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Succesfull of Artificial Insemination by Using Chilled Semen on Brahman Cross Cows

Aulia Puspita Anugra Yekti[1, a)*], Enike Dwi Kusumawati[2, b)], Kuswati[1, c)], Aswah Ridhowi[1, d)], Herni Sudarwati[1, e)], Nurul Isnaini[1, f)] and Trinil Susilawati[1, g)]

[1]*Animal Husbandry Faculty, University of Brawijaya, Jl. Veteran Malang 65145, Indonesia*
[2]*Animal Husbandry Faculty, University of Kanjuruhan Malang, Jl. S.Supriadi No. 48 Malang 65148, Indonesia*

[a)]Corresponding author: aulia_pay@yahoo.com
[b)]enikedwikusumawati@ymail.com
[c)]kuswati_bx44@yahoo.com
[d)]dhowi_fapetub@yahoo.com
[e)]herni_32@ub.ac.id
[f)]nurulisna@ub.ac.id
[g)] trinil_susilawati@yahoo.com

Abstract

Successfull of artificial insemination by using frozen semen is relatively low, because of decreasing of sperm motility after frozen process. In this study, the chilled semen was used to increase the succesful of artificial insemination. The purpose of this study was to evaluate the successful of artficial insemination by using chilled semen on Brahman Cross cows. In this study the semen was diluted with two kinds of extender were CEP without BSA + egg white albumin and tris aminomethane without raffinose. The material used on this study were 45 brahman cross x cows wich were inseminated by chilled semen using CEP without albumin +egg white (T1) and 45 brahman cross X cows were inseminated with chilled semen using Tris Aminomethane Without raffinose (T2). The chilled semen have the individual motility at least 40% to be inseminated to the female recipient which were stored at 4°C. The parameter observed were non return rate (NRR) and conception rate (CR). The data was analyzed by using chi-square analysis and t-test analysis. T-test result showed that there were significant differences ($P<0.01$) between T2 and T1 on conception rate and NRR. Then chi square result showed that there were significant differences T1 ($P<0.05$) and T2 ($P<0.01$) on conception rate wichs is the CR for T1 Was 66,7%, while on T2 was 62,2 % where the expected pregnancy rate was 60%. it indicates that chilled semen both using tris aminomethane extender and CEP extender can be used to be inseminated to the cows . In conclusions, the results clearly showed that chilled semen can be successfully inseminated to the Brahman cross cows.

Keywords: *artificial insemination, conception rate, non return rate, chilled semen, tris aminomethane*

1. INTRODUCTION

Artificial insemination is one of technology that has been proved can increase the population of cattle and also increase the genetic quality especially in Indonesia. This technology could be

maintaned by using frozen semen and liquid semen in which semen will be diluted with using extender material wich could provided the physical and chemical need to sperm life capacity. The frozen semen allows the storage of semen for a longer time, unfortunately it will cause the damage of sperm that will decrease the motility of sperm (Borges, S, *et al*, 2015) (Situmorang, 2002). Borges, S, *et al* (2015) has mentioed that the frozen semen had a less spermatozoa functionality compared to liquid semen when it is evaluated by sperm motility, thermoresistance test and hypoosmotic swelling test. Therefore inseminated semen by using liquid semen can also improve the efficiency of reproduction. Furthemore the liquid semen can persist in the female reproductive tract so that can give the higher rates of fetilization (Bucher *et al*., 2009). the conception rate of cows which is inseminated with liquid semen on 24 h is higher than frozen semen (Crespilho *et al*. 2012).

CEP-2 is a diluter with consist of many components such as fructose as a energy source, citric acid as the buffer sources, sorbitol to increase the osmolarity and bovine serum albumin (BSA) as macro-molecular component. The CEP-2 have the ionic composition mostly like liquid of bull cauda epipidymal (Verbercmoes *et al*. 2005). in this research we used the CEP-2 without BSA with the additional of egg albumin which is containing 54% of total egg protein (Alleoni dan Antunes 2004). While the tris aminomethane extender is also used to compared with CEP-2 because, this extender has been proved can maintaned the sperm life with low toxicity in high concentration (Kaeoket K, *et al* , 2011). Nolasco *et al* (2016) mentioned that the tris amino methane + 20% of egg yolk can maintaned the sperm life until 7 day after dilution. While Ducha et al (2013) showed that semen diluted with CEP-2 + 10% egg yolk could stored for 8 days after dilution.

The purpose of this study was to evaluate the successful of artficial insemination by using chilled semen in CEP-2 extender without BSA + egg albumin and tris aminomethane extender without raffinose on Brahman Cross cows.

2. MATERIALS AND METHODS

Bulls Handling

The bulls used in this study was three bulls which were raised at PT. Pasir Tengah, Cianjur, Indonesia. The bull were housed at individual housing during the experiment. The bulls were feed with additional feed on two weeks before the collected semen. Semen was colected by using Artificial Vagina with the temperature of artificial vagina was around 45-50^{o} C (Susilawati T 2013). After colected, the tube with semen colected was placed on water jacket with the temperature was 33-36^{o} C. Fresh semen with the individual motility at least 70% and mass motility was + 2 can be processed based on standart of SNI. Fresh semen was evaluated both macroscopically and microscopically which is included of individual motility, mass motility, concentration, concistency, colour, volume and pH (Susilawati, 2013).

Semen dilution

The selected semen was diluted with egg yolk caudal epididymal CEP without BSA + egg albumin (T1) and egg yolk tris aminomethane without raffinose (T2). The semen were diluted gradually until the temperature reached to 5^{o}C. Semen was placed in a straw with the volume was 0.25 ml and concentration number was 25 millions in each straw, the semen diluted was stored in temperature 5^{o}C for around 5 days. Before used for insemination, the motility semen was observed to confirmed that the motility sperm was about more than 40% based on SNI standard.

Cows Handling

The cows used in this experiment was 45 cows in each treatment both T1 and T2. The cows were housed at the group housing. The cows has been free for pregnancy by palpation rectal methods. Oestrus were observed everyday on the cows housing. The standing estrus cows were moved to mating housing and then inseminated by chilled semen. After inseminated the cows were housed on the new group housing together with other cows inseminated.

Statistic analysis

The parameter observed were Non return rate (NRR) and conception rate (CR) which is can analyzed the percentage of pregnancy of cows. Then the T test analysis was used to compare between the conception rate between T1 and T2.

3. RESULTS AND DISCUSSIONS

The Quality of Fresh Semen

The average of fresh semen were 6,4 ± 0,89 ml, this can be considered as a normal category based on Garner and Havez (2008) who stated the normal volume semen of bull is around 5-8 ml/ejaculated. The percentage of individal motllity were 70% and mass motility was ++ which is include in category normal based on Michael, et (2010). The semen colour was cream colour and pH were , while the sperm concentration was about $2470x10^6$ ± 277,488. The average of fresh semen quality used in this research were included on good category based on on Garner and Hafez (2008), Susilawati (2013). Those parameter showed that fresh semen used in this studies can be processed for chilled semen and frozen semen. The charasteristic of fresh semen that used in this experiment can be seen on the Table 1.

Table 1. Characteristics of fresh semen used in the experiment (n=5)

Parameters	Mean ± SD
Volume (ml/head)	6,4 ± 0,89
pH	6,4 ± 0,89
Concentration (10^6/ml)	$2470x10^6$±277,488
Mass motility (%)	++
Individual motility (%)	70± 0.0

Non Return Rate

The percentage of non return rate showed the number cows that did not have a sign of oestrus after the first insemination which means the cows is pregnant.The NRR can be observed in 28-3 day after inseminated or 60-90 day after inseminated (Susilawati, 2013). In this research, the NRR-1 was observed on 28 days after inseminated.

Table 2. the Non return rate percentage of cows

Treatments	Cows inseminated	NRR-1(**)
T1	45 cows	62,2%
T2	45 cows	66,7%

Note: * showed the very significant different (P<0.01)

The percentages of NRR were shown at the table. 2 where T1 or cows which were inseminated with chilled semen using CEP extender without BSA + egg albumin was about 62,2 %, while the NRR on T2 or cows which were inseminated with chilled semen by using tris aminomethane without raffinose was about 66,7%. Based on T-tets results showed that there was vsignificant differences (P<0.05) between T2 and T1 with the number T2 was higher than T1. because tris aminomethane contains more nutrions for maintaned the spermatozoa so that the quality of spermatozoa in this extender is higher than CEP-2 extender (Nolasco, *et al*, 2016).

Conception Rate

The percentage of conception rate showed the percentage of pregnancy cows that pregnant after the firs insemination. The conception rate can be evaluate which around 40-60 days after inseminated (Susilawati, 2013). In this study the conception rate was counted based on the NRR number and after that by using rectal palpation to observed the pregnancy.

Table 3. the Conception rate percentage of cows

Treatments	Cows inseminated	CR (*)
T1	45 cows	62,2%
T2	45 cows	66,7 %

Note: * showed the significant different (P<0.01)

From the Table 3. Can be seen that the conception rate of T1 and T2 both more than 60%, the conception rate value was within the normal range based on Touchberry opinion (2003) who mentioned that the conception rate value must be 60% in order to maintain the calving interval on 365 days. The T test result showed the significant differences between T1 and T2 with the T2 value was higher than T1. Furthemore the chi-Square analysis results showed that the conception rate value was significant differents(P<0.05) on T1 and very significant differences (P<0.01) on T2 compared to expected conception rate value. Based on Crespilho, *et al* (2012) mentioned that the cows which is inseminated with liquid semen on 24 h have a higher conception rate compared to frozen semen. The previous study from Nisaus, S *et al* (2016) showed that the motility of sperm with CEP-2 extender without BSA + egg albumin is more than 40% until 6 days after diluted. the liquid semen has a high motility of sperm which will increases the conception rates on cows (Borges, S *et al*, 2015).

4. CONCLUSIONS

The successful of artificial insemination by using chilled semen with extender egg yolk tris aminomethane withour raffinose give the higher result of non return rate and conception rate compared to the cilled semen with extender CEP-2 without BSA + egg albumin.

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