower CR in P1 supports evidence that FMD impairs conception through hormonal disturbance and embryonic loss (Shaban et al., 2022; Syah et al., 2024). A cow's ability to conceive upon the first insemination is also influenced by environmental variables such as ambient temperature, because heat stress disrupts cow reproduction (Sammad et al., 2020).

CR values are correlated with Non-Return Rate (NRR) and S/C values; specifically, a higher CR corresponds to a higher NRR and a lower S/C, whereas a lower CR corresponds to a lower NRR and a higher S/C. This is because an increasing number of livestock returning to estrus after artificial insemination (AI) raises the number of AI services required, resulting in a low conception rate (CR). A lower conception at first service increases the number of inseminations and days open, and therefore the feed and replacement costs borne by the farmer (Tadesse et al., 2022).

3. Conclusions

The study concludes that artificial insemination (AI) with frozen semen sexing in cattle with no history of FMD yields a higher success rate compared to AI in cattle with a history of FMD. The success rate in cattle with a history of FMD indicates that the post-FMD period impairs reproductive performance, evidenced by prolonged estrus cycles, extended intervals between calving and the subsequent pregnancy, a higher Service per Conception (S/C) ratio, and a lower conception rate.

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