Successful Pregnancy Results from Double Dosage Artificial Insemination in Different Estrous Characteristics in Madura Cattle

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Abstract. This study aims to determine the relationship between oestrus character and the success of double dosage AI at the 2 and 8 h AI intervals in Madura cattle. The research was conducted in Lenteng, Sumenep Regency. The material used was 45 cows Madura cattle which were selected by purposive sampling. The semen used was frozen semen from male Madura cattle produced by the Singosari Center for Artificial Insemination (SCAI), Malang. The method used in this study was observational; namely, the primary data collection was done by direct observation, including oestrus, signs of heat, feed, and direct interviews with farmers. At the same time, secondary data on livestock reproduction was obtained from data recording by inseminator officers. The results showed that the best percentage of red vulva color was NRR-1 93.33%, NRR-2 88.89%, CR 77.78%, and PR 82.22%. The percentage of vaginal temperature > 38 ℃ is the value of NRR-1 and NRR-2 68.89%, CR 64.44%, and PR 68.89%. The pH indicator value of 8 showed the percentage of NRR-1 75.56%, NRR-2 71.00%, CR 62.00%, and PR 66.67%. There are many conditions of cervical mucus, namely NRR-1 48.89%, NRR-2 44,44%, CR 46.67%, and PR 48.89%. The categories of vulvar swelling were very swollen NRR-1 62.22%, NRR-2 60.00%, CR 55.56%, and PR 60.00%. Vaginal smear values for the oestrus category were NRR-1 84.00%, NRR-2 82.22%, CR 68.00%, and PR 70.00%. In conclusion, A high percentage of pregnancy was indicated in oestrus conditions with an evenly red vulva color, temperature > 38 ℃, a pH value of 8, and there was much mucus in the condition, the vulva had a very swollen swelling value, and a vaginal smear value in the oestrus category.

Keywords: Artificial Insemination · Double Dosage · Oestrus Quality

1 Introduction

The increase in population and income influenced the growth of public demand for livestock products, especially meat. The growing public awareness of the importance of

consuming nutritious feed also increased the demand for meat. The artificial insemination program is a powerful way to increase livestock populations and production [1]. Madura Island is an area with a significant contribution (about 21%) to the beef cattle population in East Java [2]. Based on statistical data from the Livestock Service Office of East Java Province 2020, the population of beef cattle in East Java increased by 3.123%, from 4,511,613 in 2017 to 4,823,970 in 2020. The population of beef cattle in 2020 in Sumenep Regency was 377,124 cows, Pamekasan Regency as many as 194,286 cows, Sampang Regency as many as 216,450 cows, and Bangkalan Regency as many as 259,92 cows [3].

AI variables that can be used as benchmarks to evaluate the reproductive efficiency of female beef cattle are Non-Return Rate 1 and Non-Return Rate 2, Conception Rate (CR), and Pregnancy Rate (PR). All of these variables are an evaluation of the role of AI technology which is known to affect the character of estrus, and the success of increasing the beef cattle population, which will be able to increase meat production. Based on the statement [4] that the factors that affect the success of CR (Conception Rate) are the CR value is determined by male fertility, female fertility, and insemination techniques, besides that semen deposition and timing of AI are one of the factors that affect the success or failure of AI. Semen deposition at position 4 coincided at (corpus uteri) and position 4 + (cornua uteri). Semen deposition carried out in the study at position 4 + was better than position 4 [5]. Factors that influence the success of AI include: (1) the quality of the cement used; (2) oestrus detection; (3) BCS (Body Condition Score); (4) The level of inseminator ability and body weight. [6] stated that the S/C of Madura cattle is 1.46 while the S/C of AI of Madura cattle is 1.56. Service per Conception of Madura cattle with AI, natural and mixed mating (AI and natural mating) were 1.6 ± 0.3, 1.6 ± 0.0, and 3.3 ± 0.6, respectively. High and low CR is influenced by livestock conditions, oestrus detection, oestrus detection, and reproductive management, affecting livestock fertility and conception value [4]. This study aims to increase AI's.

2 Materials and Methods

2.1 Materials

The material used in this study was 45 Madura cattle which were taken by purposive sampling with the criteria of adult female cows having a BCS of at least 3, with an age of more than 1.5 years identified by dental poel, and having a health condition based on the working area of the insemination in Lenteng District, Sumenep Regency, East Java Province. The semen used in this study was frozen semen of Madura cattle produced by the Singosari Center for Artificial Insemination (SCAI), Malang.

2.2 Methods

The method used in this study used the observational method with primary data retrieval carried out by direct observation, which included oestrus, signs of oestrus, and feed as well as direct interviews with farmers. In contrast, secondary data on livestock reproduction were obtained from data recording by inseminator officers, Lenteng District, Sumenep.

The research sample was taken by purposive sampling, namely the selection of subjects based on at least one farmer who had Madura cattle that had used Artificial Insemination by selecting female cattle according to the criteria and having good oestrus quality. The thawing method was carried out in the field using tap water for 30 s. AI is carried out in double dosage at the 2nd and 8nd hour of oestrus. At the 2nd hour, AI is carried out 2 h after the inseminator gets a call from the farmer (if you get a call in the morning, then in the afternoon, AI is done), while at 8 h is 6 h after the inseminator gets the initial call.

3   Results and Discussion

3.1   Vulvar Color Characteristics Against Non-return Rate-1, Non-return Rate-2, Conception Rate, and Pregnancy Rate AI Double Dosage

In this study, the NRR was observed on days 19–21 and 38–40 after AI was performed, then rectal palpation was performed on the 60th day after AI. Evaluation of the results of NRR Madura cattle is shown in Table 1.

In Table 1, dosage 1 showed that Madura cattle with evenly red vulva conditions experienced re-estimation as much as two heads and made NRR-1 and NRR-2 high, namely 93.33% and 88.89%. In contrast, at dosage two, it showed Madura cattle with vulvar conditions. 2 cows were evenly red in heat and made NRR-1 and NRR-2 51.11% and 46.67%, respectively. The condition of the uneven red vulva has a percentage at dosage 1 of 6.67% and 6.67%, while at two were 40.00% and 40.00%. This is for the NRR value in the excellent category because the assessment of the color of the red vulva is evenly distributed, and the vulva is uneven if the NRR-1 and NRR-2 > 50% are added up, respectively [7]. The increase of NRR-1 and NRR-2 values at both dosages was due to differences in acceptor numbers in each vulva color condition. The condition of the vulva with a uniform red color indicates that the cattle are experiencing peak oestrus, which is influenced by the hormone estrogen, which causes swelling of the peripheral blood vessels in the vagina. The uneven red vulva indicates that the cattle are in the oestrus to metestrus phase.

Table 1. Percentage of vulvar color characteristics before AI against non-return rate at Madura cattle AI double dosage

Category Vulva Color	Number of Samples		NRR-1		NRR-2		Number of Samples		NRR-1		NRR-2	
			2nd Hour Dosage						8nd Hour Dosage			
	head	%	head	%	head	%	head	%	head	%	head	%
Even Red	42	42	42	93,33	40	88,89	25	25	23	51,11	21	46,67
Uneven Red	3	3	3	6,67	3	6,67	20	20	18	40,00	18	40,00
Total	45	45										

Table 2. Percentage of vulvar color characteristics before AI against conception rate and Pregnancy rate in double dosage AI Madura cattle

Category Vulva Color	2nd Hour Dosage						8nd Hour Dosage					
	Number of Samples		CR		PR		Number of Samples		CR		PR	
	head	%	head	%	head	%	head	%	head	%	head	%
Even Red	42	42	35	77,78	37	82,22	25	25	17	37,78	19	42,22
Uneven Red	3	3	3	6,67	3	6,67	20	20	19	42,22	20	44,44
Total	45	45										

Table 2 showed at dosage one that the lowest CR value in the red vulva was uneven at 6.67%, while the highest occurred in the uniform red vulva at 77.78%. The lowest PR value in the uneven red color of 6.67%, and the highest in the evenly red vulva color is 74.00%. At dosage 2, it was found that the lowest CR value in the red vulva was evenly distributed by 37.78%, while the highest was in the condition of the uneven red vulva color at 42.22%. The lowest PR value in the uniform red vulva color of 42.22%, while the highest occurred in the uneven vulvar color condition at 44.44%. CR and PR values have good values at dosage one and dosage 2, this follows [8] added that a good CR value based on conditions in Indonesia is 60–70% and based on the consideration of natural conditions with management and distribution of livestock spread is considered good if the CR value reaches 40–50%. Based on [9] stated that the low value of CR and PR was caused by many things that could affect the female cow before AI or after AI. in the study, there were five cows with ovarian hypofunction and persistent corpus luteum (CLP) which were detected during rectal palpation. Ovarian hypofunction is the leading cause of reproductive failure in beef cattle, especially in traditional rearing systems. Postpartum ovarian hypofunction in cows is caused by a hormonal imbalance characterized by low levels of gonadotropin hormones, especially FSH.

3.2 Effect of Vaginal Temperature on Non-return Rate-1, Non-return Rate-2, Conception Rate, and Pregnancy Rate AI Double Dosage

According to [10] showed the highest temperature compared to the proestrus, metoestrus, and dioestrus phases, which was between 37.4–38.4 °C. The results of vaginal temperature on NRR are shown in Table 3.

Table 3 showed a difference in the percentage results between the two temperature indicators at dosages 1 and 2. A comparison of the percentages between the vaginal temperatures results in almost the same high percentage of vaginal temperature. This was reinforced by [7] stated that the NRR value > 50% is still in the excellent category at a dosage of 1% at a vaginal temperature of 37.0–37.9 ºC, the NRR-1 value of 22.22%, and the NRR-2 20.00% were equally low. While the percentage of NRR in livestock that has a temperature > 38.0 ºC has a value of NRR-1 and NRR-2 which is 68.89%, higher

Table 3. Percentage of vaginal temperature before AI against non-return rate in double dosage AI Madura cattle

Category Vaginal Temperature			2nd Hour Dosage						8nd Hour Dosage					
	Number of Samples		NRR-1		NRR-2		Number of Samples		NRR-1		NRR-2			
	head	%	head	%	head	%	head	%	head	%	head	%		
37,0–37,9	10	10	10	22,22	9	20,00	19	19	17	37,78	15	33,33		
≥ 38	35	35	31	68,89	31	68,89	26	26	24	53,33	23	51,11		
Total	45	45												

Table 4. Percentage of vaginal temperature before AI on conception rate and pregnancy rate in double dosage AI Madura cattle

Category Vaginal Temperature			2nd Hour Dosage						8nd Hour Dosage					
	Number of Samples		CR		PR		Number of Samples		CR		PR			
	head	%	head	%	head	%	head	%	head	%	head	%		
37,0–37,9	10	10	9	20,00	9	20,00	24	24	17	37,78	18	40,00		
≥ 38	35	35	29	64,44	31	68,89	21	21	20	44,44	21	46,67		
Total	45	45												

than the temperature indicator 37.0–37 ℃, while at a dosage of 2 the percentage of vaginal temperature is 37.0- 37.9 ℃ has a value of NRR-1 37.78% and NRR-2 33.33% lower than the percentage of NRR in livestock that has a temperature > 38.0 ℃ with a value of NRR-1 53.33% and NRR-2 51, 11%, higher than the temperature indicator 37.0–37 ℃. The temperature indicator > 38.0 ℃ has a good percentage due to the detection of oestrus, which is carried out right in the oestrus phase. The oestrus phase has a basal level of progesterone and estrogen reaching a peak, giving signs of heat with the characteristics of a swollen, red, and warm vulva.

Table 4 showed that dosage 1 at a vaginal temperature of 37.0–37.9 ℃ had a CR value of 20.00% and a PR of 20.00%, while at a temperature > 38℃ it had a CR value of 64.44% and a PR value of 68.89%. Dosage 2 at a vaginal temperature of 37.0–37.9 ℃ had a CR value of 37.78% and a PR of 40.00%, while at a temperature > 38℃ it had a CR value of 44.44% and a PR value of 46.67%. The CR value of this study was higher by obtaining a percentage at a dosage of 1 84,44% and a dosage of 2 82,22% than the study [11], which showed the best average CR value was obtained in thick mucus conditions, namely CR 68.00% in composite cows.

Table 5. Percentage of pH value before AI against non-return rate in AI Madura cattle double dosage

			2nd Hour Dosage						8nd Hour Dosage			
Category Cervical Mucus pH	Number of Samples		NRR-1		NRR-2		Number of Samples		NRR-1		NRR-2	
	head	%	head	%	head	%	head	%	head	%	head	%
7	11	11	11	24,44	11	24,44	16	16	13	28,89	15	33,33
8	34	34	34	75,56	32	71,11	29	29	28	62,22	26	57,78
Total	45	45										

3.3 Effect of Cervical Mucus pH Value on Non-return Rate-1, Non-return Rate-2, Conception Rate, and Pregnancy Rate AI Double Dosage

The higher the estrogen hormone in the blood during oestrus, the volume of cervical mucus will be more and more with an increasingly alkaline pH so that it can be used as a reference for the success of Artificial Insemination [12]. The results of the Non-Return Rate from the pH value of cervical mucus of Madura cattle which were in double dosage AI, can be seen in Table 5.

Dosage 1 showed that cervical mucus with a pH value of 7 has a percentage of 24.44% in NRR-1 and NRR-2. Meanwhile, cervical mucus with a pH value of 8 has a percentage of NRR-1 of 75.56% and NRR-2 of 71.11%. Dosage 2 showed that cervical mucus with a pH value of 7 had a percentage of 28.89% at NRR-1 and 33.33% at NRR-2. While at a pH value of 8, it had a percentage of NRR-1 of 62.22% and NRR-2 of 57.78%. This showed that cervical mucus with a pH value of 8, as seen from a better NRR at dosage one, while a pH value of 8, seen from a better NRR, indicates a high chance of pregnancy. This is supported by the research [10] who explained that the higher pH of eight percent was caused by the discharge of a lot of thick and thick mucus, causing the condition of the vulva to become alkaline. Besed on [12] also mentioned that high levels of the hormone estrogen in the blood during oestrus will affect the condition of cervical mucus to an alkaline pH, which can be used as a reference to assess the success rate of AI.

Table 6, dosage 1 explained that the cervical mucus at pH 7 has the same CR and PR values, 22.22%. While the cervical mucus pH 8 has a CR value of 62.22% and a PR value of 66.67%. Dosage 2 showed that cervical mucus at pH 7 had a CR value of 33.33% and PR 37.78%, while at pH eight, cervical mucus had a CR value of 51.11% and PR 53.33%. The results supported the highest pregnancy percentage in this study, Dosage 1 at pH 8, which stated that in the pH range of 7.8–8.0, the pregnancy rate was relatively high at 75%. Based on [13] stated that the difference in mucus pH is influenced by the biophysical and biochemical conditions of the mucus produced by the cervix, which is controlled by hormones that play a role in the oestrus cycle. There may be factors that influence the difference and influence of pH on pregnancy.

Table 6. Percentage of cervical mucus pH value before AI against conception rate and pregnancy rate in double dosage AI Madura cattle

Category Cervical Mucus pH	Number of Samples		CR		PR		Number of Samples		CR		PR	
	2nd Hour Dosage						8nd Hour Dosage					
	head	%	head	%	head	%	head	%	head	%	head	%
7	11	11	10	22,22	10	22,22	21	21	15	33,33	17	37,78
8	34	34	28	62,22	30	66,67	24	24	23	51,11	24	53,33
Total	45	45										

Table 7. Percentage of cervical mucus condition before AI against non-return rate in double dosage AI Madura cattle

Category Cervical Mucus	Number of Samples		NRR-1		NRR-2		Number of Samples		NRR-1		NRR-2	
	2nd Hour Dosage						8nd Hour Dosage					
	head	%	head	%	head	%	head	%	head	%	head	%
There are many	22	22	22	48,89	20	44,44	9	9	8	17,78	7	15,56
There is a Little	10	10	8	17,78	7	15,56	12	12	12	26,67	13	28,89
There is not any	13	13	12	26,67	12	26,67	24	24	24	53,33	23	51,11
Total	45	45										

3.4 Characteristics of Cervical Mucus Against Non-return Rate-1, Non-return Rate-2, Conception Rate, and Pregnancy Rate AI Double Dosage

When Madura cattle are in normal condition, in the oestrus cycle, they will secrete mucus. However, cattle may secrete mucus other than in a state of heat because the amount and consistency of mucus will change depending on the phase of the oestrus cycle and depending on variations in hormonal levels [11]. The following results of observing the characteristics of cervical mucus before double dosage AI are listed in Table 7.

In Table 7, dosage 1 showed that in cows that are in heat by excreting mucus (there is a lot) the NRR-1 is 48.89% higher, and NRR-2 is 17.78%, while in the condition of cows in oestrus by secreting mucus (there is a little) has an NRR-1 value of 17.78% and an NRR-2 of 15.56%. In the condition of cows in heat with excreting mucus (none) has a value of NRR-1 and NRR-2 of 26.67%. At dosage 2, it showed that cows with excreting mucus (there was a lot) showed a lower NRR-1 of 17.78% and NRR-2 of

Table 8. Percentage of cervical mucus condition before AI against conception rate and pregnancy rate in double dosage AI Madura cattle

Category Cervical Mucus	Number of Samples		2nd Hour Dosage CR		PR		Number of Samples		8nd Hour Dosage CR		PR	
	head	%	head	%	head	%	head	%	head	%	head	%
There are many	22	22	21	46,67	22	48,89	9	9	8	17,78	8	17,78
There's a Little	10	10	7	15,56	8	17,78	15	15	13	28,89	14	31,11
There isn't any	13	13	9	20,00	9	20,00	21	21	18	40,00	19	42,22
Total	45	45										

15.56%, while in the condition of cows in oestrus by excreting mucus (there was little) the NRR-value was lower. 1 26.67% and NRR-2 of 28.89%. In the condition of cows in the heat with excreting mucus (none) has a value of NRR-1 53.33% and NRR-2 51.11%. The reoccurrence of oestrus indicates that the AI did not result in fertilization between the spermatozoa and the ovum, so the next cycle will show signs of oestrus again, such as secreting mucus. The decrease in the percentage of NRR-1 to NRR-2 was due to the timing of the AI implementation carried out by the inseminator on cows that were in the condition of cervical mucus (there, wet, a little) before the AI had not entered the peak oestrus condition so that the estrogen hormone in the cattle's body was not produced maximally [10].

In Table 8, dosage 1 showed that the mucus condition (there are many) has a CR value of 46.67% and a PR of 48.89%. The mucus condition (a little) had a CR value of 15.56% and a PR of 17.78%, while the mucus condition (none) had a CR value of 20.00% and a PR of 20.00%. Dosage 2 showed that the mucus condition (there are many) has a CR and PR value of 17.78%. The mucus condition (there are few) has a CR value of 28.89 and a PR of 31.11%. The mucus condition (none) had a CR value of 40.00% and a PR of 42.22%. The high percentage of success in the condition that there is much mucus compared to there is little mucus because there is much mucus that will facilitate the transportation of spermatozoa to the ovum for fertilization. Much mucus is produced during peak oestrus conditions, so the low percentage of pregnancy in low mucus conditions is due to hormonal conditions that have not been maximized. The condition of mucus coupled with double dosage AI at the 2nd hour AI and 8th hour AI makes the PR pregnancy rate high [10].

Table 9. Percentage of vulvar swelling before AI against non-return rate in double dosage AI Madura cattle

Category Vulvar Swelling	2nd Hour Dosage						8nd Hour Dosage					
	Number of Samples		NRR-1		NRR-2		Number of Samples		NRR-1		NRR-2	
	head	%	head	%	head	%	head	%	head	%	head	%
Very Swollen	28	28	28	62,22	27	60,00	17	17	16	35,56	14	31,11
Slightly Swollen	17	17	16	35,56	16	35,56	28	28	28	62,22	29	64,44
Total	45	45										

3.5 Effect of Vulvar Swelling on Non Return Rate-1, Non Return Rate-2, Conception Rate and Pregnancy Rate AI Double Dosage

Signs of oestrus in cows are characterized by restlessness, swelling and redness of the vulva, decreased milk production, discharge, or clear translucent mucus from the vulva [14] The effect of vulvar swelling on NRR in this study is shown in Table 9.

Dosage 1 showed that the vulvar swelling in the very swollen category has a percentage of 62.22% in NRR-1 and in NRR-2 it has a value of 60.00%. Meanwhile, in the slightly swollen vulva, the percentage of NRR-1 and NRR-2 was 35.56%. Dosage 2 showed that the vulvar swelling in the very swollen category had a percentage of 35.56% on NRR-1 and on NRR-2 it had a value of 31.11%. Meanwhile, in the slightly swollen vulva, the percentage of NRR-1 was 62.22% and NRR-2 was 64.44%. This is supported by statements [15] states that increased levels of estrogen are associated with reddening and swelling of the vulva during heat which stimulates blood flow to the reproductive tract and related genital organs, increased mucus secretion is initiated by thickening of the vaginal wall and increased vascularity by stimulation of the hormone estrogen. This condition causes changes in the condition of the vulva to swell and become red. The presence of the male influences the parent's sexual behavior as a stimulator. A parent who does not show signs of oestrus and then reared together with males will show these signs.

In Table 10, dosage 1 showed that the vulva with very swollen conditions has a CR value of 55.56% and PR 60.00%. Meanwhile, the slightly swollen vulva has a CR value of 28.89% and PR 28.89%. Dosage 2 showed that the vulva with very swollen conditions had a CR value of 31.11% and PR 31.11%. The slightly swollen vulva had a CR value of 55.56% and PR 60.00%. The highest percentage of pregnancy in this study was in the vulva which was very swollen at dosage 1, this is also supported [16] that high estrogen causes the increased blood circulation in the female reproductive organs, making the vaginal appearance swell which causes changes in vulvar fissure during oestrus cattle [15] that increased mucus secretion was initiated by thickening of the vaginal wall and increased vascularization by stimulation of the hormone estrogen. This condition causes changes in the condition of the vulva to swell and become red and hormone production

Table 10. Percentage value of vulvar swelling before AI against conception rate and pregnancy rate in double dosage AI Madura cattle

Category Vulvar Swelling	Number of Samples		2nd Hour Dosage				Number of Samples		8nd Hour Dosage			
			CR		PR				CR		PR	
	head	%	head	%	head	%	head	%	head	%	head	%
Very Swollen	28	28	25	55,56	27	60,00	17	17	14	31,11	14	31,11
Slightly Swollen	17	17	13	28,89	13	28,89	28	28	25	55,56	27	60,00
Total	45	45										

Table 11. Criteria for determining the Oestrus cycle based on the picture of changes in cell shape

Number	Oestrus Phase	Epithelial Cell	Cell Shape
1.	Diestrus	Parabasal Cell	Small, round cells with large nuclei [17].
2.	Diestrus-Proestrus	Intermediate Cell	Round cells, larger than parabasal cells with smaller nuclei [17].
3.	Proestrus-Oestrus	Intermediate/superficial cells	Large cells form a tiny polygamous nucleus or without a nucleus [17].
4.	Oestrus	Superficial/cornified cells	Large cells form a tiny polygamous nucleus or without a nucleus and keratinization occurs [17].
5.	Metestrus	Parabasal Cell	Has a large nucleus and round cytoplasm [18].

affects the body's physiology of livestock, in oestrus broodstock, among others, affects the smell and the external appearance of the body changes. Reproductive hormones influence the intensity of oestrus, so indirectly, the oestrus intensity rate (AAI) is also strongly influenced by the nutritional status of the livestock itself (Table 11).

3.6 Effect of Vaginal Smear Value on Non-return Rate-1, Non-return Rate-2, Conception Rate, and Pregnancy Rate AI Double Dosage

Every day, 5–8 vaginal smears were taken. The vaginal epithelium that was taken was dried and dipped in methanol. Then, staining was carried out with Giemsa for 1 h (Najamudin, et al. 2010) [17] (Fig. 1 and Fig. 2).
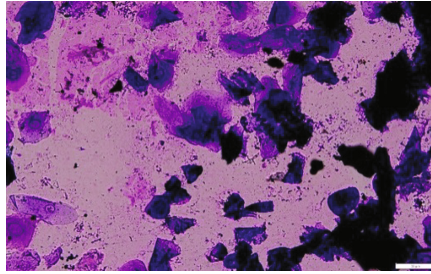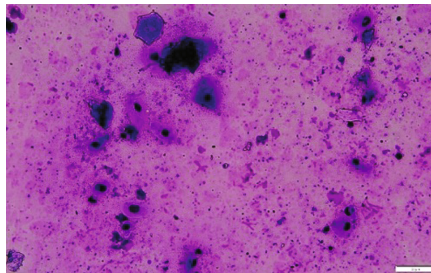
Fig. 1. Anuclear cells (Oestrus)

Fig. 2. Intermediate cells (Metestrus)

Table 12. Percentage of vaginal smaer value before AI against non-return rate in double dosage AI Madura cattle

Value Vaginal Smear	Number of Samples		NRR-1		NRR-2		Number of Samples		NRR-1		NRR-2	
			2nd Hour Dosage						8nd Hour Dosage			
	head	%	head	%	head	%	head	%	head	%	head	%
Oestrus	38	38	38	84,44	37	82,22	30	30	28	62,22	26	57,78
Metestrus	7	7	6	13,33	6	13,33	15	15	15	33,33	14	31,11
Total	45	45										

In Table 12, dosage 1 shows that the oestrus category has an NRR-1 value of 84.44% and an NRR-2 of 82.22%, while in the metestrus category, the NRR-1 and NRR-2 values are 13.33%. Dosage 2 showed that the oestrus category had an NRR-1 value of 62.22% and an NRR-2 value of 57.78%, while the metestrus category had an NRR-1 value of 33.33% and an NRR-2 of 31.11%. This shows that the oestrus category indicates that cattle are in a condition of increasing pregnancy because in the oestrus phase, the hormone estrogen will increase the activity of the uterine wall causing hypersecretion and keratinization of uterine and vaginal epithelial cells so that the cells involved in the review are superficial cells or horn cells. [17] Su and Zn supplementation were able to

Table 13. Percentage of vaginal smear value before AI against conception rate and pregnancy rate in double dosage AI Madura cattle

Value Vaginal Smear	2nd Hour Dosage						8nd Hour Dosage					
	Number of Samples		CR		PR		Number of Samples		CR		PR	
	head	%	head	%	head	%	head	%	head	%	head	%
Oestrus	38	38	34	68	35	70	30	30	26	52	27	54
Metestrus	7	7	5	10	5	10	15	15	13	26	13	26
Total	45	50										

affect the development of granulosa cells. The granulosa cells are one of the cells that produce the hormone estrogen. Estrogen can increase the secretion of cervical mucus, which is one indication of cattle in heat. The cervical mucus that comes out contains protein, NaCl, and mucin. The de Graff follicle enlarges and undergoes maturation so that if fertilization occurs, the animal will become pregnant [19].

Table 13, dosage 1 shows that the oestrus category has a CR value of 68.00% and PR of 70.00%, while the metoestrus category has a CR and PR value of 10.00%. Dosage 2 showed that the oestrus category had a CR value of 52.00% and a PR of 54.00%, while the metestrus category had a CR and PR value of 26.00%. This shows that the oestrus category has a high value in determining pregnancy. Cows in heat will show the quality of oestrus with characteristic features, namely reddened labia minora, swollen vulva, increased body temperature, and slimy (mucus or mucus coming from the cervix is transparent or clear). If the male is climbed, the male will be silent [8]. The quality of oestrus, in this case, is the best time through changes in the vulvar cleft, vaginal temperature, vulvar color appearance, and mucus properties, as well as the dominant proportion of vaginal epithelial cells in determining the accuracy of AI in Madura cattle with large cells with microscopic polygonal nuclei or without nuclei and occurs Austin.

4 Conclusions

In conclusion, AI's success in Madura cattle in double dosage at the 2nd and 8nd hours has a relationship with the character of estrous. The Non-Return Rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR) has a high percentage of pregnancy at dosage 1 is indicated in the condition of oestrus with an evenly red vulva color, temperature > 38 ℃, has a pH value of 8, there is much mucus in the condition, the vulva has a very swollen swelling value and the vaginal smear value is categorized as oestrus. In contrast, at dosage 2, it was shown in oestrus with uneven red vulva color, temperature >38 ℃, pH 8, no mucus condition, slightly swollen vulva, and vaginal smear value with oestrus category

References

1. Kastalani, H. Torang and A. Kurniawan.: Success Rate of Artificial Insemination (AI) in Beef Cattle Farming in Kalampangan Village, Sabangau District, Palangka Raya City. Journal of Tropical Animal Science 8(2), 82–88 (2019).
2. Siswijono, S.B., V.M.A. Nurgiartiningsih and Hermanto.: Development of an institutional model for the conservation of Madura cattle. Journal of Animal Sciences 24(1), 33–38 (2013).
3. Central Bureau of Statistics of the Livestock Service Office of East Java Province, http://disnak.jatimprov.go.id/web/ last accessed 2022/01/30.
4. Siagarini, V.D., N. Isnaini, and S. Wahjuningsih.: Service Per Conception (S/C) and Conception Rate (Cr) of Simmental Cross-breed Cattle at Different Parities in Sanankulon District, Blitar Regency. Research journal 1(1), 1-6 (2015).
5. Wiranto, Kuswati, R.Prafitri, A.N.Huda, A.P.A.Yekti and T.Susilawati.: Reproductive Efficiency of Beef Cattle in Mojokerto Regency. Agripet Journal 20(1), 17–21 (2020).
6. Kutsiyah, F.: Analysis of Beef Cattle Breeding on Madura Island. Wartazoa 22(3), 113-126 (2012).
7. Rosita, E.A,. T. Susilawati and S. Wahyuningsih.: Success of AI Using Frozen Semen from Sexing with Egg White Sedimentation Method in PO Cross Cattle. Journal of Animal Sciences 24(1), 72–76 (2014).
8. Rachmawati, A., Ismaya, B.P. Widyobroto, S. Bintara and T. Susilawati.: Application of Artificial Insemination in Beef Cattle Using Liquid Semen of Ongole Cross-breed Cattle with Cauda Epidymal Plasma-2 Diluent + 0.6% Bovine Serum Albumin. Journal of Animal Sciences 28(3), 247–258 (2018).
9. Yekti, A.P.A., E.A.Octaviani, Kuswati, T. Susilawati.: Increasing the Conception Rate by Artificial Insemination Using Double Doses of Semen Sexing in Ongole Cross Cattle. Journal of Tropical Livestock 20(2), 135–140 (2019).
10. Kusuma, H.R., Kuswati, A.N. Huda, R. Prafitri, A.P.N. Yekti and T. Susilawati.: Pregnancy rate of dairy cows with persistent corpus luteum after administration of a combination of prostaglandin F2α and gonadotropins. Journal of Animal Science 21(2), 94–101 (2021).
11. Irfan, S. Wahjuningsih and T. Susilawati.: The Effect of Characteristics of Cervical Mucus Before Artificial Insemination (AI) on Pregnancy Success in Composite Cattle. J. Tropical Livestock 18(1), 24–28 (2017).
12. Rizki, A., P. Srianto, E. Suprihati, T. Sardjito, Ismudiono and M.A.A. Wise.: The Effect of pH of Vaginal Mucosal Mucous During Esti on Conception Rate in Dairy Cows at KUD Tani Wilis, Tulungagung Regency and KSU Tunas Setia Baru, Pasuruan Regency. Ovozoa 8(2), 154–158 (2019).
13. Prasdani, W. A., S. Rahayu, dan M.S.Djati.: Level of Estrogen and Cervical Mucous pH as Indicator of Oestrus After Calving Towards The Provision of Selenium-vitamin ETM on Diary Cow Frisian Holstein (FH). International Journal of ChemTech Research 7(1), 190–195 (2015).
14. Pemayun, T.G.O. and I.G.N.B.T.M.K.Budiasa.: The Right Time of Artificial Insemination in Bali Cattle and Progesterone Levels in Pregnant Cattle. Veterinary Journal 15(3), 425–430 (2014).
15. Baliarti, E., Panjono, TSM., DEY. Widy, MH. Ali, B.A. Atmoko, H. Maulana, J. Effendhy, P.W. Prihandini and D. Pamungkas: Sexual Behavior of Bulls and Cows of Ongole Breeds with a Limited Colony Cage System. Proceedings of the National Seminar on Animal Husbandry and Veterinary Technology: 65–73. Retrieved January 27, 2022 at 08.27 WAI (2019).
16. Saputra, D., Sumartono and N. Humaidah.: The Relationship of Oestrus Quality Based on Cytological Profiles of Vaginal Swabs and Oestrus Symptoms on the Success of Intracervical AI in Etawa Peranakan Goats. Engineering Dynamics 2(2), 1–9 (2017).

17. Najamudin, Rusdin, Sriyanto, Amrozi, S. Agungpriyono, T. L. Yusuf.: Determination of the Oestrous Cycle in Mouse Deer (Tragulus javanicus) Based on Cytological Changes in the Vagina. Veterinary Journal 11(2), 81–86 (2010).
18. Setiadi, DR, M. Agil, L. Arifiatini, D. Sajuthi, J. Manansang, Y.T. Hastuti, S.R. Liwa.: Cytological Characteristics of the Vagina During the Oestrus Cycle and Clinical Symptoms of Oestrus in Bulls (Bos javanicus d'Alton 1823) 8(3), 40–47 (2020).
19. Muktiani, A. and E. Kusumanti.: Effect of Soybean Meal and Zinc, Selenium and Vitamin E Supplementation on Oestrous Performance in Etawah Peranakan Goats. Dipenogoro University, Semarang (2017).