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Characteristics and Kinematics of Bali Bull Sperms after Thawing Using Tris Soy Lecithin

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Abstract

Soy functions as an extracellular cryoprotectant, which can maintain the integrity of spermatozoa cell membranes with its main content is lecithin. Lecithin from soybeans protects sperm cells from cold stress and reduces the effects of oxidative stress during cryopreservation. This study aimed to analyse the effect of various levels of lecithin diluent on the quality of Bali bull semen during cryopreservation. Semen collection of Bali bull was carried out once a week during four times consecutively using an artificial vagina. The semen was then diluted using the essential ingredient Tris Aminomethan with the addition of powdered soy lecithin; P1 (1 %), P2 (3 %) and P3 (5 %), respectively. Andromed® (K1) as a positive control and Tris without soy lecithin (K2) as a negative control. The parameters observed were motility, progressive motility, kinematics, viability, membrane integrity, and acrosome integrity. The results of this study showed that the dilution of semen with soy lecithin before and after thawing the semen quality was not significantly different (P< 0.05) in motility, viability, plasma membrane integrity, and acrosome integrity. Meanwhile, the kinematics of VAP, VCL, VSL, DAP, DCL, and DSL showed that the average quality increased at P3 compared to K1, K2, P1, and P2, which decreased after thawing or were significantly different (P< 0.05). It can be concluded that Bali bull semen diluted with 3% and 5% of tris soy lecithin produces good characteristics and kinematics, can protect spermatozoa from cold shock.

Keywords: *Bali Bull, Soy Lecithin, Cryopreservation, Characteristics, Kinematics*

1. Introduction

The biotechnology livestock reproduction was experienced rapid development, especially in bull breeding efforts. One method that has been widely implemented is artificial insemination (AI), where AI allowed the distribution of semen from livestock with superior genetics, potentially increasing livestock productivity significantly, both in terms of quality and quantity (Rahmatuzzahra et al., 2022). One of the crucial stages in implementing AI is storing semen, which in its preserved form is called frozen semen. The main advantage of frozen semen is its ability to overcome time and distance limitations so that it can be used flexibly without being limited by geographic location (Herdis, 1998). The success of AI really depends on the quality of the frozen semen that will be used (Garner & Hafez, 2016).

Soybean is an example of a cryoprotectant, which is included in the category of extracellular cryoprotectants that are free from pathogens. Naturally, soybeans contain lecithin in a concentration range of between 1.48% and 3.08% and can be used as a source of quality lecithin in making diluent solutions (Aku et al., 2007). The study conducted by Thun et al., (2002) reported that the lecithin found in soybeans, similar to the lecithin found in egg yolks, can protect sperm from the effects of cold stress and reduce damage caused by oxidative stress during the cryopreservation process (Ogbuewu et al., 2010). Other study findings stated by (White, 1993) and Toelihere, (1985), show that the lecithin contained in the diluent can interact with the sperm plasma membrane, maintaining the integrity of the plasma membrane during the cryopreservation process.

The use of soy lecithin as a component in diluent has been tested on various types of livestock for cryopreservation purposes. Findings from study by Veerasingam & Kusumawati, (2018) and (Xin Yi & Kusumawati, 2018) indicated that the addition of soy lecithin to the diluent produced the highest percentage of motility and viability compared to eeg yolk. However, this study evaluating the optimal concentration of soy lecithin in Tri's diluent, especially in the context of the characteristics and kinematics of Bali bull spermatozoa to maintain semen quality during the cryopreservation process, is still limited. Therefore, further study needs to be carried out to gain a deeper understanding.

2. Materials And Methods

The study was conducted from August - September 2023 at the Semen Processing Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar, Indonesia, and the Samata Integrated Farming System, Gowa, Indonesia. In this study, semen samples were obtained from a 6 years old Bali bull weighing 250 kg, collected four times through an artificial vagina with a weekly frequency. The bull was housed separately, and fed with a diet comprising 20% concentrate and 80% elephant grass in the morning and evening. All procedures in this study were approved by the Hasanuddin University Animal Ethics Committee, Makassar, Indonesia.

Semen Evaluation

The evaluation of sperm quality encompassed both macroscopic parameters (volume, pH, color, odor, and consistency) and microscopic parameters (motility, progressive motility, kinematics, concentration, abnormality, viability, membrane integrity, and acrosome integrity).

Macroscopic Evaluation

The macroscopic evaluation includes the assessment of volume, odor, color, consistency, and pH. The volume was evalauted by observing the scale on the reservoir tube. Color was evaluated by visually examining the shade from milky white to cream. Consistency was measured by tilting the collection tube and then evaluating the semen returning to the tube's base. The assessment is based on three categories: thin (semen rapidly returns to the tube's base), moderate (semen slowly returns to the tube's base, leaving some on the tube wall), and thick (semen very slowly returns to the tube's base). The pH is evaluated using pH indicator paper within the range of pH 6.0 to 8.0 (Diansyah et al., 2022).

Microscopic Evaluation

Sperm Motility, Progressive Motility and Kinematics : Sperm motility, progressive motility, and kinematics were evaluated by applying a 10 µl drop of spermatozoa on a object glass. The evaluation of spermatozoa was conducted using the CASA system (Vision VersionTM 3.7.5 by Minitube, Germany) as described by (Raafi et al., 2021).

Sperms Concentration: The sperm concentration was assessed using an SDM 6 photometer (Minitub, Germany). Sperm samples were prepared by filling a cuvette with 3,5 ml of physiological NaCl solution, which was then inserted into the apparatus with the line facing forward, followed by pressing the zero button. Subsequently, the cuvette was removed and replaced with a new cuvette containing physiological NaCl solution and 30 µl of fresh semen. Afterward, the results button was pressed to determine the concentration of spermatozoa per milliliter (Saputra et al., 2017).

Sperms Viability: The sperm concentration was assessed using an SDM 6 photometer (Minitub, Germany). Sperm samples were prepared by filling a cuvette with 3 ml of physiological NaCl solution, which was then inserted into the apparatus with the line facing forward, followed by pressing the zero button. Subsequently, the cuvette was removed and replaced with a new cuvette containing physiological NaCl solution and 30 µl of fresh semen. Afterward, the results button was pressed to determine the concentration of spermatozoa per milliliter (Diansyah et al., 2020).

Plasma Membrane Integrity (PMI) : The analysis of sperm plasma membrane integrity was conducted through the Hypo-Osmotic Swelling Test (HOST Test). This was achieved by adding 100 µl of sperm mixed with an HOS solution, followed by an approximately 30-minute incubation at a temperature of 37°C. Subsequently, the assessment of the swelling ability of 200 sperm was performed using the coiling tail method (Cahyani et al., 2020).

Acrosome Integrity (AI): Semen samples were diluted with a 1:4 ratio of formol-saline solution. Subsequently, 10 µl of this solution was applied to a glass slide, and the sperm were examined using a trinocular microscope (Primo Star, Zeiss, Germany) at 400x magnification, observing from 10 different perspectives. 200 sperm were assessed, and a dark-colored head sperm tip identified intact acrosomal caps (Cahyani et al., 2020).

Preparation of Diluent and Cryopreservation Media

The positive control diluent (K1) was prepared using Andromed (Minitube, Germany), following the method outlined by (Mardiana, 2017), which involves a 1:4 ratio with distilled water. The negative control diluent (K2) consisted of tris (hydroxymethyl) aminomethane (Merck KGaA, Germany) at 3.634 grams, supplemented with 6% glycerol, 0.75 grams of glucose, 1.99 grams of citric acid, 0.1 grams of streptomycin sulfate, and 0.1 grams of penicillin-G, all dissolved in 100 mL of aquabidest. Additionally, soybean lecithin used was commercial soybean lecithin powder (Baktifood, Indonesia) and was dissolved in tris solution at different concentrations: 1% (w/v) (P1), 3% (w/v) (P2), and 5% (w/v) (P3). Subsequently, the mixture was centrifuged for 10 minutes and then filtered. Semen that has been diluted and has been assessed for quality can be immediately put into a straw with a capacity of 0.25 ml (Minitube, Germany) which contains at least 50 million sperm that have motility capabilities. The next step is to equilibrate it according to the method described in study by Setyawan et al. (2019), then stored it at 5°C for 3 hours. After the equilibration process at a temperature of 5°C, the semen was placed in a container containing liquid nitrogen at a distance of 5 cm above the nitrogen surface for 15 minutes, and finally storaged in liquid nitrogen at a temperature of -196°C.

Post-Thawing Semen Evaluation

Semen that has been stored in nitrogen is then thawed at 37°C for 30 seconds, and then its quality is checked, including motility, viability, membrane plasma integrity, acrosome integrity and sperm kinematics.

Data Analysis

The results of this study obtained was arranged using one-way analysis of variance (ANOVA) at P< 0.05. The significant differences found among the treatment was then differenciated using Duncan's Multiple Range Test. All calculation of the data were estimated using SPPS.

3. Results and Discussion

The fresh semen is one of parameters to predict the quality of semen before being processing to obtain freeze semen. The quality of semen obtained from the Bali bull can be seen in Table 1.

Table 1. The quality of Bali bull fresh semen

Parameter	Means ± SD
Volume (mL)	4.00 ± 135
Odor	Spesific
Colour	Cream
Consistensy	Moderate
pH	6.5 ± 0.00
Concentration (million/mL)	3759.5 ± 1608.42
Motility (%)	87.87 ± 3.97
Progressive Motility (%)	71.17 ± 4.73
Viability (%)	82.25 ± 5.18
Membrane Integrity (%)	85.50 ± 5.12
Acrosome Integrity (%)	87.25 ± 2.50

Based on Indonesian National Standard 4868.1:2007 and Regulation of the Indonesian Minister of Agriculture Number: 10/Permentan/PK.210/3/ (Diansyah et al., 2022), the results of this study indicate that the quality of fresh semen, which was evaluated both macroscopically and microscopic, generally meets the requirements of quality parameters and is suitable to proceed to the semen freezing stage.

Characteristic of Semen Before and After Cryopreservation

The results in table 2 show that the motility of Bali bull semen before freezing, K2 is significantly different (P<0.05) from P2, while it is not significantly different between K2 and K1, P1 and P4. The post-thawing semen produced by K2 was significantly different (P<0.05) from all treatments K1, P1, P2 and P3. The decreased in motility quality after thawing P1 was the lowest followed by K1, P3, K2 and P2. Diluents without additional soy lecithin in Tri's diluent can reduce motility after thawing due to the absence of additional membrane protection from soy lecithin as an extracellular cryoprotectant.

The average percentage of viability observed in semen before freezing was not significantly different between treatments K1, K2, P1, P2, and P3. Post-thawing semen showed a significant difference (P<0.05) in treatment K2 with P1, P2, and K1 but not significantly different from P3. Meanwhile, K1

is not significantly different from P1. The lowest decreased in viability quality after thawing was P1, followed by K1, P3, P2, and K2.

The average percentage of acrosome integrity before freezing was significantly different in treatments (P<0.05), namely K1 and K2, while treatments P1, P2, and P3 were not significantly different. Post-thawing semen showed significantly different treatments (P<0.05) in K2 with K1, P1, P2, and P3. Treatments K1, P2, and P3 did not show significant differences. The decreased in acrosome integrity quality with the lowest value was in treatment K1, followed by P2, P3, P1, and K2.

The fresh semen then diluted using soy lecithin and the quality of diluted semen before and after being processing into freezing semen. The quality of diluted semen from Bali bull can be seen in Table 2.

Table 2. The quality of Bali bull semen before and after cryopreservation

Parameter	Treatment	Before Freezing (%)	After Thawing (%)	Decreased (%)
Motility	K1	76.55 ± 2.96^{a,b}	47.00 ± 2.94^{b}	29.00
	K2	70.22 ± 4.67^{a}	39.17 ± 1.39^{a}	31.05
	P1	72.82 ± 5.91^{a,b}	48.25 ± 4.52^{b}	24.57
	P2	80.25 ± 4.96^{b}	48.40 ± 4.29^{b}	31.85
	P3	72.15 ± 5.98^{a,b}	45.77 ± 3.15^{b}	26.38
Viability	K1	76.25 ± 5.12^{a}	50,00 ± 2,94^{b,c}	26.25
	K2	70.50 ± 3.10^{a}	44,00 ± 1,41^{a}	26.50
	P1	72.75 ± 4.99^{a}	50,75 ± 3,59^{b,c}	22.05
	P2	78.75 ± 4.99^{a}	54,00 ± 0,81^{c}	24.75
	P3	72.50 ± 7.85^{a}	48,25 ± 4,11^{a,b}	24.25
Membrane Integrity	K1	82.50 ± 1.29^{b}	50.50 ± 1.29^{b,c}	32.00
	K2	79.75 ± 1.25^{a}	44.25 ± 0.95^{a}	35.50
	P1	82.50 ± 0.57^{b}	45.42 ± 1.47^{a}	37.08
	P2	84.25 ± 0.95^{a}	51.80 ± 1.69^{c}	32.45
	P3	79.50 ± 1.29^{b}	49.72 ± 0.98^{b}	25.78
Acrosome Integrity	K1	84.75 ± 1.70^{c}	49.55 ± 2.10^{c}	35.20
	K2	81.00 ± 1.82^{a}	41.87 ± 1.73^{a}	39.13
	P1	83.75 ± 1.25^{b,c}	46.00 ± 1.52^{b}	37.75
	P2	84.50 ± 1.29^{a,b}	52.67 ± 2.26^{c}	31.83
	P3	81.25 ± 2.21^{c}	49.37 ± 2.67^{c}	31.88

a, b, c Different superscripts in the same column indicate significant differences (P<0.05). K1 = (Andromed); K2 = (Tris); P1 = (Tris Soy Lecithin 1%); P2 = (Tris Soy Lecithin 3%); P3 = (Tris Soy Lecithin 5%).

Membrane integrity before freezing was not significantly different (P<0.05) between K2 and P2 treatments but was significantly different (P<0.05) from K1, P1 and P3. After thawing, there was no significant difference between treatments K2 and P1, significantly different from K1, P2, and P3. The lowest decreased in membrane integrity quality after thawing was in treatment P3, followed by K1, P2, K2, and P1.

Kinematics of Bali Bull Semen After Freezing

Based on the result of this study, Table 3 shows no significant differences (P<0.05) in the components of spermatozoa velocity parameters, such as VCL, VSL, and VAP, between treatment groups K1, K2, P1, P2, and P3. both before the freezing process and after the thawing. Components of spermatozoa swimming pattern parameters, such as STR, LIN, and WOB, did not show significant differences (P<0.05) between treatment groups K1, K2, P1, P2, and P3, both before freezing and after the thawing stage. In addition, regarding parameter components of spermatozoa kinematics, such as ALH and BCF, there were no significant differences between treatment groups K1, K2, P1, P2, and P3, both before freezing and after the thawing stage.

The spermatozoa kinematics which measures parameters such as distance, velocity, and other specific motion criteria using CASA. The observations of the motion pattern can be seen in the Table 3.

Tabel 3. Kinematics of Bali bull semen before and after thawing

Paramaters	Treatments	Type of Diluent				
		K1	K2	P1	P2	P3
VAP (µm/sec)	Before Freezing	49.70 ± 4.88^{a}	52.71 ± 11.11^{a}	50.05 ± 11.04^{a}	52.03 ± 6.49^{a}	48.45 ± 4.50^{a}

	After Thawing	42.07 ± 2.78[a]	39.67 ± 5.22[a]	50.58 ± 13.45[a]	42.53 ± 5.23[a]	56.73 ± 32.13[a]
VCL (µm/sec)	Before Freezing	94.81 ± 17.62[a]	91.95 ± 17.05[a]	95.36 ± 21.86[a]	101.57 ± 12.51[a]	86.02 ± 14.66[a]
	After Thawing	76.49 ± 11.09[a]	72.73 ± 4.25[a]	81.82 ± 19.50[a]	87.55 ± 25.98[a]	92.95 ± 36.06[a]
VSL (µm/sec)	Before Freezing	49.70 ± 4.88[a]	52.71 ± 11.11[a]	50.05 ± 11.04[a]	52.03 ± 6.49[a]	45.95 ± 3.39[a]
	After Thawing	30.86 ± 4.38[a]	28.93 ± 7.40[a]	38.34 ± 1272[a]	29.00 ± 3.27[a]	46.96 ± 33.50[a]
STR % (VSL/VAP)	Before Freezing	0.68 ± 0.02[a]	0.41 ± 0.06[a]	0.67 ± 0.08[a]	0.54 ± 0.31[a]	0.70 ± 0.02[a]
	After Thawing	0.73 ± 0.07[a]	0.72 ± 0.09[a]	0.74 ± 0.10[a]	0.69 ± 0.08[a]	0.78 ± 0.10[a]
LIN % (VSL/VCL)	Before Freezing	0.35 ± 0.02[a]	0.41 ± 0.60[a]	0.35 ± 0.06[a]	0.36 ± 0.01[a]	0.38 ± 0.05[a]
	After Thawing	0.41 ± 0.10[a,b]	0.39 ± 0.08[a,b]	0.46 ± 0.09[a,b]	0.35 ± 0.09[a]	0.51 ± 0.09[b]
WOB % (VAP/VCL)	Before Freezing	0.52 ± 0.02[b]	0.55 ± 0.07[b]	0.53 ± 0.06[b]	0.51 ± 0.01[b]	0.38 ± 0.05[a]
	After Thawing	0.56 ± 0.08[a]	0.54 ± 0.04[a]	0.61 ± 0.05[a]	0.50 ± 0.08[a]	0.59 ± 0.12[a]
ALH (µm)	Before Freezing	5.59 ± 028[a]	5.31 ± 0.38[a]	5.56 ± 0.72[a]	5.73 ± 0.51[a]	5.24 ± 0,42[a]
	After Thawing	5.20 ± 0.29[a]	4.75 ± 0.98[a]	6.04 ± 2.67[a]	5.02 ± 0.74[a]	6.26 ± 3.69[a]
BCF (Hz)	Before Freezing	21.60 ± 2.41[a]	21.72 ± 4.04[a]	23.50 ± 2.31[a]	20.97 ± 1.77[a]	21.14 ± 1.26[a]
	After Thawing	19.45 ± 2.63[a]	23.70 ± 9.68[a]	18,94 ± 4,49[a]	23.79 ± 3.52[a]	22.66 ± 3.96[a]

Different Superscripts In The Same Column Show Significant Differences (P<0.05). : VAP = Velocity Average Path; VCL = Velocity Curviliner; VSL = Velocity Straight Line; STR = Straightness (VSL/VAP); LIN = Linearity (VSL/VCL); WOB = Wobble (VAP/VCL); ALH = Amplitude of Lateral Head Displasemen; BCF = Beat Cross Frequency. K1 = Andromed; K2 = Tris; P1 = SL 1%; P2 = SL 3%; P3 = SL 5%.

This study results in Table 4 show that the DAP, DSL, and DCL parameters did not reveal significant differences between the various treatment groups, both before and after the thawing process. However, treatment P3 showed a significant increase in the distance traveled by spermatozoa after the thawing stage compared to treatment groups K1, K2, P1, and P2.

Table 4. Movement distance of Bali bull semen before and after thawing

Parameters	Treatments	Types of Diluents				
		K1	K2	P1	P2	P3
DAP (µm)	Before Freezing	20.42 ± 2.00[a]	21.21 ± 3.89[a]	20.38 ± 4.18[a]	20.87 ± 3.00[a]	18.69 ± 1.71[a]
	After Thawing	17.57 ± 1.47[a]	15.45 ± 1.95[a]	20.26 ± 5.82[a]	16.84 ± 2.19[a]	22.92 ± 11.82[a]
DSL (µm)	Before Freezing	13.84 ± 1.98[a]	15.54 ± 3.97[a]	13.72 ± 3.73[a]	14.52 ± 1.83[a]	13.10 ± 1.00[a]
	After Thawing	12.86 ± 2.38[a]	11.23 ± 2.80[a]	15.25 ± 5.17[a]	11.53 ± 1.35[a]	18.78 ± 12.74[a]
DCL (µm)	Before Freezing	39.34 ± 2.80[a]	38.91 ± 3.04[a]	38.42 ± 9.05[a]	41.26 ± 5.60[a]	35.39 ± 5.47[a]
	After Thawing	31.94 ± 3.71[a]	28.71 ± 0.96[a]	33.68 ± 8.83[a]	34.95 ± 10.48[a]	38.72 ± 13.14[a]

Different Superscripts In The Same Column Show Significant Differences (P<0.05). DAP (Distance Curve-Line); DSL (Distance Straight-Line); DAP (Distance Average Path. K1 = Andromed; K2 = Tris; P1 = SL 1%; P2 = SL 3%; P3 = SL 5%.

Based on the data listed in Table 1, the average semen volume of Bali bull in macroscopic observations reached 4.00 ml. These results indicate that the semen volume of Bali bull in this study was lower compared to the findings of previous study by Wijayanti et al., (2023), which recorded a volume of 6.28 ml. However, this semen volume is almost close to reported by Mujahidurrohman et al., (2023), which was 4.92 ml and was higher than the findings reported by Leo et al., (2023) of 3.20 ml. Differences in semen volume during shelter can be influenced by several factors, such as differences in body size,

offspring quality, climate, age, type of feed, frequency of shelter, and other factors (Afiati F & Said, 2013).

The odor of the semen produced was specific. According to Inonie et al., (2016), normal semen generally has a distinctive aroma, indicating the absence of damage to the semen. In addition, the semen color of Bali bull in this study was, on average, creamy white. These results align to (Mukminat et al., 2014), which noted that in healthy cows, semen generally has a white or cream color.

The semen consistency observed in Bali bull semen samples during the study tended to be somewhat liquid or showed a moderate consistency. The quality of semen consistency is related to sperm concentration and semen color. If the consistency is less thick, the sperm concentration can decrease, and the semen color will become paler. On the other hand, in semen with a medium to thick consistency, the sperm concentration will tend to be high, which is followed by a semen color that is closer to milky white or creamy white (Umami et al., 2015).

The quality of fresh semen from Bali bull in microscopic observations shows that the average spermatozoa concentration is around 3759.5 million per milliliter (ml). These results indicate that the spermatozoa concentration in this study was higher compared to several previous study, state by Mujahidurrohman et al., (2023) was 1684.4 million/ml and by Wijayanti et al., (2023), was 1646 million/ml. However, this result is lower than the study by Tethool et al., (2022), which recorded a concentration of was 7404.94 million/ml. Spermatozoa concentration is influenced by various factors such as sexual development and maturity, feed quality, reproductive organ health, testicular size, age, and male ejaculation frequency, as explained in study by (Vásquez et al., 2003).

The percentage of individual motility produced from collecting fresh semen from Bali bull reached an average of 87.87%. This finding is in line with Susilawati, (2011) statement, which indicates that the motility of fresh cow semen is generally in the range of 70-90%. This may indicate that individual motility is within the normal range and meets the requirements for the semen freezing process. According to Hapsari et al., (2018), the cryopreservation process will result in a decrease of around 20-30% in the motility of Bali bull, so it is recommended that at least 60% motility in fresh semen is required to continue the process further.

The progressive motility percentage obtained was around 71.17%. The results of study conducted by Sarastina et al., (2007) show that the average percentage of progressive motility in Bali, Madura, and Simmental bulls exceeds 70%, thus meeting the requirements to continue the processing process until it becomes frozen semen. Toelihere, (1993) stated that most fertile males have around 50-80% of spermatozoa that have active motility and move progressively.

The viability of the spermatozoa obtained was around 82.5%. The results of this study show that the viability rate is lower when compared to the results of study stated by Mujahidurrohman et al., (2023), which reached 98.60%. However, in general, the viability percentage value is similar to the study by Siahaan et al., (2012), was 85%, and Blegur et al., (2020), was 81.07%. It is important to note that for the dilution process to become frozen semen, spermatozoa viability should at least reach the range of 60% -75% of viable spermatozoa, according to the guidelines stated by (Garner & Hafez, 2000).

The results of this study show that the average percentage of plasma membrane integrity from fresh semen from Bali bull is around 85.50%. In comparison, the average percentage of acrosome integrity is around 87.5%. This plasma membrane integrity percentage value is almost similar to the average results of study stated by Bebas et al., (2021), which reached around 83.55% but is still lower than the results of study conducted by Marawali et al., (2019), where membrane plasma and acrosome intgerity reached 90.16% and 90.12% respectively. Based on the evaluation of the macroscopic and microscopic characteristics of Bali bull semen, it can be concluded that the collected semen is of good quality and meets the requirements for further processing into frozen semen.

After using andromed, tris with no lecithin, and tris with lecithin (containing 1%, 3%, and 5%) showed lower quality in tris with no lecithin before and after thawing or K2. This is because, in the K2 treatment, there is no additional soy lecithin, which causes a lack of additional protection for sperm in maintaining its quality during freezing. Tris diluent with the addition of soy lecithin indicates superior quality compared to the Andromed, which contains soy lecithin. This may happen because soybeans contain phospholipids as the main component in their phosphate fraction. The composition of these phospholipids includes phosphatidylcholine 17.50%-23.00%; phosphatidyl ethanolamine 15.00%-20.00%; glycolipids 13-16%; other phospholipids 14-18%, and triglycerides 2-4% (Aku et al., 2007). Although there is no detailed explanation regarding the mechanism of action of lecithin, based on study Campbell et al., (2002), phospholipids tend to aggregate and form aggregates that protect their

hydrophobic components. On the cell surface, phospholipids form a double layer that interacts with the water environment, both inside and outside the cell, and with the surrounding environment. Therefore, the addition of lecithin from soybeans can maintain the integrity of the sperm lipoprotein sheath from stress due to low temperatures, and most studies state that sperm quality can be maintained through the use of soy lecithin.

The motility in this study post-dilution and thawing showed the lowest quality reduction at P1, only 24.57%. The lecithin content in soybeans, similar to that in egg yolks, has the potential to protect cell membranes from the negative impacts of cold shock, as reported in study by (Qureshi et al., 2014). Cold shock can cause a decrease in flagellar activity in spermatozoa, reduce the amount of motility, and trigger the release of enzymes, according to findings from study by (Ogbuewu et al., 2010). During the cryopreservation process, the formation of ice crystals can result in the release of intracellular enzymes and damage cell organelles, such as lysosomes and mitochondria. As a result, energy formation and metabolism stop, resulting in a decrease in spermatozoa motility, as mentioned in study by (Gazali & Natal Tambing, 2002).

The quality of viability obtained in this study showed the lowest reduction in post-thawing is P1, namely only 22.05%. The results of study stated by Forouzanfar et al., (2010) a higher level of viability when using Tris-based diluent enriched with 1% soy lecithin compared with Tris diluent containing 20% egg yolk. The cryopreservation process can cause damage to cell membranes due to the loss of phospholipids. However, the phospholipids contained in soy lecithin can replace this loss, thereby maintaining the structure and function of sperm membranes after the freezing process (Graham & Foote', 1987).

In terms of measuring plasma membrane integrity and integrity after the dilution and thawing process, the most significant reduction in quality was seen in the P2 and P3 treatments. Plasma membrane integrity reached the best level in the P3 treatment, with a decrease of only 25.78%. In comparison, acrosome integrity reached the best level in the P2 treatment, with a decrease of only around 31.83%. The integrity of the plasma membrane function is vital in sperm freezing and fertilization Rasul et al., (2001). The plasma membrane controls intracellular processes as a protective barrier for cell organelles and a filter to exchange substances (Nofa et al., 2017). The integrity of the acrosome needs to be maintained because it is related to the process of capacitation and acrosome reactions; the enzymes contained in the acrosome are needed for penetration so that sperm can penetrate the zona pellucida and fertilization can occur (Alçay et al., 2019).

VAP (Velocity Average Path) refers to the speed of sperm in one minute on an average path, and VCL (Velocity Curve Linear) measures the speed of sperm in one minute on a winding path. In comparison, VSL (Velocity Straight Linear) records sperm speed in one minute on a straight line (Sarastina et al., 2007). Although initial observation results showed no significant differences in VAP, VCL, and VSL, between the various treatments, changes were seen after the thawing process, where treatment P3 experienced an increase, while K1, K2, P1, and P2 decrease. Previous study stated by Brown et al., 1988) reported that VAP, VSL, and VCL values were more effective for fertilization if they were above 50% compared to below 50%. With an average of VAP, VSL, and VCL meet good criteria, by the categorization suggested by (Inanç et al., 2018), namely VCL > 70 m/s, VSL > 45 m/s, and VAP > 45%.

LIN (Linearity) refers to a parameter that measures the level of straightness of the linear movement trajectory, and STR (Straight) indicates the average straightness of the trajectory. At the same time, WOB (Wobble) is a measure of the actual oscillation of the trajectory, which indicates how strongly the sperm sways for one second (Ratnawati et al., 2019). The results in Table 6 showed that sperm movement in a linear trajectory meets, on average, the criteria described by Oliveira et al. in 2013, with LIN > 35% and STR > 50%. The straightness of linear movement, as measured by LIN > 35%, indicates linear movement, while LIN < 35% indicates non-linear movement, according to the explanation given by (Susilawati, 2011).

Some of the parameters used to measure sperm head kinematics include ALH (Amplitude of Lateral Head Displasemen), which reflects the average width of sperm head oscillations while swimming (Kathiravan et al., 2011), and BCF (Beat Cross Frequency), which measured the frequency sperm trajectories across the groove per second on average (Sarastina et al., 2007). According to study stated by (Belala et al., 2019), the ideal ALH value is 2.5 to 6.5 µm, while the BCF must be more than 20 Hz for spermatozoa to have good mobility and fertility. In the context of this study, treatments K1, K2, P1, P2, and P3 showed adequate averages and met the expected standards.

The parameters DAP (Distance Average Path), DSL (Distance Straight-Line), and DCL (Distance Curve-Line) showed no significant differences among the treatments, either before and after thawing. DAP measures the distance that spermatozoa can travel in one minute on a mean path, DSL measures the distance that spermatozoa can travel in one minute on a straight path, and DCL measures the distance that sperm can travel in one minute on a winding path (Ratnawati et al., 2019). Among the various treatments K1, K2, P1, P2, and P3, there was an increase in the distance travelled by spermatozoa after the thawing process, especially in treatment P3. According to a study stated by (Massányi et al., 2008), sperm is considered progressive if it meets the criteria for DAP: 19.23 - 24.44 m/s, DCL: 37.43 - 47.20 m/s, and DSL: 14.27 - 18 .92m/s. The results of this study showed that several treatments, namely K1, K2, P1, P2, and P3, have typical average values for several parameters that indicated good quality especially after the thawing process.

In the results of this study, it can be seen that the addition of soy lecithin is almost matches the quality of sperm produced from andromed. Based on sperm kinematics, it shows that Balinese bull sperm fall into the hyperactive category in P2 and P3 sperm showing an increase in VCL and ALH. There are 5 parameters that are commonly used to categorize sperm hyperactivation, including increased VCL and ALH (Marquez & Suarez, 2004). While the decrease in VSL, LIN, and STR can also be used as a marker of sperm hyperactivity, but the resulting value cannot be always used as a benchmark because there is a decrease when the sperm dies (Hinrichs & Loux, 2012).

4. Conclusion

Based on this study, the semen of Bali bull obtained in the normal category, while soy lecithin can improve sperm quality and kinematics with the addition of 3% and 5% soy lecithin.

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Authors Contribution

Zahra Jinan Fadilla contributed to data collection, methodology, analysis, original manuscript writing, review, and editing. Muhammad Yusuf contributed to data analysis, original manuscript writing, review, and reading of the original manuscript and review, and finally approved the final manuscript. Abdul Latief Toleng contributed to data analysis, original manuscript writing, review, and reading of the original and review manuscripts, and approved the final manuscript. Atthar Manabi Diansyah contributed to data analysis, original manuscript writing, review, and reading of the original manuscript and review manuscript, and approved the final manuscript.

Conflict of Interests

The authors declare that they have no conflicts of interest.

Ethical Consideration

The authors have confirmed ethical issues, such as plagiarism, misconduct, fabrication and/or falsification of information, consent to publish, duplication of publication and/or submission, and redundancy.

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