The effect of 0.6 and 0.8% Bovine Serum Albumin (BSA) levels in the Cauda Epididymal Plasma-2 (CEP-2) diluent to maintain Ongole Crossbred post-thawing motility sperm

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Abstract. Decreasing in post-thawing sperm motility of Ongole Crossbred (OC) influences the success of conception through artificial insemination (AI). Sperm membrane damaged during frozen storage is assumed as the cause of a decrease in post-thawing motility. Sperm motility is one of the critical indicators of frozen semen processing AI purposes. This study was aimed to assess the best BSA levels in Cauda Epididymal Plasma-2 (CEP-2) diluent in maintaining sperm post-thawing motility. This study used fresh ejaculate of a three-year-old bull, collected once a week using artificial vagina. The experiment was based on BSA level 0; 0.6 and 0.8% in CEP-2+10% egg yolk. The results showed that different BSA levels in CEP-2+10% egg yolk diluents were able to maintain post-thawing sperm motility: 42±2.58; 44±2.11, and 44±2.11% for control (0%), 0.6% and 0.8% BSA level, respectively. It is concluded that CEP-2+10% egg yolk + 0.6% BSA level effectively maintained sperm post-thawing motility.
sperm. For that reason, the study in using of CEP-2 diluent with different level of BSA on, the motility of post-thawing frozen semen of Ongole Crossbred, was conducted.

2. Material and methods

2.1. Material
The material was fresh semen from three-year old Ongole Crossbred bull, reared in Beef Cattle Research Station, Grati Subdistrict, Pasuruan Regency. Semen was collected once a week. The bull was placed in an individual stall and clinically healthy. The bull was given concentrates (Sukamakmur KUTT, Grati District, Pasuruan Regency), Indigofera spicata, elephant grass and straw, water was given in ad libitum. Estrus cows were used as teasers.

2.2. Methods
Semen collection. The bull was cleaned at all part of stomach and prepuce to avoid contamination which had a negative impact on the quality of ejaculate and sperm. Ejaculate collection used the artificial vaginal method.

2.2.1. Sperm motility assessment

2.2.1.1 Sperm mass motility. Assessment of mass motility could be determined by dripping semen over glass objects and observed using a microscope (100x magnification). If there were large, dark and thick waves like clouds, then the assessment of sperm was excellent (+++). If there were small waves, thin, rare, unclear and slow-moving, the assessment of spermatozoa was functional (++). If there were only progressive active individual movements, the assessment was moderate (+), and if there was no movement at all, then the assessment was terrible (0) [7].

2.2.1.2 Individual motility. Determination of the percentage of individual motility was done by dripping semen into the glass of the object and calculating sperm whose movements were progressively forwards ahead of those who did not move as much as 100 sperm in units of a per cent (400x magnification)[8, 9].

Semen extender. Semen was diluted at room temperature, with CEP-2+ 10% egg yolk (control), CEP-2 + 10% egg yolk + 0.6% BSA (P1), CEP-2 + 10% egg yolk + 0.8% BSA (P2). Fresh semen with a volume of 0.5 ml added to control diluent, P1 and P2 at 37°C (semen was inserted into a 15 ml test tube and soaked in a water jacket) as much as 1: 1 (Extender A1). If the semen temperature reached 30, 25 and 20°C, then extender A2 was added. The semen was incubated at 20°C for 15 minutes, and individual motility was examined. Extender B (containing 7% glycerol) was added which reached 5°C.

Equilibration. The equilibration lasts for two hours in a cool top (5°C). Individual motility assessment was observed under a light microscope using a 400x magnification. Filling and Sealing Straw. The diluted semen was packed into a 0.25 ml (mini straw) with 25 million motile sperm per straw using automatic filling and sealing machine. Freezing. Straw was evaporated on liquid nitrogen at 4 cm for 9 minutes (-140°C) to adjust the sperm temperature before freezing (-196°C). Straw was put into a container that was filled with liquid nitrogen and arranged in a canister[10].

Post-Thawing Motility (PTM). Straw was stored in containers containing liquid nitrogen (-196°C) for 24 hours. Post thawing and individual motility were observed using 400x magnification.

Observation Variables. The best BSA level and sperm motility after dilution, before freezing and post thawing. Data Analysis. The experiment was arranged according to Randomized Block Design (RBD) and the data was analyzed using anova (alpha = 5%) [11].
3. Result and discussion

3.1. Sperm motility on fresh ejaculate. Mass motility resulted was 3+, which was characterised by the movement of sperm, quickly forming waves of greyish black clouds. These results were in the normal range, due to the standard sperm mass motility in bull semen between 2+ to 3+ [7]. Individual motility or progressive sperm movement was one of the essential indicators of the quality of fresh semen for further processing. The results of individual motility amounted to 75% and included in the range of individual motility that met the requirements for processing liquid or frozen semen. The individual motility of bull sperm was generally at 40-75% [12].

3.2. Sperm motility during freezing process. Sperm individual motility showed no difference between treatments 0; 0.6 and 0.8%. The pattern of decreasing the percentage of spermatozoa motility during the freezing process was shown in Table 1.

| BSA Level (%) | Freezing Process | After Diluting (%) | Before Freezing (%) | PTM (%) |
|---------------|------------------|--------------------|---------------------|---------|
| 0             |                  | 69±2.11b           | 64±2.11b            | 42±2.58b |
| 0.6           |                  | 68±2.58a           | 63±2.58a            | 44±2.11b |
| 0.8           |                  | 69±2.11b           | 64±2.11b            | 44±2.11b |

Note: Different superscripts in the column and row indicate a significant difference between treatments (P <0.05).

The results showed that individual motility had decreased at all BSA levels during the freezing process. The highest motility after dilution (69±2.11)% and before freezing (64±2.11)% in CEP-2 diluents level 0 and 0.8%. On the other hand, the highest post-thawing motility at BSA levels was 0.6 and 0.8% (44±2.11)%.

Post-thawing motility results were by Indonesian National Standards for AI (≥40%) and fulfilling the requirements according to the Indonesian National Standard to produce 95% pregnancy. Based on variance analysis showed that different BSA levels did not affect the decrease in motility during the freezing process. BSA level at 0.6 and 0.8% were able to maintain the best percentage of motility during frozen semen processing and 0.6% BSA level was used for commercial frozen semen production for cost efficiency. Motility decreasing during freezing caused sperm to lose aggressive potential and progressive motility by releasing the enzyme aspartate-aminotransferase (AspAT) into the plasma membrane, then ATP products stopped and caused cell death [2].

The use of CEP-2 diluent and the addition of 10% egg yolk were able to maintain motility and minimise damage to sperm membranes. The 10% egg yolk in CEP-2 played a role in maintaining sperm motility during the freezing process. Low-Density Lipoprotein (LDL) fraction, especially phospholipid contained in egg yolk, was material and very effective in protecting sperm from cold shock related to its function as intracellular cryoprotectant. The addition of egg yolk aimed to maintain and protect the integrity of the sperm lipoprotein sheath while the addition of glycerol as an extracellular cryoprotectant to prevent cold shock and damage to sperm due to the formation of ice crystals in sperm during freezing [13].

Bovine Serum Albumin could act as an extracellular cryoprotectant compound that protects and bind to plasma membranes and phospholipid groups that bind to proteins and glycoproteins which cause intra-membrane particles to accumulate due to the influence of cold shock during storage at cold temperatures. Cold shock during storage could cause damage to the cell plasma membrane and sperm death [14]. Bovine Serum Albumin was a globular protein with a molecular weight of 66 kDa having an amino acid composition of 20 kinds and BSA more complete than plasma semen. Bovine serum albumin as extracellular cryoprotectant functions to maintain motility during cold storage and freezing processes [15].
4. Conclusion
The conclusion is the addition of 0.6% BSA onto CEP-2 diluents was able to maintain individual motility (≥40% ) of post-thawing frozen semen according to Indonesian National Standard and suitable for AI.

References
[1] Aboagla E M E and Terada T 2004 Theriogenology 62 1160–72
[2] Sahara Y, Horie S, Fukami H, Goto-Matsunoto N and Nakanishi-Matsui M 2015 J. Oral Biosci. 57 102–9
[3] Verberckmoes S, Van Soom A, Dewulf J, De Pauw and De Kruij F A 2005 Reprod Dom Anim 39 410–416
[4] Delgado P A, Lester T D and Rorie R W 2018 Adv. Reprod. Sci. 06 12–21
[5] Kaeoket K, Chanapai P, Junchiaphoom P and Chandapiwat P 2011 Thai J. Vet. Med. 41 283–8
[6] Osman K 2012 Int J Biol Med Res. 3 1670–1679
[7] Cooper T G, Noonan E, von Eckardstein S, Auger J, Baker H W G, Behre H M, Haugen T B, Kruger T, Wang C, Mbizvo M T and Vogelsong K M 2009 Hum. Reprod. Update 16 231–45
[8] Ax R L, Dally M R, Didion B A, Lenz R W, Love C C, Varner D O, Hafez B and Bellin M E 2008 Semen Evaluation. In Reproduction in Farm Animal ed D Balado (Maryland: Lippincott Williams & Wilkins)
[9] Kirkman-Brown J and Björndahl L 2009 Fertil. Steril. 91 627–31
[10] Arifiantini I, Supriatna I, Klinik D and Patologi R 2007 Media Peternak. 30 100–5
[11] Steel R G D and Torrie J H 2004 Prinsip dan Prosedur Statistika Suatu Pendekatan Biometri (Jakarta: Gramedia)
[12] Garner D L and Hafez E S. 2000 Spermatozoa and Seminal Plasma Reproduction in Farm Animal ed D Balado (Maryland: Lippincott Williams & Wilkins) pp 96–109
[13] Ducha N, Susilawati T, Aularni’am, Wahjuningsih S and Pangestu M 2012 Pakistan J. Biol. Sci. 15 979–85
[14] Peris S I, Morrier A, Dufour M and Bailey J L 2004 J. Androl. 25 224–33
[15] Gadea J 2003 Spanish J. Agric. Res. 1 17