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Motility, Viability, and Abnormality of the Spermatozoa of Bali Bull with Andromed® and Egg Yolk-Tris Diluents Stored at 4 °C

Research Article

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ABSTRACT

Bali bull has great potential to be developed as a national meat source in Indonesia which can address concerns of meat importation. Bali bull farming only requires small holding system, has high fertility and low calf mortality as its advantages and is a growing enterprise by way of artificial insemination (AI) with semen collected from phenotypically superior bull sires. However, efficient means of preserving Bali bull semen quality using diluents still requires exploration at a national level. The purpose of this study was to determine which of the selected diluents will best preserve the quality of local Bali bull semen over a period of five days at 4 ˚C. Andromed® and egg yolk-tris diluents were compared in preserving quality of semen collected from seven-year old bulls with 70% minimum motility. Spermatozoa motility, viability, and abnormality were observed and compared. This research used factorial complete randomized design with two factors in which each factor has 2 levels and 5 levels with three-times treatment. Results of the study showed Bali bull spermatozoa demonstrated 51.66% motility, 59.13% viability, and only 22.68% abnormality until day five in egg yolk-tris diluent. In comparison, Andromed® diluent demonstrated 23.33% motility, 45.59% viability, and 23.15% abnormality under similar conditions. To conclude, egg yolk-tris is a superior diluent when compared to Andromed® in preserving local Bali bull semen quality by better maintaining spermatozoa motility, viability and lower percentages of abnormalities when stored for five days at 4 ˚C. This data provides useful information for bull farmers, in particular Bali bull farmers in Indonesia in the practice of AI to consider carefully the choice of diluent relative to storage time and temperature in improving cattle farming practices for increased meat productivity in the region.

KEY WORDS abnormality, Andromed®, Bali bull semen, egg yolk-tris, motility, viability.

INTRODUCTION

A method utilized to increase performance of local beef production is by way of artificial insemination (AI) technology (Mohammed, 2018; Mohanty *et al.* 2018). Utilization of AI technology helps in improving quality of offspring by careful selection and crossing of parents (Malik *et al.* 2012). However, the success of the AI application in livestock does not only depend on the quality and quantity of semen produced by a bull ejaculate but also depends on the ability to maintain quality and viability of semen for longer periods after ejaculation so that more females can be inseminated (Parker and McDaniel, 2006; Banday *et al.* 2017; Kubkomawa, 2018).

The dilution process is very closely related to the use of frozen semen for AI (Mara *et al.* 2007). The semen dilution itself aims to increase the volume of semen without reducing the quality of the semen (Udeh and Oghenesode, 2011). Toelihere (1993) stated that the use of semen diluents must be able to maintain the viability of spermatozoa for a period of time prior to its use. Requirements for diluents are: (a) to be able to provide nutrients for the needs of spermatozoa during storage, (b) allowing the spermatozoa to move progressively, (c) not toxic to spermatozoa, (d) support viability of spermatozoa, and (e) to protect spermatozoa from cold shock (both for frozen and liquid semen).

The main challenge often faced in the use of semen diluents is that not all diluents show the same ability to maintain spermatozoa from the same cattle variety or livestock coming from different breed. Therefore, this study was carried out to compare the quality of Bali bull semen stored at 4 ˚C for 1-5 days where motility, viability, and abnormalities of the spermatozoa were measured and analyzed. Thus, later it can be known which diluent can better preserve Bali bull semen quality to aid farmers in improving performance in local meat production in resource-limited settings.

MATERIALS AND METHODS

This study was conducted in the Technical Implementation Unit for Artificial Breeding and Insemination Service, Penajam Paser Utara Regency, East Kalimantan Province, Indonesia. This study was an experimental study designed to follow a factorial completely randomized design with two factors. The first factor is the type of diluent (P) and the second factor is the time of storage (T). Each factor consists of two levels and five levels with three times treatment with the dependent variable as the specified storage temperature of 4 ˚C.

Bali bull acclimatization and semen collection

The Bali bulls used have been intensively maintained in terms of (1) bull sanitation every day, (2) cage sanitation and its environment, (3) monitoring nutrition of feeds and drink, (4) weighing every week, (5) recording of age, (6) daily exercise, (7) bull handling and heat stress management, (8) cage maintenance, (9) health monitoring of bulls and bull breeding soundness evaluations that have fulfilled the four main requirements of the Indonesian national standard (Ministry of Agriculture of the Republic of Indonesia, 2006) which have clear pedigree, sperm motility > 70%, and free of 12 diseases namely bovine viral diarhea, spira spira, jembrana, bovine rhinotracheitis infectious, enzootic bovine leucosis, brucellosis, paratuberculosis, campylobacteriosis, trichomoniasis, babesiosis, theileriosis, anaplasmosis.

Feeding Bali bulls includes forages (leaves of *Zea mays*, *Pennisetum purpureum*, *Cynodon plectostachyus*, *Calliandra callothyrsus*, *Gliricidia sepium*), hay, silage and supplementary feed (minerals and concentrates). Bali bulls that have an average body weight of 500 kg are given 30-40 kg/day of forage, silage has been given as much as 5-7.5 kg/day in the morning after forages, concentrates as much as 7 kg/day, and as a complement nutrients have also been given minerals mixed with concentrates in the amount of 100-120 g/day. This study has used seven-year-old Bali bulls (Prastowo *et al.* 2018b). Five Bali bulls were used to collect the semen through artificial vagina once in a week. Ejaculated Bali bulls were permitted to mount a teaser bull. Standard procedure for cleaning shelters, prepuce and allowing bulls to carry out false mounting (3-5 times) is done on all bulls before being accommodated. The purpose of the false mounting treatment is to maximize sexual stimulation resulting in maximum semen production (Ministry of Agriculture of the Republic of Indonesia, 2006). Ejaculated semen was observed macroscopically for volume, color, consistency and pH; and microscopically for motility of mass and individual spermatozoa, viability, concentration, and abnormality.

Diluent material preparation

The egg yolk-tris diluent was made three days before the shelter with the following ingredients: 3,634 g tris (hydroxymethyl) aminomethane, 1.99 g citric acid, 20 mL egg yolk, 0.50 glucose, 1000 IU/mL penicillin and 100 mg streptomycin (Asher *et al.* 2000). While Andromed® (manufactured by Minitube International company products, Germany) diluents consist of two ml of Andromed® added to four mL of Aquadestilata, following manufacturer protocol.

Evaluation of fresh semen parameters

Bali bull semen was collected using graduated polyethylene screw-capped containers. Semen volume was recorded directly from the graduated semen collection tubes. The average volume of semen collected from Bali bulls ranged from four to ten mL. Semen color was recorded immediately after collection. Semen pH was measured against a pH paper thru observation of color change and intensity when compared to manufacturer's color scale (manufactured by Micro Essential Laboratory Inc, USA). Semen consistency was observed by shaking the semen-filled container and evaluating the semen flow rate against the container wall which were graded thin, medium, or thick as observed. Semen motility was evaluated by observing both individual and mass motility of spermatozoa in semen mixed with the elected diluents in the study using a GXM L1500 light microscope (GX Microscopes, UK).

Semen concentration was checked using an evolution TM 220 UV-Visible spectrophotometer (Thermo Fisher Scientific Inc, USA).

Preparation of semen with diluent treatments

Each fresh semen sample was divided into two equal portions in separate sterile test tubes and added with equal amounts of the elected diluents where P1 was mixed with Andromed® and P2 was mixed with egg yolk-tris diluent. Both P1 and P2 were divided into five equal parts and each tube further divided into three equal parts each to enable triplicate testing. The tubes were covered with aluminum foil and placed in a beaker containing warm water (37 ˚C) and stored in a refrigerator for observation for a period of five days at 4 ˚C.

Microscopic evaluation of liquid semen

Microscopic evaluation of spermatozoa motility, viability, and abnormalities in Bali bull semen was performed once every 24 hours for five days. Motility was observed using 1000 × magnification objective lens of GXM L1500 light microscope equipped with LCD monitor (GX Microscopes, UK).

Less than 40% motility of spermatozoa indicates poor quality and is associated with infertility. Freshly collected bull semen that has not been mixed with diluent is assessed for individual and mass movements of spermatozoa. Observation of spermatozoa viability was carried out by spermatozoa staining using 2% eosin dyes. This stain is useful for the identification of viable and dead spermatozoa. Viable spermatozoa have low affinity to the dye and resist being stained, while dead spermatozoa easily take up the stain. Viable or living spermatozoa are characterized by a white head, while for dead spermatozoa the head is stained red or pink color (Toelihere, 1993) due to the permeability (damage) of the spermatozoa cell wall. Spermatozoa abnormalities were done by observing eosin stained spermatozoa and noting the type of abnormality.

Data analysis

Data recorded in this study was statistically analyzed by observing differences between treatments using Duncan Multiple Range Test (DMRT) at the level of 5% with the SPSS 21 program (SPSS, 2011) and variance analysis in cases where there are only two factors to compare.

RESULTS AND DISCUSSION

Macroscopic and microscopic evaluation of Bali bull fresh semen

Fresh semen collected from Bali bull at the time of shelter had an average volume of 5.30 mL which was lower than that stated by Said *et al.* (2014) which was at 6.47 mL but was close to that reported by Prastowo *et al.* (2018a) which was at 5.10 mL. Whichever the case maybe, fresh semen collected from Bali bull fell within the range of ejaculated bull semen which was reported by John *et al.* (2015) which was within the 5-8 mL range. Macroscopic and microscopic results of fresh semen from the first ejaculate of seven-year-old Bali bull are shown in Table 1, where macroscopic requirements still met international standards even after the addition of diluents.

The pH of collected fresh semen collected from Bali bull in this study was 6.40 with milky white color which met the normal value for bull semen pH and is also in accordance with reports provided by Prastowo *et al.* (2018a) which reported Bali bull fresh semen as having a pH of 6.49. Generally, the pH of bull and sheep semen ranges from 6.20 to 6.80 (Toelihere, 1993).

Semen pH is essential in maintaining spermatozoa viability, too high or too low of which will cause death of spermatozoa (Zhou *et al.* 2015). The milky white color of the freshly collected semen in this study also concurred with reports by Toelihere (1993) where it was also stated that it is considered a normal phenomenon for 10% of bulls to produce yellowish semen.

Fresh Bali bull semen collected from this study had the average concentration of 749 × 10^6 cells per ml ejaculate which in comparison was lower than that reported by Haryani *et al.* (2016) which was at 803.6 × 10^6 cells per ml. The fresh semen concentration from this study was in agreement with standards set for use in AI as stated by Toelihere (1993) where minimum standard for quality of fresh semen fit for use in AI was reported at 500 × 10^6 spermatozoa per ml of ejaculate, 50% of which should be live and active.

The percentage of individual spermatozoa motility demonstrated in this study was observed at 70% and mass motility with a grade of ++ which are similar to published reports of Yendraliza *et al.* (2019) and fit Indonesia AI office standards (Secretariat General Ministry of Agriculture of the Republic of Indonesia, 2000) of 70% individual motility and ++ to +++ mass motility. Motility results of Bali bull spermatozoa in this study were also in agreement with results published by David *et al.* (2015) and Hafez (2000) which stated that the percentage of live spermatozoa must be greater than 50% and where mass motility ++ was characterized by medium waves of motion and has a percentage of live of spermatozoa of greater than 70% (O'Brien *et al.* 2018; Kunowska-Slósarz, 2015; Ratnawati *et al.* 2018).

In fine, macroscopic and microscopic semen quality namely: pH, color, concentration and motility of spermatozoa obtained from Bali bull in this study were all in accordance with published data, and both local and international

standard providing evidence that Bali bull fresh semen is fit for use in AI applications for increasing performance of meat productivity.

Percentage of motility of spermatozoa in Bali bull liquid semen

Comparison of the average percentage of spermatozoa motility between Andromed® and egg yolk-tris diluents stored at 4 ˚C are shown in Table 2 where analysis showed that the type of diluent exerted a significant difference (P<0.01) on Bali bull spermatozoa motility. In addition, storage times also demonstrated significant difference (P<0.01) as a consequence for modulating spermatozoa motility.

The results of DMRT at the level of 5% on the treatment of the type of diluent cannot be displayed because in this study, there are only two treatments, namely Andromed®, and egg yolk-tris diluents (comparators, a and b). However, the analysis of variance has explained the results, that the type of diluent showed a significant difference (P<0.01) on affecting the motility of the Bali bull spermatozoa. Similarly, the storage time of liquid semen (T) showed that the treatments of T1 and T2 were not significantly different (P>0.05), T2 and T3 were not significantly different (P>0.05), and T4 and T5 were also not significantly different (P>0.05). However, T1 is significantly different (P<0.05) from T3, T4, and T5. T2 is significantly different (P<0.05) from T4 and T5. T3 is significantly different (P<0.05) from T1, T4, and T5. T4 and T5 are also significantly different (P<0.05) from T1, T2, and T3. Thereby providing evidence that storage time is also a determinant in modulating spermatozoa motility, and that fresh semen used for AI as soon as collected would is more beneficial than using semen from prolonged storage conditions.

At the time of the study the results of the average percentage of spermatozoa motility were highest from those obtained from egg yolk-tris diluents: D1 (70.00%), D2 (68.33%), D3 (65.00%), D4 (56.66%), and D5 (51.66%), where the motility was still above 40% even after five days of storage at 4 ˚C. These results corroborated the findings of Solihati and Kune (2009) where egg yolk-tris maintained motility of Simmental cattle (*Bos taurus*) spermatozoa that are still worthy of AI even after prolonged storage of an average of 4.67 days. Andromed® demonstrated an average percentage of motility that was similar to egg yolk-tris from 1st to 3rd day but showed drastic decrease during the 4th and 5th day: D1 (70.00%), D2 (66.66%), D3 (63.33%), D4 (26.66%), and D5 (23.33%).

The consistently higher values obtained from egg yolk-tris throughout the course of five days can be attributed to higher concentrations of yolk lipoprotein and lecithin which is essential in maintaining the integrity of the lipoprotein sheaths of the spermatozoa (Rakha *et al.* 2013).

In addition, egg yolk also contains glucose which is preferentially used by bovine spermatozoa over fructose which is naturally found in semen (Toelihere, 1985; Thun *et al.* 2002; Lopes *et al.* 2015). The drastic decrease in spermatozoa motility observed during the 4th and 5th day with Andromed® diluent may be attributed to the presence of glycerol as an ingredient. Glycerol has a cryoprotectant ability which aids in the cryopreservation of spermatozoa but extended contacts have also shown cytotoxic effects (Han *et al.* 2005). Glycerol is hydrophilic and will attract water from spermatozoa cells, which causes spermatozoa cells to become dehydrated (Sieme *et al.* 2004; Vera-Munoz *et al.* 2011; Gharajelar *et al.* 2016). During the equilibration process, glycerol will enter into the cells of the spermatozoa and replace some of the water in the cell, but with a longer equilibration time, the contact with glycerol is toxic, in that it damages the plasma membrane of spermatozoa and causes disruption of motility and viability (Kubkomawa, 2018). In relation to these published reports, this study provides valuable evidence of the consideration of storage temperature when choosing a diluent for preserving bull spermatozoa. It may seem that although the components of Andromed® provided similar benefits to maintain spermatozoa viability when compared to egg yolk-tris during the first three days, storage at 4 ˚C and not cryotemperature of -196 ˚C may have contributed to the drastic decrease in spermatozoa motility as this diluent was designed with cryopreservation in mind. In comparison, five days storage at 4 ˚C in egg yolk-tris diluent-maintained spermatozoa viability within accepted AI standards because it did not contain glycerol. In fine, egg yolk-tris has provided superior results over Andromed® in terms of spermatozoa motility under specific conditions of a duration of five days at 4 ˚C.

This may not hold true however, if cryopreservation is considered as components of Andromed® are specifically formulated for such storage conditions enabling way longer periods of storage and preservation of bull semen for AI applications.

Percentage of viability of spermatozoa in Bali bull liquid semen

The results of the comparison of the average percentage of spermatozoa viability between Andromed® and egg yolk-tris is tabulated in Table 3 where variance analysis shows a significant difference (P<0.05) exerted by the diluents relative to spermatozoa viability. Similarly, storage time also exerted a significant difference (P<0.05) between the treatments while stored at 4 ˚C. However, the combined effects of type of diluent and storage time did not show any significant difference (P>0.05) as a consequence relative to Bali bull spermatozoa viability.

Table 1 Quality of Bali bull fresh semen with expected normal values

Parameters	Average value	Normal value
Volume (mL)	5.3	5-8 (John *et al.* 2015)
pH	6.4	6.2–6.8 (Toelihere, 1993)
Color	Milky white	Like milk or whitish cream and cloudy
Consistencies	Aqueous	Aqueous
Concentration	749 million cell/mL	Minimum 500 million cell/mL
Motility of mass spermatozoa	++	++ ~ +++
Motility of individual spermatozoa (%)	70	More than 50 (Hafez, 2000)

Source: this study.

Table 2 Comparison of average percentage of motility with Andromed diluents and tris egg yolk stored at 4 ˚C

Treatment	Day savings					
	1	2	3	4	5	Average
			%			
Andromed®	70.00±0.00	66.66±2.88	63.33±7.63	26.66±2.88	23.33±2.88	50.00±21.54^{a}
Egg yolk tris	70.00±0.00	68.33±2.88	65.00±5.00	56.66±2.88	51.66±2.88	56.16±17.10^{b}
Average	70.00±0.00^{a}	67.50±2.73^{ab}	64.16±5.84^{b}	41.66±16.63^{c}	37.50±15.73^{c}	-

Source: this study.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 3 Comparison of the average percentage of spermatozoa viability with Andromed diluents and tris egg yolk stored at 4 ˚C

Treatment	Day savings					
	1	2	3	4	5	Average
			%			
Andromed®	82.55±3.36	80.02±3.07	74.54±10.95	54.34±3.41	45.59±1.74	67.41±15.96^{a}
Egg yolk tris	83.67±3.69	80.73±2.99	78.22±8.08	67.50±6.03	59.13±1.46	73.85±10.39^{b}
Average	83.11±3.21^{a}	80.37±2.74^{ab}	76.38±8.84^{b}	60.92±8.43^{c}	52.36±7.55^{d}	-

Source: this study.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

The results of DMRT at the level of 5% on the type of diluent cannot be displayed because in this treatment there are only two treatments, namely andromed®, and egg yolk-tris so that there are only two comparators, a and b. However, the analysis of variance demonstrated a significant difference (P<0.05) between the choices of diluent relative to the viability of semen spermatozoa. Based on the results of the DMRT at the level of 5% (Table 3) the treatment of storage time of liquid semen (T) showed that the treatments of T1 and T2 were not significantly different (P>0.05), T2 and T3 were also not significantly different (P>0.05). However, T1 is significantly different (P<0.05) from T3, T4, and T5. T2 is significantly different (P<0.05) from T4 and T5. T3 is significantly different (P<0.05) from T1, T4, and T5. T4 is significantly different (P<0.05) from T1, T2, T3, and T5, while for T5 it is significantly different (P<0.05) from T1, T2, T3, and T4. As seen in Table 3. Andromed® and egg yolk-tris can both maintain the viability of spermatozoa at levels of > 40% over a period of five days when stored at 4 ˚C. However, egg yolk-tris diluents have a higher percentage of viability than Andromed® starting from day 1 to day 5, which is 73.85%, and Andromed® only demonstrated an average viability percentage of 67.41% which again provides evidence of the beneficial use of egg yolk-tris over Andromed® when stored up to five days at 4 ˚C. Both Andromed® and egg yolk-tris demonstrated a daily decrease in the percentage of viability of spermatozoa which can be attributed to the decreasing amount of food that serves as an energy source for metabolic processes and the increasing concentration of lactic acid formed due to the metabolic processes of the sperm cells, whereby acid-base balance in semen is disrupted (Zen *et al.* 2001). However, from both diluents, it was revealed that Andromed® had a lower percentage of viability, this could be due to the toxic effects of glycerol contained therein in relation to the choice of storage temperature of 4 ˚C as stated earlier. According to Papa *et al.* (2015) the longer the time of equilibration, the more glycerol diffuses and affects spermatozoa, but cattle spermatozoa that are exposed to longer equilibration temperature tend to run out of energy and accumulate lactic acid subsequently impacting spermatozoa viability. In fine, the less notable performance of Andromed® diluent in preserving spermatozoa viability may be attributed to suboptimal storage temperature conditions of 4 ˚C with consideration of its glycerol component which recommends storage temperatures of -196 ˚C in liquid nitrogen in order to effectively preserve semen quality for longer periods of time.

Percentage of spermatozoa abnormalities in Bali bull liquid semen

The results of the comparison of the average percentage of abnormalities of spermatozoa in liquid semen of Bali bull for 1-5 days can be seen in Table 4. The results of analysis of variance in spermatozoa abnormalities in liquid semen from Bali bull showed that the type of diluent used provided no significant difference (P>0.05), whereas storage time exerted a significant difference (P <0.05). In addition, no significant difference (P>0.05) was observed from the interactions of type of diluent and storage time.

The results of the DMRT at the level of 5% on the type of diluent used cannot be displayed because in this treatment there are only two factors, namely Andromed® and egg yolk-tris diluents. However, the analysis of variance results showed no significant difference (P>0.05) between the types of diluent tested relative to the survival spermatozoa. Based on the DMRT at the level of 5% on the treatment of storage time of liquid semen (T) showed that the treatment of T1 was not significantly different (P>0.05) from T2. T2 is not significantly (P>0.05) different from T3. T3 is not significantly different (P>0.05) from T2 and T4 and for T4 it is not significantly different (P>0.05) from T5. T1 is significantly different (P<0.05) from T3, T4, and T5. T2 is significantly different (P<0.05) from T4 and T5. T3 is significantly different (P<0.05) from T1 and T5. T4 is significantly different (P<0.05) from T1 and T2, whereas for T5 it is significantly different (P<0.05) from T1, T2, and T3. Table 4 shows that the Andromed® and egg yolk-tris diluents were able to maintain abnormal spermatozoa levels at < 25% for three days with > 25% abnormalities observed during the 4th and 5th day. As this may be the case, Da-Costa *et al*. (2016) has reported that spermatozoa abnormalities that reach 25% have not affected the fertility of spermatozoa and that semen can still be utilized for AI. Spermatozoa abnormalities data from this study provide evidence that there is a direct relationship between storage time and spermatozoa abnormalities. This can be caused by osmotic pressure imbalances due to accumulation of waste products from metabolic processes (Solihati *et al*. 2008).

In this study, egg yolk-tris diluent demonstrated lower spermatozoa abnormalities for five days (22.68%) when compared to Andromed® (23.15%.). The high percentage of spermatozoa abnormalities in Bali bull semen diluted with Andromed® may have been contributed the cytotoxic properties of glycerol as mentioned earlier in particular if samples are not stored in the recommended cryoprotective temperature range (Papa *et al*. 2015).

The most common spermatozoa abnormalities encountered in this study were secondary abnormalities characterized by broken tails, a head without a tail, a middle part that folds and lose droplets of distal protoplasm and acrosome (Toelihere, 1993). In addition, secondary abnormalities may be caused by treatment when making screw preparations (Kubkomawa, 2018). As shown in Figure 1, observed spermatozoa abnormalities in this study were folded spermatozoa (Figure 1A), curved tail spermatozoa (Figure 1B), and broken tail spermatozoa (Figure 1C).

Table 4 Comparison of the average percentage of spermatozoa abnormalities with Andromed diluents and egg yolk-tris stored at 4 °C

Treatment	Day savings					
	1	2	3	4	5	Average
			%			
Andromed®	12.35±6.51	19.44±9.52	21.94±6.38	27.12±5.25	34.90±8.0	23.15±9.95^{a}
Egg yolk tris	12.95±3.56	18.48±6.17	22.21±3.82	27.81±10.09	31.96±6.82	22.68±8.87^{b}
Average	12.65±4.71^{a}	18.96±7.19^{ab}	22.07±4.70^{b}	27.46±7.20^{bc}	33.43±6.84^{c}	-

Source: this study.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

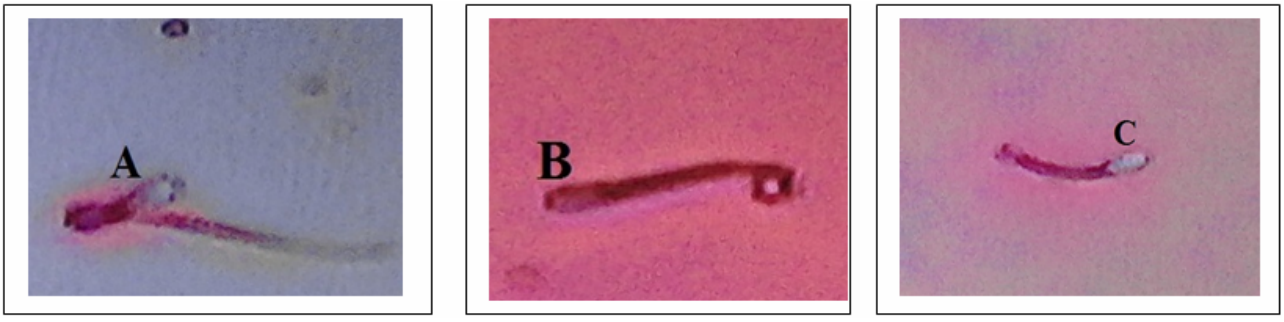

Figure 1 Abnormalities of spermatozoa
A: folded spermatozoa; **B**: curved tail spermatozoa and **C**: broken tail spermatozoa

CONCLUSION

To conclude, the results of the study showed superior performance of egg yolk-tris diluent in preserving overall semen quality of Bali bull over Andromed® in storage conditions of up to five days at 4 ˚C. Maintenance of spermatozoa motility, viability, and abnormalities were kept within acceptable AI standards and was more positively pronounced in treatments using egg yolk-tris which provides the additional benefit of being cost-efficient and ability to store Bali bull semen in non-freezing conditions or refrigerator temperature for up to five days. Finally, in improving meat productivity performance among bull farmers utilizing AI, it is important to consider the type of diluent used in relation to the storage duration and temperature. Optimal storage temperatures recommended by manufacturers of commercial diluents must be followed for better preservation of semen stock for AI.

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