

Original Article

Intracervical Sperm Deposition and Body Condition Score on the Differences in Conception Rate after First Artificial Cow Insemination

Mirajuddin ^{1*}, Padang Padang ¹, M. H. Husain ¹, Mohammad Ismail ¹

¹ Affiliation 1 Department of Animal Husbandry, Faculty of Animal Husbandry and Fisheries, Tadulako University, Tondo, East Palu, Palu, Central Sulawesi, Indonesia.

*Correspondence: mirajuddinm@gmail.com

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Abstract

Objective: This study specifically aimed to determine the method of sperm deposition during intracervical insemination for the artificial insemination (AI) of Donggala cattle (local cattle from Donggala district).

Methods: This study was conducted in the Donggala district, Central Sulawesi province Indonesia from July to November 2017. The AI receptor was productive for Donggala cow with a body condition score (BCS) of 2-4. The success of the first AI was determined based on the conception rate (CR).

Results: The results showed that the variation in BCS and the intracervical deposition location significantly affected ($p < 0.05$) the CR of the first AI in Donggala cattle. Cows with a BCS of 4 had a higher CR (100%) when compared to a BCS of 3 (76.24%) and a BCS of 2 (74.19%).

Conclusions: compared to III (87.27%), II (75.00%), and I (41.02%). It suggests that the success rate of AI can be increased if the sperm deposition was placed in the cervical ring IV and III.

Keywords: Artificial insemination; body condition score; conception rate; cow; intracervical sperm deposition.

INTRODUCTION

Achieving the national target for self-sufficiency of beef by 2026 and the development of domestic farms in potential regions, such as Central Sulawesi province Indonesia with the goal of *Sulawesi Sejuta Sapi* (S3) – One million cows in Sulawesi, needs a strategic improvement in farm development and beef provision. The government has stressed the importance of the utilization of the potential local, Indonesian-endemic, and beef cattle population acceleration program to achieve these goals [1-2]. Indonesian endemic beef cattle are cattle that can only be found in

Indonesia, while local cattle are imported cattle from outside of Indonesia but are commonly bred in Indonesia and have their distinct traits [2-3]. Donggala cattle are domestic cattle from Central Sulawesi which have been established as the germplasm for national beef cattle according to Decree of Minister of Agriculture of the Republic of Indonesia No. 666/Kpts/SR.120/6/2014 [2].

Donggala cattle have a square head, a short front protruding hump (<11 cm), a short screw (<7 cm), and various hair colors: white, gray-white, reddish-white, red, black, or striped (a combination of those colors) [4]. Donggala cattle spreads in Central Sulawesi

province Indonesia, with the highest populations recorded in the Donggala district, Sigi district, and Palu city [5]. However, the body condition and reproductive efficiency are low due to genetic erosion and adverse selection (Donggala database). The research on Donggala cattle has continued to restore its genetic excellence, economic, and social status. These cattle have good adaptability toward heat, parasites, and low-quality feed [5]. The national goals can be achieved with a good selection and mating management, followed by excellent control of cattle maintenance. The target population can only be obtained with the practical application of science and technology in every aspect of its breeding.

The commonly used artificial insemination (AI) is the most conventional technology, in which using frozen semen in the application. However, frozen semen from Donggala cattle is not readily available [4]. Therefore, a comprehensive study related to the AI in Donggala cattle is necessary. The process of AI includes the preparation of progressive motile sperms, which can be stored for a long time without freezing, have a high fertility rate, and easy to spread out [6]. The failure of AI practice in Central Sulawesi is indicated by the low service per conception ratio (S/C), which is >3 , it usually should be around 1-1.4 [7]. The low S/C is thought to be caused by the low quality of frozen semen used and the inappropriate timing of AI. The low quality of frozen sperm in this area is due to the lack of continuous availability of liquid nitrogen as a storage medium, so an appropriate solution is needed to increase the success of AI [8].

The location of sperm deposition influences the success of AI during insemination, time, and the condition of the female receptor [7,9-10]. The study objective was to determine the effect of intracervical sperm deposition and the variation in body condition score (BCS) in the success of AI using liquid semen from Donggala cattle.

MATERIALS AND METHODS

Materials

The diagram flow of the research was shown in Figure 1. This research was conducted on a local cattle farm in Donggala

District; this was chosen as the location to implement AI technologies. The study was conducted for 5 months from July to November 2017.

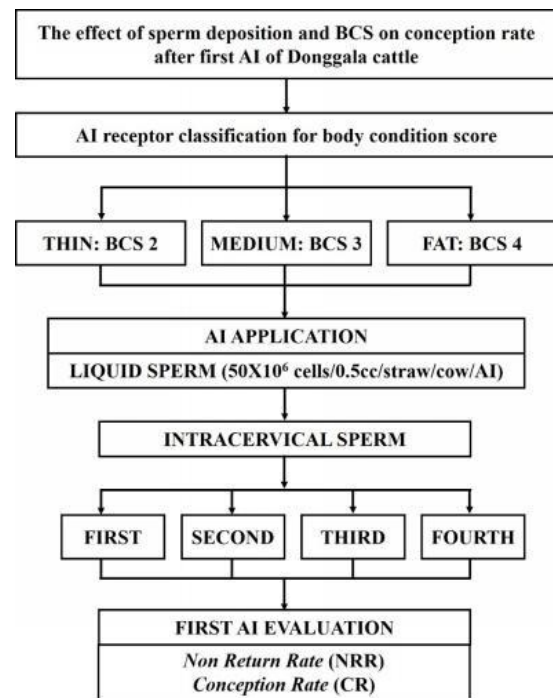


Figure 1. Research Layout

Cattle and Oestrus Synchronization

As many as 200 local female cows with a BCS score of 2, 3, or 4 based on their morphology were chosen to be the receptors of AI. The female cows were procured from Toaya, Dalaka, Lero tatar, Amal, and Enu village in Sindue sub-district; Nupabomba village in Tanantovea sub-district; Toposo, Lelea, and Panimba village in Labuan sub-district, Lumbutarombo, Bambarimi, Salunkenu, and Tanamea village in Banawa Selatan sub-district; and Kolaka village in Banawa Tengah sub-district, Donggala District, Central Sulawesi Province Indonesia.

Oestrus synchronization is performed using a single PGF2 α injection method (one time). The single injection method is performed by considering the presence of a normal or active corpus luteum in the female whose oestrus will be synchronized. Examination of the presence or absence of corpus luteum is performed using the rectal palpation method. Females with normal or active corpus luteum conditions are given PGF2 α hormone.

PGF2 α is known to have the ability to regress the corpus luteum [11]. Thus, the use of PGF2 α is carried out by a single injection with the intra muscular (IM) method at a dose of 5cc/head/IM; taking into account its effectiveness, efficiency, and economic value. The choice of a protocol which fits the specific situation under consideration is the base for the success of any prostaglandin-based oestrus synchronization programs [11]. The onset of oestrus with a single PGF2 α injection compared with a double injection, did not differ significantly ($P>0.05$) with values of 68.00 \pm 18.33 and 72.00 \pm 24.00 hours, respectively [12].

The application of AI using liquid sperm was conducted three days after the infusion once the receptor had shown the “in heat” conditions after the hormone injection (Figure 1).

Liquid Semen - AI Method

The AI was conducted 30-36 hours after the oestrus onset was observed or during the mid and late oestrus stages [7,10]. The procedure was performed in the morning, at around 08.00-10.00 AM, local time. The liquid sperm for AI was extracted from the Donggala bull using the artificial vagina method. The liquid sperm was stored for 4-7 days at 10-12 °C before it was used for the AI procedure [8]. In principle, the AI method with liquid sperm is similar to the technique with frozen sperm. The difference is that the liquid method doesn't have any thawing process before the application. The method was based on a method described by Mirajuddin [13] the AI was carried out using the rectovaginal method [14]. The receptor, which was in an oestrus state, was prepared in a special cage with clamps. The inseminator carried out rectal palpation using their left hand and reached the cervix. After that, the insemination gun was loaded with liquid sperm straw (0.5 cc semen with a sperm cell concentration of 50 \times 10⁶ cells) and covered in a plastic sheet; it was inserted into the female reproductive tract via the vagina until it reached the four cervical rings (I-IV) according to the treatment group. Then, the liquid sperm was deposited and inseminated. Finally, the insemination gun was removed from the reproductive tract, and the inseminator's hand was removed from the

rectum. The plastic sheet and plastic glove were discarded after each procedure.

Observed Parameters

To determinate the success of the first AI application, two parameters were evaluated: The Non-Return Rate (NRR) and the conception rate (CR). The non-return rate (NRR) was calculated as the percentage of females that did not return to the oestrus state after the first application of AI [7, 15]. The NRR was evaluated by directly observing the oestrus signs from the receptor or by putting a bull in the receptor's enclosure and seeing whether the receptors were asked for mating from the bull [15]. The evaluation was conducted for three oestrus cycles after the AI, which was on the (I) 18th-21st day, (II) the 39th-42nd day, and (III) the 60th to 64th day after AI. The receptors, which were not returning to an oestrus state or were not asking for mating, were counted and divided with the total number of AI receptors, using the following formula (1):

$$NRR = \frac{\Sigma \text{receptor with no estrous or no mating after AI}}{\Sigma \text{AI receptors}} \times 100\%$$

The conception rate is the percentage of female cows that got pregnant after the first AI. The CR value was calculated from the NRR of the first AI application and the total number of AI receptors; it was expressed as a percentage (%). To determine the valid pregnancy of the AI receptor the pregnancy test was conducted using fetus palpation using the rectal palpation method at a minimum pregnancy age of three months or 100 days after the AI procedure. The experiment aimed to validate the NRR value further and to avoid the bias present from observing females with silent heat, sub-oestrus signs, or after finding females that were not showing the end of oestrus because of the premature death of the embryo during the first stages of conception. Thus, the opposition attaching and adhesion of blastocyst to endometrium did not occur [10,16]. The NRR and CR data were analyzed using a Chi-Square test to check the relationship between sperm deposition and BCS to the liquid sperm first AI success rate in Donggala cattle [17].

RESULTS

BCS of AI Receptors and Cow-raising Ground Condition

The body condition of the Donggala cow, expressed as the BCS, as the AI receptor in Donggala district was summarized in Table 1. The cow raising ground condition for AI application based on the location was presented in Table 1 as well.

The receptor body condition from the various villages in the Donggala district was reasonably diverse, namely 93 cows (46.5%) were thin, with plainly visible rib and hip bone (BCS=2); 101 cows (50.5%) were medium-sized, with invisible rib but visible hip bone (BCS=3); and 6 cows (3.0%) were fat with invisible rib and hip bone (BCS=4) from a total of 200 cows, shown in Table 1. These observational results suggested that the breeding management, mainly the feed as the

primary necessity, is an essential point in developing cattle farming and other ruminants in the Donggala District. However, the condition is not only caused by the farmer's lack of knowledge in a good feeding system, but also the critical state of the cattle raising ground. It was observed that 9 out of 12 villages (75%) had a dry farm without a sufficient water source shown in Table 1. These adverse conditions lead to a low conception rate and a small reproduction efficiency of local cattle. It can be the primary challenge in developing of "Sentra Peternakan Rakyat (SPR)" – local farm center, which is proposed by the Minister of Agriculture to be produced in Central Sulawesi. It calls for the need for serious attention to be paid to developing the Donggala cattle or other local beef cattle.

Table 1. AI receptor Donggala Cow BCS and Cow Raising Ground Condition in Donggala Regency

| No. | AI location | Body Condition Score (BCS) ^{a)} | | | Breeding condition | ground |
|-----|---|--|-----------------|--------------|--|--------|
| | | Thin (BCS= 2) | Medium (BCS= 3) | Fat (BCS= 4) | | |
| 1 | Toaya village, Sindue district | 4 | 3 | 0 | Farm – dry and lack of feed | |
| 2 | Dalaka village, Sindue district | 5 | 8 | 0 | Farm – dry and lack of feed | |
| 3 | Lero Tatari village, Sindue district | 20 | 8 | 0 | Farm – dry, scarce feed | |
| 4 | Amal village, Sindue district | 4 | 9 | 0 | Farm – sufficient water and feed | |
| 5 | Enu village, Sindue district | 3 | 3 | 0 | Farm – dry, scarce feed | |
| 6 | Toposo village, Labuan district | 9 | 17 | 1 | Farm and rice field, sufficient water and feed | |
| 7 | Lelea village, Labuan district | 8 | 2 | 0 | Farm – dry, lack of feed | |
| 8 | Panimba village, Labuan district | 14 | 12 | 0 | Farm – dry, lack of feed | |
| 9 | Nupabomba village, Tanantovea district | 11 | 3 | 0 | Farm – dry, scarce feed | |
| 10 | Lumbutarombo, Bambarimi, and Salung Kenu village, Banawa Selatan district | 11 | 20 | 3 | Farm – sufficient water and feed | |
| 11 | Tanamea village, Banawa Selatan district | 7 | 11 | 2 | Farm, sufficient green grass | |

| | | | | | | |
|--------------|--------------------------------|--------------------|-----------|------------|----------|-------------------------|
| 12 | Kolakola Banawa district | village, Tengah | 5 | 5 | 0 | Farm – dry, scarce feed |
| Total | | | 93 | 101 | 6 | |

Intracervical Sperm Deposition in AI of Donggala Cattle

The data on sperm deposition during intracervical insemination based on the BCS of Donggala cattle was presented in Table 2. The location of sperm deposition during the intracervical insemination was diverse, corresponding to the BCS and the cervix condition of the acceptors. The AI was performed in the receptors in the last oestrus stage. The acceptors with a BCS of 2 tended to

have smaller cervical rings when compared to BCS of 3 and 4, but this study didn't measure the cervix size for every BCS in this study.

The variation of BCS of the AI receptors is caused by the bad management of cattle in the Donggala district. Eventually, this will lead to inefficient and low production. Moreover, the AI implementation that was intended to repair the production efficiency had a fluctuating success rate corresponding to the various BCS.

Table 2. Sperm Deposition Location During Intracervical Artificial Insemination Based on the BCS of Donggala Cattle.

| No. | Body Condition Score (BCS) ^{a)} | Intracervical sperm deposition | | | | N | Percentage (%) |
|-----------------------|--|--------------------------------|-------------|-------------|-------------|--------------|----------------|
| | | Door I | Door II | Door III | Door IV | | |
| 1 | 2 = Thin | 18 | 30 | 25 | 20 | 93 | 46.5 |
| 2 | 3 = Medium | 20 | 32 | 28 | 21 | 101 | 50.5 |
| 3 | 4 = Fat | 1 | 2 | 2 | 1 | 6 | 3.0 |
| Total | | 39 | 64 | 55 | 42 | 200 | 100.0 |
| Percentage (%) | | 19.5 | 32.0 | 27.5 | 21.0 | 100.0 | |

BCS Effect to Conception Rate of Donggala Cattle

The conception rate was calculated based on the formula and was presented as a percentage [7,10]. The AI receptor was included in the NRR if it didn't show any returning oestrus signs 42, 49, and 60 days after the AI procedure. The result was presented in Table 3.

BCS increases the conception rate significantly ($p < 0.05$) based on our similarity analysis. The chi-square analysis showed a significant difference in CR according to the AI receptor's BCS. The highest CR was BCS 4 at 100% when compared to BCS 3 ($76.24 \pm 24.76\%$) and BCS 2 ($74.19 \pm 24.28\%$). It implies that the condition of the receptor influences the success rate of liquid sperm AI. Besides, some other factors that also influenced the success rate was: the location of sperm deposition during the AI procedure; natural oestrus or synchronized oestrus; time frame since the last pregnancy with the AI application; cow age;

insemination time; feed factor; air temperature; cattle stress level and the farmer's knowledge of the oestrus signs and zoonosis disease [7,18].

Intracervical Deposition Location Affect the CR Value in Donggala Cattle

The chi-square analysis showed that the location of intracervical sperm deposition significantly increases ($p < 0.05$) the CR percentage in Donggala cattle after liquid sperm AI (Table 3). The CR in cervical ring IV deposition was higher ($95.23 \pm 2.82\%$) than in cervical ring III ($87.27 \pm 8.15\%$), cervical ring II ($75.00 \pm 14.94\%$) and cervical ring I ($41.02 \pm 34.97\%$). It was also observed that there were significant differences between cervical ring III, II, and I (Table 3). It implies that the location of sperm deposition during the AI can significantly affect CR in Donggala cattle.

Due to the low CR after AI application in the cervical ring I, shown in Table 3, it is suggested that the AI application should be in

the cervical ring III or IV. It minimizes the limiting factors for the spermatozoa to reach the oviduct and fertilize the ovum.

Table 3. Conception Rate after Liquid Sperm Artificial Insemination According to BCS and Cervical Door Deposition in Donggala Cattle.

| No | Body Condition Score (BCS) | $\Sigma \text{♀ pregnant} / \Sigma \text{♀ AI receptors}$ Based on the sperm deposition location | | | | Total | CR (%) |
|---------------|----------------------------|---|------------------------------|-----------------------------|-----------------------------|---------|-------------------------------|
| | | Door I | Door II | Door III | Door IV | | |
| 1 | 2 = Thin | 7/18 | 22/30 | 21/25 | 19/20 | 69/93 | 74.19± 24.28 _a |
| 2 | 3 = Medium | 8/20 | 24/32 | 25/28 | 20/21 | 77/101 | 76.24± 24.76 _b |
| 3 | 4 = Fat | 1/1 | 2/2 | 2/2 | 1/1 | 6/6 | 100.00± 00.00 _c |
| Total | | 16/39 | 48/64 | 48/55 | 40/42 | 152/200 | 76.00 |
| CR (%) | | 41.02± 34.97 _a | 75.00± 14.94 _b | 87.27± 8.15 _c | 95.23± 2.82 _d | 76.00 | |

DISCUSSION

Overcoming the conditions observed in Table 1 requires addressing several key priorities in the development of cattle farms in Donggala District. A critical step involves establishing Hijauan Makanan Ternak (HMT), or green livestock feed, within the targeted farm grounds of Donggala cattle. Complementing this is the need for effective agricultural waste management. Equally important is improving cattle mating management, which hinges on determining optimal birthing and mating timelines. Based on local food production cycles, the most effective mating period is suggested to be February to March, aligning with the peak of feed availability in November and December. Advancing productivity further requires the strategic application of science and technology. Finally, self-sufficiency in high-quality Donggala cattle sperm supply must be prioritized. By adopting the Low External Input Sustainable Agriculture (LEISA) concept, sustainable breeding systems can be established where seeds and feed are locally sourced, ensuring long-term autonomy and reducing dependency on external inputs [18].

The differences in CR in response to the receptor's BCS, shown in Table 2 is closely related to the fertility and reproduction efficiency of cattle. The body condition reflects the individual's metabolic state but, the details are not discussed in this paper. Good cattle

condition determines the reproduction efficiency [7]. The efficiency was considered good if the BCS was at 3 or 4 for the Donggala cattle, this increased the rate of conception and pregnancy. However, the influence of the hormonal factor and ovulated ovum quality and quantity on the CR rate in every BCS is not discussed in this paper.

Another aspect that affects the variation in CR is the bio-physiological and the reproductive organ of the receptor. Receptors with a low BCS tend to have a small, tight, and rigid reproductive tract which can inhibit the spermatozoa progress in the oviduct, especially in the AIJ and ampulla where the fertilization occurs typically. Therefore, the number of spermatozoa that can fertilize declines and results in a low CR when compared to other BCS [19]. The minimum number of fertile spermatozoa inside the ampulla region was around 100 cells [20]. The low quality of oestrus and the ovulated ovum may also affect the low CR in a low BCS receptor. Several other factors that may affect the low CR value are the possibilities of anatomical and functional disruption or other reproductive disorders [21]; pregnancy loss after the AI application; maternal, genetic, and environmental factors [17]. It is also caused by a lack of breeder knowledge in the maintenance of cattle during pregnancy all play a significant part in fetus mortality [22].

The deposition of sperm during AI can affect the sperm count, capacitation, and the sperm's ability to move in the female reproductive tract to reach the oviduct, where the fertilization occurs [21, 23]. Deposition on the cervical ring III and IV has a higher CR than II and I, this may be caused by the closer proximity of cervical ring III and IV to the oviduct, especially the AIJ and ampulla, this facilitates the sperm to increase capacitation and to reach the ovum [10, 20]. The sperm transport inside the reproductive tract cannot be separated with the contraction motion of the reproductive tract; this is controlled by the central nervous system, hormonal activity, and the role of the liquid and cilia in the oviduct, cervix, utero-tubal junction, and ampulla-isthmic junction (AIJ) [10]. The mechanical stimulation from the mating process or the AI procedure also affects the uterine contraction, spermatozoa distribution, and capacitation [24]. Another potentially limiting factor is the obstacle, phagocytosis and the mortality rate for the sperm during the movement to reach the AIJ/Ampulla [25].

Sperm deposition at the cervical ring I during the AI procedure is not only caused by the small size of the cervix, but also the rough opening of the cervix. Estrogen content in the system is not adequate to induce oestrus and open the cervical ring. The low estrogen content also decreases the possibility of ovulation. The narrow cervical ring and the viscous liquid inside the reproductive tract inhibit the spermatozoa to penetrate the cervical ring rings, killing the spermatozoa, even if they manage to reach the oviduct, the surviving spermatozoa may not be fit to carry out fertilization. The contraction motion during the non-oestrus condition is opposite to the direction of the spermatozoa. Another main factor in the low CR after the AI procedure is the long waiting time for the ovulation (10-14 days in pro-oestrus) due to the hormonal factor and oestrus' role in opening the cervical ring and the dilution of the liquid inside the cervix [21].

CONCLUSION

The AI procedure using liquid sperm from Donggala cattle showed a significant success rate based on the receptor's BCS. The

cows with the highest BCS 4 had a 100% CR when compared to BCS 3 (76.24%) and BCS 2 (74.19%). Sperm deposition location plays a significant role in the success rate of the AI. Cervical ring IV had the highest CR (95.23%) when compared to III (87.27%), II (75.00%) and I (41.02%).

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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