Evaluation of Pasundan Cattle semen quality in three different types of extender

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Abstract. One of the important genetic resources of livestock in West Java is Pasundan Cattle. The name of Pasundan Cattle has been determined by Minister of Agriculture of the Republic of Indonesia by Decree No. 1051/Kpts/RI/SR.120/10/2014 as uniformity for local cattle that previously had different names. The productivity of Pasundan Cattle until now has not optimum, especially in the community farmers and research centers. Many factors could be influenced of Pasundan Cattle productivities, including the semen quality of bull as a source and play important role in the breeding program of Pasundan Cattle. Based on this background, it was necessary to conduct this research on the exploration and evaluation of Pasundan Cattle semen quality in three different types of extender. The aim of the research is to find out the best of extender of Pasundan Cattle semen quality. This research was conducted to compare three types of semen extender (TRIS, Citrate and Skim Milk extenders) with addition 20% Egg yolk on each extender. Semen collected two times a week from 9 Pasundan Cattles (3-5 year old), consisted of 9 ejaculated semen and has been evaluated macroscopic and microscopic. Parameter of this research is motility, abnormality, intact plasm membrane and viability of Pasundan Cattle semen. Data analyzed with Anova and advance analysis of different between treatment followed with Duncan test. Result of this research showed that there was no significantly differences between TRIS and Citrate (p> .05) on motility but significant different with Skim milk extender. Otherwise there was significantly different between TRIS and SKIM on intact membrane plasm (p< .05) but not significant different with citrate extender. Meanwhile for abnormality and viability, there were no significantly different on each extenders. As an conclusion, TRIS as the best extender for Pasundan Cattle semen quality.

1. Introduction

Pasundan cattle is one of any local cattlees that under developing in Indonesia. Based on Minister of Agriculture of the Republic of Indonesia by Decree No. 1051/Kpts/RI/SR.120/10/2014, Pasundan Cattle was generated and adapted from 10 generation from/between Bos sundaicus (Bali Cattle) with Javanese/Madura/and Sumba ongole cattle. Pasundan cattle in Indonesia used to meat production. In term of development of Pasundan Cattle population in Indonesia, reproductive sector as the main of any factors to complete the Pasundan cattle needs, such as Artificial Insemination (AI) technology in effectiveness of population developments. Artificial Insemination technology has used to give any female cows from one bull semen, different with natural mating that one bull just only for one female cow. To have a good semen quality of AI, semen extender solutions is the main factor to maintain sperm quality. Semen Extender or diluent is a chemical medium used for preservation, extension
and protection of sperm cells against various shocks during processing, storage and transportation used for artificial insemination [1]. Some extender solutions such as Tris-Egg yolk, Citric Acid-Egg yolk, and Skim Milk-Egg yolk. The main of sperm extender solution is to keep secure sperm membrane from cold shock caused by temperature decrease.

2. Material and methods

2.1. Material
Semen as an research object was obtained from nine Pasundan Cattle (3-5 years old) and reared in Center of Beef Cattle Breeding Development and Artificial Insemination Institute Cijeungjing, Ciamis.

2.2. Methods
Semen was collected from each cattle twice a week on Tuesday and Thursday, by means of artificial vagina.

Immediately after collection, the ejaculates were placed in a water bath (37ºC) and aliquots were taken for the assessment of semen quality. After individual examination, only ejaculates with at least 85% estimated progressive motility, were used for treatment.

Three extenders were prepared as follows: TRIS (Trishydroxymethyl aminomethane), Citric acid and Skim Milk with addition 20% Egg yolk on each extender. Dilution was done to their extenders and followed by microscopic analysis.

Parameter of this research is motility, abnormality, intact plasm membrane and viability of Pasundan Cattle semen. Data analysed with Anova and advance analysis of different between treatment followed with Duncan test.

3. Result and discussion

3.1. Effect of different extender on motility of Pasundan Cattle Semen
Motility of semen after addition of three different extenders could be seen in table 1.

| No. | Treatment                  | Percentage ± SD |
|-----|---------------------------|-----------------|
| 1   | TRIS-Egg Yolk             | 73.89 ± 3.29    |
| 2   | Citric-Egg Yolk           | 69.85 ± 5.08    |
| 3   | Skim Milk-Egg Yolk        | 64.80 ± 5.03    |

Table 1 showed that the addition of extender TRIS to the sample semen in dilution process have bigger motility compared with other extender (Citric and Skim Milk). However result of statistical analysis showed that there were no significantly different (p> .05) between TRIS and Citric, and different between TRIS and Skim Milk. TRIS is the one of compounds used for semen extender that called as buffer solutions. Based on [2], TRIS is the semen extender that forming two hydrogen (H) bounds to maintain protein bases. Protein is the complex molecules and the traits has affected by any specific interactions. Strengthness of TRIS has an interaction with peptides chain. Egg yolk composed by complex polypeptides chain, so the interaction between TRIS and egg yolk has a positive impact to the maintenance of sperm plasm membrane. Intact Plasm Membrane affects to spermatozoa quality like motility. This correlation happened because mitochondrion in mid-piece of the sperm has produce optimum energy for sperm metabolism and movement. TRIS has a low toxicity value and maintain sperm pH, osmotic pressure, and electrolyte balance [3, 4].

Based on this research, TRIS-egg yolk affects sperm motility. Motility of the sperm affected by energy product from mitochondria called as ATP for sperm metabolism and movement by ATP distribution to the sperm tail. Combination on TRIS-egg yolk as semen extender can maintain the
intact plasm membrane which contain mitochondria inside the plasm membrane. Good mitochondria can produce optimum ATP for the sperm metabolism and motility. Technically, TRIS-egg yolk has a good cost efficiency. The motility of sperm is the most important criterion used for semen assessment, both for chilled and frozen semen.

3.2. Intact membrane plasm of Pasundan Cattle sperm

Result of Intact Plasm Membrane of Pasundan Cattle spermatozoa after addition of three types extender showed in table 2.

| No. | Treatment          | Average ± SD     |
|-----|--------------------|------------------|
| 1   | TRIS-Egg Yolk      | 73.53 ± 9.76     |
| 2   | Citrate-Egg Yolk   | 75.70 ± 4.61     |
| 3   | Skim Milk-Egg Yolk | 68.22 ± 5.58     |

There was no significantly different between treatments. It because extender containing 20% egg yolk. Egg yolk is the one of extender compounds as generally used. Egg yolk as cryoprotectant agent because inside of egg yolk contains low density lipoprotein (LDL), phospholipid, and cholesterol [5,6,7]. Fraction of LDL in egg yolk contains lipid (83% - 89%) and protein (11% - 17%). Lipid constituent of LDL have 69% triglyceride, 26% phospholipid, and 5% cholesterol [8,7]. As maintaining element of spermatozoa, phospholipid on egg yolk bounds sperm membrane and reconstruct the changes of membran structure after cryopreservation.

3.3. Effect of different extender on abnormality of Pasundan Cattle Semen

Result of abnormality value in sperm after three types extender addition showed in table 3 below.

| No. | Treatment          | Average ± SD |
|-----|--------------------|--------------|
| 1   | TRIS-Egg Yolk      | 1.28 ± 0.36  |
| 2   | Citrate-Egg Yolk   | 1.33 ± 0.35  |
| 3   | Skim Milk-Egg Yolk | 1.44 ± 0.39  |

Abnormality of sperma is the important criteria to analysed the quality of the semen and it was indicator that liquid semen could be followed for frozen semen process. The maximum value of abnormality is less then 20% when that liquid semen will followed for frozen semen process. In this study, there was no significantly different (p > .05) between treatments. It occurs in every extender consist of egg yolk and this could be protect spermatozoa.

Major sperm abnormalities come about genetic in origin and hereditary [9,10]. The other factor cause sperm abnormality were by environment. As the result of this study, sperm abnormalities from each extender categorized as normal and accepted to the next process because the abnormal sperm does not reach 20% from total (200) sperm counted [9], especially for AI program.

3.4. Effect of different extender to sperm viability

Result of sperm viability after addition of three types extender, showed in table 4.

There was no significantly different sperm viability by addition with three types extender. Sperm viability correlation with sperm metabolism. Metabolism of the sperm to maintain itself by using ATP from energy resources processed in mitochondrion. Maintained mitochondrion by intact plasm membrane could as the main factor. It correlated between viability and intact plasm membrane by using mixture of 20% egg yolk with TRIS/Citrate/and skim milk. It was analogous with [11], there was not significantly differences between extender because containing cryoprotectant from egg yolk.
Table 4. Sperm Viability after addition of three types extender

| No. | Treatment               | Average (days) ± SD |
|-----|-------------------------|---------------------|
| 1   | TRIS-Egg Yolk           | 4.22 ± 0.44         |
| 2   | Citrate-Egg Yolk        | 4.44 ± 0.53         |
| 3   | Skim Milk-Egg Yolk      | 4.00 ± 0.50         |

4. Conclusion
It was concluded that the best extender for Pasundan Cattle was TRIS-Egg Yolk because has the highest motility value than Citrate-Egg Yolk and Skim milk-Egg Yolk. Although the similar value between three types of extender to the abnormality, intact plasm membrane, and viability.

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