

The Difference of Artificial Insemination Successful Rate of Onggole Filial Cattle Using Cold Semen with Different Storage Time with Tris Aminomethane Egg Yolk Dilution Agent

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Abstract: This study was aimed to find out the artificial insemination successful rate of Onggole filial using liquid semen stored at first and fifth day diluted with tris aminomethane + 20% egg yolk. The method used in this study was field experiment and for each treatment 30 cows used for AI, hence total cows used in this study were 60 heads with following criteria have already delivery experience, age between 4 – 7 years. The successful parameter was determined using NRR, CR and S/C. The research results concluded that AI result using semen in tris aminomethane + 20% egg yolk dilution agent and cold stored for the first and fifth day of storage resulted CR 86.67% and 83.33% while S/C were 1.31 and 1.44 respectively.

Key words: *Artificial Insemination, Semen dilution agent, NRR, CR and S/C.*

a. INTRODUCTION

One of the effort to improve Onggole filial cattle genetic quality is using Artificial Insemination (AI). Senger (2003) stated that AI play an important role to improve cattle genetic quality. For supporting this genetic improvement, good quality liquid semen could be used (Hafez, 2008). High quality semen need dilution agent which could maintain spermatozoa during cooling process. The good quality of liquid semen for AI could be obtained through several process (Paola *et al.*, 2015). Tris aminomethane added with egg yolk contained components which could protect from cold shock, prevent the bacterial growth and also maintain pH stability is dilution agent commonly used (Anna Shaliutina *et al.*, 2015). The research results of Ducha *et al.*, (2011) indicated that dilution agent CEP2 added with 10% egg yolk could maintain upto 8 days. While according to Wiratri *et al.*, (2015) the utilization of tris aminomethane + 20% egg yolk are more better to maintain sperma quality during cooling process compared to CEP2 + 10% egg yolk and Skim 10% egg yolk. The previous research result which was conducted by Da Costa and not yet published showed that PO filial semen if stored in tris aminomethan could stand upto seventh day with progressive motility (45.75%), viability (49.25%) and membrane integrity (42.70%). This means that semen added with tris aminomethane egg yolk dilution agent could maintain spermatozoa quality during cooling process which have the capability as good buffer with low toxicity.

Dilution agent should have characteristics such as semen plasma i.e could imitate condition which make spermatozoa possible to stay alive against artificial condition in related to storage process. The aim of this research was to find out artificial insemination successful rate of Onggole filial cows using cold semen in Tris and egg yolk dilution agent.

II.MATERIAL AND RESEARCH METHOD

This research was carried out in public farm, Grati, Pasuruan, Indonesia from October 2014 upto June 2015. Research material used were (1) Onggole filial bull semen in tris aminomethane dilution agent stored for 1 and 5 days. (2). 60 heads of Onggole filial cows which already experienced delivery at the age between 4 and 7 years which traditionally raised by local farmers.

2.1.Research Method

Field experiment was used as research method and with following treatments:

1. Artificial insemination using liquid semen stored at first day
2. Artificial insemination using liquid semen stored at fifth day.

Each treatment was applied to 30 heads of cow hence total cows were 60 heads.

Parameters observed were:

1. Non Return Rate
2. Conception Rate
3. Service per conception

2.2. Liquid Semen Preparation With Tris Aminomethane Egg Yolk Dilution Agent

The liquid semen preparation was carried out following the method of Evans and Maxwell (1987) i.e. 1.568 g Tris-(Hydroxymethyl Amino Methane), 0.876 g monohydrate citric acid, 1.411 g fructosa and 2.540g raffinosa were put into erlenmeyer flask and added with 100 ml aquabidest. Then it was heated by putting the erlenmeyer flask on hot plate until the temperature reached 97–100°C for few minutes and lift up until solution temperature decreased to 37°C and 0.1 g penicillin and 0.1g streptomycin antibiotic and 30 ml egg yolk were added before homogenized using magnetic stirrer for 5 minutes; the amount of egg yolk added in control dilution agent based on the research result of Kulaksiz *et al.*, (2012) and Schulze *et al.*, (2016) i.e as much as 20%, followed by covering the erlenmeyer flask containing dilution agent with aluminium foil and stored at 5°C until separation between supernatant and its residue supernatant occurred and taken out and stored at 5°C until it was used.

2.3. Research Stages

Semen obtained from PO bull with motility percentage > 70% was then diluted with tris aminomethane egg yolk at concentration 100 million/ml before stored in refrigerator with temperature between 3 -5°C. At the first day and fifth day, liquid semen were put into 0.25 ml capacity straw before AI was carried out, semen was test its motility.

Liquid semen which had been put in the straw were stored in refrigerator until it will be used for AI in cows already with heat stage. Cows used were the one that already experienced pregnant and normal delivery and AI technique at position 4+.

a. SEMEN QUALITY EVALUATION

Progressive motile percentage of spermatozoa was determined based on spermatozoa movement straight to the front. Sample were dropped on the surface of object glass, covered with covering glass and observed under light microscope with magnification 400x (Susilawati, 2011). Spermatozoa concentration were counted using Neubauer counting room by diluting sample 100 times using NaCl 3%. Counting was done at 5 big counting room started from top left, top right, bottom left and bottom right. Total spermatozoa concentration at 5 counting room times 10^7 (Evans and Maxwell, 1987).

3.1. Non Return Rate (NRR) included NRR 1, NRR 2 and NRR3

NRR number was obtained by conducting heat observation on the 21st, 42nd and 62nd day after AI. If there was no heat indicator observed on those days then it was assumed that the cattle was pregnant. According to Garner and Hafez (2008) who stated that Non Return Rate (NRR) is percentage of cattle number with no oestrous cycle detected again between 60th–80th day after mating. These numbers are known as NRR number at 28 upto 35 days or NRR number at 60–90 days. NRR is a general criteria used widely for determining pregnancy.

3.2. Service per Conception

S/C number was determined by counting the number of AI services to the acceptor until pregnancy occurred, the S/C formula was as follow:

$$S/C = \frac{\text{Number of artificial insemination until pregnancy occurred}}{\text{Number of cows acceptor of artificial insemination}}$$

Caraviello *et al.*, (2006) stated that Service per Conception (S/C) is a number which indicated number of insemination to result pregnancy from a number of insemination services needed by a cow until pregnancy occurred.

Conception Rate

CR number was obtained by counting pregnant cows at first AI following this formula:

$$CR = \frac{\text{Number of pregnant cows at first AI diagnosed by rectal method}}{\text{Number of all cows inseminated}} \times 100\%$$

Susilawati (2011) and (Caraviello *et al.*, 2006) stated that Conception Rate or Conception Number is the amount of pregnant acceptor at the first AI divided by all acceptors times 100.

3.3. Data Analysis

Data analysis in this study was using ANOVA and the experimental design used was Randomized Block Design (RBD) with ten replication. If the results showed a significant effect then it was continued using Duncan Multiple Range Test. Probability less than 1% stated as significant. Data were presented as Mean and Standard Deviation (Steel and Torrie, 1991).

III. RESULTS AND DISCUSSION

3.1. Motility Percentage of Cold Semen Spermatozoa For AI

Motility percentage of spermatozoa which used for AI of Ongole filial cows are as presented in Table 1.

Table 1. Means \pm SD of spermatozoa motility in liquid semen at the 1st and 5th day using Tris Aminomethane + 20% egg yolk dilution agent

Treatment	Means \pm SD
1 st Day	66.25 \pm 13.25
5 th Day	53.75 \pm 10.75

Based on Table 1. Individual motility percentage during this study showed that addition of tris aminomethane 20% egg yolk dilution agent produced a positive result on Ongole Filial bull spermatozoa which cold stored process upto 5th day (53.75 \pm 10.75%), the analysis of variance showed a highly significant difference ($P < 0.01$), however at the 5th day still fit with Indonesian National Standard (SNI) for used in AI i.e. Motility 40%. This condition indicated that tris aminomethane + 20% egg yolk dilution agent composition have the capability to maintain spermatozoa motility percentage. Tris have the advantage as dilution agent because it had the good buffer capacity and could maintain osmotic pressure due to the salts and amino acid contents (Singh *et al.*, 2014).

While in egg yolk, phospholipid are present as an effective component to protect spermatozoa motility percentage from cold shock, beside egg yolk also contained lipoprotein, lechitin and glucosa which have the capacity to protect spermatozoa membrane integrity. Other researchers already proved that egg yolk added into dilution agent could extend shelf life of cattle spermatozoa (Vera-Munoz *et al.*, 2009).

4.2. Diagnosis comparison of pregnancy result based on Non Return Rate and rectal palpation.

Pregnancy diagnosis after NRR observation which was carried out in this study, using general method i.e palpation per rectal conducted after insemination. Pregnancy evaluation based on NRR observation and Rectal palpation are one of measurement tool to find out artificial insemination successful accuration rate.

Table 2. Pregnancy diagnosis result compared between NRR and rectal palpation.

Treatment	NRR 1 Observation		Rectal palpation	
	Pregnant	Not pregnant	Pregnant	Not pregnant
	Number of cows (heads)	Number of cows (heads)	Number of cows (heads)	Number of cows (heads)
H1	27 (90)	3 (10)	26 (86.6)	4 (13.3)
H5	25 (83.3)	5 (16.6)	25 (83.3)	5 (16.6)

The successful artificial insemination evaluation by NRR and rectal palpation observation at H1 treatment was 27 or (90%) cows with positive pregnancy and no remating from 30 heads of cattle, while only 3 was not pregnant. However reevaluation after 90 days post artificial insemination to find out more accurate pregnancy through rectal palpation the one that positive pregnant were 26 heads or (86.6%) and not pregnant 4 heads or (13.3%). A similar research result was obtained by previous researcher that beef cattle after AI evaluated using NRR method showed positive pregnancy on 28 heads of cattle and proved by rectal palpation the success were 25 heads of cattle (López-Gatius, F. 2011). This statement was also proved by other researcher that pregnancy percentage based on NRR method and evaluated by rectal palpation the result percentage was not much different (Jainudeen and Hafez, 2000). The difference of success percentage by NRR and rectal palpation occurred are due to silent heat hence difficult to be detected. The utilization of this phenomena could be used as indicator for cattle pregnancy. Evaluation based on NRR through rectal palpation easily done in pregnancy detection. However, experiences and training of officer in charge are needed hence an accuracy in pregnancy diagnosis observation with this method could be one of descent and fast monitoring and proofing the result of artificial insemination. This new technique could be conducted at pregnancy period after 30 days (Garmo *et al.*, 2008).

While observation on treatment H5 after AI evaluated using NRR and rectal palpation method the positive pregnancy cows were 25 heads from 30 cows, while 5 other cows were not pregnant as presented in Table 2. Karina *et al.*, (2013) reported that NRR 21, 41,63 days were as a comparison of first AI number of cows/NRR1 with number of cows requested to be artificial inseminated again after 90 days followed with rectal palpation detection. The result of this study were higher if compared to some studies conducted by some previous researcher (Jarnette *et al.*, 2008) who stated that dairy cow pregnancy number could reach upto 70–80% and on beef cows 66% (Jainudeen and Hafez, 2000).

Pregnancy detection efforts which are carried out for understanding the cattle reproduction status after AI is one thing urgently needed to shorten offspring interval. The successful of AI during this study was reasonably good because pregnancy number achieved was 86.67%. This figure is above the range reported by Ricardo *et al.*, (2004) where conception rate after artificial insemination of cattle were in the range of 60–73% with an average 71%. The AI successful on first conception was 55% with scale of 34–75%. Pregnancy diagnosis was more accurate conducted by Non Return Rate (NRR), rectal palpation, Conception Rate (CR) and S/C (Leutert *et al.*, 2013).

4.3. Based on Conception Rate (CR)

Conception Rate (CR) is a cow pregnancy percentage on first insemination application, CR is used as indicator for measuring cattle fertility rate. CR measurement is conducted by determining the number of cows and heifer with no reheat after 30 – 60 or 60 – 90 days after first mating or artificial inseminated. The measurement result of CR in this study are presented in Figure 1.

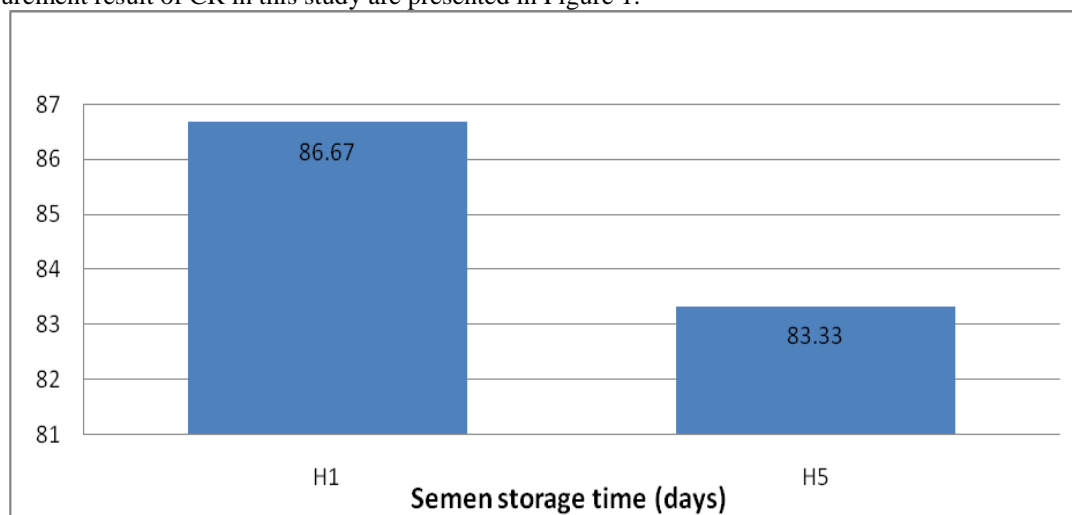


Figure 1. CR Percentage on Tris Aminomethan + 20% Egg Yolk Dilution agent during this study.

Evaluation based on CR number of cattle during the first day of study was 86.67% and CR number on the fifth day was 83.33%. This condition indicated the result could be classified as very good, because almost all of acceptor were pregnant on first artificial insemination. According to Garmo *et al.*, (2008) pregnancy number 80% as a satisfaction result and need to be maintained. In general to obtain CR number determined by clinic pregnancy diagnosis which gave a fact from around 50 days after mating. Reproduction efficiency could be stated as good if CR reaching 65–75% (Kaufmann *et al.*, 2009). The CR number result in this study especially cows with natural heat without using hormone was 86.67% which was much better compared to the research result reported by Fair *et al.*, (2004) with successful rate of 40–62% on AI method and using frozen semen. A similar result with the research result of Hill *et al.*, (1998) by using liquid semen with CR number reaching 70–80%. Reproduction efficiency of AI program result according to Pursley *et al.*, (2012) was measured from number of no return for insemination request, interval of first mating post delivery the offspring, vacant period, offspring number, offspring interval and offspring harvest number.

The high or low reproduction efficiency are series of measurements of successful AI program. Reproduction efficiency in cattle population could not be determined only by non capable producing offspring cattle proportion during its lifetime, but how big percentage of cows could give optimum reproduction characteristic performance achieved and consistent as long as their raising, because reproduction characteristics such as : offspring interval, vacant period, and number of mating per pregnancy with ideal terms in number or achievement time for reaching the reproduction efficiency.

Beside good raising management system will give an off spring number of cattle from mating result by AI reaching to 90% (John *et al.*, 2015). Beef cattle productivity could be upgraded by improving reproduction efficiency such as get better calves birth, shorten offspring interval, prolong production period and

optimization AI program management (Berry et al., 2003; Spengler et al., 2016). According to the farmers in the field during this study indicators for pregnant cows are increased appetite, no sign anymore of oestrous and more calm behaviour.

Cows inseminated with liquid semen at incorrect semen deposition will also affected the successful insemination rate (Seidel and Schenk, 2006). In this AI study 4+ position was conducted and will give CR number bigger than 4 position.

4.4. The successful of AI based on S/C

Number or frequency of mating for each pregnancy is known as Service per conception abbreviated as S/C, is one of efficiency series of beef cow's reproduction, hence measurement of S/C at AI time is absolutely needed (Ayres et al., 2014). S/C is number of AI service until a cow becoming pregnant. The research result of this study as showed in Figure 2.

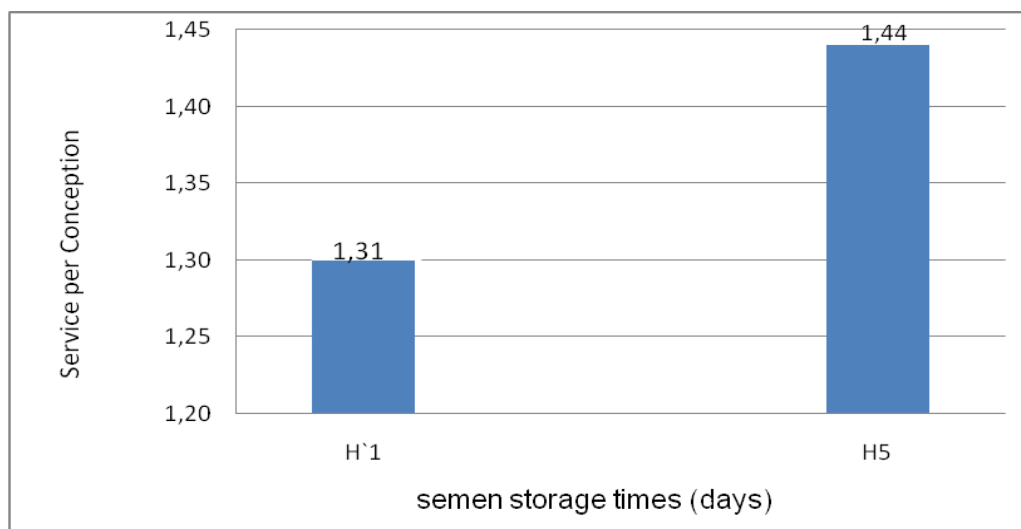


Figure 2. S/C number using Tris Aminomethan + 20% Egg Yolk during this Study.

Overall S/C evaluation in this study resulted that the best one was on the first day with means in the range of 1.31, while H5 resulted S/C of 1.44. The values obtained in this study were still below the one reported by Norman *et al.* (2011) that S/C number of cattle was 2.0–2.2. S/C number more lower than 2 means more higher cow fertility in cattle population development in one area S/C could be considered as normal i.e around 1.6–2.0. The more lower S/C number more higher fertility rate of cows and vice versa more higher S/C number more lower cow's fertility rate (Abdollahi *et al.*, 2013).

Based on S/C number obtained it could be stated that to yield once pregnancy of ongole filial cow treated 1.31 times AI (straw). This indicated that fertility rate of cattle in research location based on S/C number are adequately good. This condition could be stated as logic by considering that the farmers raised their cattle for a long time already, the farmers are well experienced in oestrous detection and artificial insemination is conducted at the right time followed by inseminator's skill in doing their job.

IV. CONCLUSION

Tris aminomethan + egg yolk dilution agent affected the spermatozoa motility of ongole filial during cold stored process. Tris aminomethan + 20% egg yolk have the capacity to give an in vivo test result of ongole filial bull liquid semen which was stored for 1 day and resulted in pregnancy rate of 86.67% and on the fifth day could yield a pregnancy rate of 83.33%. Beside the highest service per conception on the first day obtained was 1.31 and on the fifth day was 1.44.

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