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Effect of Semen Leaching and Soybean Lecithin Levels in Tris Extenders on the Quality of Preserved Sheep Spermatozoa at 5°C Temperature

Salmin[1] Marsudi[2] Deka Uli Fahrodi[3] Hendro Sukoco[4]
Animal Husbandry Study Program, Faculty of Animal Husbandry and Fisheries, Tadulako University, Palu City, Central Sulawesi Province, Indonesia[1]
Animal Husbandry Study Program, Faculty of Animal Husbandry and Fisheries, University of West Sulawesi, Majene Regency, West Sulawesi Province, Indonesia[2,3,4]
Email: salmin.bouato@gmail.com[1]

Abstract
This study aims to study the leaching of semen and the level of soy bean lecithin (Soybean lecithin) in relation to the quality of sheep spermatozoa preserved at 5OC. The study used a Complete Randomized Design (RAL) factorial pattern of 2 x 5 x 3. As the first factor is cement washing (P), consisting of P0 = Fresh cement without washing and P1 = Fresh cement undergoes a washing process. The second factor is the level of soy bean lecithin (L), consisting of L0, L1, L2, L3, and L4. Independent variables are cement washing (P) and soybean lasitin levels (L). As a dependent variable is the quality of spermatozoa at a storage temperature of 5^0C, which consists of progressive motility, viability, abnormality and intergrity of the spermatozoa membrane. The results of the study obtained that the treatment of cement leaching and soy bean lecithin levels together did not show any noticeable interaction, however, single-seeded lecithin levels showed markedly different influences (P≤0.05) and the washing treatment showed no noticeably different influence on all quality parameters of sheep spermatozoa during five days of storage at 5^0C. The use of soy bean lecithin as a component of sheep cement extenders is effective against samples of unwashed cement or washed cement. The best quality of sheep spermatozoa was obtained at the treatment of 3% soy bean lecithin levels in Tris extenders against washed and unwashed semen preserved for five days of storage at 5oC with an average progressive motility percentage of 63.18%; viability 72.20%; abnormality 12.43%; and membrane integrity of 72.92%.
Keywords: Semen Washing, Lecithin, Soy Bean, Spermatozoa

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INTRODUCTION

One of the protective materials that has the potential to be developed in protecting spermatozoa from the adverse effects of preservation and cryopreservation is soy bean lecithin (soybean lecithin). This material is one of the vegetable lecithins that contains phospholipids which are very important for protecting the spermatozoa membrane in the preservation process and cryopreservation. Naturally, the lecithin content in soybeans is 1.48–3.08%. The main components of soy bean lecithin are phospholipids consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositols and glycolipids (Shurtleff and Aoyagi, 2007; Erickson, 2002). Hygienically, soy bean lecithin does not contain microogranism which can adversely affect spermatozoa or the female reproductive tract (Bousseau et al., 1998).

The effectiveness of the use of soy bean lecithin as a component of sheep cement extenders is not yet known with certainty. The presence of the enzyme phospholipase A in sheep plasma seminals (Scott and Dawson, 1968; Roldan and Fragio, 1993) produced by the bulbourethralis gland are thought to interfere with the survival of spermatozoa. The enzyme

fosolipase A can catalyze the hydrolysis of lecithin into fatty acids and lysolecithin which can coagulate the extender medium and is toxic to spermatozoa (Chemineau et al., 1991; Rizal et al., 2008; Iritani and Nishikawa, 1972 in Ashmawy et al., 2010). Especially in goat cement, seminal plasma removal by washing and centrifugation immediately after cement storage is a routine procedure if diluted with an extender containing egg yolk lecithin (Ayu, 2012; Chemineau et al., 1991; Evans and Maxwell, 1987). In sheep semen, the effectiveness of seminal removal of plasma in this way before being diluted with soy bean lecithin has not been explained. Accurate information regarding the effect of washing of semen and lecithin soybeans on the quality of sheep spermatozoa is needed to test the usefulness of kalelai bean lecithin as a component of sheep cement extenders. As explained above, it has been studied about the effect of semen washing and soybean lecithin (Soybean lecithin) lecithin on the quality of sheep spermatozoa preserved at 5^{0} C temperature.

RESEARCH METHODS

The place where the research was carried out was the Laboratory of Physiology and Reproduction of Livestock, Faculty of Animal Husbandry UGM Yogyakarta with a duration of about four months. Some of the ingredients used for research purposes are fresh lamb cement, soy bean lecithin (soybean lecithin) 10% (CENTROL 3 flub, sertificate number: TSC 04020, USA), Tris (hydroxymethyl) aminomethane, citric acid, glucose, aquadestilata, aquabidestilata, penicillin, streptomycin, Krebs- Ringer phosphate glucose solution, hypoosmotic swelling test (HOS-test) solution, physiological NaCl, hayem solution, eosin Y/Negrosin, alcohol 70%. The equipment used in the study was an artificial vagina, thermometer, ice flask, scaled tube, test tube, eppendorf tube, measuring cup, erlenmeyer, cup cup, aluminum foil, blender machine (Miyako BL-101 PL), stirring rod, micropipette (Transferpette®), drip pipette, centrifugation device (MLW T 52.1), light microscope (Tension), optilab camera (Optilabpro viewer®), object glass, cover glass, haemocytometer, Neubauer counting chamber, pH meter (Sentron 501 pocket FET®, Netherlands), refrigerator (Sanken CN),® hand tally counter (Laboratory Dc Counter: DBC-9. K Gemini Ind. Corp., USA), analytical scales. ®

The study was conducted experimentally with a Complete Randomized Design (RAL) factorial pattern of 2 x 5 x 3. The first factor is cement washing (P), consisting of: P0 = Fresh cement without washing. P1 = Fresh cement undergoes washing process The second factor is soy bean lecithin content (L), consisting of: L0 = 0% soy bean lecithin + 100% Tris L1 extender = 1% soy bean lecithin + 99% Tris L2 extender = 2% soy bean lecithin + 98% Tris L3 extender = 3% soy bean lecithin + 97% Tris L4 extender = 4% soy bean lecithin + 96% Tris extender.

Independent variables are cement washing (P) and soybean lasitin levels (L). As a dependent variable is the quality of spermatozoa stored at 5^{0}C, as follows: Motility of spermatozoa, calculated based on the percentage of spermatozoa that are progressive motile (moving actively forward). The number of progressive motile spermatozoa is calculated by subtracting the total spermatozoa observed with a progressive non-motile number of spermatozoa on ten observation objects under a microscope (Salmin, 2000; Salmin, 2002). Viability or percentage of life of spermatozoa, calculated based on the percentage of living spermatozoa through differential staining of the total observed spermatozoa (Toelihere, 1985b; Bearden et al., 2004) and expressed in percent. Spermatozoa abnormality, calculated based on the percentage of abnormal spermatozoa from the total observed spermatozoa (Toelihere, 1985b; Bearden et al., 2004) and expressed in percent. The integrity of the spermatozoa membrane, calculated based on the percentage of spermatozoa that have an

intact membrane layer of the total observed spermatozoa (Jayendran and Zaneveld, 1986) and expressed in percent.

Experimental sheep cement shelter was carried out at the time of the study using an artificial vagina (Toelihere, 1985b; Bearden et al., 2004). The cement storage time is carried out every three days which starts at 07.00 WIB. After finishing the cement reservoir, the ejaculate is immediately taken to the laboratory to be immediately evaluated and processed. Information from the evaluation results is very necessary, especially to ensure the feasibility of cement and determine the level of extender used. Post-reservoir cement evaluation is carried out on the basis of macroscopic and microscopic assessments. Macroscopic assessments include examination of the volume, pH, color, and consistency (viscosity) of cement (Toelihere, 1985b; Evans and Maxwell, 1987). Microscopic evaluation includes spermatozoa motility (mass and individual movements), spermatozoa concentration, spermatozoa viability and spermatozoa abnormalities (Sasmita, 2017; Ayu, 2012; Toelihere, 1985b; Ax et al., 2000; Bearden et al., 2004) and the integrity of the spermatozoan membrane (Jayendran and Zaneveld, 1986).

The process viable cement sample is separated into two tubes with the same volume is many, namely the P0 sample (cement without washing) and the P1 sample (cement through the washing process). Furthermore, each tube is divided into five parts, namely P0L0 (cement without washing + extender Tris-lecithin soybeans 0%), P0L1 (cement without washing + extender Tris-lecithin soybeans 1%), P0L2 (cement without washing + extender Tris-lecithin soybeans 2%), P0L3 (cement without washing + extender Tris-lecithin soybeans 3%), P0L4 (cement without washing + extender Tris-lecithin soybeans 4%) and P1L0 (cement through the washing process + extender Tris-lecithin soybeans 4%) and P1L0 (cement through the washing process + extender Tris-lecithin soybeans 0%), P1L1 (cement through washing process + extender Tris- soy bean lecithin 1%), P1L2 (cement through washing process + extender Tris- soy bean lecithin 2%), P1L3 (cement through washing process + extender Tris- soy bean lecithin 3%), P1L4 (cement through washing process + extender Tris- soy bean lecithin 4%).

Before the dilution process is carried out, each cement sample in five tubes to be washed, namely P1L0, P1L1, P1L2, P1L3, and P1L4, was first carried out the washing process with Krebs-Ringer phosphate glucose solution according to the procedure of Chemineau et al. (1991) as follows; Krebs-Ringer phosphate glucose washing solution is prepared one day before the washing process is carried out. The ratio of 1 : 9 between the cement and the washing solution is centrifuged at a speed of 1800 rpm for 15 minutes at a temperature of 20^0C, then the supernatant (upper clear liquid) is discharged. Next, the washing solution is added again with the same ratio and centrifuged a second time with the same speed, time and temperature, then the supernatant is discarded. After the supernatant is removed, the rest is in the form of a portion containing spermatozoa (spermatozoa pellets) then diluted with an extender which is tried as needed in this experiment. Cement samples in five other tubes, namely P0L0, P0L1, P0L2, P0L3 and P0L4 were not carried out the cement washing process but immediately added extenders that were tried as needed in this experiment.

The preparation of Krebs-Ringer phosphate glucose solution was carried out the day before the cement reservoir with a composition according to the instructions of Chemineau et al. (1991) as follows:

Table 1. Krebs-Ringer Phosphate Glucose Solution Preparation

0,9 % NaCl	100,0
1,15% KCl	4,0
1,22% CaCl2	3,0

2,11% KH2PO4	0,4
3,82% MgSO4.7H2O	1,0
Phosphate buffer pH 7,4	12,0
5,34% Glucose anhydrous	4,5

The cement base extender is prepared the day before the cement shelter is carried out. The basic extender used in the study was the Tris-citrate-glucose solution (Evans and Maxwell, 1987), hereinafter referred to as the Tris extender, which consists of Tris (hydroxymethyl) aminomethane, citric acid and glucose. The procedure for making Tris basic extenders is as follows: A total of 3,634 grams of Tris (hydroxymethyl) aminomethane, 1.99 grams of citric acid and 0.50 grams of glucose are dissolved with acuadestilata until it reaches a total volume of 100 ml then put into a clean and dry erlenmeyer. The solution is stirred in a water bath with a temperature of 100^0C for 15 minutes until homogeneous (clear-looking) then cooled to room temperature and antibiotics are added in the form of penicillin 1,000 IU / ml extender and streptomycin 1 mg/ml extender. Next, the solution is stored at a temperature of 3-5^0C in the refrigerator and if it will be used for extenders, the addition of soy bean lecithin is carried out according to the level of treatment to be tried (L0, L1, L2, L3, and L4).

Extender Tris-lecithin soybeans for the purpose of liquid cement is made one hour before the cement reservoir. This is intended so that the cement dilution process time can be accelerated, so that the quality of cement immediately after macroscopic and microscopic evaluation is maintained. The Tris base extender that had been prepared the day before was divided into five tubes and then soy bean lecithin was added according to the level of treatment tried as follows: L0 or control (0% soy bean lecithin + 100% Tris extender), L1 (1% soy bean lecithin + 99% Tris base extender), L2 (2% soy bean lecithin + 98% Tris base extender), L3 (3% soy bean lecithin + 97% Tris base extender), and L4 (4% soy bean lecithin + 96% Tris base extender). Tris base extender mix and soy bean lecithin in each of these tubes is whipped with a stirring rod until it becomes a homogeneous extender solution then covered with aluminum foil and ready for use.

RESULTS OF RESEARCH AND DISCUSSION

Characteristics of Fresh Cement Sheep Research

Before the dilution process is carried out for preservation purposes, the quality of cement samples is evaluated both macroscopic and microscopic. The results of the evaluation of the quality of fresh cement of sheep research are listed in Table 2.

Table 2. Average Results of Quality Evaluation of Fresh Cement of Lamb Research

Characteristics of Fresh Cement	Sheep Cement Group		
	1	2	3
Volume (ml)	0,80±0,14	0,82±0,18	0,83±0,16
Color	Kream	Kream	Kream
Degree of Acidity (pH)	5,99±0,12	6,01±0,13	5,99±0,11
Consistency (Viscosity)	Kental	Kental	Kental
Mass Movement	+++/++	+++/++	+++/++
Spermatozoa Concentration (x107 cells/ml)	297,56±37,45	285,22±25,51	281,67±46,55
Spermatozoa motility (%)	75,56±5,27	76,11±6,01	76,67±5,59
Viability of Spermatozoa (%)	89,93±1,64	90,38±1,78	90,03±1,43
Spermatozoa abnormalities (%)	8,63±1,47	9,68±1,77	9,35±1,44
Membrane Integrity of Spermatozoa (%)	90,96±1,69	91,54±2,06	91,51±1,73

Based on the results of the evaluation of the quality of fresh semen in sheep research

(Table 2) it appears that several main parameters on which the dilution rate is determined (to calculate the need for extender volume) such as semen volume, concentration, and percentage of spermatozoa motility are eligible for further processing. Similarly, the quality parameters of fresh cement are other before being diluted for subsequent processing. These three groups of fresh cement samples are within the "normal standard" range as reported by many researchers. Macroscopically, the normal standard volume of fresh semen of lamb is 0.5–2.0 ml (Ax et al., 2000; Rizal and Herdis, 2008), viscous consistency, cream-colored (Toelihere, 1985b; Evans and Maxwell,1987), acidity pH 5.9–7.3 (Bearden et al., 2004; Garner and Hafez, 2000). Microscopically, the concentration of spermatozoa of various types of sheep ranges from 1500–3800 million/ml, spermatozoa motility 75.0–89.8%, spermatozoa viability 87.33–94.2%, spermatozoa abnormality 4.80–5.47% and spermatozoa membrane integrity 81–94% (Ayu, 2012; Rizal and Herdis, 2008).

According to Toelihere (1985b), basically sheep cement has a low volume but high concentration so that it reveals a thick consistency and is creamy or milky-colored. The low volume of cement is actually not detrimental because the high and low volume of semen that is ejaculated is generally not related to fertility or stud sterility. Furthermore, according to Ax at al. (2000) that the volume of sheep cement varies according to the method of shelter, and the volume of ejaculate obtained is due to many factors including the age of the rams, conditions, season, frequency of shelter and the skills of the collector. Semen reservoirs that use an artificial vagina by streamlining stimuli (false mounts) can increase the volume of ejaculates. If ram semen is accommodated three or more times per day or throughout the period it will decrease ejaculate volume. Further stated, the normal color of sheep semen is milky-white (milky-white) or whitish cream (pale creamy). Deviations from the color are suspected that the semen has been contaminated with blood (pink color) or urine (yellow color and foul smell) or the presence of a reproductive tract infection (gray or brownish color) in which such cement samples are not suitable for further processing.

The microscopic parameters of all sheep fresh semen samples in the study were also within the normal standard limits recommended by several other experts, namely concentrations of 2000 – 3000 million/ml (Toelihere, 1985b), motile spermatozoa 70 – 90% (Ax et al., 2000), abnormal spermatozoa 8 – 10% (Bearden et al., 2004) and membrane integrity of more than 60% (Jayendran and Zaneveld, 1986). According to Bearden et al. (2004) that the concentration, motility and morphology of spermatozoa are important criteria in evaluating fresh semen before further processing or before use for IBs. There is a positive relationship between the normal morphology of spermatozoa and their motility. Sheep semen motility of more than 85% and abnormalities of less than 10% are classified as high quality (Ax et al., 2000) where the range of 8 – 10% of abnormal spermatozoa has no detrimental effect on fertility (Bearden et al., 2004). Furthermore, Ax et al. (2000) assert that fresh semen that cannot be used for artificial insemination is that which contains abnormal spermatozoa of more than 15%.

Other microscopic parameters such as plasma membrane integrity in this study were also above the normal standard range. According to Jayendran and Zaneveld (1986), if the integrity percentage of the spermatozoa membrane reaches 60% or more then the fresh semen sample is considered normal, if it is less than 50% it means that it is abnormal and if the value is between 50 – 60% it is doubtful of its normality. Furthermore, it is stated that a high percentage of plasma membrane integrity has positive correlation with the ability of spermatozoa to penetrate the egg, because with an intact membrane the spermatozoa can still maintain its viability and motility and can protect its acromosomes.

Quality of Sheep Spermatozoa Post-Washing Research

The quality parameters of post-wash spermatozoa include concentration, motility, viability, abnormality and integrity of the spermatozoa membrane. The results of the evaluation of sheep spermatozoa post-washing research are listed in Table 3.

Table 3. Average Results of Evaluation of Spermatozoa Quality of Sheep Post-Washing Research

Characteristics of spermatozoa	Sheep Cement Group		
	1	2	3
Spermatozoa concentration (x107/ml)	295,56 ± 42,06	280,89 ± 37,99	280,11 ± 41,62
Motility of spermatozoa (%)	75,00 ± 3,54	77,22 ± 3,63	75,56 ± 4,64
Viability of spermatozoa (%)	89,07 ± 1,30	89,60 ± 1,23	88,59 ± 1,61
Spermato- zoa(%) abnormalities	9,62 ± 1,21	9,74 ± 1,13	9,55 ± 1,26
Membrane integrity of spermatozoa (%)	89,27 ± 1,57	90,05 ± 1,58	89,48 ± 1,36

Similar to fresh sheep cement before washing (Table 2), the results of the evaluation of the quality of fresh cement after washing (Table 2) are also still in good condition so that it is feasible for further processing. The success of cement washing has been widely reported by researchers with a variety of methods (Marcus, 2010; Lee et al., 2009; Councel et al., 2004; Somfai et al., 2002). Cement washing is a routine procedure that has long been practiced in the application of assisted reproductive technology (Perez-Pe et al., 2001). The principle of semen washing is to separate spermatozoa from seminal plasma to improve spermatozoa motility (Councel et al., 2004), or get rid of bacteria, debris and other chemicals that can cause infection and irritation and improve capacity (Marcus, 2010) or to isolate motile spermatozoa from non-motile spermatozoa and free from contamination of various components seminal plasma (Mc Clure et al., 1989) which is detrimental.

Effect of Cement Leaching and Soybean Lecithin Levels on Sheep Spermatozoa Quality at 5^{0}C Storage Temperature

The average quality of spermatozoa of lambs treated with leaching and soy bean lecithin levels during five days of storage at 5^{0}C is listed in Table 4.

Table 4. Average Quality Yield of Sheep Spermatozoa For Five Days of Storage at 5^{0}C Based on Washing Treatment and Soy Bean Lecithin Levels (%)

Spermatozoa Quality	Cement Washing (P)	Soy Bean Lecithin Levels (L)					average
		L0	L1	L2	L3	L4	
Progressive Motility	P0	52,22 ±6,37	58,46 ±4,66	60,93 ±5,69	63,16 ±4,10	54,92 ±7,63	57,94 ±6,98
	P1	51,04 ±8,07	58,46 ±5,47	60,97 ±3,79	63,21 ±3,13	54,98 ±5,83	57,73 ±6,96
Viability	Average	51,63 ±8,18[a]	58,46 ±5,03[b]	60,95 ±4,78[c]	63,18 ±3,61[d]	54,95 ±6,72[e]	57,83 ±6,96
	P0	70,62 ±1,88	71,61 ±2,89	71,94 ±2,23	72,28 ±2,36	72,04 ±2,12	71,70 ±2,36
Abnormalities	P1	70,64 ±2,10	71,57 ±2,43	71,85 ±2,47	72,12 ±2,14	71,48 ±2,52	71,53 ±2,36
	Average	70,63 ±1,97[a]	71,59 ±2,64[b]	71,89 ±2,47[b]	72,2 ±2,23[b]	71,76 ±2,32[b]	71,61 ±2,35
Membrane Integrity	P0	18,69 ±1,74	14,24 ±1,05	12,93 ±1,08	12,18 ±1,19	13,59 ±1,42	14,32 ±2,64
Spermatozoa	P1	18,98	14,79	13,05	12,67	14,4	14,78

Quality		±1,88	±1,27	±1,02	±0,89	±1,02	±2,58
	Average	18,84 ±1,8[a]	14,52 ±1,19[b]	12,99 ±1,04[c]	12,43 ±1,07[d]	14,00 ±14,00[b]	14,55 ±2,61
Progressive Motility	P0	71,80 ±2,44	71,40 ±2,86	72,03 ±2,40	73,05 ±2,26	72,11 ±2,34	72,08 ±2,49
	P1	71,60 ±2,40	71,82 ±2,35	72,02 ±2,52	72,79 ±2,67	71,90 ±2,46	72,03 ±2,48
Viability	Average	71,70 ±2,40[a]	71,61 ±2,60[a]	72,03 ±2,44[a]	72,92 ±2,45[b]	72,01 ±2,38[a]	72,05 ±2,48

Ket. : a, b, c, d, different eSuperscript on the same line show a noticeable difference (P≤0.05); L0, L1, L2, L3 and L4 = Soy bean lecithin content of 0%, 1%, 2%, 3% and 4%; P0 = Unwashed cement and P1 = Washed cement.

Based on the results of the diversity analysis, the washing of cement and the dose of soy bean lecithin together did not show a noticeable interaction, but singularly the dose of soy bean lecithin showed a markedly different influence (P≤0.05) and cement leaching showed no noticeable difference on all quality parameters of sheep spermatozoa for five days of storage at a temperature of 5^0C. This means washing treatment, i.e. unwashed semen (P0) and washed semen (P1), give the same effect to all quality parameters of spermatozoa, namely the percentage of progressive motility, viability, abnormality and integrity of the membranes of sheep spermatozoa for five days of storage at a temperature of 5^0C.

The phenomenon hints that sheep semen does not need to be washed to get rid of its plasma seminal if diluted with soy bean lecithin The use of soy bean lecithin as an extender component does not have any negative effects that can interfere with the function, impulse, viability, morphology and structure of the sheep spermatozoa membrane in both unwashed semen and washed cement. This is thought to be due to the enzyme phospholipase A in sheep plasma seminals (Scott and Dawson, 1968; Roldan and Fragio, 1993) can tolerate the levels of soy bean lecithin used in this study, so as not to catalyze the hydrolysis process of soy bean lecithin into fatty acids and lysolecithin which are toxic to spermatozoa and cause coagulation of the extender medium (Chemineau et al., 1991; Rizal et al., 2008; Iritani and Nishikawa, 1972 in Ashmawy et al., 2010). Spermatozoa are still very likely to perform mobile activities and maintain their viability in an extender medium enriched with soy bean lecithin.

The positive effect of soy bean lecithin in sheep cement extenders can be seen from the trend chart as Figure 1. The higher the level of soy bean lecithin in the Tris extender, the more the quality of spermatozoa increases to the level of 3% soy bean lecithin then decreases in the level of 4% soy bean lecithin. This indicates that the 3% level of soy bean lecithin in the Tris extender is the optimal level in improving progressive motility, viability, and membrane integrity and lowering the percentage of abnormalities of sheep spermatozoa during storage at 5^0C.

The results of further tests on the single effect of soy bean lecithin levels, found the percentage of progressive motility (63.18%) and membrane integrity (72.92%) of sheep spermatozoa at real L3 treatment was higher (P≤0.05) and the percentage of abnormalities of spermatozoa (12.43%) was noticeably lower (P≤0.05) than other treatments attempted. The percentage viability (72.20%) of sheep spermatozoa at the real L3 treatment was higher (P≤0.05) than the control treatment (L0) but did not differ markedly from other treatments (L1, L2 and L4).

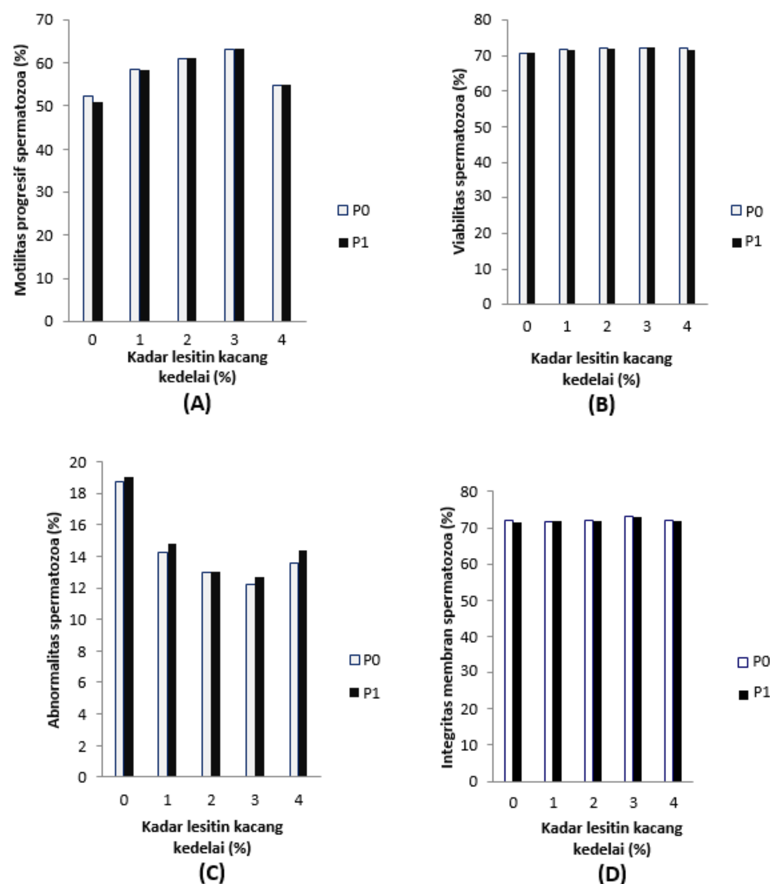

Figure 1. Effect of Semen Leaching and Soybean Lecithin Levels On Motility (A), Viability (B), Abnormality (C) and Membrane Integrity (D) of Sheep Spermatozoa During Five Days of Storage at 5^0C.

High progressive motility, viability, membrane integrity and low abnormality of sheep spermatozoa at 3% soy bean lecithin (L3) levels are thought to be because at these levels soy bean lecithin is relatively effective at protecting spermatozoa from the influence of cooling at a temperature of 5^0C so that movement activity proceeds normally, life force is maintained, death can be suppressed, morphology is not disturbed and the spermatozoa membrane remains intact. At this level, spermatozoa are protected from the adverse influence of 5^0C temperature cooling in the form of cold shock. Spermatozoa that experience cold shock result in a rolled tail, circling motion and backward motion that interferes with and decreases its progressive motility (Immelda, et al., 2019; Ayu, 2012; Evans and Maxwell, 1987). In addition, cold shock also results in disruption of the function and structure of cell membranes that are irreversible and in turn cause the death of spermatozoa. Morphologically, spermatozoa are Experiencing cold shock is characterized by a rolled tail or a bent tail which is one of the parameters of spermatozoa abnormality. According to Partodihardjo (1992), the shape of the tail of spermatozoa such as a rolled or bent tail is categorized as a secondary abnormality.

CONCLUSION

As the results of the study mentioned above, the following things were concluded: The cement washing treatment and soy bean lecithin levels together showed no noticeable interaction, but singularly the soy bean lecithin levels showed a markedly different influence (P≤0.05) and the washing treatment showed no noticeable difference on all quality parameters of sheep spermatozoa for five days of storage at 5^0C. This means treatment washing, that is, unwashed cement (P0) and washed cement (P1), exerts the same effect on

the percentage of progressive motility, viability, abnormality and integrity of the membranes of sheep spermatozoa during five days of storage at a temperature of 5^0C. The use of soy bean lecithin as a component of sheep cement extender is effective against samples of unwashed cement or washed cement. Thus, there is no need to wash sheep cement if it is diluted with soy bean lecithin in the cement preservation process at a temperature of 5oC. The best quality of sheep spermatozoa was obtained at the treatment of 3% soybean lecithin levels against washed and unwashed semen preserved for five days of storage at a temperature of 5oC with an average progressive motility percentage of 63.18%; viability 72.20%; abnormality 12.43%; and membrane integrity of 72.92%.

Suggestions in the study: it is necessary to conduct research from other aspects on the use of soy bean lecithin to improve the quality of sheep spermatozoa preserved at a temperature of 5oC. The aspects that need to be studied as soon as possible are mainly cryopreservation at the appropriate temperature, duration and method of equilibration, cooling speed, ratio between soy bean lecithin and other extender components such as Tris-citrate-fructose.

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