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The Effect of Using Soybean Extender on the Movement Patterns of Spermatozoa and Plasma Membrane Integrity in Bali Bull

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Abstract

Selection of good extender quality greatly affects the quality of spermatozoa. One of the natural ingredients that can be used as a semen extender is soybean, which is known to maintain the quality of spermatozoa by preventing cold shock. This study was conducted to determine the effect of using soybean extender on the movement pattern and plasma membrane integrity (PMI) of spermatozoa. This study was conducted at the Samata Integrated Farming System (SIFS), Samata Village, Kab. Gowa. The processing of semen was carried out at the Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University. A Bali bull was used to collect the semen for five times using an artificial vagina. Four types of extenders as treatments were used to extend the semen; T0 (andromed) for control, T1 (tris), T2 (soybean), and T3 (tris-soybean) The parameters measured were the plasma membrane integrity (PMI) and movement patterns of spermatozoa. The results showed that the average motility of fresh semen obtained was 94.22% ± 2.37, viability 96.06% ± 1.09, concentration 1596 x 10^6/mL ± 1472, and MPI 95.11% ± 1.82.

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The average percentage of MPI in the use of tris soybean extender was higher (97.79 ± 1.14) compared to other extenders and there was no significant difference (P>0.05) was found between the four treatments. In the movement pattern parameters, all treatments in each parameter (VCL, VAP, VSL, DCL, DAP, DSL, LIN, and STR) did not show any significant difference to the control. Based on the results of study, it can be stated that the use of soy extender was able to maintain the quality semen and can be classified as good for further processed. As conclusion, the use of soybean extender was able to maintain the quality of Bali bull semen indicated that soybean extender was classified as very good and could be used further.

Keywords: Soybean; Extender; Bali bull; Plasma membrane integrity (PMI); Movement pattern.

1. Introduction

Improving the genetic quality of Bali cattle is supported by the development of reproductive technology, in which, one of them is the use of Artificial Insemination (AI). AI has been well known as a reproductive technology in which a straw containing high quality sperms is inserted into the female reproductive tract using special equipment. One of the factors that support the success of genetic quality improvement through the AI program is the high quality of semen. The high percentage of live sperms is caused by the role of the extender media used [1]. Therefore, an extender is needed that can minimize the decrease in the quality of sperms.

Selection of a good extender can affect in maintaining the quality of sperms. One of the natural ingredients that is possibility good to use as a semen extender is soybean, which is known to maintain the quality of spermatozoa by preventing cold shock. Soybean is one of the natural ingredients that can be used as a semen extender. Some of the nutritional content possessed by soybeans including protein, minerals, fat, and carbohydrates where these components are known to be present in semen and of course needed by spermatozoa. In addition to these ingredients, soybeans are also known to contain lipoproteins and lecithin which can protect spermatozoa from cold stress or known as cold shock. This thing which causes soybeans to be considered capable of maintaining the quality of spermatozoa. Evaluation of the quality of spermatozoa is considered very important, especially motility, viability, abnormalities, movement patterns and the plasma membrane integrity (PMI) because it is directly related to the level of fertility.

Several physiological processes during fertilization (capacitation, acrosomal reaction, union of spermatozoa and egg) require an active membrane and cannot be fertilized with an inactive membrane condition [2]. Therefore, this study was arranged to determine how the effect of the use of soybean extender on the movement pattern of spermatozoa and the plasma membrane integrity (PMI) of spermatozoa.

2. Materials and Methods

This study was conducted from February 2021 to April 2021. The semen of a Bali bull was collected at the Samata Integrated Farming System (SIFS), Samata Village, Gowa Regency. Processing of semen after collection was performed at the Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University, Makassar.

2.1. Preparation of extender and treatments

Tris crystals (Hydroximethyl aminomethane) of 3.634 g, glucose crystals 0.50 g, citric acid 1.99 g, penicillin and streptomycin 0.1 g, and aquabidest up to 100 ml were mixed as a tris solution (T1). Peeled soybeans 37.5 g, penicillin and streptomycin 0.1 g, sodium citrate 5 g, and aquabidest of 250 mL were mixed as an extender for soybean. After mixing each of above solution, the three different extenders were made; solution 1, soybean extender (T2), solution 2, and tris-extender, (soybean extender + tris extender with a ratio of 50 : 50). In the present study, T0 as control was used commercially extender; andromed.

2.2. Collection and evaluation of semen

The semen of a Bali bull was collected for five times in the morning using an artificial vagina. The semen that had been obtained was then examined macroscopically and microscopically. Microscopic examination included examination of volume (ml), consistency, pH, color and odor. Microscopic examination included semen concentration, motility, viability, abnormalities, plasma membrane integrity (PMI), and movement patterns of spermatozoa.

2.3. Processing of semen

Semen dilution was performed by mixing semen into each treatment. The semen was diluted to obtain a spermatozoa concentration of 25 million/straw (0.25 ml). Calculation of dilution to extend the volume of semen was based on Tambing and his colleagues [3]. The semen was then inserting into the straw using a syringe and clamping the straw using heated tweezers and kept at a temperature of 4 - 5°C for equilibration during 4 hours. The freezing process was conducted by means of a straws were gradually cooled for 15 minutes before dipping in liquid nitrogen.

2.4. *Parameters measured*

- Plasma Membrane Integrity (PMI)

Plasma Membrane Integrity (PMI) was observed by inserting 10 µl of semen sample into HOST solution (0.179g NaCl in 100 ml of aquabides), then incubated for 1 hour at 37ºC. Spermatozoa with intact plasma membranes were characterized by coiled tails and damaged sperm were characterized by straight tails. The evaluation was conducted with a 400x magnification microscope by counting 200 spermatozoa cells using the formula below.

$$\text{PMI} = \frac{\text{Number of spermatozoa with coiled tail}}{\text{Sperm count circular tail + number of straight tail spermatozoa}} \times 100\%$$

- Movement Pattern

Parameters such as velocity (VCL, VAP, VSL), linearity (LIN), and distance (DCL, DAP, DSL) tested using the Computer Assisted Semen Analysis (CASA) which is intended to overcome subjectivity evaluation.

2.5. Data analyses

The data obtained in the study were tabulated and calculated in Excel program for windows. Descriptive statistic was used to obtain average and standard deviation (SD). Plasma membrane integrity (PMI) and movement patterns of the sperms at different extender treatments were arranged using one-way analysis of variance (ANOVA) and calculated using SPSS program for windows. Furthermore, least significant difference (LSD) test was used to compare each different treatments.

3. Results and discussion

3.1. Macroscopic and microscopic evaluation of Bali bull fresh semen

The degree of acidity (pH) of bovine semen obtained in this study within within the normal pH range; 6.6 ± 0.54. The pH value was in accordance with the opinion of Butar [4], the pH of fresh semen of Bali bull was 6.4 - 7.8. Nursyam [5] added that the pH of good quality semen is 6.8 – 7.6. However, Feradis [6] stated that each breed of cattle has a different pH value of fresh semen. The pH value of bull semen that is too low or tends to be acidic can be caused by high levels of lactic acid which is the result of metabolism. In addition, the pH of semen that is too acidic can also be caused by damage to the plasma membrane or acrosome [7]. A pH value that is too high or tends to be alkaline is caused by excess fluid production by the accessory glands. In addition, the pH of semen that is too alkaline can also be caused by the large number of dead spermatozoa [8].

The smell of semen in this study had a distinctive odor. According to Susilawati [9] that semen with a distinctive smell indicates is normal and there is no contamination. Rizal and his colleagues [10] added that normal semen, in general, has a distinctive fishy odor accompanied by the smell of the animal itself. A foul odor usually occurs when the semen contains pus caused by an infection of the male animal's reproductive organs or tract.

The color of the semen in this study was specific. This showed that the results in this study were still in normal conditions, this was because the semen produced did not have red spots, if there were red spots it indicates the presence of blood in fresh semen. These results were in accordance with the opinion of [5,6] which stated that normal bull semen is milky white or creamy white and cloudy.

The consistency of the semen obtained in this study was moderate. Consistency is a parameter to determine semen quality which is closely related to concentration. Consistency is defined as the viscosity of semen, and concentration is the number of spermatozoa cells per ml of semen. The highest of concentration of spermatozoa could be the thickest of consistency of the semen. Meanwhile, if the concentration is low, the consistency of the semen will also be more watery [11]. The consistency in this study was in line with the average value of fresh semen concentration obtained, which was 1596 x 10^6/mL ± 1472. The concentration obtained from this observation was quite good. This was supported by the opinion of [12] which stated that good spermatozoa have concentrations ranging from 2000 – 2200 x 10^6/mL. According to Dewi and his colleagues [13] that one of the factors that affect the viscosity of semen is the quality of the feed given.

The individual motility produced in this study was 94.22 ± 2.37. From these results, the semen obtained was

suitable for further processing. According to Gunawan and his colleagues [14], that the Indonesian National Standard requires that semen that meets the requirements used in the AI program must have a minimum motility of 40%. Susilawati [15] stated that the motility of fresh bull semen ranged from 70% - 90%.

The viability obtained in this study was 96.06% ± 1.09. From these results, the semen obtained was suitable for further processing. This was in accordance with Toelihere [16] statement that good semen has a viability percentage above 50%. The results showed that the average percentage of fresh bull semen plasma membrane integrity (PMI) was 95.11% ± 1.82. The high percentage of PMI could be caused by the high percentage of viability of the fresh semen. According to [17], viability has a correlation with the strength of the plasma membrane of spermatozoa.

Movement patterns of Bali bull sperms are shown in Table 1. Based on Table 1, it can be seen the results of observing the movement pattern of fresh semen of Bali bull. Sarastina and his colleagues [18] stated that the LIN and STR values can be used as indicators of progressive motility and swimming pattern. Meanwhile, the ability to fertilize has a correlation with VSL and VCL parameters that contribute to the characteristics of spermatozoa function [19].

Table 1: Movement patterns of Bali bull fresh semen

Movement Pattern	Average (± SD)
VCL (µm/det)	153.38 ± 29.41
VAP (µm/det)	76.28 ± 6.96
VSL (µm/det)	47.98 ± 1.63
DCL (µm)	62.80 ± 10.87
DAP (µm)	31.12 ± 2.27
DSL (µm)	19.46 ± 0.79
LIN (VSL/VCL)	0.32 ± 0.05
STR (VSL/VAP)	0.63 ±0.05

SD = Standard Deviation; VCL = Velocity Curviliner; VAP = Velocity Average Path; VSL = Velocity Straight Line; DCL = Distance Curviliner; DAP = Distance Average Path; DSL = Distance Straight Line; LIN = Linierity; STR = Straightness

3.2. Plasma Membrane Integrity (PMI) of Bali Bull Sperms at different extenders

The plasma membrane is a part of spermatozoa that functions to regulate the traffic in and out of all substrates and electrolytes from cells that are needed in the metabolic process [20]. The percentage of plasma membrane integrity (PMI) of Bali bull sperms are shown in Table 2.

Table 2: Plasma membrane integrity (PMI) of Bali bull sperms using andromed, tris, soybean, and tris-soybean extenders.

Extender	Average (%)	SD	Min	Max	95% CI	
					Lower bound	Upper Bound
Andromed (T0)	92.02	4.16	84.84	95.10	86.84	97.19
Tris (T1)	93.36	1.88	91.28	96.24	91.02	95.69
Soybean (T2)	89.33	4.27	85.35	94.14	84.02	94.63
Tris-soybean (T3)	97.79	1.14	91.12	93.81	91.36	94.22
P-value	0.235					

P = Probability; SD = Standard Deviation; Min = Minimum; Max = Maximum; CI = Confidence Interval

Table 2 shows plasma membrane integrity of Bali bull sperms at different extenders. The average of plasma membrane integrity did not differed (P=0.235) among different treatments ranging from 89% to 98%. This suggests that the use of tris or soybean alone or their combination are able to maintain plasma membrane integrity of Bali bull sperms.

Based on the results of the study, it is known that the lowest percentage of PMI was with the use of soybean extender. This indicates that the more soybean was added, the lower the percentage of intact plasma membranes of Bali bull spermatozoa so that it could cause toxic effects. Pleated on the plasma membrane is very vulnerable against the peroxidation reaction [21]. The intact spermatozoa plasma membranes obtained were still in the good category with an average percentage of each treatment <80%. This is in accordance with the opinion of [22] which stated that the category of infertile semen is less than 60%.

3.3. Movement patterns of Bali Bull Sperms at different extenders

Spermatozoa movement patterns were analyzed using CASA. The pattern of movement is divided into two; the speed of movement of the spermatozoa and the distance traveled by the spermatozoa. The speed of spermatozoa includes VAP = Velocity Average Path, VCL = Velocity Curviliner, VSL = Velocity Straight Line. Meanwhile, the distance traveled by spermatozoa includes DAP = Distance Average Path, DCL = Distance Curviliner, and DSL = Distance Straight Line. The movement pattern of Bali cattle spermatozoa can be seen in Table 3.

Table 3: Movement patterns of Bali bull sperms at different extenders

Parameter	Extender				P-value
	Andromed (T0)	Tris (T1)	Soybean (T2)	Tris-soybean (T3)	
VCL (µm/s)	113.47± 20.44	142.10 ± 23.08	125.14 ±36.80	139.84 ± 31.27	0.379
VAP (µm/s)	59.69 ± 6.80	69.83 ± 7.27	60.27 ± 13.62	66.93 ± 11.13	0.334
VSL (µm/s)	40.21 ± 3.67	45.06 ± 3.03	37.52 ± 6.78	42.50 ± 5.03	0.128
DCL (µm)	46.97 ± 7.54	58.16 ± 8.94	50.99 ± 14.56	56.41 ± 12.65	0.406
DAP (µm)	24.63 ± 2.37	28.43 ± 2.83	24.62 ± 5.57	26.77 ± 4.46	0.395
DSL (µm)	16.54 ± 1.40	18.23 ± 1.15	15.11 ± 2.63	16.89 ± 1.96	0.114
LIN (VSL/VCL)	0.36 ± 0.04	0.32 ± 0.03	0.31 ± 0.05	0.31 ± 0.03	0.283
STR (VSL/VAP)	0.67 ± 0.03	0.65 ± 0.03	0.62 ± 0.03	0.64 ± 0.03	0.222

VCL = Velocity Curviliner; VAP = Velocity Average Path; VSL = Velocity Straight Line; DCL = Distance Curviliner; DAP = Distance Average Path; DSL = Distance Straight Line; LIN = Linierity; STR = Straightness

Based on Table 3, it can be seen that the VCL value in each treatment did not show a significant difference (P > 0.05) against T0 with a value of 113.47 ± 20.44 µm/s, where the values of each treatment were T1 = 142.10 ± 23.08 µm/s, T2 = 125.14 ± 36.80 µm/s and T3 = 139.84 ± 31.27 µm/s. The VAP value in each treatment did not show a significant difference (P > 0.05) against T0 with a value of 59.69 ± 6.80 µm/s, where the values of each treatment were T1 = 69.83 ± 7.27 µm/s, T2 = 60.27 ± 13.62 µm/s and T3 = 66.93 ± 11.13 µm/s. This indicated that the progressive motility of spermatozoa had a high value as reported by Suzuki and his colleagues [19] that, VAP values >25.0 µm/s indicated progressive motility of spermatozoa. The results of another study stated that the percentage of spermatozoa that had a VAP <25 µm/s. While the percentage of progressive motility of spermatozoa is the percentage of spermatozoa with a VAP value >25 µm/s [23]. The VSL value in each treatment did not show a significant difference (P > 0.05) against T0 with a value of 40.21 ± 3.67 µm/s, where the value of each treatment were T1 = 45.06 ± 3.03 µm/s, T2 = 37.52 ± 6.78 µm/s and T3 = 42.50 ± 5.03 µm/s.

One of the factors that affect the speed of spermatozoa is the extender used. As it is known that the four extenders used have different consistency, where the tris and andromed extenders have a watery consistency, soybeans with a thick consistency, and tris soybeans with a medium consistency so that the extender with a watery consistency will support the speed of movement of spermatozoa more freely [24].

The DCL, DAP, and DSL values in each treatment did not show a significant difference (P>0.05) in comparison to T0 Table 3). This indicated that the distance achieved of sperms during traveling had similar among the treatments. Therefore, the use of tris or soybean or their combination were able to move with similar distance. Furthermore, these extenders can be used to extend the semen of Bali bull semen.

Reference [25] stated that LIN is a straight line curve linear. The LIN value was obtained by dividing VSL and VCLL multiplied by 100 and expressed in %. Furthermore, Bahrawy [26] stated that the LIN value in spermatozoa can indicate the characteristics of the direction of movement or swimming straightness of

spermatozoa. The LIN and STR values in each treatment did not differed (P>0.05) among the treatments. However, the LIN values shown in the present study for all treatments showed a lower value than that stated by Sarastina and his colleagues [18] which stated that buffalo sperm has a LIN value of 0.533 [25]. The STR value in each treatment did not show a significant difference (P>0.05) against T0 with a value of 0.67 ± 0.03, where the value of each treatment were T1 = 0.65 ± 0.03, T2 = 0.62 ± 0.03, and T3 = 0.64 ± 0.03.

Based on the results of the study and discussion, it can be concluded that the use of soybean extender was able to maintain the quality of Bali bull semen indicated that soybean extender was classified as very good and could be used further.

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