Conception rate of artificial insemination using sexed frozen semen with albumin sedimentation method on Friesian Holstein cow

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Abstract. This study aimed to determine the success of Artificial Insemination (AI) using unsexed and sexed semen of albumin sedimentation method based on Non-Return Rate, Conception Rate, reproduction performance, estrus characters, and feed nutrition. This research was conducted from August to December 2022 in Bendosari Village, Pujon District, Malang Regency. The material used in this study was 76 Friesian Holstein (PFH) cows with the criteria of having a minimum Body Score Condition (BCS) of 2.5 (scale of 1-5), normal reproductive organs and showing signs of heat. Thirty-eight cows were inseminated with unsexed frozen semen and 38 with albumin sedimentation sexed semen. The results showed that the percentage value of CR in AI using unsexed frozen semen was 55.23%, while CR in AI using albumin sedimentation sexed was 60.53%. In conclusion, AI's success using frozen albumin sedimentation sexed semen shows better results than AI using unsexed frozen semen.

1 Introduction

Dairy farms in Indonesia are still small-scale and traditional, which has led to low livestock productivity. One step to increase livestock productivity is to improve reproductive performance. Efforts are made to increase livestock productivity by using Artificial Insemination (AI) [1]. Artificial insemination is one of the reproductive technologies compatible with and has succeeded in improving the genetic quality of livestock so that in the future, it can produce good quality offspring in large numbers by using superior males as much as possible [2]. The utilization of sexing or separation of X and Y spermatozoa is the technology that supports AI in increasing the efficiency of livestock breeding. Various sexing methods have been carried out, including sedimentation, albumin column, percoll density gradient centrifugation, electrophoresis, H-Y antigen, flow cytometry, and filtration with sephadex column [3]. The success of pregnancy and the accuracy of the genital sex of the calves born are the final results of the application of AI with sexed sperm. In the

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research conducted by [4] at the AI center of Lembang, the sex suitability of X sperm reached 87% and Y sperm reached 89.5%. This study aims to determine the success of AI in friesian holstein cow dairy cows in terms of Non-Return Rate (NRR) and Conception Rate (CR) using sexed frozen semen through the albumin sedimentation method.

2 Materials and methods

This research was conducted from August to December 2022. This research was conducted in Bendosari Village, Pujon District, Malang Regency, East Java. The materials used in this study were 76 female friesian holstein females with at least 1,5 years old criteria, BCS value of at least 2.5 (scale 1-5), normal reproductive organs and showing signs of estrus. Samples were selected by purposive sampling with the criteria of female cows, vulva color, swollen vulva, vagina temperature, mucus cervix. The cows were divided into 38 cows inseminated with unsexed frozen semen (T0) produced by the AI center of Singosari Malang, then compared with 38 cows inseminated with sexed frozen semen using the albumin sedimentation method (T1) produced by the AI center of Lembang.

3 Variable

3.1 Non-Return Rate

The NRR method is based on the assumption that cows that have been inseminated and no longer in heat are considered pregnant [5]. NRR-1 observations are made by looking at signs of heat, including vulva condition (vulva swelling), vulva colour, and riding or being ridden by cows on the 19 to 22 days after AI. Cow that are not in heat will continue to NRR-2 observation, which includes vulva condition (vulva swelling), vulva color, and climbing and riding other cows on the 39 to 42 days after AI [6] with the following formula:

$$\text{NRR} = \frac{\text{Total inseminated cows} - \text{total cows in heat}}{\text{Total inseminated cows}} \text{ X } 100\%$$

3.2 Conception Rate

The number of pregnant cows can obtain the CR value at the first AI [7]. [8] states that CR is the percentage of cows that become pregnant at the first IB. The CR formula is:

$$\text{CR} = \frac{\text{Number of cows pregnant at first insemination}}{\text{Number of acceptors}} \text{ X } 100\%$$

4 Results and discussion

4.1. Body Performance of Friesian Holstein Breeders

The reproductive physiology of female cows is influenced by various factors, including body weight, Body Condition Score (BCS), and age. Body weight measurements are used to estimate BCS so that phenotypes are better in cows because BCS directly affects dairy cows' nutritional, health, and reproductive status [9]. Underweight body weight will have reproductive disorders commonly referred to as subfertile [10], and BCS that is <2.5 experiences nutritional deficiencies resulting in the reproductive system not functioning optimally and if livestock are mated and too young, will cause suboptimal body weight [11]. Management in optimizing BCS can affect reproductive performance [12]. Low body weight is caused by nutritional deficiencies. The composition of the feed ration is low and does not meet the daily feed needs and nutrient deficiencies in the body of livestock resulting in the reproductive system will not run normally [10]. [13] added that when feed consumption cannot meet the daily needs of livestock, it will cause a decrease in BCS and will interfere with their reproductive status.

One of the factors that affect the low percentage of pregnancy in livestock is the age factor. This is reinforced by the opinion of [3] which states that the older the age of the livestock, the lower the pregnancy rate. According to [14] female cattle fertility will increase continuously until the age of 4 years and will flatten at the age of 6 years until finally it will decrease gradually when the old mother is older.

Table 1. Body Weight, Body Condition Score, Age of Friesian Holstein Cattle

Treatment	Age	Weight	BCS
T0	3.89±1.34 years	336.94 ± 34.16 kg	3.52±18.67
T1	4.07±1.82 years	323.44 ± 38.07 kg	2.66±0.20

The acceptors used in this study are 76 acceptors divided into three groups, namely acceptors weighing 300, 350, and 400. The average weight of acceptors in this study for T0 is 336.94 ± 34.16 kg and T1 is 323.44 ± 38.07 kg. The BCS range used in this study was BCS 2.5-3.5. This study's mean BCS of acceptors was 3.52±18.67 for T0 and 2.66±0.20 for T1. The age of acceptors in this study ranged from 1.5-7 years. The average age in this study was 3.89±1.34 years in T0 and 4.07±1.82 years in T1.

4.2. Estrus Performance

Table 2. Estrus Performance on Artificial Insemination Success Rate Using Unsexed Semen and Albumin Sedimentation Sexed Semen Before First Artificial Insemination

Treatment	Vulva Colors			Cervical Mucus	
	Evenly Red (%)	Red Unevenly (%)	Pale (%)	Much Mucus (%)	No Mucus (%)
	Head (%)	Head (%)	Head (%)	Head (%)	Head (%)
T0	17 (80,95 %)	1 (4,76 %)	3 (14,29 %)	20 (95,2 %)	1 (4,8 %)
T1	19 (82,61 %)	0 (0,0 %)	4 (17,39 %)	23 (100 %)	0 (0,0 %)

Estrus or heat is a condition where female cow have shown heat, namely swelling of the vulva, reddening of the vulva color, warmth, and willingness to ride. Estrus in livestock is characterized by mucus discharge and changes in the physical appearance of the vulva, which is influenced by estrogen hormone levels [15]. [16] added that cows show signs of estrus, including increased restlessness and activity, mucus discharge from the vagina, willingness to ride other cows, or standing heat. Table 2 shows the percentage of estrus appearance in the condition of evenly red, unevenly red, and pale vulva, as well as the quality of mucus with mucus and traces of mucus. Differences in vulval color can be caused by the hormonal activity of each individual cow. Vulvar color is used as an indicator because an increase in the hormone estrogen in cattle increases will cause blood to flow to the vulva, so the vulva will turn red. The color of the vulva turning reddish is one of the signs of heat because the blood flow to the reproductive tract will be higher, so it will have an impact on the condition of the vulva to be swollen, evenly red and the temperature will increase [17]. The amount of blood supply to the vulva can cause swelling of the vulva and vestibule area, so that it will become bright red due to congestion of blood vessels, while the vulva that shows an uneven red color indicates cattle in estrus cycle conditions towards metestrus and at the end of estrus cattle will show a pink or pale color [18].

The results of the research showed that acceptors who were positive for post-AI pregnancy with red mucus color were evenly distributed in the T0 and T1 treatments as many as 17 heads and 19 heads with a percentage of 80.95% and 82.61%, for uneven red vulva color only in the T0 treatment as many as one heads with a percentage of 4.76%, for pale vulva color in the T0 and T1 treatments as many as three heads and four heads with a percentage of 14.29% and 17.39%. In addition to the color of the vulva, the appearance of estrus observed in this study is cervical Mucus, where the results showed that acceptors who were positive for post-Al pregnancy with a description of cervical Mucus had Mucus in the T0 and T1 treatments as many as 20 heads and 23 heads with a percentage of 95.2% and 100 % and for the description of former Mucus there was only one head in the T0 treatment with a percentage of 4.8%. Based on Table 1, it was found that the CR value in cows with evenly distributed red vulva color was higher than uneven and pale red, which was 80.95% in P0 and 82.61% in T1, while the CR value in cows with cervical Mucus was higher than mucus marks, which was 95.2% in T0 and 100 % in T1.

The quality of heat shown by each acceptor at the time of AI is closely related to the success of pregnancy, which includes vulvar reddening and transparent (clear) mucus [19]. Cows in normal and healthy heat will indirectly secrete cervical mucus with a large volume, thick and watery during estrus. The success of pregnancy is positively correlated with transparent mucus secreted from the cow's vulva [20]. According to [21] stated that cervical mucus secreted is influenced by estrogen which makes the hormone adrenaline and the hormone oxytocin secreted, the hormone oxytocin will make blood vessel endothelial cells become permeable which increases the activity of goblet cells so that water accumulation occurs so that high goblet cell pressure results in goblet cells rupturing and cervical mucus will come out. [22] say the hormone estrogen stimulates thickening of the vaginal wall, increased vascularization, causing the external genitals to swell, reddish in color, and increased vaginal secretions characterized by mucus hanging on the vulva or sticking around it.

4.3. Feed Quantity and Quality

Table 3. Observation results of Non-Return Rate-1 and Non-Return Rate 2 of filial Friesian Holstein Artificially Inseminated Using Unsexed Semen and Albumin Sedimentation Sexed Semen.

Treatment	Number of Acceptor	NRR_{0-22}		NRR_{23-43}	
		Head	%	Head	%
T0	38	4	89.47%	9	76.32%
T1	38	2	94.74%	4	89.47%

Farmers play a significant role in detecting heat in female cow, and maintenance management also determines the success of AI. One of the things that is considered is the feed given to livestock. The types of feed given in this study are *Pennisetum purpureum* and corn stover. According to [23], the type of feed and nutritional content will affect the physiology of livestock. Lack of feed intake can result in less visible conditions because of inhibited production of reproductive hormones. [24] stated that nutrition is essential in cow reproductive performance that affects fertility, especially at various stages of the reproductive cycle. [25] added that feed nutrition received by cows before and after calving also affects CR because nutritional deficiencies can cause delays in the estrus cycle.

Adult cows that are deficient in nutrients for a long time can cause the ovaries to become inactive, resulting in irregular cycles and anestrus. Low quality feed such as lack of fat and carbohydrates can affect ovarian activity, suppressing follicle growth rate and encouraging anestrus [26]. According to [5] the lack of feed nutrition will cause the basic needs not to be fulfilled, so that milk production will decrease and reproductive efficiency is low. The protein intake of cattle given irregularly will result in disrupted fat metabolism, decreased LH, cholesterol enzyme activity and estradiol which will affect follicular development, so that it can reduce reproductive performance.

4.4. Pregnancy Rate Based on Non-Return Rate

Non-return rate is the first step to determine the success rate of Al with the understanding that 78 acceptor cows are routinely observed on the 22 and 43 days. Susilawati (2011) [5] explained that NRR is the percentage of female AI acceptors that are no longer in heat for 20-60 days or 60-90 days after the implementation of Al.

Based on Table 3, the percentage of T0 unsexed semen NRR-1 and NRR-2 is 89.47% and 76.32%. The NRR value in the T1 sexing treatment using the albumin method obtained the observation values of NRR-1 and NRR-2 of 94.74% and 89.47%. The decrease in NRR value may be caused by silent heat in female cattle. Cattle affected by silent heat are unable to show signs of heat due to low levels of the hormone estrogen, so farmers will have difficulty in detecting and eventually will be considered not in heat [27]. Silent heat is a condition where cows do not show clear signs of heat but when checked using per rectal palpation there is ovarian activity in the form of follicular development or development of the corpus luteum which is characterized by ovulation [3]. Follicle Stimulating Hormone (FSH) during silent heat is able to encourage the development of follicles in the ovaries until ovulation occurs, but cannot encourage granulosa cells to be able to synthesize the hormone estrogen, so that signs of heat cannot appear [28]. According to [29] one of the causes of silent heat is due to disturbances from ectoparasites and endoparasites that can cause stress on acceptors.

The decrease in NRR value can also be caused by embryonic death. It is in accordance with the opinion of [30] that early embryonic death is caused by livestock disturbed by disturbances from ectoparasites and endoparasites so that livestock will be exposed to stress which will cause neurotransmitters, namely inhibition of the release of hormones caused by the influence of the five senses or stress [10]. [29] explained that early embryonic death is closely related to the implantation process that occurs in the endometrium, the process of implantation will be disrupted if there is a lack of steroid hormones. Acceptors who experience early embryonic death after 9 days of age, the next estrous cycle will experience a setback or longer than the normal cycle (long cycle), this is because the body needs time to destroy the conceptus and be absorbed by the endometrium, then the endometrium secretes the hormone PGF2α and estrus occurs again [10].

4.5. Artificial Insemination Success Based on Conception Rate

Table 4. Conception Rate Results of Filial Friesian Holstein Cows Inseminated Using Unsexed Semen and Albumin Sedimentation Sexed Semen.first AI

Treatment	Number of Acceptor	Pregnant	
		Head	%
T0	38	21	55,26%
T1	38	23	60,53%

The high level of reproductive efficiency can be measured using the conception rate method. [5] stated that CR can be measured by knowing based on pregnancy between 60-90 days after the first insemination. The value of CR is caused by many factors, one of which is detection in heat. Accuracy at the time of heat detection significantly affects the CR value. The CR value is influenced by the accuracy of heat detection and the time of implementation of AI. Silent heat can occur due to errors during heat detection. [31] added that the high and low CR and PR values are influenced by several factors, including estrus condition when the cow is inseminated and the time of AI. There are several factors that cause cows to go into heat, one of them is the lack of attention from breeders in the detection of heat and the delay in reports to the Inseminator, as a result, the implementation of AI was delayed. The low CR value can be caused by the fact that during the implementation of unsexed AI, the animals were still recovering after the Foot and Mouth Disease (FMD) outbreak. In contrast, the implementation of sexing AI was carried out after FMD recovery. [32] stated that foot and mouth disease has a negative effect, which can interfere with several reproductive hormones, which can harm cow productivity.

The ideal CR value is 65-75% [33]. The CR percentage in this study can be said to be below the normal value. The low CR value can be caused by silent heat that occurs due to ovary hypofunction and ovary cystic follicles and feed consumption that cannot meet the daily needs of livestock. [34] explained that ovarian hypofunction is a condition where ovarian function decreases, so that there is no optimal follicular development caused by impaired secretion of FSH and LH hormones, while cystic follicles are young follicles that grow on the ovary for 10 days or more caused by a lack of LH hormones so that ovulation or anovular does not occur. [35] added that the ovum produced by ovary hypofunction has low fertility, making it difficult to fertilize even though the spermatozoa are of good quality.

5 Conclusion

The success rate of AI using unsexed semen is lower than albumin sedimentation sexed semen. The non-return rate value in unsexed semen treatment was 89.47% and 76.32%, while for albumin sedimentation, sexed semen was 94.74% and 89.47%. The conception rate in unsexed and sexed semen was 55.26% and 60.53%, respectively.

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