



Evaluation The Success of Artificial Insemination Using Frozen Sexed Semen Based on Different Estrus Characters

(Evaluasi keberhasilan inseminasi buatan menggunakan semen beku sexing berdasarkan karakter estrus yang berbeda)

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ABSTRACT. Knowledge of farmers about estrus detection is one of the factors that affect fixed-time artificial insemination (AI). This study aims to evaluate the success rate of AI using Y sexing frozen semen based on the estrus character of cattle. Forty-five Limousin Crossed Cows involved in this research with a Body Condition Score of 3-5 (1-9 scale), 1.8-7 years old. Y sexing frozen semen is produced by the Singosari Center for Artificial Insemination using Percoll's Gradient Density Centrifugation method. Observation of estrus character was done before insemination with the deep insemination technique of AI. Artificial insemination was carried out using double doses at the 2nd and 8th hours after estrus. Rheinbio vitamins as BioATP+ are injected after AI. The results showed that the Conception Rate and Pregnancy Rate based on the estrus character was higher as indicated by the red colour of the vulva was 17.78% and 22.22%; very swollen vulva was 20% and 22.22%; abundant cervical mucus was 15.56% and 17.78%, and vaginal temperatures ranging from 38.0-38.5°C was 13.33% and 20%. In conclusion, the low percentage of pregnancy in this study was caused by various factors, mainly the genetic quality, early embryonic efficacy, maintenance management especially feed, and there were cows that experienced reproductive disorders.

Keywords: Artificial insemination, estrus character, pregnancy, sexed semen

ABSTRAK. Pengetahuan peternak mengenai deteksi estrus merupakan salah satu faktor yang berpengaruh terhadap ketepatan waktu inseminasi. Penelitian ini bertujuan untuk mengevaluasi tingkat keberhasilan IB menggunakan semen sexing Y berdasarkan karakter estrus ternak. Akseptor yang digunakan pada penelitian ini berjumlah 45 ekor Sapi Persilangan Limousin dengan BCS 3-5 dan umur berkisar antara 1,8-7 tahun. Semen beku sexing Y merupakan hasil produksi Balai Besar Inseminasi Buatan (BBIB) Singosari menggunakan metode Sentrifugasi Densitas Gradien Percoll (SGDP). Pengamatan karakter estrus dilakukan sebelum inseminasi. Inseminasi Buatan dilakukan dengan menggunakan double dosis pada jam ke-2 dan jam ke-8 dengan teknik deep insemination. Vitamin Rheinbio sebagai BioATP+ diinjeksikan setelah IB. Hasil penelitian menunjukkan bahwa keberhasilan kebuntingan ditinjau dari nilai conception rate (CR) dan pregnancy rate (PR) berdasarkan karakter estrus ternak lebih tinggi ditunjukkan pada kondisi warna vulva merah merata yaitu 17,78% dan 22,22%; vulva yang sangat bengkak yaitu 20% dan 22,22%; banyak mengeluarkan lendir servik yaitu 15,56% dan 17,78%, serta suhu vagina yang berkisar antara 38,0-38,5°C yaitu 13,33% dan 20%. Persentase kebuntingan yang rendah pada penelitian ini disebabkan oleh berbagai faktor, utamanya kualitas genetik ternak, kematian embrio dini, manajemen pemeliharaan khususnya pakan, dan terdapat ternak yang mengalami gangguan reproduksi.

Kata kunci: Inseminasi buatan, karakter estrus, kebuntingan, semen sexing

INTRODUCTION

Artificial Insemination (AI) is one of the reproductive biotechnology that has successfully improved the genetic quality of cows. Implementing AI for smallholder farmers is more economical because it can reduce the cost of maintaining males to mating females, prevent infectious diseases, and facilitate a more accurate recording process (Susilawati, 2013). The AI program can be increased in value by a combining of sexing technology. One of the guidelines for AI

is the appearance of signs of estrus. Breeders and inseminators need to detect estrus one period of the estrus cycle to determine the best time for AI. Signs of an estrus cow include releasing transparent or clear mucus, swollen and red vulva, restlessness, and mounting by other cows (Labetubun, Siwa, and Reressy, 2020). The hormone estrogen influences the appearance of signs estrus. The higher level of estrogen possessed by cows, the signs of estrus that appear will also be more apparent. Several factors influence the success of the AI program. These factors include the physiological condition of the female to be inseminated, human resources, fixed time of AI, estrus detection, semen handling, age, environment, and semen quality (Lamb *et al.*, 2010). The method of sexed spermatozoa can

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cause a decrease in the quality of spermatozoa so that AI using semen sexed is done with a double dose (Fatahillah, Susilawati, and Isnaini, 2017). In smallholder farms, beef cattle have been crossed with a Limousine or Simental cattle repeatedly so that the success rate of pregnancy is low with a high service per conception. Besides that, its effect on differences in the duration of estrus in each cow.

Detection of estrus, which includes estrus characters such as the color of the vulva, vaginal temperature, presence of cervical mucus before the implementation of AI, affects the fixed-time AI. Damarany (2020) stated that the percentage of pregnant cattle with thin mucus during estrus was higher by 87.5% than cattle with thick mucus, which was 25%. The cow that showed cervical mucus pH >8 had a higher percentage, namely 78.6%, than the cow with a pH of 7.5, which was 62.5%. Ansori, Kuswati, Huda, Prafitri, Yekti, and Susilawati (2021) added that cattle showing a red vulva character secreting a lot of mucus with a pH of 8 gave a higher pregnancy rate. Annasru *et al.* (2017) suggested that insemination at time intervals of 0-4 hours after estrus has a good value on pregnancy success compared to time intervals of 8-12 hours after estrus. Yekti *et al.* (2018) stated that AI using non-sexed semen is higher at 65.31% than sexed semen, which is 55.10%. Therefore, it is essential to know the success rate and evaluate AI using frozen semen sexed on Limousin cross cattle based on different estrus characters to increase the success of AI.

MATERIALS AND METHODS

Time and Location

This study was conducted from March 5th 2021 to June 20th 2021. The post-thawing spermatozoa quality test in the Reproductive Laboratory of the Faculty of Animal Science, University of Brawijaya, Malang, and the AI was in the Senggreng Village, Sub-District Sumber Pucung, Malang Regency, East Java.

Materials

The material used in this study was 45 female Limousin Crosses with the criteria : Body Condition Score of 3-5 (scale 1-9), age of 2-7 years having a health condition, showing signs of estrus, and examining their reproductive organs and ensuring that they are in good condition, not pregnant. The frozen sexed semen used was frozen Y from superior males of Limousin cattle produced by the Singosari Center for Artificial

Insemination (SCAI). The sexed semen used is the result of sexed with the Percoll Density Gradient Centrifugation method.

Methods

The method used in this study is an experimental method with a field experiment. The sampling technique used the purposive sampling method. Observation of estrus character was done before AI. The AI technique is performed by deep insemination semen on the uterine cornua (Susilawati, 2019). Insemination using a double dose at the 2nd and 8th hours after estrus, the "Rheinbio" brand of the vitamin was injected as BioATP. Pregnancy examination was carried out two months after AI using the Mindray DP-50 brand of ultrasonography (USG) and rectal palpation. The independent variable observed in this study was the character of estrus, including a) Vulva Color: The color of the vulva seen by visual observation. The criteria for the color of the vulva are assessed by point modification which consists of evenly red with 3 points, uneven red (pink) with 2 points, and pale with 1 point (Saputra *et al.*, 2018). b) Swelling of the vulva: Swelling of the vulva was assessed with a modified point consisting of very swollen with 3 points, slightly swollen with 2 points, and not swollen with 1 point (Saputra *et al.*, 2018). c) Cervical mucus: Cervical mucus is assessed with a modification of points consisting of there is abundant mucus with 3 points, moderate mucus with 2 points, and low mucus/absent at 1 point (Murtaza *et al.*, 2020). d) Vaginal temperature: Vaginal temperature before AI is measured using a thermometer with a depth of 3-5 cm. The dependent variables observed in this study include:

Non-Return Rate (NRR): the percentage of acceptors who do not show signs of estrus again within 18-21 (NRR 1) and 40-42 (NRR 2) days after insemination (Wiranto *et al.*, 2020). The NRR calculation formula based on Jaenudeen and Hafez (2000) is:

$$NRR = \frac{\text{Total inseminated cattle} - \text{Total re-inseminated cattle}}{\text{Total inseminated cattle}} \times 100\%$$

Conception Rate (CR): the percentage of pregnant acceptors from the first insemination of the total acceptors who are inseminated (Susilawati *et al.*, 2019). The CR calculation formula based on Jaenudeen and Hafez (2000) is:

$$CR = \frac{\text{Total pregnancy cattle from the first insemination}}{\text{Total inseminated cattle}} \times 100\%$$

Pregnancy Rate (PR): percentage of pregnant acceptors from the number of inseminated cows.

The PR calculation formula based on Jaenudeen and Hafez (2000) is:

$$PR = \frac{\text{Total pregnancy cattle}}{\text{Total inseminated cattle}} \times 100\%$$

Data Analysis

The data obtained were the estrus character of cattle before the implementation of AI, as well as the success parameters of AI, including the value of Non-Return Rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR). Data were analyzed using the descriptive analysis method using Microsoft Excel software.

RESULTS AND DISCUSSION

Evaluation of Vulva Color Character with Non-Return Rate, Conception Rate, and Pregnancy Rates

Rao *et al.* (2013) state that changes in vulva color characterize estrus in cows, swelling of the vulva, transparent mucus discharge from the vulva, feeling restless, and tails that are often lifted. The majority of farmers know or detect estrus in the morning. Changes in the color of the vulva are one of the physical manifestations of estrus, one of these changes being red (Lestari and Ismudiono, 2014). Based on Table 1, it can be seen that in this study, most of the cows showed a change in the color of the vulva, namely an even red color of as much as 48.89%.

Changes in the colour of the vulva in each cow are related to the levels of the hormone

estrogen in the blood. Table 1 shows that cattle with evenly distributed red vulva characters have higher NRR, CR, and PR percentages, respectively, 37.78%, 33.33%, 17.78%, and 22.22%, compared to cattle showing vulva red colour markings are uneven and pale. Various factors influence the low success rate of cows pregnancy. Laksmi and Trilaksana (2020) added that the increase in the hormone estrogen level during estrus cattle stimulates blood flow to the reproductive tract, causing the blood vessel network to increase, resulting in reddish color change in the vulva. Differences in estrus intensity in the cow are driven by individual factors related to hormonal conditions, especially estrogen levels, which play a role in stimulating the onset of estrus. The appearance of estrus in cattle that is not visible can be caused by the cattle are in silent heat. Silent heat can be caused by disruption of ovarian activity due to low consumption of nutrients in feed. This results in endocrine disorders that involve low estrogen levels so that cows cannot show signs of estrus. Gonadotropins have an essential role in the performance of ovarian functions, such as follicular growth, ovulation, and development of the corpus luteum. In addition, heat stress in cows can cause changes in steroid and hormone levels, such as producing higher progesterone levels with inhibition of LH secretion, leading to impaired growth hormone and fertilization (Naranjo-Gomez *et al.*, 2021).

Table 1. Vulva color character with AI success parameters

Vulva color before AI	Total head (%)	NRR-1 (%)	NRR-2 (%)	CR (%)	PR (%)
Evenly red	22 (48,89)	17 (37,78)	15 (33,33)	8 (17,78)	10 (22,22)
Uneven red	14 (31,11)	13 (28,89)	12 (26,67)	3 (6,67)	3 (6,67)
Pale	9 (20)	7 (15,56)	5 (11,11)	0 (0)	0 (0)
Total	45 (100)	37 (82,23)	32 (71,11)	11 (24,45)	13 (28,89)

Evaluation of Vulva Swelling Character with Non-Return Rate, Conception Rate, and Pregnancy Rates

At the smallholder farm, vulva swelling is one of the most easily recognized signs of estrus. Estrus in cattle is characterized by a swollen, red, and warm vulva. In these conditions, female cows are willing to be ridden (Indira *et al.*, 2014). Based on Table 2, it can be seen that in this study, most of the cows showed changes in vulva swelling to become very swollen as much as 60%. The condition of the vulva that did not experience swelling during estrus was not found in this study.

Elevated levels of the hormone estrogen in the blood are associated with the occurrence of

vulva swelling caused by thickening of the vaginal wall and vascularization (Frandsen *et al.*, 2009). Based on the results of the study presented in Table 2, cows that showed signs of very swollen vulva swelling had a higher percentage of NRR 1 46.67%, NRR 2 35.36%, CR 20%, and PR 22.22% higher than cows showing estrus character, slight swelling of the vulva. However, the percentage of successful pregnancies in this study is still low. The CR value is said to be good if it reaches a rate of more than 60%. The high and low CR values are influenced by cows conditions, estrus detection, and reproductive management, affecting the conception value and cows fertility (Yekti *et al.*, 2019). In addition, the presence of

reproductive disorders in cows, such as silent heat, affects the high and low success of pregnancy. The condition of the cage that is dark and not clean and the intensity of cows that gets little exposure to sunlight affect the presence of ectoparasites and endoparasites that can cause stress in cows. Susilawati (2019) states that when cows experience reproductive disorders such as silent heat, ovulation does not occur or

experiences premature embryo death. Kruse *et al.* (2017) added that nutritional deficiencies in cows before insemination could affect the low viability of oocytes, resulting in low pregnancy success. Lack of nutrients consumed by cows after mating can also affect the quality of the embryo. It can be affected by not developing embryos because the quality of the embryos produced is relatively low.

Table 2. Characteristics of vulva swelling with AI success parameters

Swelling vulva before AI	Total head (%)	NRR-1 (%)	NRR-2 (%)	CR (%)	PR (%)
Very swollen	27 (60)	21 (46,67)	16 (35,36)	9 (20)	10 (22,22)
Slightly swollen	18 (40)	16 (35,56)	16 (35,56)	2 (4,44)	3 (6,67)
Not swollen	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	45 (100)	37 (82,23)	32 (71,12)	11 (24,44)	13 (28,89)

Evaluation of Cervical Mucus Character with Non-Return Rate, Conception Rate, and Pregnancy Rates

Ihsan (2010) stated that in the estrus phase, increased estrogen levels due to the maturation of the de Graff follicles cause changes in the tubular reproductive tract at maximum conditions, such as the uterus becomes tense and the volume of cervical mucus secretion increases. Cervical mucus secretion is characterized by a state that hangs like a string. In estrus phase, the cervix will

undergo relaxation and abundantly secrete thick and slimy mucus from the cells of the cervical goblet, anterior vagina, as well as the uterine glands. The appearance of cervical mucus during estrus is clear or transparent. Based on Table 3, it can be seen that in this study, most of the cows showed estrus characters with a slight mucus condition, namely 48.89%. Cows that showed a lot of mucus had a percentage of 42.22%, lower than cows that emitted a little mucus.

Table 3. The character of cervical mucus with AI success parameters

Servical mucus before AI	Total head (%)	NRR-1 (%)	NRR-2 (%)	CR (%)	PR (%)
Abundant	19 (42,22)	16 (35,56)	15 (33,33)	7 (15,56)	8 (17,78)
Moderate	22 (48,89)	17 (37,78)	13 (28,89)	4 (8,89)	5 (11,11)
Low/Absent	4 (8,89)	4 (8,89)	4 (8,89)	0 (0)	0 (0)
Total	45 (100)	37 (82,23)	32 (71,11)	11 (24,45)	13 (28,89)

Based on the data in Table 3, it is known that AI using frozen sexed semen with estrus character of cows that secreted a lot of mucus during estrus before AI resulted in higher CR and PR values of 15.56% and 17.78% compared to cattle that secreted a little mucus. The success percentage is relatively low compared to the results of the research of Yekti *et al.* (2019) using frozen sexed semen, which achieved CR 53.13% success. The low success of AI pregnancy results can be caused by errors in estrus detection in cows due to silent heat. Yekti *et al.* (2018) stated that neurotransmitters could cause cows to experience reproductive disorders caused by stress or stress on the five senses that cause hormone release induction or inhibition. It can happen due to the low level of the hormone estrogen so that cows cannot show signs of estrus. This condition can occur in malnourished females. Susilawati (2019)

added that all cows activities could not be separated from the influence of the feed consumed, such as reproductive, metabolic, and growth activities. This study also found some cattle with endometritis. Endometritis is inflammation of the endometrium caused by a bacterial infection that results in impaired fertility of cows. Cattle that experience endometritis do not ovulate because there is a disturbance in the mechanism of the release of the hormone prostaglandin (Melia *et al.*, 2014).

Evaluation of Vaginal Temperature Character with Non-Return Rate, Conception Rate, and Pregnancy Rate

Vaginal temperature is one of the characters related to the physiological condition of cows regarding the estrus phase. Based on Table 4, it can be seen that in this study, the vaginal

temperature of cattle before AI showed more than 38.5°C was 53.33%. Vaginal temperature is initially low in the proestrus phase, increases during estrus, and decreases again in the metestrus phase. This change in vaginal temperature is caused by the hormones estrogen and LH in the blood and the vascularization process (Randi *et al.*, 2018). In the estrus phase, the average vaginal temperature of cows is 38.39±0.29°C, and during estrus, it will decrease to 38.05±0.15°C (Indira *et al.*, 2014).

Based on the data in Table 4, it is known that AI using frozen sexed semen with a vaginal temperature range of 38.0-38.5°C resulted in higher CR and PR pregnancy values was 13.33% and 20%, respectively. The increased estrogen hormone affects increasing vaginal blood circulation, followed by changes in vaginal temperature. Randi *et al.* (2018) state that changes in temperature can be caused by an increase in cow activity during estrus because GnRH and

estrogen influence the central regulation of body temperature. These results are still relatively low compared to the research of Sharma *et al.* (2018) AI using frozen sexed semens is 40%. Wiranto *et al.* (2020) added that AI using frozen semen sexing on Limousin Crossed Cattle showed a high pregnancy rate of 74.03%. The difference in the appearance of pregnant and non-pregnant cow is shown in Figure 1. The results of the pregnancy examination using the USG method revealed that many cattle had ovarian hypofunction. Ovarian hypofunction is a reproductive disorder caused by a decrease in ovarian function so that primary, secondary, and tertiary follicles cannot develop (Ervandi and Susilawati, 2021). Cattle with ovarian hypofunction cause silent heat, irregular estrus cycle, and anestrus. Kruse *et al.* (2017) added that nutritional deficiencies in adult female cows for an extended period could cause a decrease in ovarian activity so that an irregular estrus cycle is accompanied by anestrus.

Table 4. The character of Vaginal Temperature with AI Success Parameters

Vaginal temperature before AI	Total head (%)	NRR-1 (%)	NRR-2 (%)	CR (%)	PR (%)
38,0-38,5°C	21 (46,67)	18 (40)	17 (37,78)	6 (13,33)	9 (20)
>38,5°C	24 (53,33)	19 (42,22)	15 (33,33)	5 (11,11)	4 (8,89)
Total	45 (100)	37 (82,22)	32 (71,11)	11 (24,44)	13 (28,89)

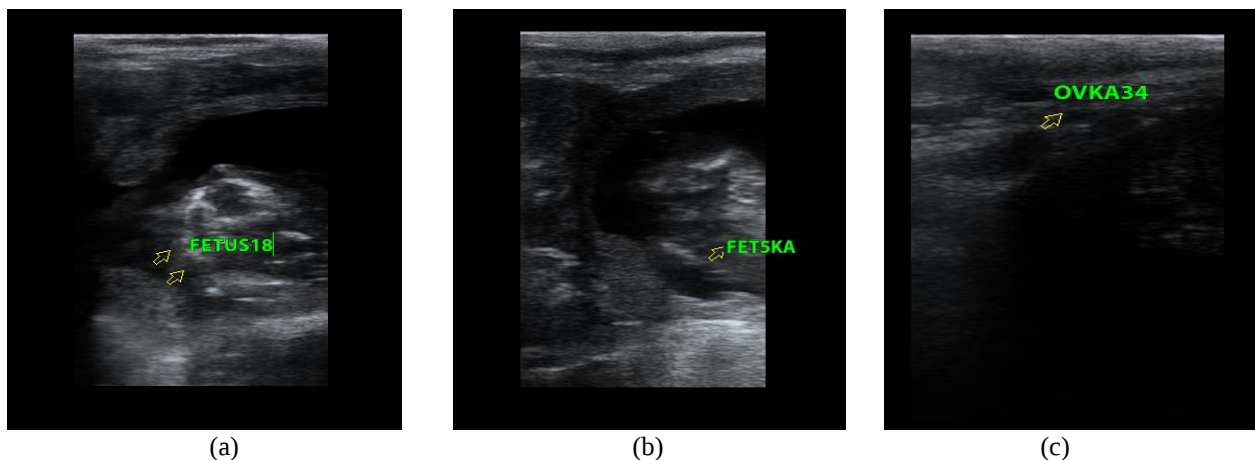


Figure 1. Results of Ultrasound Examination in Cow (a) Pregnant, Fetus Position is in the Left Uterine Cornua (b) Pregnant, Fetus Position is in Right Uterine Cornua (c) Cows Experiencing Silent Heat (Right Ovary Found Follicles and Corpus Luteum; Diestrus Phase).

CONCLUSIONS

The success of pregnancy (conception rate and pregnancy rate) based on the estrus character of cows was higher as indicated by the evenly red color of the vulva; the vulva was very swollen. It secreted a lot of cervical mucus, and the vaginal temperature ranged from 38.0-38.5°C. The low percentage of pregnancy in this study was caused by various factors, mainly the genetic quality

(crossbreed cows), early embryonic efficacy, maintenance management especially feed; nutrients contained in the feed, and there were cows that experienced reproductive disorders.

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