Research Article

The Study of Artificial Insemination with Double Doses at Different Times in Friesian Holstein Crossbred

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Abstract | The reproductive performance of dairy cows in Indonesia still needs to be improved. The method of Double-dose AI can highly improve the success of AI. The materials used were 160 cows divided into 80 cows inseminated by a single dose and 80 cows inseminated with a double dose. There were two treatments in this research; T1 was AI single dose at the 8th hour after estrus onset, while T2 AI double dose at the 2nd and 8th hour after estrus onset. The Non-Return Rate (NRR 1) was observed on the next cycle after AI. If the cows showed the estrus sign, the insemination was repeated with the same treatment and then continued to observe NRR 2 on the next cycle. After two months, the pregnancy and AI failure were observed using ultrasound. A Chi Square was performed using SAS OnDemand for Academics (ODA, Cary, NC, USA). Moreover, probability values were calculated using the least significant different testing. The results showed that NRR-1 was 83.75% and 85%, while NRR-2 was 62.5% and 68.75% on T1 and T2, respectively. Furthermore, the failure of AI was mainly caused by repeat breeders, which were 28.75% on T1 and T2. It consists of 95.65% and 87.5% of normal ovaries, Corpus luteum persistent at 4.35% and 8.34%, and ovarian hypo function at 0% and 4.16% in T1 and T2, respectively. In conclusion, Artificial Insemination using the double doses method did not significantly improve the pregnancy rate of dairy cows.

Keywords | Artificial Insemination, Friesian Holstein, Ovarium Condition, Reproductive Disorder

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INTRODUCTION

Friesian Holstein (FH) breeds are the most dairy cattle raised in Indonesia due to the good adapt to subtropical and tropical climatic conditions in Indonesia. Recently, the demand for milk in Indonesia is around 80% still imported (DITJENPKH, 2020). In contrast, subtropical country such as the United Kingdom (UK), FH cows can achieve milk production of approximately 7,609-8,548 kg for each lactation period (Albarran-Portillo and Pollott, 2008). Reproduction in dairy cattle is crucial and related to its productivity. Reproductive disorders can reduce milk production, and maintenance costs. A study in India found that reproductive disorders that often occur in dairy cows include AI (repeat breeder) failure, retained placenta, abortion, and vaginal discharge. Early pregnancy to reduce AI failure and abortion (Crowe, et al., 2018). The effects of low-dose AI on pregnancy rates have seldom been studied in lactating dairy cows. Failure of dairy cows to become pregnant after repeated AI is a source of frustration and economic loss to local farmers. A traditional protocol for noticing dairy cows in heat is by direct observation, which is a time consumable and not effective. Unfortunately, may factors caused and shorten the duration of heat and also

weaken the behavioral sign regarding to the phenomenon. However, these methods can't guarantee every detection of signal will be found and inseminated (Nowicki et al., 2017). This due to the appearance of estrus signs on dairy cows has been decreasing, as well as an ovulation disorder related to endocrine function (Endo, 2022).

The reproductive performance of dairy cows in several regions in Indonesia varies widely and still needs to be improved. Repeat breeding has long been considered one of the critical reproductive disorders in cattle. Repeat breeding is often defined as a syndrome with many possibilities including genetic, acquired defects of the ovary, sperm, or early zygote. Further, Research infections or inflammatory, endocrine dysfunctions, nutritional deficiencies were associated with embryonic loss (Yekti et al., 2023). Incidences of repeat breeding in lactating dairy cows varied among regions, environments, and management (Yusuf et al., 2010). Dairy cows with high genetic value should usually produce female offspring, while cows with low value should produce male beef-crossbred calves with high meat quality and good growth performance. Several studies on AI using low-dose frozen-thawed and low-dose sexed semen have been undertaken in heifers and beef cows (Yekti et al., 2023; Susilawati et al., 2018a; Susilawati et al., 2018b).

Previously study presented while using single doses AI was service per conception (S/C) was 2.31 ± 0.54 times, days open (DO) 161.95 ± 61.99 days, calving interval (CI) 444.47 ± 61.21 days, respectively. Furthermore, the reproductive performances in were S/C 2.11 ± 0.62 times, DO 138.38 ± 47.18 days, and CI 419.86 ± 46.95 days, respectively after injected low doses AI (Kusmayadi et al., 2020). In light of previous finding, the increase in the success of AI can be improved by using double doses AI (Yekti et al., 2019). AI using the double dose method can increasing the success of pregnancy, both single dose and double dose in Ongole crossbreed cattle (Ervandi et al., 2009). The time interval difference of the AI may also influenced the result of pregnancy. Since there is seldom using double doses in the Friesian Holstein dairy cows, this research was conducted on a smallholder farm in the Pujon sub-district, Malang Regency, Indonesia. The aim of this study was to determine the double doses AI at different time of Frisien Holstein Crossbred.

MATERIALS AND METHODS

This research was conducted in the Pujon sub-district, Malang Regency. The material used is FH crossbreed cattle belonging to *Koperasi* (KOP) SAE Pujon breeders, Malang Regency. With the criteria of having given birth and being normal, Body Condition Score (BCS) > 2.5 (rating range 1-6), aged 3-7 years based on the dental replacement. Samples of cows were 160 heads, T_1 was 80 cows, and T_2 was 80 cows. The frozen semen was produced from the Artificial insemination center of Singosari. The deposition of semen was position 4 (corpus uteri) with a double dose at the 2nd hour and the 8th hour after estrous onset.

Artificial Insemination Process

The farmer observed the estrous regularly, and if the cows showed estrous signs, the farmer called the Inseminator to inseminate the cows. The estrous condition was observed further based on the onset of estrous, and the vulva condition was swollen and red, with clear mucus. T0 will be inseminated 8 hours after estrous onset, while T1 will be inseminated two times at 2 hours and 8 hours after estrous onset to improve the pregnancy rate. The vitamin of BIO Adenosin Triphosphate (ATP) was injected before inseminating as much as 10 ml intramuscularly. The semen deposition was placed at the corpus uteri (position 4) (Yekti et al., 2022). Furthermore, Cows that did not pregnant or have repeat breeders are observed for their ovaries using Ultrasonography, namely ovarian hypo function, corpus luteum persistence, and ovarian hyper function,

Non-Return Rate

The non-return rate (NRR) was observed after one cycle (days 19-21). If there was no estrus, it was considered pregnant (NRR-1), whereas if the cows were estrous, AI was carried out according to treatment. The second cycle (NRR-2) was evaluated on days 39-42. After two months, pregnancy and ovarian conditions were evaluated by using Ultrasonography (USG).

Parameters Observed

Non-return rate (NRR) includes NRR1 and NRR2: The NRR value was obtained by observing lust on days 19-21 and days 40-42 after IB. If there are signs of lust, the AI was failed and repeats once again (Ervandi et al, 2009).

$$NRR = \frac{(AAI - EAI)}{AAI} \times 100\% \quad \text{----(1)}$$

Where: NRR is non-return rate, AAI is the number of acceptors AI, and EAI is the number of acceptors that have a sign of estrous.

Conception rate (CR): The CR value can be obtained by calculating the pregnant cows in the first AI. (Yekti et al, 2022) CR is the percentage of pregnant cows in the first AI of the conception rate, with the formula:

$$CR = \frac{(The\ Number\ of\ Pregnant\ Acceptors\ from\ First\ AI)}{(The\ Number\ of\ AI)} \times 100\% \quad \text{---- (2)}$$

Evaluation of AI failure. Repeat Breeder is a condition

where the female cows fail to conceive after being AI several times with fertile males without any observed abnormalities (Amiridis et al., 2009). The parameters for the repeat breeder were:

$$Repeat\ Breeder\ (\%) = \frac{(The\ Number\ of\ Fail\ Pregnant\ Acceptors)}{(The\ Number\ of\ Cows\ Inseminated)} \times 100\% \quad (3)$$

$$Normal\ Ovary\ (\%) = \frac{(The\ Number\ of\ Cows\ with\ Normal\ Ovary)}{(Number\ of\ Cows\ with\ Repeat\ Breeder)} \times 100\% \quad (4)$$

$$Hypofunction\ Ovary\ (\%) = \frac{(The\ Number\ of\ Cows\ with\ Hypofungtion\ Ovary)}{(The\ Number\ of\ Cows\ with\ Repeat\ Breeder)} \times 100\% \quad (5)$$

$$Persistent\ Corpus\ Luteum\ (PCL;\%) = \frac{(The\ Number\ of\ Cows\ with\ CLP)}{(The\ Number\ of\ Cows\ with\ Repeat\ Breeder)} \times 100\% \quad (6)$$

Data Analysis

The data and coding analysis was conducted using General Linear Model and Chi Square was performed using SAS OnDemand for Academics (ODA, Cary, NC, USA). The results were presented as standard error mean (SEM). Moreover, probability values were calculated using the least significant different testing. The modelling used as follows (Ardiansyah et al., 2022):

$$Y_{ijk} = \mu + \tau_j + \beta_1 X_{ij} + b_i X_{ij} + e_{ijk} \quad ----(7)$$

where: Y_{ijk} = the expected output for dependent variable, = overall mean value, = fixed effect of the j^{th}, β_1, = overall value of the linear regression coefficient of Y to X (a fixed effect) (sex) β_2, = overall value of the linear regression coefficient of Y to X (a fixed effect), X_{ij} continuous values of the predictor variable (in linear regression), and e_{ijk} = residual value from unpredictable error. $S\tau_{ij}$ dan S_i are taken to be independent variables that are chosen at random. The Chi-Square is denoted by χ^2. The chi-square formula is:

$$X^2 = \sum \frac{(O_i - E_i)^2}{E_i} \quad ----- (8)$$

where: O_i = observed value (actual value) and E_i = expected value.

Therefore, the validation test was conducted using the root mean square error (**RMSE**) and Nakagawa determination coefficient (**R²**) or or $R_{GLMM}(c)^2$.

$$RMSE = \sqrt{\frac{\sum(O-P)^2}{NDP}} \quad --- (9)$$

Note: = actual value, = estimated value, = number of data point, is the variant of a fixed factor, is the sum of all variants of the component, is the variant due to the predictor dispersion and is the specific distribution of the variant (Adli et al., 2023; Sholikin et al., 2023).

RESULTS AND DISCUSSION

In this study, the cows had a body condition score (BCS) between 2.5-3 .5, a BCS with an average of 2.86 ± 0.12 with a score between 1-5. The BCS indicated the performance, and a cow with a BCS of 2.5 showed normal reproduction. Regarding this result, it is assumed that the nutrition provided for FH was well-enough (Parmar et al, 2016). The lack of nutrition and the poor BCS condition can negatively impact cow fertility. Dairy cow reproduction fertility is influenced by the nutrition fed in, which plays an important role in the reproduction cycle (Pradhan, 2008). Adequate nutrition will affect hormonal conditions that play a role in pregnancy success. Insufficient progesterone hormone in the early stages of pregnancy can cause mortality. Nutrients, minerals, and vitamins were associated with the female reproduction and must be given in balanced amounts (Smith, and Akinbamijo, 2000). Indirectly, nutrition affects the hypothalamus gland in the brain to secrete gonadotropin hormone (GnRH), which in turn stimulates the anterior pituitary to produce FSH and LH. Further, associated with the follicular development, which leads to the emergence of estrus and successful pregnancy in cows. Previously, the reproductive cows aged 4.41 ± 0.22 years, and parity 2.53 ± 0.27 times have good physiological conditions (Hafez and Hafez, 2000). Inseminations in either horn, without ovarian palpation, gave an insignificant pregnancy rates than inseminations in the uterine body with doses of 2 million spermatozoa (Andersson et al., 2004). Another result presented insignificant difference on the pregnancy rates were achieved after preovulatory follicle palpation and deep corneal insemination into the ipsilateral horn (Andersson et al., 2004). First, spermatozoa from the contralateral horn moving through the oviduct and abdominal cavity into the ipsilateral oviduct. Second, the spermatozoa was transported within the uterus from the uterine body to the fertilization site in cows (Yekti et al., 2023). Current knowledge about the function of the reproductive tract and appropriate use of hormonal drugs affords control of the estrus cycle of cows. Use of such protocols in reproductive management allows estrus cycles to be synchronized and cows to be effectively inseminated without oestrous detection, which is time-consuming and difficult in farms with numerous cows.

The data showed that NRR-1 values at T0 and T1 were 83.75% and 65%, respectively (Table 1). The non-return rate value was calculated based on the number of cows that did not show estrus after AI, it's was assumed the dairy cows were pregnant. Based on the NRR result, injected using single dose presented 16.25%, and double dose,

Table 1: Non-Return Rate of Single and Double Doses of Frisian Holstein.

The treatment of semen used for AI	N	NRR1 (days 0-21)		RMSE	SEM	NRR2 (Days at 22-42)		RMSE	SEM
		Heads	%			Heads	%		
Single dose	80	67	83.75	0.22	3.22	52	65.00	1.31	4.33
Double dose	80	68	85.00	0.34	1.22	55	68.75	0.34	2.86

AI – Artificial Insemination; N – number of acceptors; NRR – non return rate; RMSE – root mean square error; SEM – Standard Error Mean

Table 2: Conception Rate from Artificial Insemination using Single and Double doses of Frisian Holstein.

Treatments	N	Conception Rate		RMSE	SEM	Pregnancy Rate (2nd AI)		RMSE	SEM
		Heads	%			Heads	%		
A single dose (T_1)	80	50	62.50	1.56	0.67	55.00	68.75	1.13	2.31
Double dose (T_2)	80	46	57.50	1.67	0.31	55.00	68.75	1.45	1.77

AI – Artificial Insemination; N – number of acceptors; NRR – non return rate; RMSE – root mean square error; SEM – Standard Error Mean

Table 3: Conditions of the ovaries or uterus in cows that fail to pregnant after injected by single and double doses of AI

Treatments	Repeat Breeder n (%)	SEM	Normal Ovarian n (%)	SEM	Follicle cystic n (%)	SEM	Corpus Luteum Persistence n (%)	SEM	Hypo function ovary n (%)	SEM
Single doses (T1)	23 (28.75)	2.25	22 (95.65)	2.11	0 (0)	1.22	1 (4.35)	1.12	0 (0)	2.32
Double doses (T2)	24 (28.75)	1.25	21 (87.50)	1.23	0(0)	1.45	2 (8.34)	1.34	1 (4.16)	1.45

AI – Artificial Insemination; N – number of acceptors; SEM – Standard Error Mean

35%, respectively (Susilawati et al, 2020). Susilawati et al., (2017) state that the progressive motility of spermatozoa is the most decisive in fertilization. Frozen semen allowed the storage of semen for a longer time. However, it will cause damage to sperm that will decrease its motility of sperm (Borges-Silva et al, 2015). Hafez and Hafez (2000) has mentioned that frozen semen had fewer spermatozoa functionality than liquid semen when it is evaluated by sperm motility, thermo-resistance test, and hypo-osmotic swelling test. Therefore, inseminated semen using liquid semen can also improve reproduction efficiency.

NRR1 and NRR2 decreased, indicating the occurrence of silent heat, early embryonic death, or when estrus detection is not accurate. According to opinion (Crowe et al, 2018). Farmers have an essential role in detecting estrus because it will affect the time to do insemination, thus affecting the accuracy of AI by the Inseminator. Cows that experienced repeat breeders or failed to conceive in the first AI with the second treatment at single and double doses were 28.75% and 28.75%, respectively. In cows with repeat breeders, 95.65% and 87.5% of normal ovaries were found. This happens because of several possibilities: failure in fertilization, failure of implantation, or premature embryo death. Fertilization failure is caused by low semen quality, poor oocyte quality, or inappropriate estrus detection and timing of AI. Unfavorable physiological conditions cause implantation failure and embryo death. A repeat Breeder is a cow bred several times or has not been successfully AI, caused by poor management and good ovary condition (Sing et al, 2018). Based on the Table 2, the number of conception rate presented 62.50 (single doses;%), and 57.50 (double doses;%), respectively. Furthermore, the second time after insemination was 68.75% both single and double doses (Table 2). Based on survival analysis from Yusuf et al. (2010) it took 94 d after calving for 50% of normal fertility cows to become pregnant, compared to 155 d for repeat breeders. For repeat breeders, 31.4, 50.0, and 58.1% became pregnant within 210, 300, and 435 d after calving, respectively (Yusuf et al., 2010).

Cows that were AI with single and double doses had a Persistent Corpus Luteum (PCL) incidence of 4.35%, and 8.34% (Table 3) of cattle were categorized as repeated breeders. PCL is a condition in which the corpus luteum does not regress and remains in the ovary for a long time. The size of the corpus luteum is large and has a hard texture (Struve et al, 2013). Cows in AI with a single dose and double dose with ovarian hypofunction were 0% and 4.16% (Table 3). Ovaries surface was smooth, and there was no protrusion of follicles or corpus luteum. The supporting diagnosis for ovarian hypofunction is ultrasound

(ultrasonography) to see the presence of subordinate follicles with a size of <5 mm (Hermadi et al., 2017). While the case of cystic follicles is absent of Follicular cysts, the diameter of the ovary usually reaches 2.5 in large numbers, and the surface is smooth, the walls are thin, and if pressed, there is a feeling of fluid (Bearden et al, 2004).

CONCLUSION

In conclusion, there was no difference in pregnancy success in artificial insemination using single and double doses. But, the result of non-return rate on inseminated two times at 2 hours and 8 hours after estrous onset was better.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

NOVELTY STATEMENT

A traditional protocol for noticing dairy cows in heat is by direct observation, which is a time consumable and not effective. Since there is seldom using double doses in the Friesian Holstein dairy cows. The double doses of Artificial Insemination were conducted accordingly. These method was increasing the change of pregnancy especially in the tropical condition like Indonesia.

AUTHORS CONTRIBUTION

Trinil Susilawati contributed to the concept and design of the research, supervision of the research, Aulia Puspita Anugra Yekti contributed to the concept and design of the research, writing original manuscript, project administration, data curation, and software, Amir Firdaus contributed to the collecting data, Dinda Ayu Damayanti the collecting data, Rizki Prafitri contributed critical of the manuscript, Nanang Febrianto contributed critical of the manuscript, Kuswati contributed to the supervision and revision of the manuscript, Achadiah Rachmawati contributed to the data validation, Sri Wahjuningsih contributed to the supervised of the manuscript, Nurul Isnaini contributed to the supervised of the manuscript. All authors read and approved the final version of the manuscript in the present journal.

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