

The Effect of Fresh Semen Dilution of Pesisir Cattle With Combination of Coconut Water and Duck's Egg Yolk on Spermatozoa Quality

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Abstract- This study aimed to determine the effect of dilution of fresh semen using a combination of coconut water with duck egg yolk at different lengths of storage on motility, percentage of life and abnormalities spermatozoa of pesisir Cattle. Complete Random Design in Factorial 3 x 5 with 3 replications in each treatment used in this research. Factor A was the use of diluent by combining coconut water with duck eggs, A1 = pure sperm, A2 = 75% young coconut water + 25% duck egg yolk, A3 = 75% old coconut water + 25% duck egg yolk. Factor B was the long of storage : B1 = 0 days, B2 = 2 days, B3 = 4 days, B4 = 6 days, and B5 = 8 days. The results showed a very interaction significant ($P < 0.01$) on the combination of coconut water and egg yolk with long storage on motility, life percentage and abnormalities of pesisir cattlespermatozoa. The conclusion of this study is the fact that dilution of fresh semen with a combination of coconut water with duck egg yolk can complement each other by providing a comfortable environment for spermatozoa so that they were protected from cold shocks and the fulfillment of energy resource requirements for pesisir cattlespermatozoa. The best resulted in this study was a combination of 75% old coconut water with 25% duck egg yolk with a ripening period of 6 days.

Keywords: *quality spermatozoa, Cattle pesisir, coconut water, duck egg yolk,*

I. INTRODUCTION

pesisir cattle is a breed of local Indonesian cattle that are small in size but capable of breeding well even with limited nutritional supply. Hendri (2013) stated that pesisir cattle is one of five cattle breeds native to Indonesia after Bali cows, Aceh cows, Sumbawa cows, and Madura cows, they had the ability to adapt to the conditions of the coastal environment that were poor forage, and a high percentage of carcasses. The population of cattle has been decreased from year to year because of the maintenance carried out traditionally, with a natural reproductive system, high productive cattle slaughter, limited feeding, extensive depletion of grazing, inbreeding, lack of availability of superior bulls and genetic degradation (Adrial 2010). Therefore it is very important to improve the genetic quality of these cattle through selection and reproduction improvement. One effort to improve the productivity of cattle can be done with the Artificial Insemination method. The success of artificial insemination is influenced by the quality of male semen and the treatment of semen (Wijayanti and Simanjuntak, 2006). The quality of semen in relation to fertility is determined by the morphological features of spermatozoa, the percentage of live spermatozoa concentration and

motility of spermatozoa and the ejaculated volume. The length and breadth of the sperm cells add to the quality in respect of fertilizing capacity (Akhter, 2013). The use of sperm for artificial insemination needs to be mixed with a diluent solution that guarantees physical and chemical requirements and can be stored at certain temperatures and conditions that maintain the life of spermatozoa during the desired time to then used as needed. Sperm dilution aims to lengthen the life and fertility of spermatozoa. The diluent contains food substances as an energy source and was not toxic to spermatozoa, can protect spermatozoa from cold shock, inhibiting microbial growth and be a buffer (Widjaya, 2011).

Semen dilution nowadays generally using synthetic materials such as egg yolk tris aminomethane (Aziz et al, 2018). This research aimed to find a source of natural spermatozoa dilution and easy to get it, non-commercial. The dilution in this research used coconut water and duck egg yolk. The nutritious content of young coconut water and old coconut water have different values, old coconut water has a protein content of 0.14 g, calories 18.50 cal, fat 1.50 g, carbohydrate 3.60g, young coconut water has 0,20g protein composition, 17,00 cal calories, 3,80 carbohydrates (Palungkun, 1992). Egg yolks contain lipoproteins and lecithin when be used as dilution semen it will be as food and energy sources, the protective of extracellular spermatozoa from cold shock. Research of Kulaksiz et al, (2010), Duck egg yolk was higher protein, fat, and cholesterol content than chicken egg yolks, more beneficial as cryopreservation of sheep sperm than some poultry species other than chicken. These components are beneficial for spermatozoa during the cryopreservation process because they specifically function as protective components (Kulaksiz et al, 2010; Gholami et al, 2012). Ryan's research (2015) that dilution using (25% egg yolk - 75% coconut water) was the best diluent combination to maintain the abnormalities, viability and motility spermatozoa with storage until 2-day, it could be an alternative medium of diluent frozen spermatozoa of Bali cattle. this research was conducted to evaluate the use of dilute fresh semen a combination of Coconut water and Egg Yolk Duck on Motility, Life Percentage and Abnormalities of Spermatozoa pesisir cattle.

II. MATERIALS AND METHODS

The material used is fresh semen obtained from coastal cow male, old coconut water, coconut water, and a duck egg yolk.

Implementation of this study are as follows:

The male of pesisir cattle brought to the cage and was met by a female angler to provide stimulus to the male libido before shelters the cement. The quality of fresh semen was evaluated by macrocosmic, and the quality of spermatozoa. The spermatozoa of pesisir cattle are diluted using old and young coconut water combined with egg yolk duck using a ratio of 75:25 with different storage time. The ideal storage temperature is 5 ° C spermatozoa (Toelihere, 1985).

Factorial in Complete Random Design 3 x 5 with 3 replications in each treatment used in this research.

Factor A was the using of diluent by combining coconut water with duck eggs, there were : A1 = pure sperm, A2 = 75% young coconut water + 25% duck egg yolk, A3 = 75% old coconut water + 25% duck egg yolk.

Factor B was the long of storage: B1 = 0 days, B2 = 2 days, B3 = 4 days, B4 = 6 days, and B5 = 8 days.

The Variables Observed were :

1. The percentage of spermatozoa of pesisir cattle after the addition of coconut water and egg yolk duck at 5 ° C.

The formula to calculate the percentage of spermatozoa (Toelihere, 1985).

$$\text{Percentage of life} = (\text{life cell sperm}) / (\text{collecting cell Sperma}) \times 100\%$$

2. The percentage of motility (movement power off) after the addition of spermatozoa cattle coastal coconut juice and egg yolk duck at 5 ° C.

The formula to calculate motility (Toelihere, 1985).

$$\text{motility} = (\text{cell motile sperm}) / (\text{cell Sperma observed}) \times 100\%$$

3. The percentage of abnormalities (spermatozoa defects) after the addition of spermatozoa pesisir cattle with coconut water and egg yolk duck at 5 ° C.

formula menghitung abnormal spermatozoa (Toelihere, 1985).

$$\text{abnormalities} = (\text{sperma cell abnormal}) / (\text{sperm cell observed}) \times 100\%$$

III. RESULTS AND DISCUSSION

The Macrokopis Evaluation spermatozoa of Fresh Semen pesisir cattle

The Fresh semen pesisir cattle has been accommodated, then evaluated to determine the feasibility of cement for further processing, determine the volume of diluent should be added and to know the amount of straw that can be generated in the process of freezing semen (Feradis, 2010). The macroscopic evaluation of fresh semen including color, volume (ml), consistency, pH, and microscopically is a mass movement, motility (%) and concentration (million/ ml). Fresh semen motility value data obtained during the study of the pesisir cattle are presented in Table 1. The volume semen of pesisir cattle obtained was 8 ml. This sperm volume more than the research Hartanti et al. (2012) who get Cattle Jabres semen volume range of 3.2 -7.3 ml. According to Garner and Hafez (2000) volume of cattle semen ejaculation, every single time ranges from 5-8 ml.

Table 1. The Average of Observations Fresh spermatozoa Semen pesisir cattle are Makrokopis and microscopic

observations Makrokopis	Result
Sperm Volume (ml)	8 ml
Color Spermatozoa	beige
consistency spermatozoa	thick
Sperm concentration (M / ml)	1600
(PH) spermatozoa	6
motility	80 %
Mass movement	+++

The fresh semen color is beige. According to Toelihere (1981), normal semen has a color such as milk or cream whitish and turbid. The concentration of fresh semen obtained by 1600x 106sel / ml.

The Sperm Motility Of pesisir cattle

The average of the treatment affected by the combination of coconut water and duck egg yolk on the motility of spermatozoa pesisir cattle with different long storage can be shown in Table 2.

Table 2. The average of affect coconut water combination with duck egg yolk on sperm motility of pesisir cattle(%)

factor A	factor B					Mean
	0 Day	2 days	4 days	6 Days	8 Days	
A1	88,33 ^{aA}	49,66 ^{bB}	11,33 ^{cC}	0 ^{eB}	0 ^{eB}	29.867
A2	82,66 ^{aA}	75,55 ^{aA}	55,66 ^{bB}	40,5 ^{cA}	18,66 ^{dA}	54.6
A3	84,66 ^{aA}	80,33 ^{aA}	69,33 ^{bA}	49,66 ^{cA}	28 ^{dA}	62.4
Average	85.22	68.5	45.44	30.056	15.56	

note :. Superscript (a, b, c, d) on the same line and (A, B, C) in the same column shown the high significantly affected (P <0.01). A1: pure sperm, A2: 75 young coconut water + 25% duck egg yolk, A3: old coconut water 75% + 25% duck egg yolk. B1: storage 0 hour, B2: 2day, B3: 4 days, B4: 6 days, B5: 8 days. Superscript (a, b, c, d) on the same line and (A, B, C) in the same column shown the hight significantly affected (P <0.01).

The results of the analysis of variance showed a highly significant interaction (P <0.01) between the treatment of coconut water with different long storage on the semen motility of Cattle pesisir. This is because coconut water was supplemented with egg yolk duck has a complementary role as a buffer, so that sperm can remain motionless until the storage time of 8 days, spermatozoa without diluent can only move up to long 4 days because there was no given buffer so that sperm can't live long.

Further test results by Duncan Multiple Range Test (DMRT) showed that the use of old coconut water was significantly different from the use of young coconut water in 4 days of storage, while the storage of 0, 2, 6 and 8 days showed similarly affected. This is presumably because of the sperm storage 0 days still in fresh condition so as no significant affected (P > 0.05) with the diluent. This is because on the first day of spermatozoa in fresh condition so it does not affect the coconut water diluent young and old coconut water. In Table 2 looks at the storage time 6 and 8 days semen coastal cattle without the use of diluent had no one moved, while the semen using coconut water diluent can still survive with the percentage motility of 40.5% on the sixth day and decreased to 18, 60% on the eighth day on semen cows using coconut water, while the percentage of semen motility pesisir cattle using an old coconut water with duck egg yolk sixth-day percentage is 49.66%, and decreased on the eighth day 28%. This showed that the use of coconut water diluent combination with duck egg yolk was able to provide nutrition and environment needed to keep moving spermatozoa, and protects sperm from cold shock effect so that it can survive in conditions of motile until the eighth day. This condition shows that the use of coconut water diluent combination with duck egg yolk was able to provide nutrition and environment needed to keep moving spermatozoa, and protects sperm from cold shock

effect so that it can survive in conditions of motile until the eighth day. This condition is consistent with the statement This shows that the use of coconut water diluent combination with duck egg yolk was able to provide nutrition and the environment needed to keep moving spermatozoa, and protects sperm from cold shock effect so that it can survive in conditions of motile until the eighth day. This condition is consistent with the statement Toelihere, (1993) that motility of spermatozoa was affected by metabolic capabilities supported by the environment such as temperature and the components in the medium diluent, this is in accordance with the statement Solihati and Kune (2009), the terms motility that can be used for artificial insemination is 40%. Spermatozoa results of this study more better compared with Nune et al (2004) who use coconut water as a diluent spermatozoa goat and research results Ismaya et al (2015) who used a diluent coconut water plus 30% yolk duck eggs were only able to maintain motility up to 2 days, while the results of the study Affandhy (2003) and Afiati et al. (2003) showed that sperm motility in semen of Simmental cattle and crossbreed ongol diluted with egg yolk and coconut water only lasted until the third day.

The living percentage spermatozoa of Cattle pesisir

The average affected of treatment with the combination of coconut water duck egg yolk on the percentage of spermatozoa pesisir cattleat different storage times can be seen in Table 3.

Table 3. The Average affected of Combination Coconut Water with Duck's Eggs Yolk on The living percentage spermatozoa of Cattle pesisir(%)

factor A	factor B					Mean
	0 Day	2 days	4 days	6 Days	8 Days	
A1	80,83 ^{aA}	56,33 ^{bB}	31,33 ^{cB}	0 ^{dc}	0 ^{dB}	33.70
A2	80,66 ^{aA}	71,33 ^{aA}	53,83 ^{bA}	35,5 ^{cB}	28,33 ^{dB}	53.93
A3	82,66 ^{aA}	75,83 ^{aA}	64,83 ^{bA}	54,66 ^{cA}	37 ^{dA}	63
Mean	81.389	67.83	50.00	30.056	21.78	

Note : Superscript (a, b, c, d) on the same line and (A, B, C) in the same column shows the different treatments were significantly ($P < 0.01$) A1: pure sperm, A2: coconut water 75 + duck egg yolk 25%, A3: old coconut water 75% + 25% duck egg yolk. B1: storage 0 hour, B2: 2day, B3: 4 days, B4: 6 days, B5: 8 days.

The analysis results showed an affected on significant interaction ($P < 0.05$) between the treatment combination of coconut water and duck's egg yolk with storage long on the life percentage of pesisir cattlesemen. This was because the use of coconut water with duck's egg yolk was able to provide nutrients for sperm to survive until the storage time of 8 days. Further test results Duncan Multiple Range Test (DMRT) showed that the using of old coconut water was significantly different from the use of coconut water at 6 and 8 days of storage, while storage 0, 2 and 4 days showed there was no difference ($P > 0.01$), This suggests sperm storage 0 days in the fresh conditions that no affected without or with the diluent. The results showed that the use of diluents combination of coconut water with duck egg yolk showed the no different affected ($P > 0.01$) with no diluent on days 0, whereas the subsequent storage (2, 4, 6, and 8

days) showed a significantly different effect ($P < 0.01$), where the percentage of spermatozoa without the use of diluents significantly lower compared with the use of diluents spermatozoa, according to Yani et al. (2001), the percentage of live spermatozoa were still high at the start of storage caused by the availability of energy needed substance, which is still stable buffer solution, which is isotonic osmotic pressure, and age are still fresh spermatozoa.

The average percentage of spermatozoa was 54.66% by using the old coconut water diluent, and 35.5% by young coconut water diluent. This illustrated that the diluent used old coconut water was able to provide the nutrients and energy needed spermatozoa to survive longer than young coconut water. The combination of old coconut water with duck's egg yolk was able to protect the sperm from the effects of cold shock, Reduced energy in the diluent, a decrease in pH, toxic effect on the seminal plasma, and the osmotic pressure of the diluent (Affandhy, 2003; Isnaini, 2002; Garner and Hafez 2000; Toelihere, 1981).

These results showed that the data percentage of live sperm was lower than the data Motility. These resulted indicate that the pesisir cattlesperm can still be used after being stored for 6 days using a diluent using a combination of old coconut water with duck's egg yolk. the percentage of spermatozoa pesisir cattle can be seen in the picture. 1.

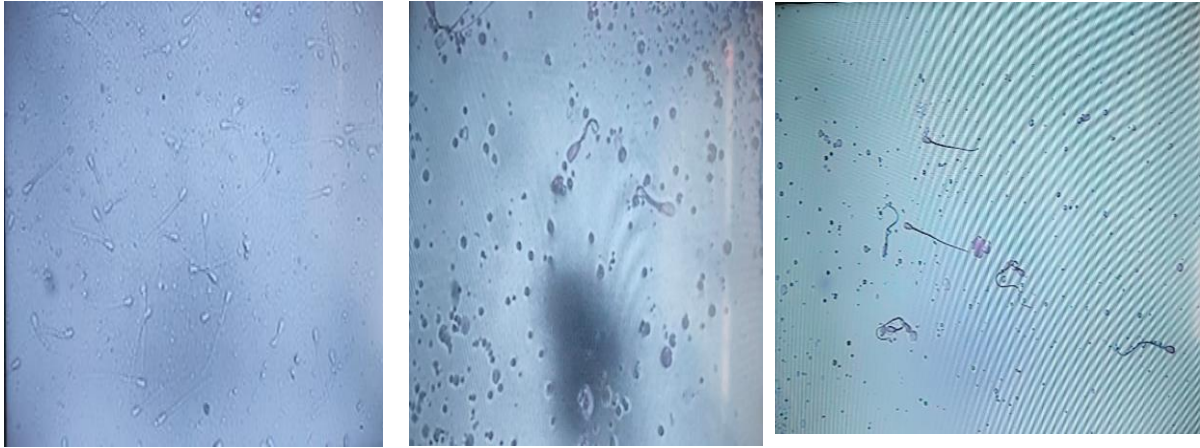
The Sperm Abnormalities of Cattle Pesisir

The average affected of treatments by the combination of coconut water duck's egg yolk to the percentage of spermatozoa pesisir cattle at different storage times can be seen in Table 4.

Table 4. The Average Coconut Water Combination with Duck's Egg Yolk on sperm abnormalities (%)

factor A	factor B					
	0 Day	2 days	4 days	6 Days	8 Days	Mean
A1	8,33 ^{aA}	15,67 ^{aA}	17 ^{aB}	18,33 ^{aB}	19 ^{aB}	15.67
A2	10 ^{eA}	16,83 ^{dA}	26,67 ^{eA}	42,83 ^{bA}	56,67 ^{aA}	30.60
A3	9,16 ^{eA}	13,83 ^{dA}	21,83 ^{eA}	35 ^{bA}	49 ^{aA}	25.77
Mean	9.17	15.44	21.83	32.06	41.56	

Information. Superscript (a, b, c, d, e) on the same line and (A, B, C) in the same column shows the different treatments were significantly ($P < 0.01$) A1: pure sperm, A2: coconut water 75 + yolk duck 25%, A3: old coconut water 75% + 25% duck egg yolk. B1: storage 0jam, B2: 2day, B3: 4 days, B4: 6 days, B5: 8 days.



Sperm that do not absorb the dye of eosin is live sperm.

Abnormal Spermatozoa
Large-headed spermatozoa, short tail, tail folded

Large-headed spermatozoa, short tail, tail folded

Based on the variance analysis of the results showed the significantly affected ($P < 0.05$) between the treatment combination of coconut water and duck's egg yolk with the storage time on the semen abnormalities of Cattle Pesisir. This was because of the content of old coconut water with duck's eggs different from young coconut water with duck's egg yolk. The Protein content of the young coconut water with duck's egg yolk more higher than the old coconut water with duck's eggs yolk.

In table 4 showed that the spermatozoa abnormalities found in storage 0 days without diluting the percentage (8.33%), while the highest abnormality found in spermatozoa using a combination of coconut water diluent with duck egg yolk on the storage time of 8 days (56.67 %). Further trials using the Duncan Multiple shows the effect of the abnormalities were no different affected ($P > 0.01$) between using or does not use a diluent at 0 and 2 days. The spermatozoa storage 4, 6, and 8 days showed significant differences affected ($P < 0.01$) between spermatozoa using diluents and without using of diluent.

Table 6. Showed pesisir cattlespermatozoa abnormalities significantly lower than that using a diluent, but each diluent combination of coconut water with duck egg yolk and old coconut water with duck egg yolk showed similar effects. The results of this study do not differ much with research Dwatmadji et al (2007) showed that in terms of Nubian goat spermatozoa abnormalities may only be used up to two days of storage.

Figures percentage of abnormalities of spermatozoa cow this coast is increasing with the increase in the old days of storage, this is due to increasing the amount of time it causes changes in acidity (pH) and the changing balance of nutrients so that the increased percentage of abnormal sperm, a condition in accordance with the opinion of Kamal et al (2005) which states abnormalities more than 20% spermatozoa can not be used for artificial insemination.

CONCLUSION

the results of this study can be concluded, dilution of fresh semen with a combination of coconut water 75% with egg yolk duck 25% and an old coconut water 75% and egg yolk duck 25% with the storage time is different effect on the percentage motility, percentage of live and abnormal spermatozoa cows coast and able to survive kept for 6 days in an old coconut water treatment with duck's egg yolk, but for an old coconut water treatment abnormalitas with duck

egg yolk most optimum can only be stored for 2 days. Coastal cattle sperm that can be used for artificial insemination is in storage for 2 days.

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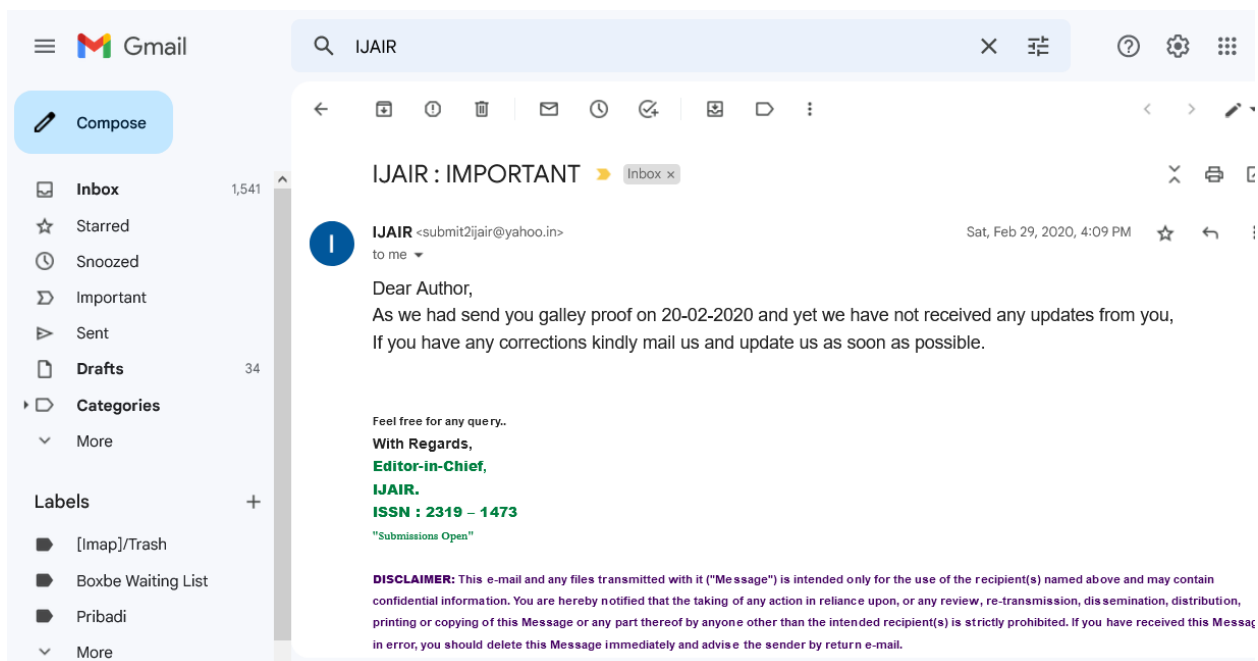
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Dear Tri Astuti, R.M Sari, S.A. Akbar, and D. Kornelio,

Paper ID : IJAIR-3140

Paper Title : **The Effect of Fresh Semen Dilution of Penisir CattleWith Combination of Coconut Water and Duck's Egg Yolk on Spermatozoa Quality**

The review process for International Journal of Agriculture Innovations and Research (IJAIR) has been completed. Based on the recommendations of the reviewers and the Technical Program Committee, we are pleased to inform you that your paper identified above has been accepted for online publication. You have to **update your paper as per reviewer's comments**, so that it can be published. You are requested to complete the formalities. This notification email serves as our formal acceptance of your paper as well as consideration for online publication provided you complete the formalities and update the paper as per reviewer's comments.

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