Kinematics motility of frozen-thawed X and Y sperm of Sumba Ongole bull

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Abstract. The aim of this study was to investigate the kinematics motility of frozen-thawed Sumba sexed sperm of Ongole bull using snake head fish albumin (SFA) column method. The SFA was made by different concentration ratio of top and bottom fraction namely 2 and 4%; 3 and 5%; 4 and 6% respectively, and BSA 5 and 10% as control. Semen pellet was diluted with Optixcell CSS extender, then sexed sperms were filled in mini straw (0.25 mL) followed with equilibration at 4°C for 2-4 hours. Prefreezing was performed for 15 minutes then freeze in liquid nitrogen. Kinematics motility was evaluated using Computer Assisted Semen Analyzer (CASA: Spermvision™ 3.7.8) in parameter of total motility, progressive motility, distance average path (DAP), distance curvilinear (DCL), distance straight line (DSL), velocity average path (VAP), velocity curvilinear (VCL) and velocity straight line (VSL). The results of showed that sperms X (top fraction) of SFA treatment after frozen have better kinematics motility parameter compared to Y sperms, in total motility 42.13-64.09%, progressive motility 38.7-62.01%, VCL 55.89-61.85 µm/s, VAP 102.53-110.14 µm/s, and VSL 42.19-45.33 µm/s. In conclusion sperms resulted using SFA treatment (X sperm) have higher kinematic reflecting higher fertility capacity compared to control group.

1. Introduction

Sperm sexing is a reproduction technology which aimed to select offspring sex according to the need of farm operation to improve the production efficiency. This technology is based on the principle of difference of DNA content between X and Y sperm [1] and difference in motility and morphometry of sperm head. Sperm X contain more DNA and larger, meanwhile sperm Y has faster motility causing higher penetration capacity to penetrate the albumin column.

Although fertility of frozen-thawed semen is generally acceptable but the efficiency of cryopreservation is still relatively low. Rapid rate of cryopreservation results in cell damage to sperm due to osmotic imbalance [2]. Cryopreservation affects the functional integrity of acrosome and mitochondria that is responsible for the generation of energy from intracellular stores of ATP [3], and also bring injuries to sperm membrane leading to changes in the sperm physiology which may reflected in sperm motility [4].

Snake head fish is source of albumin for hypoalbuminemia and wound healing post surgery. At this point its albumin, called SFA, could be used as an alternative material for X and Y sperm separation [5]. The snake head fish albumin has similar molecular weight compared to bovine serum albumin (BSA) and human serum albumin (HSA) [6]. The CASA is a method to evaluate sperm quality which
efficient, appropriate services and reliable tools for evaluating fertility objectively [7]. The aim of this study is to examine the fertility capacity of frozen-thawed X-Y sperm which sexed using snake head fish albumin (SFA) extract on Sumba Ongole bull using CASA analysis.

2. Material and methods

2.1. Material
Three certified mature bulls (4-5 years, 500-700 kg body weight) were reared at PT. Karya Anugerah Rumpin Bogor West Java Indonesia. Fresh semen was collected using artificial vagina then evaluated on its volume, concentration, motility and sperm abnormality prior to freezing. Minimum standard of sperm motility for sexing sperm is 60% [8] and abnormality sperm <20%.

2.2. Methods

2.2.1. Sperm X and Y separation using SFA column. Semen was diluted in final concentration 200x10⁶ sperm/mL using Tris buffer (Tris aminomethane 3.03 g citric acid 1.78 g, fructose 1.25 g, penicillin-streptomycin 100 µL, H₂O 100 mL). Aliquot of 1 mL semen was added in the base part of sexing tube and treatment namely T0 (control group BSA concentration at top and bottom fraction were 5 and 10 %); T1 (SFA column top and bottom fraction were 2 and 4 %) T2 (SFA column top and bottom fraction were 3 and 5 %) and T3 (SFA column top and bottom fraction were 4 and 6 %).

Semen was incubated for 30 minutes in room temperature (27°C), followed with sperm fraction collection and centrifuge at 1800 rpm for 10 minutes. Resulted sperm pellet was diluted with Optixcell CSS extender, filled in mini straw 0.25 mL. Equilibration was done at 4 °C for 2-4 hours, prefreezing for 15 minutes and then freeze in liquid nitrogen.

The bottom column fraction, predicted as Y sperm, and top column fraction was predicted as X sperm [8]. Frozen straws were thawed in water bath at 37°C for 30 seconds, then the sperm motility was performed.

2.2.2. Kinematics motility evaluation of sperm using computer assisted sperm analyzer (CASA). Kinematics post thawing motility were evaluated using CASA (Spermvision™ 3.7.8 Minitube Germany) at magnification of 200 x. Semen sample was dripped on the slide glass then covered. The following parameter were obtained that are percentage of motile sperm (TMOT %), percentage of progressive motility sperm (PMOT %), velocity average path (VAP: µm/s), velocity curve linear (VCL: µm/s), velocity straight line (VSL: µm/s), distance average path (DAP: µm/s), distance curve linear (DCL: µm/s), and distance straight line (DSL: µm/s) [1, 9, 10]

2.2.3. Statistical analysis. Data of kinematics motility of sexed sperm were presented as means ± standard deviation (SD). The statistical analysis was done by one-way analysis of variance (ANOVA) using Minitab 17 for Windows. Differences between treatments were considered statistically significant at P<0.05 by Fisher LSD analysis.

3. Results and discussion
Fertility capacity of X and Y sperm after thawing were evaluated based on motility and motion pattern of sperm by using CASA. This study showed total motility in 2% SFA treatment in the top fraction was significantly different (P <0.05) compared to 5% BSA and 3% and 4% SFA (Table 1). Whereas the bottom fraction of 10% BSA in the bottom fraction is higher than the SFA treatment (Table 2).

Likewise, the progressive motility values in the top and bottom fractions have the same trend as the total motility where the 2% SFA higher in the top fraction and 10% BSA higher in the bottom fraction (Table 1 and 2). Total motility and progressive motility in the top fraction were higher than the minimum requirements for post thawing motility (PTM) but in the bottom fraction 6% SFA was lower. Motility is a standard assessment to measure possible or not to be used for artificial
insemination programs with 40% minimum PTM and 32% progressive motility [10]. The motility and velocity of sperm were varied and influenced by several factors such as age, collection time, ejaculation time, viscosity, osmolarity, pH and ion concentration in seminal plasm [11].

### Table 1. Kinematics motility of X sperm after thawing

| Parameter | Control BSA 5% | Treatment (Mean ± SD) |
|-----------|---------------|----------------------|
| TMOT (%)  | 54.36±5.30b   | 64.09±4.37a          |
| PMOT (%)  | 52.76±5.58b   | 62.01±4.30a          |
| VAP (µm/s)| 65.58±4.47a   | 61.85±4.37a          |
| VCL (µm/s)| 124.58±6.14a  | 110.14±4.97b         |
| VSL (µm/s)| 46.82±5.00a   | 45.33±3.24a          |
| DAP (µm/s)| 29.01±1.62a   | 26.64±1.11ab         |
| DCL (µm/s)| 55.47±3.02a   | 47.70±1.37ab         |
| DSL (µm/s)| 20.69±1.93a   | 19.50±0.82a          |

| Parameter | 2% | 3% | 4% |
|-----------|----|----|----|
| TMOT (%)  | 54.55±5.39b | 42.13±3.91c |          |
| PMOT (%)  | 52.06±5.82b | 38.70±4.19c |          |
| VAP (µm/s)| 60.14±2.43ab| 55.89±10.20 |          |
| VCL (µm/s)| 109.38±8.55b| 102.53±16.05b|          |
| VSL (µm/s)| 45.35±2.86a | 42.19±9.30 |          |
| DAP (µm/s)| 25.81±2.24ab| 23.61±3.95b |          |
| DCL (µm/s)| 48.47±5.98 | 45.63±7.73b |          |
| DSL (µm/s)| 19.96±2.15a | 18.52±4.29a |          |

### Table 2. Kinematics motility of Y sperm after thawing

| Parameter | Control BSA 10% | Treatment (Mean ± SD) |
|-----------|---------------|----------------------|
| TMOT (%)  | 51.80±5.30a   | 33.02±4.13c          |
| PMOT (%)  | 50.42±5.58a   | 32.67±4.57b          |
| VAP (µm/s)| 58.99±4.47a   | 54.89±8.31a          |
| VCL (µm/s)| 122.00±6.14a  | 104.51±12.24b        |
| VSL (µm/s)| 42.35±5.00ab  | 42.68±8.45ab         |
| DAP (µm/s)| 27.17±1.62a   | 28.22±9.37a          |
| DCL (µm/s)| 56.42±3.02a   | 47.57±4.67b          |
| DSL (µm/s)| 19.54±1.93ab  | 21.48±7.35a          |

| Parameter | 4% | 5% | 6% |
|-----------|----|----|----|
| TMOT (%)  | 38.72±6.05b | 25.3±4.48d |          |
| PMOT (%)  | 36.45±6.30a | 23.09±4.95c |          |
| VAP (µm/s)| 59.28±7.98a | 46.13±9.38b |          |
| VCL (µm/s)| 108.64±8.95 | 88.04±17.24c|          |
| VSL (µm/s)| 46.98±9.95a | 34.83±19.19 |          |
| DAP (µm/s)| 26.83±3.69a | 20.59±4.64b |          |
| DCL (µm/s)| 49.43±4.03b | 38.56±8.18c |          |
| DSL (µm/s)| 21.26±4.62a | 15.52±4.35b |          |

The average velocity path (VAP) was a parameter measuring the average speed of the sperm from the beginning to the end of the period of the travel path. The optimal VAP value in the study was more than 25 µm/s in the top fraction (Table 1). This study showed that 4% SFA was lower than 5% BSA and 2% SFA, but not significantly different from 3% SFA. The same thing was also shown in the bottom fraction (Table 2) where 6% SFA was lower than BSA 10% and 4%, 5% SFA.

The curvilinear velocity (VCL) of this study have optimal sperm movement with VCL values $>70$ µm/s and VSL $>25$ µm/s (Tables 1 and 2), showed BSA control of 5% and 10% higher than SFA treatment in top and bottom fractions. The straight-line velocity (VSL) parameter in Table 1 and 2 showed no difference between BSA 5% and SFA treatment in the top fraction, but in the bottom fraction 5% SFA was higher than 10% BSA and 4% and 6% SFA. Decreased velocity parameters in the 6% SFA might be caused by the concentration of SFA was denser and the presence of collagen particles was higher so, that inhibit and influence the movement of sperm. These velocity results were in line with previous study [9] which reported that Madura and Ongole cattle had VAP values (72.54 µm/s and 65 µm/s), VCL (117.34 µm/s and 105.30 µm/s), VSL (61.60 µm/s and 63 µm/s) while other study [12] using Automated Sperm Analysis, reported that Sahiwal cattle sperm after freezing had VAP values (53.22 ± 6.67 µm/s), VCL (94.90 ± 15.31 µm/s), VSL (25.20 ± 4.36 µm/s) and LIN (28.86 ± 6.38%). The results of this study was similar with WHO [10] standards that sperm must have minimum VSL values 25 µm/s and VCL ≥ 70 µm/s.
The distance curve length (DCL) SFA treatment lower than the control group in the top and bottom fractions (Tables 3 and 4). High DCL value showed sperm move faster and correlate with VCL value where VCL height followed by a high DCL value. Other results on the parameters of average path distance (DAP), in the top fraction showed that SFA 4% lower than BSA 5% but not significantly different with 2% and 3% SFA treatment (Table 1) as well as the bottom fraction 6% SFA lower than BSA 10%, also 4% and 5% SFA treatment (Table 2).

The DAP value has a correlation with the average velocity value of sperm (VAP) and an increase in the DCL value causes a decrease in the DAP value. The DCL parameter value indicated faster movements and DCL values correlated with DAP, where high DCL caused a low DAP value. The distance straight line (DSL) in this study showed no difference between 5% BSA and SFA treatment in the top fraction (Table 1) but in the bottom fraction 6% SFA lower than 4% and 5% SFA treatment (Table 2). High concentration SFA caused the distance to be shorter and retarded movement of the sperm across the straight track. The SFA treatment in top and bottom fraction has a DAP value ranging (20.59 µm/s - 28.22 µm/s), DCL (38.56 µm/s - 49.43 µm/s) and DSL (15.52 µm/s - 21.48 µm/s) This result was lower than the earlier reported [9], which found the evaluation of motion patterns of fresh semen in Ongole cattle has mean DCL of 45.12 µm/s, DSL 22.52 µm/s, DAP 27.69 µm/s and in bali cattle DCL 51.77 µm/s, DSL 33.18 µm/s DAP 25.64 µm/s.

The VAP, VCL and VSL as sperm motion pattern were indicators for sperm fertility and have a high probability more outcome in pregnancy. This dide to VCL and VSL are parallel with fertilization ability, while VAP values indicate a high correlation with pregnancy rate [7, 13, 14]. The results of kinematics evaluation in this study showed that SFA treatment in the top fraction resulted value more than standard according to the known standard [7,10] such as total motility more than 40%, progressive motility > 32%, VCL> 70 µm/s, VAP> 45 µm/s, VSL> 25 µm/s, ALH <5 µm and BCF >15 Hz.

4. Conclusion

In conclusions the top fraction (X sperm) resulted from SFA treatment has higher kinematic value and fertility capacity compared to BSA group.

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