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Successful Artificial Insemination using Sexed and Non Sexed Semen on Limousin Crossbred Cow

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ABSTRACT

Background: Beef cattle fattening business requires male cattle because they grow faster so that by using sexed semen (Y sperm) it is expected to get a male calf. The purpose of this study was to determine the success of artificial insemination using non-sexed and sexed frozen semen using percoll density gradient centrifugation (PDGC) method.

Methods: A total of 120 female Limousin Crossbred were used in this study. The cows were raised on smallholder farms aged between 3-9 years with a body condition score (BCS) between 3-7 (score 1-9). The treatment was T0: 60 cows were inseminated using non-sexed frozen semen while T1: 60 cows were inseminated using sexed Y frozen semen. The insemination was performed with double doses at the 2nd and 8th hours after oestrus and deep insemination. The parameter measured were NRR-1, NRR-2, conception rate and pregnancy rates. Determination of pregnancy was performed using rectal palpation and ultrasonography (USG).

Result: The results showed that NRR-1 and NRR-2 at T0 were 47 (78.33%) and 42 (70%), while at T1 were 37 (61.67%) and 35 (58.33%), respectively. While conception rate at T0 and T1 was 22 (36%) and 18 (30%), pregnancy rate at T0 and T1 was 31 (56%) and 19 (31.67%) and silent heat at T0 and T1 was 24 (40%) and 26 (43.3%).

Key words: Artificial insemination, Limousin crossbred cow, Percoll density gradient centrifugation, Sexed semen.

INTRODUCTION

The beef cattle breeding business aims to produce calf as feeders from the fattening business on people's farms (Boukari *et al.*, 2018). Cattle breeding on smallholder farms mainly uses artificial insemination technology. Artificial insemination can be increased in value using sexed semen to producing calf sex as expected (Susilawati *et al.*, 2017).

One of the sexing methods that can be used is Percoll density gradient centrifugation (PDGC). Percoll is a medium consisting of colloidal particles coated with polyvinyl pyrrolidone (PVP), which can increase the motility of spermatozoa. Sexing using the Percoll density gradient centrifugation method can separate with an accuracy of more than 80% based on identifying spermatozoa head measurements (Susilawati, 2014). Hayakawa (2012) stated that the sexing process could reduce the quality of spermatozoa so that quality is lower compared to spermatozoa that are processed without sexing process. Research conducted by Kusumawati *et al.* (2017) reveals that after sexing using the PDGC method, the percentage of Y spermatozoa motility decreased by 48.55%. However, it is still feasible for artificial insemination because the minimum motility according to the Indonesian National Standard (SNI) the minimum of progressive motility of frozen semen must be at least 40%. The results of AI using semen sexing PDGC method obtained the birth of male calf as much as 28.57% and female calf 71.43% (Promthep *et al.,* 2016). This study researched artificial insemination using sexed semen by the PDGC method compared to frozen semen without sexing, using double doses and deep insemination on cows raised on smallholder farms.

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MATERIALS AND METHODS

A total of 120 Limousine crossbred cows in the Senggreng village, Sumber Pucung district, Malang Regency were selected for the present experiment. They had BCS 3-4 (on a 1-9 scale) and aged between 2-5 years. They were divided into two groups having 60 animals in each group.

The research was conducted from January until September 2021. This research methodology was experimental field treatment: AI used frozen semen sexed using the PDGC (Y Sperm) method that AI centre Singosari has produced. The treatments were T0 = AI using non-sexed frozen semen while T1= AI using sexed frozen semen. The parameter measured was the evaluation of the success of AI using NRR-1, NRR-2, conception rate

and pregnancy Rates. Determination of pregnancy was using rectal palpation and ultrasonography (USG).

The method included: 1) Selection of cows as acceptors. 2) Thawing semen using tap water with (28°C) for 30 seconds based on the inseminator thawing method, the semen quality after thawing has the minimum criteria for AI with progressive motility at least 40%. 3) Cows are inseminated according to the treatment using non-sexed frozen semen (T0) and sexed frozen semen (T1) with the double dose method at the 2nd hour and the 8th hour by deep insemination. 4) Injection of BIO ATP Vitamin (Rheinbio) 10 ml/injection before AI. 5) Observation of NRR 1, NRR2 and oestrus occur on next cycle after insemination. If the cows were experienced estrous, then AI was repeated. 6) After two months, pregnancy and ovarian and uterine status were examined using rectal palpation and ultrasonography.

Supporting variables

Vulva color: The vulva color was categorized with three scores included: Red evenly (score 3), Red uneven (score 2) and pale (score 1).

Swelling of the vulva: The swelling of the vulva was categorized as swollen (score 3), slightly swollen (score 2), not swollen (score 1).

Cervix mucus: The cervix mucus was categorized as a lot (score 3), moderate (score 2) and a little/none (score 1).

Heat detector value: Heat detector value was categorized with the value of 20-29 Ω and between 30-35 Ω.

Dependent variables

Non-return rate value (NRR): NRR-1 is the Percentage of cows that do not ask for repeated AI after 1st AI. NRR-2 is the Percentage of cows that do not ask for repeated AI after 2nd AI.

Conception rate: Percentage of pregnant cows from the first AI.

Pregnancy rate: Percentage of pregnant cows from the first and second AI.

Silent heat: Percentage of cows who do not show heat signs but are not pregnant.

Data analysis of each parameter was analyzed by using unpaired T-test and descriptive-analytic.

RESULTS AND DISCUSSION

Body weight and body condition score

The average body weight in the control treatment was 376.66±37.57 kg, while the bodyweight of cows with AI treatment using frozen sexed semen was 339.63±44.65 kg. Based on statistical tests, the bodyweight of non-sexed AI acceptors is heavier than sexed (P<0.01). Likewise, on the BCS, body score condition in T0 is 4.17±0.57, while in T1, it was 3.78±0.74. The body condition score of AI acceptors using non-sexed frozen semen was better than sexed (P<0.01). Although the initial body weight and BCS of T0 and T1 are different, they are in the range of BCS within 3-4, which is used as female criteria on this research that could have good reproduction performances. According to Hafez and Hafez (2000), in cows that have higher body weight, their reproductive conditions are better than those of lean ones. Bodyweight also represents the body condition score, a visual or tactile assessment of muscle and adipose tissue. Body condition is used as an indicator to estimate tissue reserves in body weight (William and Swecker, 2014).

Oestrus characteristic

The success of artificial insemination is determined by the appearance and detection of actual oestrus signs during the oestrus phase of the estrous cycle. Therefore, observations of the appearance of oestrus signs were carried out and the results were as follows, the vaginal temperature of non-sexed and sexed AI acceptors in the first AI was 38.25±0.50°C and 38.25±0.42°C. These results showed no significant (P>0.05) difference. Likewise, in the second AI, it was 38.41±0.59°C and 38.48±0.37°C which showed no significant difference. The pH of the vulva of AI acceptors using non-sexed frozen semen and sexed frozen semen in the first AI was 7.70±0.46 and 7.26±0.62, which showed a significant (P<0.05) difference. Likewise, in the second AI, it was 7.64±0.56 and 7.52±0.50, which were not significantly different. The vulva color of the non-sexed and sexed AI acceptors in the first AI was 2.36±0.52 and 2.09±0.76, respectively, which showed a very significant (P<0.01) difference. While in the second AI, it was 2.34±0.48 and 2.11±0.86, which do not differ significantly (P>0.05). Cervical mucus for non-sexed and sexed AI acceptors in the first AI was 2.70±0.45 and 1.89±0.49. This value showed a very significant (P<0.01) difference. Likewise, for the second AI, it was 2.64±0.48 and 2.33±0.60. The heat detector values of sexed and non-sexed AI acceptors at the first AI were 23.47±3.03 and 22.38±1.80, which do not differ significantly (P>0.05). Likewise, the second AI, 22.80±2.92 and 22.2±2.73, showed no significant difference (P>0.05). Swelling of the vulva of sexed and non-sexed AI acceptors in the first AI 46.99±7.78 cm and 51.64±7.75 cm were significantly (P<0.05) different. Likewise, In the second AI, it was 47.71±8.29 cm and 52.28±8.41 (P<0.05). Changes in the color of the vulva, swelling of the vulva and mucus discharge are related to the hormone estrogen, which tends to increase in the oestrus phase. Scolari *et al.* (2011) state that estrogen stimulates vaginal wall thickening and increases vascularity which causes the vulva to experience swelling and redness and increased mucus secretion from the vagina so that there is mucus hanging on the vulva or sticking to the buttocks. During oestrus, the high estrogen in cattle causes the blood vessel network to multiply in the reproductive tract. The study results by Verma *et al.* (2014) showed that cows with more clear mucus resulted in a pregnancy percentage of 41.25%, which is higher than that of cows with cloudy mucus resulting in a pregnancy percentage of 14.28%. Cows with watery consistency have a pregnancy percentage of 26.66%, slightly thick had

47.54% and 11.11% in thick. The high percentage of pregnancy in estrous cows with the consistency of thin cervical mucus may be due to the increased movement of spermatozoa in the female reproductive tract. The presence of mucoprotein causes disruption of spermatozoa motility and penetration of the ovum, so that the success of pregnancy is low in cows with thick cervical mucus.

Pregnancy outcome

Data in Table 1 show the value of NRR-1 and NRR-2 in cattle AI using frozen non-sexed semen and sexed semen. Our result indicates that it was higher in cattle inseminated with frozen non-sexed semen than in cattle inseminated with frozen sexed semen. However, the statistical test results showed no difference (P>0.05) between them. The value of NRR-1 was less than 100% because of the failure of fertilization and implantation after insemination. On the other hand, the decrease in NRR-1 to NRR-2 value is caused by early embryonic death and silent heat.

During pregnancy, the presence of the conceptus causes inhibition of the regression of the corpus luteum so that the hormone progesterone reaches the peak and does not give rise to the signs of estrous. Therefore the absence of signs of estrous indicated the success of pregnancy or called non-return rate (NRR) (Jaenudeen and Hafez, 2000). The NRR values of the various treatments are shown in Table 1. The NRR1 and NRR 2 values on T1 are lower than T0, indicating that the ability of non-sexed semen to fertilize eggs is higher than sexed semen. Several research results showed that the PDGC sexing method causes a decrease in semen quality and membrane damage compared to non-sexed semen. This is due to centrifugation during the separation and washing process, which causes a mechanism that can cause sperm membrane damage resulting in a decreased ability of sperm (Susilawati *et al.*, 2017).

Furthermore, Table 1 showed decreasing value from NRR1 to NRR2 on both treatments, which was on T0 decreases from 81.67% to 75%, T1 decrease 61.67% to 56.67%. The decrease of NRR-1 to NRR-2 might be due to early embryonic death or not detected estrus during the NRR-1 observation, so that the NRR-1 score was higher than NRR-2. In the opinion of Bajaj and Sharman (2011), Embryonic mortality denotes the death of fertilized ova and embryos up to the end of implantation. Early embryonic mortality accounts for most reproductive failures, with a mortality rate of up to 40% of fertilized eggs. In addition to the time of AI implementation, one of the factors that determine pregnancy success. Lack of knowledge of farmers in detecting oestrus and awareness to immediately call the inseminator resulted in delays in the implementation of AI, resulting in pregnancy failure (Van de Gucht *et al.*, 2017).

In addition, in this study, some of the female cows inseminated are still breastfeeding their calves. This is also one of the causes of the absence of oestrus. According to Jaenudeen and Hafez (2000), one of the causes of silent heat is that the cow is in breastfeeding period will result in the high of prolactin hormone, which suppresses the release of FSH so that the hormone estrogen does not come out, which causes silent heat.

Table 1: The value of non-return rate 1 and non-return rate 2.

Treatment	Number of cows	NRR-1 Head (%)	NRR-2 Head (%)
T0	60	49 (81.67)	45 (75%)
T1	60	37 (61.67)	34 (56.67%)

Table 2: Conception rate and pregnancy rate using non-sexed and sexed frozen semen.

Treatment	Number of cows	Conception rate head (%)	Pregnancy rate head (%)	Silent heat rate head (%)
T0	60	22 (36%)	31 (56%)	24 (40%)
T1	60	18 (30%)	19 (31.67%)	26 (43.33%)

Data in Table 2 show the results of this study which indicate that the success of pregnancy was still low. The success rate of pregnancy based on the conception rate on AI using frozen semen sexing was lower than AI using semen sexing. This result aligns with the NRR value on sexed semen and non-sexed semen. Producing sexed semen with the PDGC method is more complex than non-sexed semen. In sexed semen, sperm will experience the separation through percoll gradient and centrifugation. This process can decrease spermatozoa's motility, viability and concentration after sexing (Kusumawati *et al.*, 2017).

In addition, pregnancy failure based on the results of ultrasound examination showed a lot of ovarian hypofunction and persistent corpus luteum. The value of cows experiencing silent heat, *i.e.*, not showing signs of heat, but when checked for pregnancy, they were not pregnant at T0 was 24 heads (40%) while at T1 it was 26 heads (43.33%). According to Jainudeen and Hafez (2000), reproductive failure is influenced by genetic and environmental factors that impact ovarian hypofunction, embryonic death and failure in implantation of the conceptus. Inchaisri *et al.* (2011) added that AI success also depends on parity, breed, the season of insemination, frequency of insemination and timing of insemination.

CONCLUSION

In conclusion, the success of AI using frozen sexed semen is lower than AI using frozen semen without sexing based on the NRR value, conception rate and pregnancy rate. However, many factors influence this, including estrous detection by farmers, timing inseminations and female reproduction conditions. Furthermore, the failure of the pregnancies on both treatment are most affected by the silent heat.

Conflict of interest

All authors declare that they have no conflict of interest.

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