Research Article

The Quality of Bali Bull Sexed Sperms Using Freeze Dry Albumin at Different Concentrations of Sexing Medium

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Abstract | Sexing spermatozoa using freeze dry albumin with different concentrations is expected to separate spermatozoa. This study aims to identify the quality and movement patterns in Bali bull sexed sperm using freeze-dry albumin. This study was conducted five times for semen collection and using treatments; T1 = Medium concentration of 10% and 30%, T2 = Medium concentration of 15% and 45%, T3 = Medium concentration of 20% and 60%. Parameters measured in this study were motility, viability, abnormality, membrane integrity, acrosome integrity, movement pattern and proportion of spermatozoa. The data obtained were analysed using one-way analysis of variance (ANOVA). A Descriptive Statistical test used to analyze the proportion of sperms. The results showed that different concentrations of sexing media showed no significant difference (P>0.05) on motility, viability, abnormality, membrane integrity, acrosome integrity, and movement patterns of spermatozoa in treatment between T1, T2 and T3 treatments both in the upper and lower fraction. In the movement pattern of sexed sperms, There was no significant difference in the kinematics parameters (P>0.05) in the T1, T2 and T3 treatments. The proportion of X:Y with freeze-dried albumin on the upper layer showed T1 (57.00:39.13) significantly different (P<0.05) from T2 (69.61:25.49) and T3 (75.00:24.00), while the lower layer showed T1 (33.17:64.36) significantly different (P<0.05) from T2 (23.27:72.77) and T3 (22.17:75.73). The proportion showed that X sperms in the upper fraction were higher than the lower fraction in all treatments, while Y sperm in the lower fraction was higher than X sperm. Based on the study's results, it can be concluded that freeze dry albumin could be used as a sexing medium and maintain characteristics sperms with any concentrations, and can change the proportion of spermatozoa effectively from the natural proportion of spermatozoa.

Keywords | Sexing, Albumin, Freeze-dry, Spermatozoa, Kinematics, Characteristic

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INTRODUCTION

Improving science and technology plays a strategic part in expanding livestock populations. Reproductive technology that is capable of being one of the options in endeavours to increase the efficiency of livestock reproduction is artificial insemination (AI). AI may be made valuable by utilizing sexed sperm technology to produce offspring according to the desired sex. Sexing Spermatozoa is the method of separating spermatozoa with X and Y chro-

mosomes employing a separation medium (Luzardin et al., 2020). The application of reproductive biotechnology, especially for sexing spermatozoa by separating spermatozoa carrying X and Y chromosomes, is an elective used to predict the sex of livestock that will be born (Bhalakiya et al., 2018).

Several factors, including the type of medium and the concentration, influence the quality of sexed sperm. Egg albumin is one of the materials that can be used as a sexing medium. Sexing with egg white albumin is based on differences in spermatozoa motility between X and Y by making different medium concentrations (Sianturi et al., 2007). Increasing concentration differences are expected to separate spermatozoa based on their motility. Spermatozoa with high motility can penetrate a more concentrated medium concentration, while spermatozoa with low motility will remain in the medium with a low concentration (Sianturi et al., 2004).

Egg albumin was chosen as the sexing medium because it can be effortlessly made into a fraction or separate medium with different concentrations. The high protein substance in egg albumin is additionally valuable as a source of energy for spermatozoa during the separation process. Economically, egg albumin is more efficient and beneficial than other materials. When utilized as a sexing, it is cheap, affordable, and easy to obtain (Takdir et al., 2016).

Freeze drying technology has developed, which is one of the strategies to produce products with extended shelf life. Freezing dry albumin aims to achieve viscosity uniformity in the sexing media (Amaliah et al., 2023). However, the use of albumin freeze dried for sexing has not been widely carried out, so information about the quality of sexed spermatozoa and the exact concentration of egg albumin in separating spermatozoa is still limited. This study aimed to determine the quality and movement pattern of Bali bull sexed spermatozoa using freeze-dry albumin with different sexing concentrations is expected to separate spermatozoa effectively.

MATERIALS AND METHODS

Materials of the study and Semen Collection

This study was conducted between January and March of 2023 at the Samata integrated farming system, Samata, Somba Opu District, Gowa Regency, and the Laboratory of Animal Reproduction, Semen Processing Unit, Faculty of Animal Science, Hasanuddin University, Makassar. The study applied the sperm of 2 Bali bulls aged 4 years. The bull was fed 10% of its body weight in elephant grass and concentrate.

Preparation of Extender and Sexing Media

Preparation of Tris-Eggyolk (TEY) Diluent: The method used was based on Amaliah et al. (2023), which was modified; preparation of *Trishydroxyl methylamine* 3.63 g, *citric acid* 1.78 g, and *fructose* 1.25 g into a volumetric flask, then adding aquabidest until it reached 100 ml and then homogenized for 15 minutes. The next step was to put 80 ml of this solution that had been made into a volumetric flask and add 20 ml egg yolk until it reached 100 ml, then homogenize for 10 to 20 minutes.

Preparation of Freeze-dry Albumin: Freeze-drying of albumin will be using chicken eggs, the egg yolks and egg white (albumin) separated in a cup. Egg whites are stored in a room with a temperature of 30°C and 75% humidity. Then put in the freeze dryer with settings: heating temperature 45°C, freezing temperature -10°C and pressure 25 Pa for 15 hours. Egg whites are frozen at -10°C for 3 hours, then egg whites that has been frozen is put into the dryer using a heating temperature of 45°C at a pressure of 25 Pa for 12 hours. After it becomes flour, it is then packaged using a sterile container. After that it is substituted into the solution according to the treatment (Fitriyani et al, 2017).

Semen Collection and Evaluation

Semen collection: Semen collection was carried out for 5 replications. Semen that had been collected was immediately brought to the Laboratory for processing at a later stage.

Semen Evaluation: Semen evaluation was done macroscopically and microscopically.

Macroscopic Evaluation: The volume, pH, colour, odour, and consistency of the sperm were evaluated macroscopically. The volume was determined by examining the scale on the collecting tube. The colour was evaluated subjectively by visually distinguishing between pure white and cream. Shaking the collection vessel to determine its consistency. Using pH indicator paper, the pH of sperm was measured in the range of 6.0 to 8.0 (Diansyah et al., 2022a).

Microscopic Evaluation

Sperms Motility and Kinematics: The motility and kinematics of sperm were observed by pouring 10 µl of sperm onto a glass object and covering it with a cover glass with 400 magnifications. Spermatozoa were then analyzed using *Computer Assisted Sperm Analysis* (CASA) (Amaliah et al., 2023).

Sperms Concentration: The sperm concentration was measured using a Minitube SDM 6 (Germany) photometer. The cuvette containing 3 ml of physiological NaCl solution was positioned with the line facing forward be-

fore the zero button was pressed. By removing the cuvette and replacing it with one containing a physiological NaCl solution, to which 30 µl of fresh sperm was added, and then pressing the result button, the sperm concentration in amount per ml can be determined (Dianysah et al., 2022b).

Sperms Viability and Abnormality: Spermatozoa's viability and abnormality were evaluated by combining 10 µl of sperm and 10 µl of 2% Eosin above the microscope slide. The specimen was then examined with a 400 trinocular microscope. Spermatozoa that have died are crimson, while those alive are colourless. As aberrant spermatozoa, broken tails, severed tails, and anomalous head morphologies were identified. One observation reveals a minimum of 200 sperm (Diansyah et al., 2020).

Membrane Integrity: Examine membrane integrity by mixing treated sperm with HOST (*Hypoosmotic Swelling Test*) solution in a ratio of 1: 10 (100 µl of treated sperm: 1 ml HOST solution), followed by 30 minutes of oven incubation. The observations were conducted using a trinocular microscope at 400 magnification and at least 200 spermatozoa cells. Sperms with membrane integrity were characterized by a circular tail and damaged sperms were characterized by a straight tail. (Diansyah et al., 2020).

Acrosome Integrity: The acrosome integrity was determined by combining the sperm with a formal saline solution in a ratio of 1:4. Using a trinocular microscope with 400 magnifications, at least 200 spermatozoa cells were observed. The percentage of acrosome integrity that has integrity acrosomal hoods is marked by a black head tip when exposed to a formol-saline solution (Rizal, 2006).

Sexing of Bali Bull Semen

Sperm Separation (sexing): The sperm is separated by making sexing media using freeze dry albumin dissolved in distilled water. The medium, consisting of the upper and lower fraction, was made by mixing freeze dry albumin that had been diluted with TEY diluent in one tube. Furthermore, 2 ml of each layer was compiled to form a gradient. Three mediums were made according to the treatment, namely medium T1 = 10% (upper fraction) and 30% (lower fraction), T2 = 15% (upper fraction) and 45% (lower fraction), T3 = 20% (upper fraction) and 60% (lower fraction). Next, the semen that was evaluated was then diluted with TEY diluent in a ratio of 1:1. Insert 1 ml of diluted semen was placed into each tube containing sexing medium according to treatment and then incubated for 30 minutes. After the incubation process, the top and lower fractions were separated prior to centrifugation, centrifugation was carried out at 1500 rpm for 5 minutes. The centrifugation result (supernatant) was discarded while the precipitate (sediment) was taken, and then motility, concentration, viability, abnormality, membrane integrity, acrosome integrity and proportion of sexed sperms were observed.

Proportion of X and Y: The size of the spermatozoa head was measured to determine the X and Y spermatozoa using a smear preparation, measured using a trinocular microscope (AxioCam Erc 5s, ZESS, Germany), with 100x10 magnification. The size of X and Y spermatozoa in Bali bull semen was measured using the average of the spermatozoa head size of fresh bull semen, which was then used as a benchmark to calculate the ratio of X : Y spermatozoa (Haryani, 2016). Spermatozoa with larger heads than the control categorized as X spermatozoa, whereas if the head size is larger smaller than the control were categorized as Y. To determine the proportion of the number of Y and X spermatozoa, the size of the spermatozoa head was measured in the same way as fresh semen. The Calculation of the proportion of spermatozoa Y : X in semen after sexing treatment, based on the measurement results of fresh semen. The data obtained was calculated by calculating the percentage of X and Y spermatozoa. Where to get the formula below.

Spermatozoa Y ≤ Average head size of spermatozoa – SE
Spermatozoa X ≥ Average head size of spermatozoa + SE
Uncategorized Spermatozoa = Average of the spermatozoa head is between SE

Statistical Analysis

The data obtained in this study were tabulated in Microsoft Excel. Furthermore, it was analyzed by one-way analysis of variance (ANOVA) using SPSS version 25. The descriptive statistical test is used to analyze the proportion of X and Y spermatozoa.

RESULTS AND DISCUSSION

The Characteristics Of Bali Bull Fresh Semen

The characteristics of fresh semen both macroscopically and microscopically in this study can be seen in Table 1.

Based on Table 1, the volume of Bali bull's fresh semen has a value of 4.33 ml ± 0.76. Fresh semen volume in the study showed a normal range. This follows the opinion of Arifiantini (2012), which states that the range of cow semen volume is 4 to 8 ml. This study's degree of acidity (pH) was 6.10 ± 0.14. Nahriyanti et al. (2017) argue that the normal pH of semen is between 6-7. The colour of Bali's fresh semen obtained was beige, indicating that the semen was normal. This follows Nursyam (2007) that normal bovine semen is milky white or whitish beige and cloudy. The smell of semen obtained in this study can be considered normal, namely the typical smell of semen. Rizal and

Table 1: The Characteristics of Bali Bull Fresh Semen

Parameters	Mean (±SD)
Macroscopic	
Volume (ml)	4.33±0,76
pH	6.10±0.14
Colour	Creamy
Smell	Typical
Consistency	Medium
Microscopic	
Concentration (million/ml)	1,524±0.346
Motility (%)	87.99±2.22
Viability (%)	85.11±3.28
Abnormality (%)	6.15±0.22
Membrane Integrity (%)	89.86±1.28
Acrosome Integrity (%)	89.82±1.57

Table 2: Semen characteristics of sexed Bali Bull at different media concentrations

Parameters		Treatment		
		T1	T2	T3
Motility (%)	Upper layer	80.93±1.74	80.42±1.88	80.11±1.68
	Lower layer	81.72±2.68	82.18±1.34	81.38±1.53
Concentration (×10^6/ml)	Upper layer	0,393±0.41	0,420±0.34	0,405±0.49
	Lower layer	0,381±0.72	0,358±0.34	0,350±0.56
Viability (%)	Upper layer	80.88±1.68	80.12±1.55	80.16±1.99
	Lower layer	80.87±1.87	81.54±1.88	82.63±1.94
Abnormality (%)	Upper layer	8.09±0.39	8.19±0.35	8.52±0.26
	Lower layer	8.55±0.43	8.88±0.51	9.29±0.39
Membrane Integrity (%)	Upper layer	85.22±1.18	83.60±3.23	82.74±2.17[b]
	Lower layer	83.40±2.12	82.88±2.03	82,56±2,03
Acrosome Integrity (%)	Upper layer	84.81±1.70	82.64±3.51	82.14±2.05
	Lower layer	83.17±2.97	82.34±3.67	80.17±4.11

Notes: T1 = Concentrations 10% and 30%
T2 = Concentrations 15% and 45%
T3 = Concentrations 20% and 60%

Herdis (2008) stated that generally, the smell of semen is categorized as a typical smell. The consistency of the semen obtained was in the range of slightly viscous. There is a correlation between consistency and concentration; the thicker the consistency, the higher the concentration (Adhyatma et al., 2013).

The concentration obtained during the five times collection was 1,524 million/ml ± 0.346. Feradis (2010) states that normal sperm concentration is between 1,000 and 2,000 million cells per milliliter. This investigation determined that the motility of sperm was 87.99% ± 2.22. Good motility was derived from the fresh semen of a Bali bull. According to Garner and Hafez (2016), the motility value of bovine sperm ranges between 70 and 80%. The viability of the fresh sperm obtained in this study was 85.11 % ± 3.28. In the study conducted by Savitri et al. (2014), the viability of the fresh sperm of a Bali bull was determined to be 75%. This result was significantly higher. The percentage of abnormality was calculated to be 6.15 0.22. Since the abnormality standard for spermatozoa is 15% (Knox, 2011), the results of this study were deemed satisfactory.

This research determined that the membrane integrity was 89.86% ± 1.28. This value was classified as excellent. The high percentage of membrane integrity positively influences sperm motility. Sperm motility is related to membrane integrity. (Azzahra et al., 2016) Progressive sperm activity is proportional to membrane integrity. This study determined that acrosome integrity was 89.82% ± 1.57. Since it

is known that the acrosome hood is closely related to the integrity of the plasma membrane of spermatozoa, damage to the acrosome hood may increase if plasma membrane damage increases during incubation due to chemical processes (Garner and Hafez, 2008).

The Characteristics Of Bali Bull Semen After Sexing With Freeze Dry Albumin Media

The characteristics of spermatozoa show how good the spermatozoa are to move progressively, especially to fertilize the egg. The characteristics of Bali bull semen after sexing can be seen in Table 2.

Based on Table 2, the quality of semen after sexing showed a decrease in spermatozoa from fresh conditions, but did not significantly influence the quality of Bali bull spermatozoa. Sperms motility after sexing showed no significant difference (P>0.05) on between T1, T2, and T3 treatments in both the upper and lower layers. This is due to the high ability spermatozoa and the amount of energy required to move penetrate to the lower layers. This can also be due to the low viscosity of the sexing media used is albumin freeze dry so that the spermatozoa can penetrate the lower layer.The decrease in spermatozoa motility was due to the treatment during the sexing process. This is in accordance with the opinion of Susilawati (2014) states that the average motility of separated spermatozoa has decreased compared to the motility of spermatozoa before separation or fresh semen. Trilas (2003) expressed that the decrease in sperm quality may be due to spermatozoa's experience in a series of treatments ranging from the collection process to the separation process that requires a lot of energy. The concentration of sexed sperms showed no significant difference (P>0.05) with treatments T1, T2, and T3 in both the upper and lower layers. The concentration after separation has decreased,this is due to the presence of sperm left in the separator media and some of it is involved during the centrifugation process. The number of sperm that enter a semen fraction will decrease as the albumin concentration increases which can increase the viscosity of the diluent and only truly motile sperm can penetrate the media (Dixon et al., 1980). This could be due to the use of freeze dry albumin as a sexing medium which can reduce the decrease in semen quality after sexing because the viscosity of freeze dry albumin can be more evenly uniformity than fresh albumin (Amaliah et al., 2023). so that the decrease in the quality of spermatozoa is not significant.

According to Indonesian Minister of Agricultural Regulation Number:10/Permentan/PK.210/3/2016 and Indonesian National Standardization 4868.1:2007 for bull semen (Baharun et al., 2021), Based on these regulation, the quality of bali bulls sexed sperm in the present study was considered a normal category.

Figure 1: Observation of Membrane Integrity Bali bull sexed sperms; Red Arrow: Normal membrane integrity sperms; Blue Arrow: Damaged membrane integrity sperms

The percentage of viability after sexing showed no significant difference (P>0.05) in the treatment between T1, T2 and T3 treatments in both the upper and lower layers. This is caused by immotile spermatozoa remain in the upper layer whereas motile spermatozoa can penetrate the freeze dried albumin medium.Living spermatozoa have good membrane conditions, so that the dye has difficulty penetrating membrane, as a result the spermatozoa cells remain clear in color. Viability of spermatozoa in the lower layer tends to be higher because immotile spermatozoa will be filtered out and do not penetrate into the lower layer, so only spermatozoa that have high motility penetrate the lower layer (Kaiin et al., 2017). The viability decreased after treatment with albumin media because sperm spend too much energy when through a separating medium. Indriani et al. (2013) stated that the number of dead and damaged spermatozoa, which decreased viability percentage, was due to the restricted energy required by spermatozoa. Spermatozoa abnormalities showed no significant differences between treatments but abnormality in sexed spermatozoa increased due to a series of treatments during the sexing process. There was an increase in abnormalities along with the increasing concentration of the separating media layer in each treatment which would further decrease the protective function of spermatozoa. This causes the percentage of spermatozoa abnormalities and spermatozoa membrane damage to increase (Sujoko et al., 2009).

The membrane integrity is one of the vital parameters to be observed because it is related to the permeability of the plasma membrane which plays a role in protecting spermatozoa. Damage spermatozoa which appear in the tail of the spermatozoa, sperms with a good membrane can see by a circular tail and damaged sperms were characterized by a straight tail (Figure 1). The membrane integrity of sperms showed no significant differences between the treatments T1, T2 and T3, but the quality of the integrity membrane is decreased. The decrease in plasma membrane

Table 3: The Kinematics of Bali Bull Sexed Sperms at Different Medium Concentrations

Parameters		Treatment		
		T1	T2	T3
DCL (µm)	Upper layer	45.26±1.72	45.01±1.32	44.62±2.83
	Lower layer	45.97±2.78	46.38±1.85	46.61±1.50
DAP (µm)	Upper layer	24.95±1.76	23.77±1.73	23.35±1.46
	Lower layer	23.49±2.29	23.65±2.09	23.78±2.01
DSL (µm)	Upper layer	16.42±0.91	16.83±0.76	16.67±1.15
	Lower layer	16.95±0.94	17.53±1.38	17.64±1.49
VCL (µm/s)	Upper layer	118.58±3.37	117.90±2.03	116.96±2.21
	Lower layer	117.51±3.32	118.05±1.92	120.37±2.36
VAP (µm/s)	Upper layer	52.75±2.52	52.39±2.68	53.48±2.18
	Lower layer	54.92±2.36	55.43±2.93	56.08±2,30
VSL (µm/s)	Upper layer	44.41±3.56	43.13±3.77	42.62±2.19
	Lower layer	45.97±2.73	44.67± 32.15	43.10±3.13
LIN (%)	Upper layer	0.37±0.02	0.37±0.03	0.38±0.02
	Lower layer	0.40±0.03	0.41±0.01	0.42±0.01
STR (%)	Upper layer	0.71±0.02	0.72±0.03	0.72±0.02
	Lower layer	0.73±0.02	0.74±0.01	0.75±0.02
WOB (%)	Upper layer	0.46±0.02	0.45±0.01	0.45±0.01
	Lower layer	0.49±0.01	0.47±0.01	0.47±0.01
BCF (Hz)	Upper layer	21.33±0.47	21.61±0.88	21.87±0.71
	Lower layer	21.65±1.30	22.29±1.44	22.63±1.68
ALH (µm)	Upper layer	5.48±0.43	5.71±0.23	6.19±0.32
	Lower layer	5.55 ±0.24	5.61±0.21	5.98±0.45

Notes: T1 = Concentrations 10% and 30%
T2 = Concentrations 15% and 45%
T3 = Concentrations 20% and 60%

quality and acrosome integrity of spermatozoa during the sexing process is due to the friction that occurs between spermatozoa during the sexing process (Berg et al., 2005). There is a part at the acrosome that is damaged or cannot absorb the solution or a pale color is visible on the head (Figures 2) The low percentage of acrosome integrity is associated with lower percentage of membrane integrity, viability and motility (Sitepu et al., 2018). According to Triwulaningsih et al. (2003), the percentage of Intact Acrosome Hood decreases as the time of semen processing or storage increases.

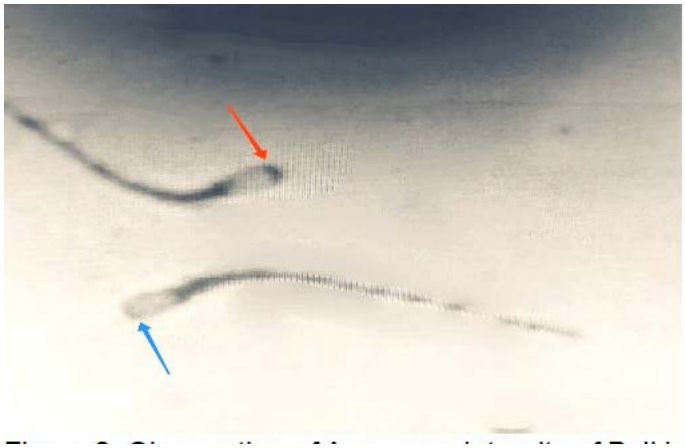

Figure 2: Observation of Acrosome Integrity Bali bull sexed sperm; Blue Arrow: Normal acrosome integrity sperms; Red Arrow: Damaged acrosome integiry sperms

The Kinematics Of Bali Bull Sexed Sperm With Freeze Dry Albumin Media

Movement patterns of Bali bull sperms after sexing were analysed using CASA (*Computer Assisted Sperm Analysis*). Movement pattern and distance travelled of sexed sperms using different egg albumin concentration gradients are shown in Table 3.

The separation results DCL, DAP, and DSL values using freeze-dry albumin media showed no significant difference (P>0.05) in the treatment between T1, T2, and T3, both in the upper and lower layers. The Distance Curve Line (DCL) is the distance that sperm may travel in one minute on the curve route. Distance Straight Line (DSL) is the straight-line distance sperm can travel in one minute.

Distance Average Path (DAP) refers to the average distance that sperm can travel in one minute (Ratnawati et al., 2019). DAP, DCL, and DSL measurements showed that the values obtained tended to decrease in the upper layer, while DAP, DCL, and DSL values increased in the bottom layer. This could be due to the selectivity of spermatozoa after the sexing process. This selectivity occurs when only spermatozoa with good movement can penetrate higher concentrations during the sexing process (Haryani, 2016). The pattern of spermatozoa velocity for each parameter of VCL VSL and VAP using freeze-dry albumin media showed no significant effect (P>0.05) with the treatment between T1, T2 and T3 both in the upper and lower layers. The sperms velocity had a good value of kinematic sperms, according to the statement Krízková et al. (2017) VCL values can be divided into: fast (>90 µm/s), moderate (45-90 µm/s), slow (10-45 µm/s), and statis or immotile 25.0 µm/s are a good predictor of in vitro fertilization ability (Suzuki et al., 2003). In the sexing process, spermatozoa that cannot penetrate the separating medium will be left behind in the low-concentration layer. Conversely, spermatozoa with higher motility can penetrate the separating medium. Suzuki et al. (2003) state that fertilization capacity depends on VSL and VCL parameters contributing to spermatozoa function characteristics. VCL is the sperm velocity in one minute of a curve, while VSL is the sperm velocity in one minute of a straight line (Sarastina et al., 2007). VAP values are less than VCL and more than VSL. The VAP value of spermatozoa with relatively regular and linear trajectory movements will be closer to VSL than VCL. According to Perreault (2002), the VCL, VAP, and VSL parameter values only indicate the intensity of sperm motility and do not provide information on sperm mobility.

The average values of LIN, STR and WOB after sexing showed no significant effect (P>0.05) in treatment between T1, T2 and T3 both in the upper and lower layers. No significant difference at treatment was found for the parameters. This means that the kinematics of Bali bull's sexed sperm, both velocity and distance, is relatively similar for each treatment. The mean straightness, linearity (LIN; VSL/VCL) and WOB (VAP/VCL), movement patterns of this shows that the average spermatozoa move linearly because spermatozoa move linearly by showing STR > 0.5 and LIN > 0.35. STR and LIN values perform a role in determining the swimming direction of spermatozoa. Because the LIN value indicates the quality of sperm movement, the lower the LIN value in spermatozoa, the higher the quality of sperm movement. Sarastina et al. (2007) state that LIN and STR values can be used as progressive indicators of swimming patterns and motility. WOB is the maximum spermatozoa oscillation per second. The ALH value in the upper layer did not differ significantly among interventions T0, T1, T2, and T3. According to Shojaei et al. (2012), when ALH > 7 µm, LIN 65%, and VCL > 80 µm, the movement of sperm transforms into hyperactive motility. Susilawati (2011) stated that spermatozoa must be hyperactivated before the acrosome reaction during fertilization. The BCF value was not substantially different (P>0.05) between the upper or lower layers treatments.

In this study, the kinematics of sexed sperms was belongs to hyperactivation. The hyperactivation is a movement pattern seen in sperm at the site and time of fertilization in mammals. This may be important for successful of fertilization, as it increases the sperm's ability to penetrate of the oviduct, to move around in the labyrinthine lumen of the oviduct, to penetrate mucous substances and, finally, to penetrate the zona pellucida of the oocyte (Suarez and Ho, 2003).

The Proportion Of Bali Bull Sexed Sperm With Freeze Dry Albumin Media

The proportion of sexed sperm at different albumin concentration gradients can be seen in Table 4.

Table 4: Proportion of Bali Bull sexed sperm at different medium concentrations

Type of Spermatozoa	Treatment (%)			
	T0	T1	T2	T3
Before *sexing*				
Spermatozoa X	49.75	-	-	-
Spermatozoa Y	47.78	-	-	-
Upper layer				
Spermatozoa X	-	57.00	69.61	75.00
Spermatozoa Y	-	39.13	25.49	24.00
Lower layer				
Spermatozoa X	-	33.17	23.27	22.17
Spermatozoa Y	-	64.36	72.77	75.73

Notes: T0 = Fresh Semen
T1 = Concentrations 10% and 30%
T2 = Concentrations 15% and 45%
T3 = Concentrations 20% and 60%

The data obtained is calculated by calculating the percentage of spermatozoa X and Y. Calculation of the proportion of spermatozoa is the percentage divided by the number of spermatozoa counted with total spermatozoa. So the proportion of spermatozoa will be obtained. Measurement of the size of spermatozoa heads (Figure 3) in fresh semen is used as a basis for determining the size of X and Y spermatozoa, which is then used to calculate the proportion of X : Y spermatozoa after sexing treatment. Determination of head size in this study categorizes X spermatozoa as having a head size of more than or equal to 9.0726 µm, while Y spermatozoa have a head size of less than or equal to 9.0238 µm, while spermatozoa that have a head size

between 9.0238 µm to 9.0726 µm can be said to be uncategorized spermatozoa.

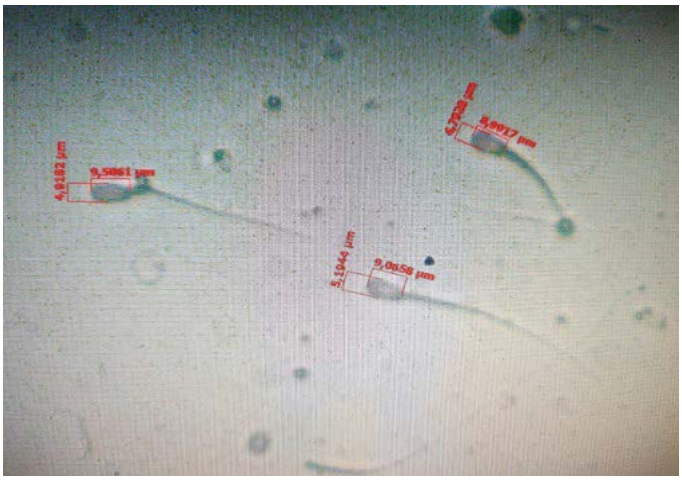

Figure 3: Measurement of the spermatozoa head

The average percentage of spermatozoa proportion in this study was obtained after sexing using freeze dry albumin media with different concentration treatments for each treatment. The percentage proportion of X spermatozoa using freeze dry albumin media in the top layer tends to be higher than in the bottom layer. The proportion of spermatozoa after sexing using freeze-dry albumin denoted that the number of X spermatozoa in the upper layer was higher than Y. X spermatozoa generally have a broader and wider head than Y spermatozoa. The difference in the size of X spermatozoa and Y spermatozoa caused their movement speed to differ. The existence of these differences made it difficult for X spermatozoa to penetrate the separating medium, whose concentration was more intense. According to Afiati (2004), Y chromosome carrier spermatozoa have higher motility than X chromosome carrier spermatozoa, which allows Y spermatozoa with higher motility to move down while X spermatozoa will remain in the upper layer. In the sexing process, spermatozoa will be naturally selected, i.e. spermatozoa with less speed and unable to penetrate the lower layer of the tube will be left at the upper layer. On the contrary, spermatozoa with higher speed will be able to reach the lower layer of the tube. The percentage of Y spermatozoa in the lower layer was higher than in the upper layer because Y spermatozoa have a smaller size and lighter mass than X spermatozoa, so Y spermatozoa have higher motility. They are more likely to penetrate the separating medium layer/faction with a higher concentration. This is in line with what was stated by Sianturi et al. (2004) that X spermatozoa will remain in a medium with a low concentration, while Y spermatozoa with high motility will be able to penetrate a more intense medium concentration.

CONCLUSION

Based on the results of the study it can be concluded that Freeze dry albumin can be used as a sexing medium and can maintain characteristics sperms with any concentrationsand can change the proportion of spermatozoa effectively from the natural proportion of spermatozoa.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interests.

NOVELTY STWTATEMENT

The novelty of this study is the optimum concentrations of freeze dry albumin for sexed sperm. on Bali bull.

AUTHORS CONTRIBUTION

All authors equally contributed and approved the manuscript.

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