

Successful artificial insemination of sexed semen using albumin sedimentation on Friesian Holstein cows

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Abstract. This study aimed to determine the success of artificial insemination (AI) using unsexed and sexed frozen semen with albumin sedimentation. This research was conducted from August to December 2022 at a farmer community in Pandesari Village, Pujon District, Malang Regency. The material used in this study was 78 heads of Friesian Holstein cattle, with the criteria of having a minimum body condition score of 2.5 (scale of 1–5), having normal reproductive organs, and showing signs of estrus. 38 cows were used as acceptors in artificial insemination (AI) with unsexed frozen semen, and 38 cows in AI with albumin sedimentation frozen semen. The variables used are non-return rate 1, non-return rate 2, and conception rate. The data obtained were then analyzed descriptively. The results showed that the percentage values of NRR1, NRR2, and CR in AI using frozen semen unsexed were 76.3%, 63.1%, and 47.3%, respectively, while the percentage values of NRR1, NRR2, and CR in AI using frozen semen sexed were 94.7%, 86.15%, and 63.15%. Based on the study's results, insemination success using albumin sedimentation frozen semen sexed showed better results than AI using unsexed semen.

1 Introduction

The demand for cow's milk in the country has increased along with the increase in population. Still, the consumption level of cow's milk in Indonesia is not supported by high milk production. Efforts can be made to increase cow's milk production by increasing the population of female dairy cows with reproductive technology, Artificial Insemination (AI). Artificial insemination has become one of the most essential techniques developed for the genetic improvement of farm animals [1]. The AI program can be improved by spermatozoa sexed, which aims to produce superior cattle with the appropriate sex for maintenance [2]. Spermatozoa sexed technology that can be used to increase the value of AI is sexed using the albumin sedimentation method. One method of sexed spermatozoa

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that is easy to use is Bovine Serum Albumin (BSA). Sexed spermatozoa using albumin is a method of separating spermatozoa that is easy to do and can produce spermatozoa with X and Y chromosomes between 71-76% [3]. Assessment of the reproductive success of AI cattle can be conducted by observing the parameters of Non-Return Rate (NRR) and Conception Rate (CR) [4]. This study aims to evaluate the success of AI using frozen semen sexed results with albumin sedimentation method in Friesian Holstein cows based on NRR1, NRR2, and CR, which are the evaluation parameters of artificial insemination success.

2 Materials and methods

This study was conducted in the working area of inseminator KOP SAE Pujon Pandesari Village, Pujon Subdistrict, Malang, East Java, and was conducted from August 13 to December 13, 2022. The materials used in this study were 76 female PFH cows. Samples were selected by purposive sampling with the requirements of BCS at least 2.5-3.5 (scale 1-5), having normal organs and showing signs of estrus. The cows were divided into 2 treatments, where 38 females were inseminated with non-sexed frozen semen, and 38 females were inseminated with sexed frozen semen using albumin sedimentation. AI was conducted by an inseminator in the local village and was done when there were reports from farmers that cattle showed signs of estrus. The inseminator inseminated using the single dose method at the 8th hour, by positioning the semen on the corpus utery. After AI, the cows were injected with 20 ml of ADE vitamin. Semen deposition treatment in position 4 in this study is expected to increase the birth of female calves in Friesian Holstein cows. Research conducted by Azmi *et al* [5] stated that AI with a single dose of frozen semen in position 4 or corpus utery obtained sex identification using the ultrasound method of 72.89% of female calves and 27.11% of male calves, while in double dose AI treatment received identification of 69.33% female calves and 30.67% male calves.

3 Variable

3.1. Non-Return Rate

Non-Return Rate 1 (NRR₁) is the percentage of cows that do not return to estrus on days 19-22 and 39-43 after AI. Cows that do not show signs of estrus are assumed to be pregnant; if the cow shows signs of estrus, it is assumed that the female is not pregnant. According to Iswoyo *et al* [6], NRR₁ can be calculated with the formula:

$$NRR_1 = \frac{\text{Total AI in cows - number of estrous cows}}{\text{Total cows in AI}} \times 100\%$$

$$NRR_2 = \frac{\text{Total AI in cows - number of estrous cows (2nd cycle)}}{\text{Total cows in AI}} \times 100\%$$

3.2. Conception Rate

Conception Rate (CR) is the percentage of mothers who become pregnant at the first insemination. The CR value is obtained from the results of acceptors who do not show

signs of estrus in NRR₁ and NRR₂ observations and have carried out pregnancy checks using the rectal palpation method, CR calculation can be obtained by the formula [4]:

$$CR = \frac{\text{Total cows pregnant at first insemination}}{\text{Total cows in AI}} \times 100\%$$

3.3. Body Condition Score

Body Condition Score (BCS) is a subjective assessment method to determine the energy reserves of cattle, this assessment is based on fat reserves in the back and flanks, the scale commonly used is 1-5 [7].

3.4. Age

Age determination can be done in two ways, based on birth records/interviews with breeders and permanent tooth replacement. The age determination method based on permanent teeth is one pair of permanent teeth = 18-24 months, two pairs of permanent teeth = 24-36 months, and 3 pairs = 38-44 months.

4 Results and discussion

4.1. Artificial Insemination Success Based on Non-Return Rate

Table 1. Observation Results of Non-Return Rate 1 and Non-Return Rate 2 of Friesian Holstein Cows Inseminated with Sexed and Non Sexed Frozen Semen

Treatment	Number of acceptors (head)	NRR ₁		NRR ₂	
		Number of acceptors (head)	%	Number of acceptors (head)	%
T1 (<i>nonsexed</i>)	38	29	76.3	25	63.1
T2 (<i>sexed SDA</i>)	38	36	94.7	33	86.15

Evaluation of the success of pregnant cattle can be seen from the appearance of signs of estrus at a certain period, the NRR value is used to measure the ability of the cow, when the cow does not show signs of estrus in the estrous cycle, then the acceptor is assumed to be pregnant [8]. Other factors include the ability of the inseminator to thaw [9], the ability of the farmer to recognize signs of estrus, and other external factors that affect the outcome of artificial insemination [10]. According to Susilawati [11], NRR observations can be observed on days 0-30 and 30-60 after AI, while in this study, NRR observations were observed on days 19-22 for NRR1 and days 39-43 for NRR2. Acceptors who showed signs of estrus in NRR1 observation were considered failed, while acceptors that didn't show signs of estrus will be observed in NRR2, if the acceptor showed signs of estrus, then the acceptor was considered failed, if it didn't show signs of estrus, then a pregnancy test will be conducted on day 60 after AI. The percentage of NRR in this study can be seen in table 1.

Based on table 1, the results of NRR observations in T1 (AI unsexed) obtained the percentage of NRR₁ and NRR₂ of 76.3% and 63.1%. Then, for the observation of NRR T2 (AI sexed), the rate of NRR₁ and NRR₂ was 94.7% and 86.15%. The lower NRR value in T1 than T2 was due to the number of old cows in T1. The increase in the number of parities causes the age of the cows to get older, leading to a decrease in the condition and

reproduction of the cows [12]. Another factor causing the lower NRR at T1 was probably due to observation error [11]. According to Iswoyo *et al* [6], the NRR value can be categorized as good if it reaches 79.53%. The percentage of NRR of unsexed and sexed frozen semen in the study was higher than the research conducted by Rosita *et al* [13], namely the NRR₁ and NRR₂ values of unsexed frozen semen of 74% and 74%, while the percentage of NRR₁ and NRR₂ of sexed frozen semen was 70% and 66%. Based on table 1. the rate of NRR values at T1 and T2 decreased. According to Yekti, *et al* [14], the decrease in NRR value is caused by silent heat and early embryonic death. Silent heat is a condition where cattle do not show signs of estrus, but have a normal reproductive cycle, thus increasing the possibility that the estrus cycle will be missed from observation [15]. This is due to low estrogen, leading to no signs of estrus [14]. Silent heat can also be caused by heat stress [16].

4.2. Artificial Insemination Success Based on Conception Rate

Table 2. Conception Rate in Friesian Holstein Cows Artificially Inseminated with Albumin Sedimentation Sexed and Unsexed Frozen Semen

Treatment	Number of receptors (head)	CR	
		Number of pregnant cows	%
T1 (<i>nonsexed</i>)	38	18	46,15
T2 (<i>sexed</i> SDA)	38	24	63,15

Conception rate (CR) is the percentage of acceptors who successfully conceive in the first AI known after rectal palpation. Costa *et al* [17] stated that CR can be used as an indicator of female cattle fertility, CR results can be measured by rectal palpation 60 days after AI. The calculation of CR value in this study is based on pregnancy examination using the rectal palpation method on days 60-90 after AI.

Table 2 shows that T2 has a better CR value than T1, Priyanto *et al* [18] stated that the CR standard ranges from 60%-70%, so T2 has an ideal value, and T1 is still below the ideal value. The CR value in this study had a higher percentage than the CR value in a study conducted by Rosita *et al* [13] for AI using sexed frozen semen, which was 59%, while for AI using non-sexed semen in this study had a lower value of 74%. The decrease in NRR2 value to CR value can be caused by factors such as acceptor age, BCS, animal parity, estrus detection management, and cow physiology [19]. In addition, the success of AI is also influenced by the quality of spermatozoa used [20].

The interesting data in this study is that the CR value of acceptors AI using sexed frozen semen is higher than acceptors AI using non-sexed frozen semen. The low CR in T1 (unsexed) was also due to the large number of old acceptors, there were eight acceptors over six years old in T1, and 7 of them suffered pregnancy failure. According to Jainudeen *et al* [4], the older the cow, the lower the pregnancy rate. Susilawati [21] added that cows aged 6-7 years have lower fertility.

4.3. Artificial Insemination Success Based on Body Weight

Based on Table 3, acceptors with the highest percentage of pregnancy are acceptors with a BW of 300-400 for T1 and 401-500 for T2, this is because the BW is the ideal weight for cattle to be mated. According to Moran [22] cows with a body weight of 300 are the ideal weight for mating. Cows that are mated when the weight is not ideal will lead to difficulties during parturition. In the first mating, it is crucial to see that the cattle are mature and have reproductive maturity so that the resulting offspring have good performance. Furthermore,

cattle that are mated when the weight is less than ideal will lead to difficulties during parturition. Mammary gland development is influenced by the hormone estrogen and progesterone produced by the ovaries under the influence of FSH and LH hormones [12].

Table 3. Percentage of Artificial Insemination Success Based on Conception Rate at Various Body Weights

Body Weight (kg)	T1 (%)	T2 (%)
300-400	39	12.5
401-500	33	58.3
501-600	22	20.8
<600	6	8.3

4.4. Artificial Insemination Success Based on Body Condition Score

Table 4. Percentage of Artificial Insemination Success Based on Conception Rate at Various Body Condition Scores

BCS	T1 (%)	T2 (%)
2,5	44	29,17
2,75	33	37,5
3,0	17	33,33
3,5	6	0

Based on Table 4, the BCS with the highest pregnancy rate is BCS 2.5 for T1 and 2.75 for T2. The high pregnancy rate of acceptors with BCS 2.5-3 is because the ideal BCS for cows to become pregnant is cows with BCS 2.5-3. Roche *et al* [23] reported that the ideal BCS of a cow is between 2.5-3.5. Cows with a BCS of less than 2.5 or more than 3.5 have a higher chance of reproductive disorders.

4.5. Artificial Insemination Success Based on Age

Table 5. Percentage of Artificial Insemination Success Based on Conception Rate at Various Ages

Age (years)	T1 (%)	T2 (%)
1,5	38,9	12,5
2,5	5,6	0
3	5,6	20,8
3,5	5,6	8,3
4	16,7	20,8
4,5	16,7	8,3
5	5,6	20,8
<6	5,6	8,3

Table 5 shows that in this study, the age of cows that experienced the most pregnancies was at the age of 1.5 years. This could be due to the ideal age of AI implementation, according to Jainudeen *et al* [4], the ideal age for FH heifers to be mated for the first time is at the age of 15 months. The age of old cows will reduce the percentage of pregnancy, in

this study, seven acceptors were more than seven years old, and all experienced pregnancy failure. Susilawati [20] states cows older than six years have lower fertility than younger cows.

5 Conclusion

The success of artificial insemination using SDA-sexed frozen semen showed better results than artificial insemination using non-sexed semen in NRR₁, NRR₂, and CR values. Artificial insemination using unsexed frozen semen obtained NRR₁, NRR₂, and CR values of 76.3%, 63.1%, and 46.15%, respectively, while the percentage of NRR₁, NRR₂ and CR values in AI using SDA sexed frozen semen was 94.7%, 86.15% and 63.15%.

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