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The test about blood serum capabilities in maintaining the quality of bull spermatozoa during storage in cep diluent at refrigerator temperature

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Abstract. The storage of spermatozoa requires a protective material from cold shock events and the presence of free radicals. In CEP diluent contain BSA, that was used as spermatozoa protection. This study aim was to examine the ability of cow blood serum in replacing BSA as spermatozoa protective in CEP diluent. Fresh semen from Limousin bull was diluted with CEP diluent + BSA as control, in the treatment group were CEP without BSA, but replaced with 3%, 5%, and 7% serum from fresh blood. Spermatozoa quality tests included motility and viability. The motility of spermatozoa was observed by two people using a light microscope with 200 X magnification at temperature of 37°C. The method of viability observation was eosin nigrosin staining, and observed under a light microscope with 400 X magnification. The results showed that the replacement of cow blood serum with various concentrations gave different effects on the quality of spermatozoa. The best motility and viability of the treatment group was at serum concentrations of 5% after eight days storage and was not significantly different from the controls. The conclusion in this study was cow blood serum can replace BSA in CEP diluents.

Keywords: blood serum, CEP diluent, quality of bull spermatozoa

1. Introduction
The storage technology of spermatozoa becomes very important for the success of Artificial Insemination (AI). It requires media to increase the volume of semen and maintain the quality of spermatozoa. The medium is referred as semen diluent. A diluent is not only contains an energy source, but also a spermatozoa protective material from the cold shock incident as well as the presence of free radicals during the storage process [1,2].

The spermatozoa has the high content of polyunsaturated fatty acids (PUFAs) in the plasma membrane makes spermatozoa sensitive to damage caused by cold shock and lipid peroxidation by free radicals that affect motility, metabolism, ultrastructure, and fertility [3,4].

CEP diluent contained electrolyte ions, energy sources such as fructose, antibiotics, and there were also Bovine Serum Albumin (BSA) as macromolecule [5,6,7]. Bovine Serum Albumin (BSA) was often added in the diluent to maintain the quality of spermatozoa [8,9].

Blood is composed of blood plasma and shaped components. In the blood plasma there is a component of blood serum. The main component in both blood serum and BSA is albumin protein
BSA is the product of the isolation of blood plasma, the price is very expensive, while the blood serum in this study was obtained from fresh blood of cows through the process of centrifugation. Based on the same major constituent between BSA and blood plasma serum, the possibility of plasma serum from cow fresh blood may provide protection against spermatozoa during storage at low temperature. The aim of this study was to examine the serum plasma capability of cow fresh blood in CEP diluents on the quality of Limousin bull spermatozoa during storage at refrigerator temperature.

2. Methods

2.1 Making of diluent media

CEP extender contained NaCl 15 mmol/L; KCl 7.0 mmol/L; CaCl$_2$(H$_2$O)$_2$ 3.0 mmol/L; MgCl$_2$(H$_2$O)$_6$ 3.0 dmmol/L; NaHCO$_3$ 11.9 mmol/L; NaH$_2$PO$_4$ 8.0 mmol/L; KH$_2$PO$_4$ 20.0 mmol/L; fructose 55 mmol/L; sorbitol 1.0 gr/L; BSA 2.0 gr/L; Tris 133.7 mmol/L; penicillin 1000 IU; streptomycin 1 gr; and citrate acid 42.6 mmol/L (Bioworld, USA) [5,6]. CEP with the addition of BSA was as a control group, and treatment group with different concentration from blood serum. All chemical were dissolved with deionized water, and sterilized with millipore membrane. Blood serum was obtained from fresh blood plasma that came healthy cow blood in slaughterhouses.

2.2 Preparation of fresh semen

The using of fresh semen was obtained from Limousin cattle at the Center for Artificial Insemination, Singosasari, Indonesia with the provision of eligible for the process of dilution and storage of motility ≥ 55%, viability ≥ 70%, morphological normality ≥ 75%. Fresh semen was diluted with CEP containing BSA for the control group, whereas in the treatment group was diluted with CEP which added blood serum from cow fresh blood 3%, 5%, and 7%. Semen dilution was determined 25 x10$^6$ spermatozoa/ml in accordance with Indonesian National Standard for storage of cow or goat spermatozoa at freezing temperatures. After the dilution process, the semen in the control group and the treatment was carried out in the refrigeratore at 4-5 °C under dark conditions.

2.3 Observation of sperm motility

The motility of spermatozoa was observed using a light microscope. The semen of all treatments was taken using an glass needle, placed on a glass object, and then observed under a 200 X magnification of light microscope at 37 °C, and the observations were made by two persons [10,11]. The motility of spermatozoa was observed daily until showed results below standard according to Indonesian national standards for storage or freezing process of not less than 40%.

2.4 Observation of sperm viability

Spermatozoa survival (viability) was observed using eosin negrosin staining [12,13]. Semen of all treatments and controls was taken using glass needles, dripped on a glass object, mixed with eosin nigrosin, and made smear preparations. The preparation were observed under a 400 X magnification of microscope, the percentage of living cells was calculated based on the counting of 100 cells. The observation of viability was performed daily to show results below the standard for storage at low temperatures was 50%.

3. Results and Discussion

3.1 Data of spermatozoa motility

The mean and repetitive standard of motility percentage of the Limousin bull spermatozoa in CEP diluent that containing and in the treatment group with BSA replacement using cow blood serum were shown in Table 1.
Table 1. Spermatozoa motility of Limousin bull daily in CEP + BSA (control) and CEP + cow blood serum (treatment group) diluents during storage at 4-5°C

| Various of diluent | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     |
|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Control           | 55.00±1.18 | 52.50±1.60 | 51.00±1.24 | 50.50±1.13 | 48.50±2.74 | 45.50±1.94 | 45.50±4.33 | 42.50±1.92 | 37.50±1.56 |
| Treatment A       | 53.50±2.43 | 53.50±1.82 | 52.00±2.61 | 50.50±0.67 | 49.50±1.31 | 46.00±1.52 | 46.00±3.13 | 41.50±1.12 | 39.00±1.23 |
| Treatment B       | 54.50±1.68 | 51.50±0.62 | 50.50±0.69 | 48.00±0.91 | 47.00±0.47 | 47.00±0.98 | 45.50±3.93 | 43.50±1.71 | 38.50±1.39 |
| Treatment C       | 51.50±0.93 | 50.50±1.02 | 49.50±0.83 | 48.50±0.84 | 46.50±3.22 | 45.50±2.33 | 41.50±2.64 | 38.50±1.06 | 35.50±1.41 |

Note : Control = CEPD + BSA ; treatment A = CEPD – BSA + blood plasma 3%; treatment B = CEPD – BSA + blood plasma 5%; treatment C = CEPD - BSA + blood plasma 7%.

Based on Table 1 showed a decrease in the percentage of motility during storage until the ninth day. Motility data that appropriate with Indonesian National Standards for artificial insemination (40%) after the storage process appeared on the 8th day of storage, storage on day nine was already below 40% in all treatments.

In the 8th day storage there was a difference of motility between treatments, the best motility showed in the 5% serum treatment, but also similar to the control group and the 3% serum treatment. 7% serum treatment showed the lowest motility. However, on day 9, the percentage of motility showed no difference in all treatments.

3.2 Data of spermatozoa viability

The mean and deviation standard of viability percentage of the Limousin bull spermatozoa in CEP diluent + BSA and CEP no BSA but replaced with cow blood serum showed in Table 2.

Table 2. Spermatozoa viability of Limousin bull daily in CEP + BSA (control) and CEP + cow blood serum (treatment group) diluents during storage at 4-5°C

| Various of diluent | 1      | 2      | 3      | 4      | 5      | 6      | 7      | 8      | 9      |
|-------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Control           | 76.37±2.45 | 74.15±1.88 | 72.42±1.73 | 71.02±2.06 | 67.49±2.74 | 66.88±2.53 | 66.56±4.33 | 57.68±2.69 | 55.10±2.22 |
| Treatment A       | 74.92±1.43 | 73.38±1.92 | 74.71±1.46 | 71.41±3.13 | 68.89±1.31 | 63.50±1.31 | 61.85±2.12 | 52.76±1.90 | 49.18±1.90 |
| Treatment B       | 72.60±0.68 | 72.40±0.82 | 70.89±0.59 | 69.75±1.46 | 68.43±0.71 | 65.66±0.47 | 62.01±0.88 | 56.97±1.39 | 53.23±1.59 |
| Treatment C       | 71.30±0.91 | 69.30±0.62 | 68.64±0.52 | 67.94±0.44 | 64.26±1.42 | 61.98±3.23 | 59.18±2.64 | 52.12±1.02 | 49.45±1.81 |

Note: Control = CEPD + BSA ; treatment A = CEPD – BSA + blood plasma 3%; treatment B = CEPD – BSA + blood plasma 5%; treatment C = CEPD - BSA + blood plasma 7%.

Based on the data in Table 2 showed decreased viability of Limousin bull spermatozoa during storage. The percentage of spermatozoa viability showed a significant difference in various treatments on daily observation. The treatment group that approached the viability of the control group was at 5% treatment of serum.
Based on the results of the study showed that the replacement of BSA with cow blood serum in CEP diluents capable of maintaining the motility and viability of spermatozoa Limousin bull up to 8 days of storage. The best concentration of the treatment when compared with the control group was 5%.

Cow blood serum can protect against bull spermatozoa as well as BSA [14,15], this was because both have the same component of albumin. Blood serum is essentially blood plasma without fibrinogen and clotting factor [16,17]. Blood plasma is a light yellow liquid composed of 91% water, 7% protein and 2% other components such as ions, nutrients, gases, electrolytes, and hormones. The main proteins contained in it are albumin, globulin and fibrinogen. Albumin is the most numerous protein of 60% [18].

The albumin protein contained in BSA or serum becomes a transition link between Fe^{2+} and Cu^{+} metal ions, thereby minimizing the formation of OH− radicals promoting peroxidation of lipid spermatozoa [19].

4. Conclusion
Bull blood serum capable to maintenance of spermatozoa quality in CEP diluent during storage at 4-5°C, so that capable to replace BSA in CEP diluent, and may be can use the other diluent.

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