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The Increase of Madura Cows Reproduction Performance with Double-Dose Method of Artificial Insemination

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Abstract: One of the potencies of Madura cow genetics is its adaptation ability to the tropical climate. Yet in most cases, the reproduction efficiency of the Madura cows has not reached the optimum point. This research examines the use of double-dose artificial insemination compared to a single-dose on Madura cows. The study was conducted in Daramista, Lenteng District, Sumenep Regency, Madura, East Java. This research utilized an experimental field method with purposive sampling on 100 Madura cows, with a body condition score of 3-4, aged about 2-6 years old. The treatments were divided into two groups, T0 and T1. T0 cows received single-dose artificial insemination treatment on the 8th h after estrus and T1 cows received double-dose artificial insemination treatment on the 2nd and 8th h after estrus. The Singosari National Artificial Insemination Centre produced Madura bull frozen semen straws used in this research. Before inseminating, frozen semen straws were thawed at 28°C for 30 sec. This research observed some variables, including; Non-Return Rate (NRR1, NRR2, NRR3), Service per Conception (S/C), Conception Rate (CR), and estrus quality condition as supporting data. The researchers analyzed the data descriptively and through chi-square analysis. The result of the study showed that NRR1, NRR2, and NRR3 of T0 were 94, 84, and 70%, while NRR1, NRR2, and NRR3 T1 were 92, 86, and 82%. The CR of T0 was 64% compared to the CR of T1 at 76%. The double dose insemination method at different times on the 2nd and 8th h after estrus can increase the reproduction performance in Madura cows.

Keywords: Madura Cow, Double-Dose, Estrus, Different Time, Conception Rate

Introduction

Madura cattle breed is one of Indonesian germplasm with superior characteristics. The breed can adopt tropical weather in Indonesia, low quality of feed, good quality of the skin, and resistance to ectoparasite and endoparasite disease. Moreover, the Madura cattle breed is vital in local culture in Madura as Karapan cattle and Sonok cattle exhibition. Therefore, the conservation and development of this breed are important (Nurgiartiningsih *et al*., 2016). Artificial Insemination (AI) is utilized as a reproduction technology to increase the genetic quality of cattle and has been widely implemented in Indonesia. In general, AI implementation aims to improve the genetic quality of the animal, prevent infectious disease prevention, provide more accurate recording and prevent accidents caused by the bull during natural breeding (Ax *et al*., 2000).

Jainudeen and Hafez (2000a) stated that the average estrus cycle of cattle is 21 days (14-29) and the average estrus period is 18 (12-30) h and the average duration between estrus and ovulation is 30 (18-48) h. The estrus cycle, estrus period, and time between estrus and ovulation are important to determine the perfect timing to implement the AI and increase fertilization change. Moreover, the implementation of double-dose AI will increase the conception percentage. Wiranto *et al.* (2020) did a previous study 2020 that proved the implementation of double-dose AI on Limousin cross breed resulted in a high percentage of conception rate up to 74,03%. In addition, Yekti *et al*. (2022) study showed that AI using a double dose method at 2 and 8 h after estrus in Ongole crossbred cattle resulted in a higher conception rate compared to AI at 8 and 16 h after estrus. Therefore, this research aims to examine the double-dose AI implementation success rate on Madura cows, which are

local Indonesian cattle with high adaptability to tropical conditions using frozen semen and deep insemination method (deposition 4+) at the 2nd and 8th h after estrus signs.

Materials and Methods

The study was performed on local farmers in Daramista Village, Lenteng District, Sumenep Regency, and Madura. The study was done from June to November 2021. The recipients were 100 cows' Body Conditioning Score (BCS; 3-4), aged between 2-6 years old (indicated by the permanent teeth), and had given birth to show the cow has normal reproduction organs. The frozen semen used was produced and purchased from Singosari National Artificial Insemination Centre, which fulfills the National standard of Indonesian that has individual motility of 45%.

The research method was a field experiment in which cows were divided into two groups, T0 and T1. The T0 cows received a single dose of AI with a deep insemination method on the 8th h after detecting estrus signs. The T1 cows received a double dose of AI on the 2nd and 8th h after the estrus signs were seen. The farmer observed the estrus sign regularly and then if the cows showed the estrus sign, the farmer would report to the inseminator. Then we will continue to monitor the estrus and interview the farmer when the onset is to determine the time of insemination of the cows. Before the AI implementation, cows were injected with the vitamin of Bio ATP Rheinbio brand (Rheinvet, Germany). The frozen semen straws were thawed with 28°C water for 30 sec, following the inseminator method and the previous research by (Yekti *et al*.,2022) (Chaudhary *et al*., 2021), then implemented with a deep insemination technique (cornua uteri position). After the insemination, the cows were fed an additional 1 kg concentrate feed per day for seven days with protein of 14%.

Estrous Observation

The estrus was observed visually based on the condition of cervix mucus, vulva color, and vulva swelling. The vulva color was classified as even red, uneven red, and white (Irfan *et al*., 2017). The temperature of the vulva was measured by using a digital thermometer Thermo One (Onemed, Indonesia), while the pH with a pH indicator strip was universal (Merck, Germany).

Non-Return Rate

The NRR1 was done on the 19th to 21st days after insemination to observe other estrus signs of the cows. If the cows did not show estrus signs, it was considered that the cow was pregnant. However, suppose the cow shows estrus signs on the 19th to 21st days after the insemination. In that case, it indicates that the cow is not pregnant and the inseminator continued with the 2nd attempt the insemination.

The NRR2 was observed on the 38th to 42nd days of AI. Cows with no estrus signs on NRR2 indicated the success of the AI. However, cows that showed estrus signs on NNR2 did not receive more AI. Pregnancy checks were done in the 2nd month after the AI implementation (Bekele *et al*., 2016). A total number of cows inseminated showed estrus signs, divided into AI done times 100% (Jainudeen and Hafez, 2000b). Furthermore, the NRR3 was observed on AI's 57th to 59th days.

First Conception Rate (CR)

The first Conception Rate (CR) is the percentage of pregnant cows on the first AI. The CR was calculated based on the rectal palpation evaluation after two months of AI (Jainudeen and Hafez, 2000a).

Data Analysis

The data obtained were then tabulated and analyzed descriptively. Furthermore, analyzed with statistical analysis using the Chi-Square test (X2), which is a test that compares the observation value and the expected value (Sudarwati *et al*., 2019).

Results

Physiological Condition of Cows

The primary evaluation of physiological cows' condition was seen from their body weight, BCS, age, and parity. The values of body weight, BCS, and age of the cows used as acceptors are reported in Table 1.

The physiological conditions of the female cows used in the study of both T0 and T1 showed uniform results and were not significantly different based on body weight, BCS, age, and parity. The results showed that the age of the female cattle used at T0 was 3.74±1.99 years on average, while at T1 was 3.94±2.12 years. Body weight and BCS conditions also showed the same thing, namely 245.02±51.84 kg on T0 and 253.54±53.85 kg for T1, while the Average BCS of T0 was 3.56±0.74 and 3.72±0.82 for T1. Parent parity at T0 and T1 was also not significantly different, 1.80±2.01 and 1.96±2.11 for T1.

The Vaginal Temperature and pH

The estrus condition, including vagina temperature and pH, were measured before the insemination. The results of the vaginal temperature and pH are described in Table 2.

Based on the results, the temperature values in treatment T0 and T1 at first AI and second AI were

38.07±0.57, 38.35±0.67, and 37.23±0.50, respectively, indicating an increase in average body temperature in cows. The temperatures in T0 and T1 in the first AI were not significantly different (P>0.05), while the temperatures of T0 and T1 in the second AI were significantly different (P<0.01).

Non-Return Rate

The non-return rate is the percentage of acceptors that did not show estrus signs after AI implementation. No estrus signs indicate the success of AI and the cattle is pregnant Jainudeen and Hafez (2000b). The value of the non-return rate can be seen in Table 3.

Based on observations, it is shown that the percentages of NRR-1, NRR-2, and NRR-3 are good. NRR-1 of T0 and T1 were 94 and 92%, NRR-2 were 84 and 86% and NRR-3 were 70 and 82%, respectively.

The First Conception Rate

The first conception rate shows the number of pregnant cows after the first insemination. The value of the conception rate can be seen in Table 4.

The first conception rate showed that T1 had higher results compared to T0. The conception rate of T1 and T0 was 76 and 64%, respectively. At the same time, the conception rate was 1,43 for T1 and 1,66 for T0.

Table 1: Values of body weight, BCS, Age, and parity of cows

Treatments	Body weight (Kg)	BCS	Age (year)	Parity
T0	245,02±51,84	3,56±0,74	3,74±1,99	1,80±2,01
T1	253,54±53,85	3,72±0,82	3,94±2,11	1,96±2,11

Table 2: The estrus condition before AI of Madura cattle

Treatments	Vagina temperature (°C)	pH
T0 (first AI at 8 h after estrus)	38,07±0,57	7,68±0,51
T1 (first AI at 2 h after estrus	38,35±0,67	7,82±0,48
T1 (second AI at 8 h after estrus)	37,23±0,50	7,60±0,53

Table 3: The non-return rate of Madura cattle

Treatments	NRR-1 head (%)	NRR-2 head (%)	NRR-3 head (%)
T0	47 (94)	42 (84)	35 (70)
T1	46 (92)	43 (86)	41 (82)

Table 4: The first conception rate and service per conception of Madura cattle

Treatments	Conception rate head (%)	Service per Conception (S/C)
T0	32 (64)	1,66
T1	38 (76)	1,43

Discussion

Physiological Condition of Cows

The study's result indicates no significant difference in the cattle condition between T0 and T1 (P>0,05). In line with Nurgiartiningsih *et al*. (2016), research showed that the average weight of the Madura cow was 200-300 kg. Cow with BCS more than 3 indicated better AI results than those with BCS less than 3. The research selection is suitable and indicates good physiology with good raising management based on the need of Madura cattle.

The Condition of Estrus before AI

According to the analysis results, the vaginal temperature will rise above the normal temperature while the animal is in estrus. The vulva color of samples during estrus before AI showed that 70% of T0 were light, compared to T1, with 90% having the stronger color of red. While the vulva's color is still evenly dispersed, the animal may be still in heat; nevertheless, if the vulva's color has changed to pale white, it's possible that the animal is either not in estrus or has passed it. Moreover, 40% of T0 and 50% for T1 indicate much cervical mucus during estrus. Cows do not always show clear signs of vulva color and serve mucus to indicate the estrus. High levels of estrogen during estrus cause the blood vessel network to multiply by developing the reproductive tract. At the same time, estrogen increases blood flow to the reproductive tract, resulting in a high vulvar temperature (Randi *et al*., 2018). Bernardi *et al*. (2016) stated that service mucus and other estrus signs, including red color vulva caused by the high level of estrogen hormone during estrus, cause the blood flow to increase in the reproductive organs. The pH value showed no significant results (P>0,05) between T0 and T1. pH is related to the quality of spermatozoa during travel through the female reproductive tract and it is an important indicator. If the pH of the spermatozoa is not appropriate, the spermatozoa will die and be unable to fertilize, resulting in repeated breeding and economic losses for farmers.

Non-Return Rate

The results of the analysis showed that the percentages of NRR-1 of T0 and T1 were not significantly different (P>0.05), NRR-2 of T0 and T1 were significantly different (P<0.05), and NRR-3 of T0 and T1 was significantly different (P<0.01). Two factors caused the decreased number of NRR. First, because farmers failed to observe the estrus signs, failure fertilization. Second, it is caused by premature embryo death. Balhara *et al*. (2013) argued that Non-Return Rate is one criterion known to indicate animal pregnancy. However, the weakness of this method is the possibility of failure of estrus signs observation. The NRR was also influenced by the animal's

condition, indicated by the Body Condition Score (BCS). Therefore, inseminators and farmers' role in observing the estrus signs is essential.

The First Conception Rate

Based on the research data, the conception rate of the sample that received double-dose AI had a higher percentage, up to 10%, compared to single-dose AI. Previous research done by Ervandi *et al*. (2019) proved that the conception rate of Brahman Crossbreed lower on single-dose AI done on the 2nd h was 25% while AI done on the 8th h was 20%. Therefore, this research confirmed that the Madura cattle breed has higher fertility than Brahman Crossbreed. In line with this, the Madura cattle breed has a better adaptation to the tropical condition than Brahman Crossbreed in Indonesia. The double dose method can increase the concentration of spermatozoa in the female reproductive organs so that with a higher number, the chances of the spermatozoa fertilizing the egg are greater. The higher the number of spermatozoa, the higher the competition for spermatozoa to fertilize the ovum (Parker, 2020).

This study showed that 15 of the T0 sample failed to be pregnant. The case of early death embryos were six cattle (40%), ovary hypofunction in seven cattle (47%), and Corpus Luteum Persistence 2 cattle (13%). Moreover, on T1, Pregnancy failure was in ten cattle, ovary hypofunction in five cattle (50,00%), and corpus luteum persistence in five cattle (50,00%). The result of the research, in line with Ervandi *et al*. (2020), is that the decreased number of NRR in the Conception and Pregnancy Rate in cattle was caused by silent heat, premature embryo, ovary hypofunction, and corpus luteum persistence. Furthermore, the value of Services per Conception (S/C) did not significantly differ ($P>0.05$) between T0 and T1, while T1 had a lower S/C than T0. T0 and T1 had S/C values of 1,66 and 1,43, respectively. The S/C results revealed that the cows have a high rate of reproduction efficiency. The S/C value shows the efficiency of cow mating management (Wicaksono *et al*., 2018). This result suggested that Madura cows have a high reproduction efficiency.

Conclusion

In conclusion, on Madura cattle, the double dose method of artificial insemination can increase the non-return rate and conception rate. Furthermore, pregnancy failure is mainly caused by early death embryo, ovary hypofunction, and corpus luteum persistence.

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Author's Contributions

Kuswati, Trinil Susilawati and Aulia Puspita Anugra Yekti: Designed and conducted the research and write the manuscript.

Wike Andre Septian and Khairul Rasyad: Conducted the research and data analysis.

Rizki Prafitri and Asri Nurul Huda: Data analysis and write the manuscript.

Ethics

The corresponding author confirms that this article is original and no ethical issues are involved.

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