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Comparison of Different Lecithin Diluents for Cryopreservation of Toraya Buffalo Semen

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ABSTRACT

The objective of this study was to compare the quality of frozen semen from Toraya buffalo with different lecithins from different commercial diluents. Fresh semen from two 6-9 years old Toraya buffaloes were collected once a week in the morning through an artificial vagina. Fresh semen was examined macroscopically and microscopically. Semen with more than 70% sperm motility was divided into three tubes, each diluted with Andromed (soy lecithin), Steridyl (egg yolk lecithin), and Bovifree (synthetic lecithin) diluents at a concentration of 100 x10^6 mL^{-1} motile sperm. The diluted sperm were placed in 0.25 mL straws and allowed to equilibrate for 4 hours. The sperm was frozen above liquid nitrogen favour for 15 minutes and stored in liquid nitrogen containers for further evaluation. The quality of frozen semen was assessed 24 hours after freezing. The parameters tested were motility, viability, abnormalities, plasma membrane integrity, and sperm recovery rate (RR). The results showed no significant differences in the values of motility, viability, abnormalities, plasma membrane integrity, and sperm RR in the three diluents used. Sperm motility values ranged from 45.93% to 47.09% after freezing. Sperm viability ranged from 56.21% to 60.27%. The values of membrane integrity of the three diluents used ranged from 57.73% to 61.36%. The values of sperm abnormalities after freezing and thawing ranged from 2.74% to 3.18%. In conclusion, three commercial diluents containing animal, vegetable, and synthetic lecithin bases can be used as diluents for freezing Toraya buffalo semen with similar results.

Keywords: frozen semen; lecithin; semen quality; Toraya buffalo

INTRODUCTION

In Indonesia, there is a unique species of spotted buffalo (*Bubalus bubalis*), called Toraya buffalo, according to the Ministry of Agricultural of Indonesia, while the local people called Tedong Bonga. This breed originated from Tana Toraja in the Province of South Sulawesi (Kaiin *et al.*, 2017). The body of the spotted buffalo is larger than the mud buffalo and has a straight and wide back. This buffalo has a distinctive skin color and a very high socioeconomic and cultural value, which is why it is sacred to the Toraja people. There are six variations of coat and eye color phenotypes in swamp buffalo, and the type of spotted coat color was classified according to the Toraja culture classification system (Yusnizar *et al.*, 2015), with tedong saleko being the most expensive and tedong toddi being the cheapest. The demand for buffalo in Toraja Regency is showing an upward trend. This is because most of the Toraja who emigrate and are financially successful when they die, their remains are brought back to Tana Toraja to be buried on their ancestral land. Therefore, the number of buffalo tends to increase the number of buffaloes slaughtered in their funeral rites. In the past, a nobleman needed only two buffaloes as standard (Sapu Randanan); these days, however, depending on finances, it can require hundreds of buffaloes to pay respects to deceased loved ones (Rasyid *et al.*, 2022). Numerous factors lead to the decline of the buffalo population, including low reproductive output and the rearing system and breeding method of natural mating. To avoid a significant decline in the population, artificial insemination with frozen semen of Toraja buffalo is required.

Frozen semen from Toraya buffalo is produced at the Technical Implementation Center for Artificial Insemination and Semen Production, South Sulawesi Provincial Livestock and Animal Health Office. The quality of frozen buffalo semen refers to the Indonesian National Standard (SNI) for frozen buffalo semen No. 4869-2:2021, including fresh semen, which must have a minimum motility of 70% and a maximum sperm abnormality of 20%. The quality guideline for frozen buffalo semen in the SNI is a sperm motility value after thawing ≥ 40% with a sperm concentration per straw of 25×10^6 (BSN, 2021). Semen from each animal has different abilities to withstand freezing (Sukmawati *et al.*, 2014). Freezing occurs at an extreme temperature change from 37 °C to -196 °C, which leads to changes in

sperm ultrastructure, especially the plasma membrane, decreased motility, and increased damage to the sperm DNA (Layek *et al.,* 2016). Lecithin is one of the components of the plasma membrane of mammalian cells. Lecithin is water and fat-soluble and helps transport lipids into and out of lipid-containing cell membranes. Lecithin is required by sperm during preservation or cryopreservation for sperm protection (Bustani & Baiee, 2021); therefore, all frozen semen diluents contain lecithin.

Currently, in Indonesia, the freezing of Toraya buffalo semen uses homemade diluents such as Skimmed milk-egg yolk, a combination of Skimmed milk and Tris egg yolk diluents (Arif *et al.,* 2022), and Andromed, a commercial diluent (Iskandar *et al.,* 2022). There are several brands of commercial semen diluents for bovine, including Andromed (Minitube), Optixelll (IMV), Biladyl (Minitube), Steridyl (Minitube), BioXcell (IMV), Triladyl (Minitube), and BoviFree (Minitube). Andromed and BioXcell (IMV) contain soy lecithin (vegetable lecithin). In contrast, Steridyl and Triladyl contain egg yolk lecithin (animal lecithin), and the other commercial diluents, Bovifree and Optixell, contain synthetic lecithin. Andromed has proven effective in freezing bovine (Baharun *et al.,* 2017), caprine (Silvestre *et al.,* 2021), and buffalo semen (Yulnawati *et al.,* 2010). The use of Steridyl diluent has been reported for freezing sperm of stallions (Nikitkina *et al.,* 2020), camels (Swelum *et al.,* 2019), and leopards (Mulia *et al.,* 2021).

Commercial liposome-based diluents have been described by Kumar *et al.* (2015) for frozen buffalo semen and Amal *et al.* (2019) for frozen bovine semen. The advantages of commercial diluents are ease and convenience of preparation (Tahar *et al.,* 2022) and minimization of the potential risk of contamination with microorganisms (Stewart *et al.,* 2016). Moreover, commercial semen diluents contain antibiotics following international trade regulations for frozen semen (Morrell & Wallgren, 2014). Commercial diluents for frozen semen from Toraya buffalo use only Andromed, a vegetable lecithin commercial diluent. The conception rate of artificial insemination results in 2020-2022 with very limited data, 496 heads, reached only 16.33% with this diluent (Isihknas, 2023; unpublished data). Therefore, this study aimed to compare animal lecithin (Steridyl), vegetable lecithin (Andromed), and synthetic lecithin (liposome; Bovifree) for freezing Toraya buffalo semen.

MATERIALS AND METHODS

Ethical Clearance

The use of animals in this study was approved by the Ethics Committee on the Use of Animals for Research Purposes of the Institute for Research and Community Empowerment of IPB University under number 239-2022.

Date and Location of the Research

The study was conducted from October 2022 to January 2023 at the Technical Implementation Unit for Artificial Insemination and Frozen Semen Production (UPT-PIBPS) of the South Sulawesi Provincial Livestock and Animal Health Office in Pucak Village, Tampobolu Sub-district, Maros District, South Sulawesi Province, Indonesia.

Source of Fresh Semen

Fresh semen was obtained from two 6-9 years old Toraya buffaloes classified as Bonga Tenge with a body weight of 400-500 kg. Both buffaloes were intensively housed in a 2.5 x 4 m pen equipped with feeding and drinking stations. Feeding was twice daily in 40-45 kg of forage, 4 kg of concentrate, 1 kg of green bean sprouts, and drinking water ad libitum.

Preparation of Diluent

The commercial diluents Andromed 1:4, Steridyl 1:1.5, and Bovifree 1:4 were diluted with distilled water by following the instructions for using the diluent on the packaging. The ingredients from each commercial diluent are presented in Table 1. The diluents were then stored at 34 °C.

Semen Collection and Fresh Semen Quality Evaluation

Semen collection was performed once a week using an artificial vagina by a bull master. A female buffalo was used as a teaser. The freshly collected semen was stored at 34 °C and examined macroscopically and microscopically, according to Herbowo *et al.* (2020). The macroscopic examination was based on the volume, pH, consistency, and color of the semen. Microscopic evaluation was based on the mass movement, motility, viability, plasma membrane integrity, sperm concentration, and morphology of sperm. Microscopic examinations were performed using a phase contrast microscope (Olympus CX31RTSF), and concentration was determined using a photometer (SDM 6, Minitube, Germany).

The mass movement was determined by dropping 10 μL of sperm onto a slide glass and observing it under a microscope with 100x magnification. The value of mass movement is determined based on the velocity of the moving sperm cloud with a value of +++ (positive 3/very good), ++ (positive 2/good), and + (positive 1/poor). Sperm motility was tested by diluting 10 μL of semen with 40 μL of diluent. Both solutions were homogenized, and 10 μL was withdrawn, placed on a sliding glass and covered with a coverslip. Comparison of progressive and non-progressive sperm motility was evaluated under a microscope with 400x magnification in five fields of view. The values were expressed as percent (%). Sperm viability was assessed by eosin-nigrosin staining. A total of 10 μL of semen were mixed with 40 μL of eosin-nigrosin. Both solutions were homogenized, smear, and dried at 37 °C. Observations were performed using a microscope at 400x magnification and a minimum number of 200 sperms.

The normal and abnormal sperm morphology was evaluated by eosin-nigrosin staining, corresponding to

Table 1. The ingredients of diluents for frozen Toraya buffalo semen with different lecithin sources

Ingredients		
Andromed*	Steridyl**	Bovifree***
Phospholipid (1% soya lecithin)	Irradiated sterile egg yolk	Phospholipid (liposome-based formula and animal-free protein)
-	-	Proprietary membrane protectants
Tris	Tris	-
Citric acid	Citric acid	Citric acid
Sugar (fructose)	Sugar (fructose)	Sugar (fructose)
Antioxidants	-	Antioxidants
Buffers	Buffers	Proprietary buffers
Glycerol	Glycerol	Glycerol
Purest water	Purest water	Aquabidest
Antibiotics (tylosin, gentamicin, spectinomycin, lincomycin)	Antibiotics (tylosin, gentamicin, spectinomycin, lincomycin)	Antibiotics (tylosin, gentamicin, spectinomycin, lincomycin)

Source: *https://www.minitube.com/catalog/en/andromed-200-ml-p1497/
**https://www.minitube.com/catalog/en/steridyl-p1484/
***https://www.minitube.com/catalog/en/bovifree-200-ml-p7806/

the viability test. Observation of normal and abnormal sperm was performed under a microscope with 400x magnification and a minimum cell count of 200 sperms. Then, plasma membrane integrity testing of sperm was performed using a hypoosmotic swelling solution (0.735 g sodium citrate and 1.351 g fructose in 100 mL double-distilled water). Ten microliters of semen were added to a microtube containing 1 mL of hypoosmotic swelling (HOS) solution. The mixture was incubated in a water bath at 37 ^{o}C for 60 minutes. The sperm that responded or did not respond to the hypoosmotic solution were counted in 10 fields of view with a total of 200 cells.

Preparation of Frozen Semen

Fresh semen with sperm motility of >70% was divided into three parts, and each was diluted with Andromed, Steridyl, and Bovifree diluents. The semen was diluted to 100×10^{6} mL^{-1} and then filled into mini-straws with a volume of 0.25 mL (concentration 25×10^{6} straw^{-1}) using an automatic filling and sealing machine. The straw containing the semen was placed in a freezing rack and equilibrated at 5 °C for 4 hours. The freezing rack was placed 10 cm above the liquid nitrogen vapor for 15 minutes. The frozen sperm was stored in liquid nitrogen containers at -196 °C. The test of frozen semen quality was performed 24 hours after freezing.

Frozen Semen Evaluation

Frozen semen was thawed before evaluation. Thawing of frozen semen was done individually in 37 °C water for 30 seconds. The thawed semen was placed into a microtube and stored at 37 °C. After that, the quality of frozen semen was tested for sperm motility, viability, morphology, plasma membrane integrity, and recovery rate (Arif *et al.*, 2022). The test procedures for frozen semen were performed as for fresh semen with slight modifications. Observation of sperm motility was performed without dilution. Sperm viability and morphology were tested using eosin-nigrosin with a ratio of 1:2. Plasma membrane integrity was determined by adding 50 μL of sperm to 1 mL of HOS solution. The recovery rate was calculated by multiplying sperm motility after thawing with fresh sperm motility and multiplying by 100%.

Statistical Analysis

The fresh semen data from two buffaloes were analyzed using a T-test for independent samples at a 95% significance level. The frozen semen data were analyzed using an ANOVA analysis of variance at a 95% significance level. Data were processed using the Statistical Package for the Social Sciences (IBM SPSS 25). The experimental design was a 3×2 factorial completely randomized design (CRD) with three commercial diluents (Andromed, Bovifree, and Steridyl) as the first factor, two Toraya buffaloes as the second factor, and eight collections as replicates.

RESULTS

Fresh Semen Quality of Toraya Buffalo

Volume, color, consistency, pH, mass movement, motility, concentration, live sperm, plasma membrane integrity, and sperm abnormalities of Toraya buffalo semen showed good quality (Table 2).

Quality of Frozen Semen of Toraya Buffalo in Various Commercial Diluents

Statistical tests revealed no interaction between diluent types and individuals. There was no difference in sperm motility between the three diluents and the two individual buffaloes, with sperm motility values ranging from 45.93% to 47.09% after freezing (Table 3). Sperm viability ranged from 56.21% to 60.27% in the study (Table 4), and there was no interaction between the type of diluent and the two individual buffaloes. Plasma membrane integrity of sperm in the commercial

diluents Steridyl, Andromed, and Bovifree showed no interaction with the two buffaloes used. The statistical test showed no difference in sperm plasma membrane integrity after freezing between the three diluents and the two buffaloes used. The value of membrane integrity of the three diluents used was 57.73%-61.36% (Table 5). The results of this study on sperm abnormalities in the commercial diluents Andromed, Steridyl, and Bovifree showed no interaction with the animals. The statistical analysis showed no difference in sperm abnormality after freezing between the three diluents and the two animals used. The values of sperm abnormalities after freezing and thawing ranged from 2.74% to 3.18% (Table 6).

DISCUSSION

The quality of fresh semen from Toraya buffalo in this study supports the results of Kaiin *et al.* (2017) on the same breed of buffalo and Herbowo *et al.* (2020) on mud buffalo. The statistical analysis showed that the quality of fresh semen of the two Toraya buffaloes was not different. The quality of fresh semen showed that Toraya buffaloes are suitable for processing frozen semen because they have fresh semen motility according to Indonesian National Standard 4869-2:2021, which is ≥70%. Good semen quality in Toraya buffaloes that passed the selection stage and are raised in a bull-rearing system according to the standards of artificial insemination centers.

It is reported that buffalo semen is more sensitive to freezing and thawing than bull semen, resulting in lower fertilization potential (Dessouki *et al.*, 2022). The value of sperm motility after thawing in this study follows SNI for frozen buffalo semen No. 4869-2:2021, and it reaches sperm motility of at least 40% after thawing, which is suitable for insemination. The sperm motility value does not differ because the three diluents contain the same buffer composition. All three contain tris (hydroxymethyl) aminomethane, fructose, and citric

Table 2. Fresh semen quality of Toraya buffalo

Variables	Bull code		Mean ± SE
	131602	131303	
Semen Volume (mL)	1.95 ± 0.54	2.45 ± 0.39	2.20 ± 0.32
Color	Creamy	Creamy	Creamy
pH	6.4	6.4	6.4
Consistency	Moderate	Moderate	Moderate
Mass movement	+++	++	++ - +++
Progressive sperm motility (%)	80.00 ± 3.53	73.75 ± 2.39	76.88 ± 2.30
Sperm viability (%)	88.55 ± 2.05	85.15 ± 1.99	86.85 ± 1.80
Intact plasma membrane of sperm (%)	87.37 ± 2.53	82.07 ± 1.37	84.72 ± 1.66
Sperm concentration (x10^6 ml^{-1})	1234 ± 285.26	990 ± 69.33	1112 ± 143.50
Sperm abnormality (%)	2.17 ± 0.21	2.82 ± 0.21	2.49 ± 0.36

Note: Data are expressed as mean ± SE of 8 ejaculations. The results of the t-test analysis showed no significant ($p>0.05$) in both males.

Table 3. Sperm motility percentage of frozen semen of Toraya buffalo in diluents with different lecithin sources (Mean±SE)

Bull code	Diluents		
	Andromed®	Steridyl®	Bovifree®
131602	47.12 ± 2.41	47.41 ± 1.63	48.28 ± 1.90
131303	45.11 ± 1.37	46.78 ± 1.94	43.59 ± 0.92
Mean	46.12	47.09	45.93

Note: Data are expressed as mean ± SEM of 8 ejaculations. The results of the ANOVA analysis showed no significant in both males in diluents with different lecithin sources.

Table 4. Sperm viability percentage of frozen semen of Toraya buffalo in diluents with different lecithin sources (Mean±SE)

Bull code	Diluents		
	Andromed®	Steridyl®	Bovifree®
131602	54.07 ± 0.88	60.06 ± 2.20	55.21 ± 1.44
131303	58.73 ± 3.52	60.46 ± 3.46	57.21 ± 3.12
Mean	56.4	60.27	56.21

Note: Data are expressed as mean ± SEM of 8 ejaculations. The results of the ANOVA analysis showed no significant in both males in diluents with different lecithin sources.

Table 5. Plasma membrane integrity percentage of Toraya buffalo sperm in diluents with different lecithin sources (Mean±SE)

Bull code	Diluents		
	Andromed®	Steridyl®	Bovifree®
131602	56.62 ± 0.74	60.17 ± 2.64	55.03 ± 0.85
131303	59.83 ± 4.09	62.56 ± 5.14	60.43 ± 2.54
Mean	58.23	61.36	57.73

Note: Data are expressed as mean ± SEM of 8 ejaculations. The results of the ANOVA analysis showed no significant in both males in diluents with different lecithin sources.

Table 6. Sperm abnormalities percentage of frozen semen of Toraya buffalo in diluents with different lecithin sources (Mean±SE)

Bull code	Diluents		
	Andromed®	Steridyl®	Bovifree®
131602	3.03 ± 0.28	2.93 ± 0.37	2.77 ± 0.26
131303	3.28 ± 0.35	3.43 ± 0.16	3.11 ± 0.29
Mean	3.16	3.18	2.74

Note: Data are expressed as mean ± SEM of 8 ejaculations. The results of the ANOVA analysis showed no significant in both males in diluents with different lecithin sources.

acid. Fructose is the major carbohydrate source in bovine, including buffalo semen (Juyena & Stelletta, 2012). Tris (hydroxymethyl) aminomethane is a universal buffer suitable for preserving semen from various livestock (Namula *et al.*, 2019). The results showed that the three commercial diluents with different lecithin sources had the same ability to protect the sperm plasma membrane during freezing and thawing. This result is evident from the lack of differences in sperm viability and plasma membrane integrity in three commercial diluents. The integrity of the plasma membrane is vital for protecting the cell interior. During freezing and thawing, extreme temperatures damage the cell membranes; therefore, semen diluents must contain ingredients that can protect cell membranes. This study shows the increased sperm abnormalities from fresh semen to semen after freezing and thawing. However, the value is very small, ranging from 0.25% to 0.69%. This result is caused by the effect of diluent to protect the cell membrane from damage, and the freezing techniques are well established.

The recovery rate can also assess the success of sperm freezing. Recovery rate is the ability of sperm cells to recover after freezing. The recovery rate value of sperm in the three commercial diluents showed no interaction between males. Also, it showed no difference in the recovery rate of sperm after freezing between the three diluents and the two males used. The recovery rate values of Andromed, Steridyl, and Bovifree diluents were 60.03%, 61.35%, and 59.73%, respectively.

Several researchers have reported Buffalo semen freezing with various commercial diluents. Kumar *et al.* (2015) and Singh *et al.* (2018) reported that Murrah buffalo semen in OptiXcell diluent (commercial diluent with synthetic lecithin) had higher sperm motility after thawing compared to those frozen with Andromed, BioXcell, and Tris egg yolk diluent. Studies by Chaudhari *et al.* (2015) on frozen semen from Surti buffalo showed that sperm motility was equally good in OptiXcell and Tris egg yolk diluent. Meanwhile, sperm viability and plasma membrane integrity were better in OptiXcell than in BioXcell and Tris egg yolk. OptiXcell also showed the highest motility, plasma membrane integrity, and normal sperm morphology after thawing in Nili Ravi buffalo compared to Triladyl and Tris egg yolk diluents (Naz *et al.*, 2018). These results suggest that soybean, egg yolk, and liposome lecithin can protect cell membranes equally. OptiXcell contains liposomes similar to those in the Bovifree in this study. The different results reported in this study may be due to the different breeds of buffalo used. Breeds and individual animals exhibit differences in their abilities to resist freezing (Sukmawati *et al.*, 2014; Indriastuti *et al.*, 2020). However, the results of this study suggest that no individual differences were observed in the Toraya buffalo.

Commercial diluents were used to freeze semen from different animals with different results. Freezing of semen from FH bull is better with Triladyl or Andromed diluents than with OptiXcell and BioXcell (Miguel-Jimenez *et al.*, 2020). Other researchers reported that the motility of bovine sperm after thawing was equally good in Andromed and OptiXcell diluents (Lima-Verde *et al.*, 2018). The plasma membrane integrity of bovine sperm was higher in OptiXcell, followed by Triladyl, and the lowest was found in Andromed (Fleisch *et al.*, 2017). Freezing of ram semen with Triladyl was better than with Tris egg yolk diluent (Rekha *et al.*, 2016). Swelum *et al.* (2019) reported that freezing camel sperm with Triladyl was better than with SHOTOR, Steridyl, Andromed, and OptiXcell. In leopards (Mulia *et al.*, 2021), the quality of frozen semen in Steridyl and Andromed diluents was equally suitable. Andromed, OptiXcell, and Ovine red diluents were equally good in white-tailed deer semen (Stewart *et al.*, 2016).

The mechanism of the three sources of lecithin for sperm protection in Steridyl, Andromed, and Bovifree are almost the same. Lecithin from three different sources has the same main component, phosphatidylcholine. Therefore, the results of the studies did not differ. Phospholipids, as a major component of membranes, play important physiological roles in lowering the freezing point, thus avoiding the formation of large ice crystals to reduce potential mechanical damage to the sperm membrane. Soy lecithin is a natural mixture of phosphatidylcholine and various fatty acids, such as stearic, oleic, and palmitic acids, that provide structural stability to the cell membranes (Oke *et al.*, 2010).

Compounds derived from soybeans, particularly soy lecithin, are effective substitues for egg yolk because it is not of animal origin. The components of egg yolk lecithin include phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine. Phosphatidylcholine is the main component of lecithin, the content of which is ~73.0%. The content of lecithin in egg yolk is three times higher than lecithin in soybean. It has been shown that the contents of lecithin in chicken egg yolk and duck egg yolk are large, accounting for ~10% of total lecithin (Zhao *et al.*, 2023).

In addition, liposomes are spherical or multilayer spherical vesicles produced by the self-assembly of phospholipids with diacyl chains (lipid Bilayer) in aqueous solution. Liposomes can be formed from both natural and synthetic phospholipids. For example, liposomes composed of naturally unsaturated phosphatidylcholine, such as egg or soybean phosphatidylcholine are formulated to have high permeability and low stability properties (Nsairat *et al.*, 2022).

Lecithin from egg yolk, soy, and liposomes acts in two ways: First, it forms a protective layer on the surface of the sperm (Emamverdi *et al.*, 2013). This protective layer protects the sperm plasma membrane from forming ice crystals during freezing. Second, the sperm plasma membrane releases phospholipids into the environment during freezing (Drobnis *et al.*, 1993). The lecithin in the diluent replaces the phospholipids in the sperm membrane that are lost or damage during freezing (Mehdipour *et al.*, 2018).

Commercial diluents are available in two varieties. Ready-to-use diluent containing buffer, lecithin, cryoprotectant, and antibiotics, with only distilled water added during preparation. Others are buffers containing glycerol and antibiotics but no lecithin, such as Triladyl and Optidyl. Commercial diluents that do not contain lecithin in their preparations need to be added with egg yolk. Adding egg yolk to commercial diluents that do

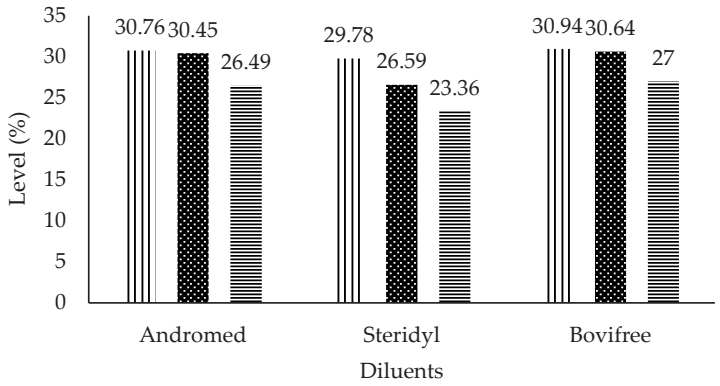

Figure 1. The decrease of Toraya sperm quality after freezing in diluents with different lecithin sources.
Sperm motility Sperm viability Sperm plasma membrane intact

not contain lecithin leads to inconsistent results, similar to homemade diluents, e.g., egg yolk tris. The inconsistent quality of frozen semen with homemade diluents mainly concerns the integrity of the plasma membrane (Tarig *et al.,* 2017).

Freezing decreases sperm quality; the decrease in sperm motility from fresh to frozen sperm ranged from 29.78% to 30.94%. Sperm viability and intact plasma membrane (IPM) decreased from 26.59%-30.64% and 23.36%-27.00%, respectively (Figure 1). This decrease in quality is almost comparable to that observed in bovine semen freezing (Baharun *et al.,* 2017). Frozen semen from Toraya buffalo showed almost the same quality in this study. Motility, viability, IPM, and sperm abnormalities in Steridyl, Andromed, and Bovifree diluents after freezing did not differ, and there was no interaction with the males used. A limitation of this study is that only two samples of Toraya buffalo were used. Due to their high economic value, only two Toraya buffalo are available at the research location.

CONCLUSION

Vegetable, animal, and synthetic lecithin sources in Andromed, Steridyl, and Bovifree commercial diluents have the same ability to protect sperm from cold shock during freezing. All three commercial diluents can be used as frozen semen diluents for Toraya buffalo with similar results.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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