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The Success of Double Dose Artificial Insemination at Different Times in Ongole Crossbred Cattle

Aulia Puspita Anugra Yekti, Rizki Prafitri, Kuswati, Asri Nurul Huda, Kusmartono and Trinil Susilawati

Faculty of Animal Science, Universitas Brawijaya, Malang, Indonesia

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Corresponding Author:
Trinil Susilawati,
Department of Animal Production, Faculty of Animal Science, Malang, Indonesia
Email: tsusilawati@ub.ac.id

Abstract: Artificial insemination technology has been proved to increase cattle productivity. This research aims to evaluate the success rate of double-dose artificial insemination at different times in Ongole crossbred cattle. This research was done in cattle household farms in Senggreng Village, Sumber Pucung District, Malang Regency, East Java, Indonesia. Fifty cattle involved randomly in this research met the criteria; Ongole crossbred, 4-11 years old, minimum Body Condition Score 3 points (1-9 points scale), reproductive sound, have given birth. Twenty-five cattle (T1) were inseminated 2 and 8 h after the estrus sign, while the other 25 (T2) were inseminated 8 h and 16 h after the estrus sign. After the first insemination, the cattle were injected with vitamin Bio ATP 10 mL and 3 kg concentrate feed (protein 12%) for three days. The next estrus sign was observed for the first and second cycles (NRR1 and NRR2). Re-insemination was done to cattle with an estrus sign-on NRR1. Palpation per rectal and ultrasonography was performed to determine the conception rate, pregnancy rate and services per conception. The study showed that double-dose artificial insemination at 2 and 8 h (T1) had better results than T2 (8 and 16 h). NRR1 for T1 = 84 and T2 = 76%; NRR2 for T1 = 80 and T2 = 76%; Conception Rate of T1 = 44% and T2 = 32%; Pregnancy Rate of T1 = 64 and T2 = 52%; services per conception of T1 = 1,32 and T2 = 1,24. In conclusion, double dose artificial insemination given at the 2nd and 8th h after estrus sign had better result on pregnancy than double dose insemination given at the 8th and 16th h.

Keywords: Ongole Crossbred, Artificial Insemination, Double Dose, Conception rate, Non-Return Rate

Introduction

Artificial Insemination (AI) technology has been proved to elevate the reproductive efficiency of beef cattle. Every year, reproductive efficiency and calf production indicate the success of beef cattle production (Moorey and Biase, 2020). The implementation of AI in Indonesia was popularized in 1970 by using Limousine and Simmental frozen semen on cross local cattle breeds (Ongole Breed) to produce new crossbreed. The new crossbreed was widely distributed for farmers across Indonesia.

Ongole breed is Bos Indicus cattle originally from a tropical area with better adaptation to feed with high dietary fiber, hot climate, ectoparasite and endoparasite. In contraries, Limousine and Simental breeds are Bos Taurus breeds of the subtropical area with low adaptation to hot weather, high dietary fiber feed, ectoparasite and endoparasite (Susilawati, 2017). Yekti *et al*. (2017) stated that Bos Taurus, which live in hot weather, would have silent heat or an ovular estrus governed by neurotransmitters. The high temperature triggers the gonadotropin neuro system to release Gonadotropin Inhibiting Hormone (GnIH) and the adenohypophysis to stop the production of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). As a result, the cattle are experiencing silent heat and ovulation failure.

The success rate of artificial insemination Ongole in Crossbred is relatively low in services per conception, days open and calving interval (Suyadi *et al*., 2014). The success rate of artificial insemination is affected by many factors, including semen quality, reproductive status and efficiency of the estrus detection, as well as the inseminator skills such as techniques of thawing for the semen straw, insemination and disposition of the semen (Lamb *et al*., 2010). The implementation of

double dose artificial insemination at one time increases the success of artificial insemination and improves the pregnancy rate, (Yekti *et al*., 2019). Therefore, this research will apply double-dose artificial insemination at different times to enhance the pregnancy rate in the Indonesian Ongole cattle breed.

Materials and Methods

The research materials were Crossbred cattle which are selected through a purposive sampling method with the following criteria: Age of 4 -11 years and Body Condition Score (BCS) range of 3-7 points on 1-9 points scale (Rasby *et al*., 2014), no reproductive problems indicated by given birth before normally. The total population eligible as a sample of the research in Senggreng Village was 250 cows. Due to the limitation of permission from farmers involved in this research, the total research sample was 50 Ongole crossbred cows.

Samples were randomly divided into two groups of equal numbers. The first group (T1) were 25 cows inseminated at the 2nd and 8th h after farmers reported estrus signs to the inseminator. Another group (T2) were 25 cows inseminated at the 8th and 16th h after the exhibition of the estrus signs. Inseminations were done using frozen produced by the Singosari Insemination Center. Singosari Insemination Center is one of Indonesia's licensed national insemination centers with frozen semen that fulfill the national standard of frozen semen with minimum motility is 40% and concentration of 25 million/straw. Moreover, the inseminator involved in this research is a professional inseminator provided by the Department of Animal Husbandry and veterinary of Malang Regency.

The inseminator thawed frozen semen at tap water (25°C for 20-23 seconds) before applying it to the cattle. These methods were chosen to increase the possibility of pregnancy because the estrus period happened up to 12-18 h (Yekti *et al*., 2017). After the first AI, the cattle were injected with vitamin of Bio ATP "Rheinbio" brand 10 mL/injection to increase the energy metabolism and fed with an additional 3 kg concentrate with protein 11,20% for three days.

The observation of NRR-1 was done on days 19-22. Re-insemination with a similar method was done to cattle that indicated estrus signs. The insemination was only repeated for the cow which experiences estrous on NRR-1 (19-22 days after inseminated). Cows with no estrus signs after 19-22 days will be assumed as pregnant (NRR1). A veterinarian did palpation per rectum and Ultrasound after two months of the last insemination to ensure the pregnancy (Jainudeen and Hafez, 2000). The ultrasound equipment was the ultrasound from Mindray DP-50. The Non-Return Rate (NRR), Conception Rate (CR), Pregnancy Rate (PR) and Services per Conception (S/C) were estimated. The results were analyzed descriptively and formulated as follow.

Non- Return Rate (*NRR*)

NRR measurement was including the cows that did not show any estrus signs at 21-60 days after the insemination. stated that the percentage of AI acceptors which did not show any estrus signs at 18-21 days (NRR1) and day 40-42 after AI (NRR2). Jainudeen and Hafez (2000) stated the NRR equation as:

$$\frac{Total\ inse\min ated\ cattle - total\ re\ inse\min ated\ cattle}{Total\ inse\min ated\ cattle} x100\%$$

First Service Conception Rate (CR)

First service conception rate is total pregnancy indicated from the rectal palpation of the first insemination divided into total inseminated cattle times 100% Jainudeen and Hafez (2000)

$$\frac{Total\ pregnancy\ cattel\ \ from\ the\ first\ inse\min ation}{Total\ inse\min ated\ cattle\ of\ \ first\ services} x100\%$$

Pregnancy Rate

Pregnancy Rate (PR) is the number of total pregnant cattle from the first and second insemination divided to total inseminated cattle in the herd. Based on Jainudeen and Hafez (2000), PR is calculated:

$$\frac{Number\ of\ cows\ pregnant}{Total\ cows\ in\ herd} x100\%$$

Services per Conception (*S/C*)

Services per Conception (S/C) is calculated from total pregnancy in the population. Based on Jainudeen and Hafez (2000), S/C is calculated:

$$\frac{Number\ of\ services\ in\ all\ \ pregnant}{total\ conceptions} x100\%$$

Results

Non- Return Rate

Non-Return Rate indicates the success of AI based on the failure of implants. The result of double dose of AI at different times in this research can be seen in Table 1.

The result of the study showed NRR1 and NRR2 of T1 were 84 and 80%, while both NRR1 and NRR 2 of T2 were 76%.

Conception Rate and Pregnancy Rate

Palpation per rectal and Ultrasound 2 months after double dose AI indicate the conception rate and pregnancy rate. The conception rate and pregnancy rate results of the research were presented in Table 2.

Table 1: Non -Return rate of double dose AI at different times

Treatments	Not estrus (Cows)	NRR-1 (%)	Not estrus (Cows)	NRR-2 (%)
T1	21	84	20	80
T2	18	76	20	76

Table 2: Conception rate and pregnancy rate of double dose AI at different times

Treatments	Total pregnant of 1st AI (Cows)	Conception rate (%)	Total pregnant of 1st and 2nd AI (Cows)	Pregnancy rate (%)
T1	11	44	16	64
T2	8	32	13	52

Table 3: services per conception of double dose AI at different times

Treatment	Total AI	S/C
T1	33	1.32
T2	31	1.24

Fig. 1: The USG from 1-month pregnancy (A) and 2-month pregnancy (B); A. 1-month pregnancy; B. 2 Months pregnancy: Backbones identified

The conception rate is calculated from the total number of pregnant female of the first insemination. The pregnancy rate was measured from the pregnant cows of all samples. Result of this study showed that conception rate on T1 was 44%, it was higher than T2 which only 32%. The pregnancy rate also showed the same pattern, 64% for T1 and 52% for T2. Figure 1 showed the USG results of the first month pregnancy and second month pregnancy where the backbones of the fetus can be identified clearly.

Evaluation of Reproduction Efficiency Based on the Services per Conception

The result of Service per Conception of the study is presented in Table 3.

Table 3 showed the number of Services per Conception (S/C) in T1 treatment was 1.32, while the S/C value of T2 was 1.24.

Discussion

Evaluation of AI Success Based on Non-Return Rate

Jainudeen and Hafez (2000) stated that fertilization failure was caused by several factors, including abnormal eggs, structural barrier, abnormal sperm and gamete transport. The low result of T1 was possibly caused by silent heat, embryo death, or farmers' failure to indicate estrus signs. However, the NRR result of the research was still in a good range based on Tas *et al.* (2007) stated that NRR in cattle has a range of 66.7-79.9%. Therefore, the results are in a suitable category. According to the opinion of Hafez (2008) mentioned that in general, the value of NRR is 70%.

However, the result of the study indicated that the NRR result of double-dose AI using a similar method on Ongole crossbred cows was lower than Brahman cornbred. Research by Ervandi *et al.* (2019) indicated that the result of single-dose AI in Brahman crossbred with insemination interval 0-2 h after estrus signs were NRR1 75 NRR2 85 and NRR3 85%, while AI with insemination interval 8-10 h after estrus signs was NRR 1 75 NRR2 65 and NRR3 65%.

The decrease of NRR1 and NRR2 values is possibly caused by silent heat and early embryonic death. Silent heat is generated by ovarian hypofunction, while embryo death might be caused by low progesterone hormone so

that the conceptus cannot implant or survive and eventually dies (Susilawati *et al*., 2019) (Yekti *et al.*, 2017). Moreover, other factors includ inseminators, farmers' ability to recognise estrus signs and other external factors affecting the result of Artificial insemination.

Evaluation of AI Result Using Conception Rate and Pregnancy Rate

Based on previous research by Yekti *et al*. (2019), double dose AI with Bio ATP injection and additional 3 kg concentrate feed with 16% protein for three days resulted in a conception rate of 37, 50% and Pregnancy Rate 53,13%. Moreover, research conducted by Susilawati *et al*. (2019) using single-dose AI resulted in a conception rate of 40, 62%. Similar research done by Ervandi *et al*. (2019) indicated the success of AI in Brahman crossbred at 0-2 h after estrus signs resulted in a conception rate of 25 and 20% for AI at 8-10 h after estrus signs. The result of the study also indicated hypofunction ovary for most Ongole cows. Moorey and Biase (2020) also revealed that the appropriate nutritional status is essential for reproductive success in cattle. The success of pregnancy until the occurrence of parturition is influenced by several factors including hormonal conditions, adequacy of nutrition and energy, environmental factors and the presence of infectious diseases (Parmar *et al*, 2016).

Hafez (2008) stated that the absence of signs of estrous or called silent heat is caused by a failure of ovarian growth caused by LH and or GnRH hormone deficiency, persistent corpus luteum is generally due to reproductive tract disease resulting from PGF2α resulting in corpus luteum persistent. The mortality of the embryo is caused by the lack of progesterone hormone produced by the corpus luteum, so it cannot keep the embryo's life (Parmar *et al*., 2016), (Rani *et al*., 2018). Susilawati *et al*. (2019) stated that the death of embryos could occur due to the livestock experienced reproduction disorders caused by stress with the ectoparasite and endoparasite and cows too thin or have a low body condition score.

Yekti *et al*. (2017) revealed that silent heat is the process of not achieving the conditions of estrus signs that the hormone progesterone reaches the basal and 17 β estradiol hormone does not reach the peak. Hormonal conditions are not maximal are caused by several things: Genetic and environmental factors. Genetic factors for Ongole crossbreeding are Ongole breeding cows with Limousin, or Simental derived from sub-tropical regions that are not resistant to hot temperatures. In addition, the feed factor is given to livestock, which can be seen from the Body Condition Score (BCS), which varies between 2-6.

Factors affecting the success of AI are including the breed, body condition score, feed and the age of the cattle, which was up to 15 years old. Moreover, the inseminator's skill also plays a role in the success of the AI, including the thawing method used by the inseminator. The previous research conducted by Yekti *et al*. (2019) applied 37°C for 10 sec while this research applied tap water 25°C for 20-40 sec Salim *et al*. (2012) stated that the most effective thawing method is 37°C for 10 sec.

Services per conception result of the study indicated higher than previous research conducted by Yekti *et al*. (2019) using double dose with vitamin injection and additional concentrate feed resulted in S/C = 1,09. The S/C value of T1 was higher than T2, however this score is still on normal range for S/C value.

The number of S/C indicates the success or failure of fertilization and represents the efficiency of cow mating management (Wicaksono *et al*., 2018). Based on the study, the S/C value of T1 and T2 shows a normal range and indicates that both treatments effectively get the pregnancy.

Conclusion

The study showed that the implementation of double dose Artificial Insemination at the 2nd and 8th h after estrus signs resulted better than insemination at the 8th and 16th h after estrus signs. However, many factors affect the result, including the inseminator's skill, farmers' ability to recognize estrus signs and the variety of cows' conditions at the farmers' level.

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Author's Contributions

Aulia Puspita Anugra Yekti and Trinil Susilawati: Designing and conducting the research and writing the manuscript.

Rizky Prafitri, Asri Nurul Huda, Kuswati, Kusmartono: Collaborating in field research and interpreting the obtained data.

Ethics

The corresponding author confirms that this article is original and no ethical issues involved with certificate ethical clearance No: 128-KEP-UB-2021.

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