The Pregnancy Evaluation on Ongole Crossbred Cows by using Liquid Semen and Frozen Semen

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Abstract. Artificial Insemination using liquid semen is needed for areas where there is no liquid nitrogen and the high price of liquid Nitrogen and equipment for storing frozen semen. The purpose of this study was to determine the success rate of Artificial Insemination using Frozen semen and liquid semen on different diluents in cattle Ongole crossbred. The research method used was Field experiment design. The material used was 81 Ongole Crossbred cows. Each treatment was 27 head. Control was T0 = 27 Cows in AI use frozen semen, T1 = 27 Cows in AI uses Liquid semen with CEP-3 + 10% egg Yok diluter and T2 = 27 Cows in AI use Liquid semen with Tris Amino methan + 20% egg Yok diluter. Cows were selected by purposive sampling which was normal reproduction and showing normal signs of estrus. AI methods was Recto vaginal method with 4+ semen deposition (Uterus Position). The parameter observed were Non Return Rate and Conception rate. The pregnancy determination using the per rectal palpation method.

The results showed that the value of NRR-1, NRR-2 and NRR-3 in T0 = 81,48%, 81,48% and 81,48%; of T1 = 81,48%, 81,48% and 70,37% while T2= 96,30%, 88,89% and 81,48%. Whereas the Conception rate was at T0,T1 and T2 = 62,96%, 51,85% dan 59,26%. In conclusion, the success rate of liquid semen AI using CEP-3 + 20% egg yolk diluent and Tris Aminomethan + 20% egg yolk gave the same effect compared to AI using frozen semen.

1. Introduction

Ongole Crossbred Cattle is one of Indonesia's local beef cattle that have good productivity [1]. Local cattle can be increased in productivity by using Artificial Insemination technology [2]. Currently the popular AI program is using frozen semen, but the disadvantage is that not all locations have liquid nitrogen and expensive. [3] explained that spermatozoa after freezing and thawing cause damaged membranes. The use of frozen semen faced several problems, about 30% of spermatozoa died during freezing and spermatozoa survive during freezing has low fertility. Beside the price of liquid nitrogen is quite expensive and it is not always found outside Java or small cities.

To overcome these problems, liquid semen technology has been developed, The results showed the pregnancy rate in the use of liquid semen at 1 day storage was 86.67% higher than the use of frozen semen which was only 63.33% [4]. [1] mentioned that extender of CEP-2 + 10% egg yolk which has the best extender to maintain quality Artificial Insemination (AI) is a reproductive of stored semen at refrigerator temperature (4-5°C) technology that can improve the genetic quality of that is suitable for artificial insemination with the livestock.
Another advantage of liquid semen technology is the easy method and does not require expensive equipment. So that it can be used for industrial-scale nursery units or on farms [5]. Liquid semen is made by adding diluent into the semen then cooled. The diluent provides an environment for spermatozoa that physically and chemically resembles plasma semen, does not contain toxic substances, and does not reduce fertility. One of the diluent that is currently used is CEP-2 (Cauda epididymal Plasma). CEP-2 has chemical compositions like NaCl, KCl, CaCl2 (H2O) 2, MgCl2 (H2O) 6, NaHCO3, NaH2PO4, KH2PO4, fructose, sorbitol, Bovine Serum Albumin, Tris, penicillin, streptomycin, citric acid [6] plus 10% egg yolk [7]. In use CEP-2 diluent obstacles that Bovine Serum Albumin (BSA) as one of the ingredients in the CEP-2 diluent is imported products are difficult to obtain and very expensive precious, so it takes diluent replacement which can be obtained easily and inexpensively and can maintain the quality of the spermatozoa properly, CEP-3 diluent is one of the semen diluents modified from CEP-2 diluents, namely BSA (Bovine Serum Albumin) component in CEP-2 replaced with egg white. research result [8] BSA is able to replace egg whites in CEP-2 diluent for liquid semen storage until day 5, hereinafter called CEP-3. use egg yolks as a diluent is also known to protect sperm from cold shock.[3] explain egg yolks contain amino acids, lipoprotein and lechitin which function to protect spermatozoa from cold shock, because it has the ability to protect the integrity of the spermatozoa membrane. This study aims to determine the success rate of AI using liquid semen with CEP-3 + 20% egg yolk diluents and Tris aminomethan + 20% Egg yolk in Cow Ongole crossbred. [9] reveals that there is a constraint in some area in Indonesia is the absence of liquid nitrogen, therefore it needs AI technology the sperm motility better than other diluent for Bali using liquid semen [10] which cattle sperm and supported by [11] has some storage techniques for AI applications that the usage of CEP-2 diluent in the processing of liquid semen can support sperm motility on Ongole Crossbred.

2. Materials and methods

2.1 Data collection and animal criteria
This research was conducted at the Sumber Sekar Field Laboratory, Dau District, Malang Regency for processing semen and cattle in the people’s farms in the District of Tumpang, Malang Regency. The material used is frozen semen from AI center Singsosari as a control and Liquid semen was collected from two bull Ongolle Crossbred and 81 cows from Ongole Crossbred selected purposively sampling with the criteria of having a normal birth and good estrus conditions and on AI using the rectovaginal method in the position of deep insemination (4+) [9].

2.2 Treatments of research
Field experimental research methods, T0 = 27 Head in AI with frozen semen (control); T1 = 27 head on AI with liquid semen using CEP-3 diluter + 20% Egg Yolk and T3 = Tris Amino Methan + 20% Egg Yolk. The liquid semen used was in which has been stored for 3 days [12].

2.3 CEP-3 diluents preparation
CEP -3 diluter have chemical compositions such as NaCl, KCl, CaCl2(H2O)2, MgCl2(H2O)6, NaHCO3, NaH2PO4, KH2PO4, fructosa, sorbitol, Tris aminomethan , penicilin, streptomisin, citric acid, egg whites, aquades + 10% egg yolk , while tris aminomethan have chemical compositions such as tris aminomethan, citric acid, lactose, fructose, Penicylin, streptomycin, Aquades + 20% egg yolk.

2.4 Tris aminomethane diluents preparation
Preparation of trisaminomethan + 10% egg yolk was for 100 mL as Susilawati [3] with the composition were tris amino methan 1.363 g, citric acid 0.762 g, lactose 1.5 g, fructose 0.5 g, egg yolk 10 g, raffinosa 2.7g, streptomycin, 0.1, aquabidest 80 mL, penicillin 0.1. all the materials were homogenized and mixed with antiAliotics. After that egg yolk was added as much as 10% then homogenized. The diluents were taken as much as 5 mL, then centrifuged for 2 times in 30 minutes
with 1500 rpm. The last, the supernantant was taken as much as 3 mL.

2.5 Variable of research
The research variable observed in this study was the Non Return Rate (NRR-1, NRR-2 and NRR-3). NRR-1 observations Estrus were carried out on the 19-21st, NRR-2 observations Estrus were carried out on 39 - 42nd and NRR-3 observations Estrus were carried out on 63rd days after the AI. The Conception Rate is the percentage of pregnancy in the first AI, pregnancy detection used is palpation per rectal [13]. The collected data was analyzed descriptively and then continued with the chi-square test.

3. Result and Discussion

3.1 Pregnancy evaluation based on Non Return Rate (NRR)
One parameter of the success of AI is Non Return Rate (NRR). The results of the NRR analysis are presented in Table 1.

| The treatment of semen used for AI | Number of acceptors | NRR1 (day 0-21) | NRR2 (day 22-42) | NRR3 (day 43-63) |
|-----------------------------------|---------------------|-----------------|-----------------|-----------------|
|                                    | Head %              | Head %          | Head %          | Head %          |
| Frozen semen                       | 27                  | 22 81,48        | 22 81,48        | 22 81,48        |
| Liquid semen diluent CEP3 + 10% egg yolk | 27                  | 22 81,48        | 22 81,48        | 19 70,37        |
| Liquid semen diluent Tris Aminomethan + 20% egg yolk | 27                  | 26 96,30        | 24 88,89        | 21 81,48        |

Based on Table 1, it is known that the NRR-1, NRR-2 and NRR-3 in AI frozen semen showed greater results than the NRR on AI values of liquid semen in 2 kinds of diluents, but the chi-square test results showed no significant differences (P > 0.05), The low NRR value of AI frozen semen and liquid semen is probably due to the low quality of semen, the inaccuracy of AI time and imperfect quality as well. [14] the accuracy of AI time is determined by the timeliness of the detection of heat by the breeder, because the accuracy of estrous and the time of the AI greatly determine the success of pregnancy. Furthermore [15] explaining the accuracy of AI time is before ovulation, that is, if the cow shows signs of morning sickness then on the AI in the afternoon and if it shows estrus in the afternoon then on the morning AI the next day. However, each cow has a different time range. The NRR-2 AI value of liquid semen showed the same value as the NRR-1 value of 81.48%. This is likely to occur because some animals experience a silent heat. The event of silent heat results in inaccurate detection [16, 17]. [15] further explained that NRR's observations were not guaranteed to be 100% correct, because there were cows that were not pregnant but showed no signs of lust. So that more accurate examination is done by rectal palpation. Silent heat can occur due to hormonal disorders and stress due to disruption of ectoparasites and endoparasites. [15] adding factors that cause pregnant cows to experience early embryo death are abortion and mummification [18] further explained that most pregnancy failure in cattle occurs during the first three weeks after conception, especially during the period of pre-implantation pregnancy.

3.2 Pregnancy evaluation based on Conception Rate (CR)
Conception rate as a way of determining the success of AI was observed using rectal palpation [19]. Rectal palpation is a method of pregnancy examination that is practical, easy to implement and can be believed to be true. The results of the CR observations are presented in Table 2.
Table 2. Conception Rate on Artificial Insemination Using Frozen semen and Liquid semen

| The treatment of semen used for AI | Number of acceptors | Conception Rate % |
|-----------------------------------|---------------------|-------------------|
| Frozen semen                      | 27                  | 17                | 62.96              |
| Liquid semen CEP3 + 10% egg yolk  | 27                  | 14                | 51.85              |
| Liquid semen Tris Aminomethan + 20% egg yolk | 27 | 16 | 59.2 |

The value of CR on AI liquid semen is lower than the SNI set at 60%. In addition, the CR value in AI liquid semen was also lower than that of [4] regarding the success of AI using frozen semen and liquid semen the percentage of CR was 63.33% for AI frozen semen and 86.67% for AI liquid semen. The percentage value of CR is influenced by many things including the quality of semen used. Post thawing motility when going to AI will decrease to 20-40%. The low quality of semen in liquid semen is one of the causes of low pregnancy success. The motility parameters of spermatozoa are the most important parameters in the semen quality test [20, 21] the quality of bull semen collected was determined by genetic factors, age, season, temperature, libido, feed, collection method and collection frequency. In addition, the cause of the decrease in the quality of semen is that when storage of spermatozoa has lost energy to life and motility. Added by [3] Factors influencing the success of AI in addition to semen quality are female physiological conditions, inseminator skills and the accuracy of heat detection. Based on Saha et al [22], mentioned that the CR using liquid semen is lower than frozen semen on local breed and crossbred of local and frisian cow because of temperature fluctuation when semen was brought to the insemination field.

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 [23, 24]. [23] has mentioned 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. Furthermore the liquid semen can persist in the female reproductive tract so that can give the higher rates of fertilization [25]. the conception rate of cows which is inseminated with liquid semen on 24 h is higher than frozen semen [26].

[27] reveals that dilution based on tris aminomethane + egg yolk can maintain the semen quality including sperm motility, viability and cattle sperm membrane integrity during cooling process. AI 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 [28]. The liquid semen has a high motility of sperm which will increases the conception rates on cows [23].

4. Conclusion
The success rate of liquid semen AI using CEP-3 + 20% egg yolk diluent and Tris Aminomethan + 20% egg yolk gave the same effect compared to AI using frozen semen. Conception Rate on frozen semen AI, Liquid Semen using CEP-3 + 10% diluent egg yolks and Tris Aminomethan + 20% egg yolk diluent were 62.96%, 51.85% and 59.2% respectively.
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