Quality of Bali bull cryopreserved sperm using different extenders and equilibration times on pregnancy rate of Bali cows

by Yendraliza Yendraliza

Submission date: 04-Aug-2020 02:18PM (UTC+0700)
Submission ID: 1365787796
File name: Scopus.pdf (664.81K)
Word count: 6008
Character count: 31558

Songklanakarin J. Sci. Technol.
42 (3), 652-659, May - Jun. 2020

http://www.sjst.psu.ac.th

Original Article

Quality of Bali bull cryopreserved sperm using different extenders and equilibration times on pregnancy rate of Bali cows

Yendraliza[1*], Anwar Efendi Harahap[1], Jully Handoko[1], Muhamad Rodiallah[1], and Chairusyuhur Arman[2]

[1] *Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Riau, 28293 Indonesia*

[2] *Faculty of Animal Science, Mataram University, Mataram, Nusa Tenggara Bar, 83125 Indonesia*

Received: 24 January 2018; Revised: 29 October 2018; Accepted: 21 March 2019

Abstract

This study aimed to investigate the effects of three different semen extenders (TEY, AND®, and TSY) and three equilibration periods (2, 4, and 6 h) on the storage of Bali bull spermatozoa. This research used a completely randomized block design with two factors (extenders and equilibration time) and 12 replications. The measured variables were fresh semen quality of Bali cattle, motility, viability, abnormality, plasma membrane integrity (PMI), recovery rate (RR), pregnancy rate (PR), and NRR. The results showed that the spermatozoa of the extended semen with TEY and AND® within 4 h equilibration period was able to show better motility, viability, PMI, and RR than the spermatozoa of the extended semen with TSY within 2-h and 6-h equilibration periods. The conclusion of this research is that the TEY, AND®, and TSY extenders on Bali bull semen with a 4-h equilibration time yielded 90% PR and 100% NRR.

Keywords: motility, plasma membrane integrity, non-return rate, recovery rate, pregnancy rate

1. Introduction

Artificial insemination (AI) is one of the technologies to increase a local breed population. One of the critical success factors of AI is the quality of the frozen semen used. Frozen semen is affected by the quality of the diluent and the freezing method. The type of extender required is preferably locally available, fast, cheap, and able to maintain the motility and the survival of spermatozoa. The time required in the process of preserving semen at low temperatures (equilibration) will determine the quality of the spermatozoa because at that time the spermatozoa were preparing to enter a cold shock. The cold shock could damage the plasma membrane of the cells which results in death of the spermatozoa. According to Tsutsui *et al.* (2003), egg yolk serves to protect the spermatozoa from cold shock during storage. Moreover, the contents of lipoprotein and lecithin in egg yolk also serve to maintain and protect the intensity of the envelope and lipoprotein of the spermatozoa from cold shock and stabilize the plasma membrane (White, 1993). Some extenders have been widely used to increase the survival of spermatozoa after freezing such as Tris-egg yolk, skim milk-egg yolk, and lactose-egg yolk. Some of commercial extenders are Andromed®, Bioexcell®, Triladyl®, Biladyl®, and Biochips plus (Gil *et al.,* 2003; Rothe, 2003; Minitub Germany, 2005). Andromed® is a commercial extender composed of phospholipids, tris-(hydroxymethyl)-aminomethane, citric acid, fructose, glycerol, tylosin tartrate, gentamicin sulfate, spectinomycin, and lincomycin. The use of Tris-egg yolk and skim milk have been widely reported by previous researchers (Afriantini & Purwantara, 2010) in Friesian Holstein (FH) cow (Wiratri, Susilawati, & Wahjuningsih (2014) Limousin bulls, and buffalo (Sukhato, Thongsodseang, Utha, & Songsasen, 2001).

The Andromed® extender is a commercial diluent already used in Simmental, Limousin, and buffalo semen

*Corresponding author
Email address: yendraliza@uin-suska.ac.id

(Yendraliza, 2014), yet it has not been used on Bali cattle. Therefore, a quality test of Bali cattle was conducted to obtain the quality of Bali cattle semen in several extenders with the best equilibration time. Watson (1995) argues that the success of the cryopreservation sperm process is influenced by species-specific selection, freezing method, thawing rates, and diluents. Further, Paulenz *et al*. (2002) stated that this type of cement diluent varies greatly in providing sperm value. Aligned with this idea, Kulaksiz *et al*. (2010) confirmed that a suitable diluent is a basic necessity for the successful preservation of spermatozoa and for obtaining higher conception rates in field trials using diluted semen.

2. Materials and Methods

The research was performed in two stages: diluting and semen freezing. Semen was collected using artificial vagina (IVM, France), at 42 °C from three Bali bulls with similar ages (4−5 years) for six week periods. The only ejaculates collected had a sperm concentration greater than 800×10^6 sperm/mL, >70% motility, and normal morphology in >80% of the sperm. Six ejaculates were individually processed for preservation. The next stage was AI on 60 Bali cows. The female breed used has given birth and has healthy reproductive organs. All of the selected Bali cows were synchronized using GnRH (Fertagyl®) on the first day with a dose of 3 mL and PGF2α (2.5 mL, Lutalyse®) on the 7th day after GnRH injection. The cows showed estrus after the injection of PGF2α. AI was performed with the frozen semen from the first stage of the research. The pregnancy rate (PR) was determined by two tests during the 60 days following the AI. The first pregnancy test was conducted on day 21 following insemination which indicated the non-return rate (NRR). Based on this test, all cows were observed as to whether or not they returned to estrus. The second pregnancy test was conducted on day 60 following the AI. The pregnancy was determined using rectal palpation methods instead of estrus detection (Hafez & Hafez, 2016).

2.1 Preparation of extender

Tris-citric egg yolk (TEY) extender was prepared using 3.0 g tris-(hydroxymethyl-aminomethane) and 1.56 g citric acid, fructose 0.2% (w/v), glycerol 7.0 mL (Merck, Germany), and egg yolk 20% in 74 mL distilled water. All chemicals used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA). Antibiotics were added to the TEY extender: 1000 IU/mL of benzylpenicillin (Pharmacia & Upjohn, Belgium) and 1000 µg/mL of streptomycin sulphate (Pharmacia & Upjohn, Belgium). Tris-skim milk-egg yolk (TSY) extender was prepared using 3.0 g tris-(hydroxymethyl-aminomethane) and 1.56 g skim milk, fructose 0.2% (w/v), glycerol 7.0 mL (Merck, Germany), and egg yolk 20% in 74 mL distilled water. Andromed® (AND®) extender was prepared according to the manufacturer's instructions (IVM, France).

2.2 Semen evaluation and processing

Sperm progressive motility was determined microscopically (×400, Olympus BX20, Tokyo, Japan) and sperm concentration was determined using a digital photometer (IVM, France). Each pooled sample was split into two aliquots and diluted with AND® extender or TEY or TSY at 37 °C in a single step for a final concentration of 25×10^6 sperm/mL. After dilution, the semen was maintained in a water bath for 10 min at 35 °C for stabilization. Thereafter, it was cooled from 37 °C to 25 °C in approximately one h at room temperature. Straws designated for the same duration of equilibration time transfer used liquid nitrogen (freezing rates −20 °C/min, from 5 to −120 °C, duration: 10 min), varying only for the equilibration times at 5 °C: 2 h, 4 h, and 6 h for a total of two factors and three treatments. French straws (IVM, France) with a suction pump at 4 °C in a cold cabinet unit (IVM, France) were placed in liquid nitrogen vapors 5 cm above the level of the liquid nitrogen. Straws were then plunged and stored under liquid nitrogen (−196 °C). After 72 h, four frozen straws from each group were thawed individually at 37 °C for 30 sec in a water bath for evaluation. The parameters measured in the first stage of the study were viability, motility, abnormalities, and integrity of the plasma membrane of the fresh semen of the Bali cattle and the quality of the Bali cattle sperm after thawing. The second stage of the research measured the recovery rate (RR), PR, and NNR.

2.3 Viability

The fresh and treated semen samples were dripped onto microscope glass slides. Eosin-nigrosin was dripped onto the samples and mixed. The semen mixed with eosin-nigrosin was smeared with the end of a microscope glass slide until it was spread along the surface of the glass slide. It was then allowed to air dry. Then the sample was observed with a light microscope with 400x magnification.

2.4 Motility

The sperm was prepared by mixing the semen gently and placing a 10 µL drop of diluted semen on a warm microscope slide and then covered with a glass coverslip (18x18 mm). The motility of the sperm was evaluated from five representative fields. The mean of the five estimations was recorded as the final motility score.

2.5 Abnormality

The percentage of abnormal sperm (detached heads, tailless, acrosomal aberrations, abnormal mid-pieces, or tail defects) was recorded by counting a total of 200 spermatozoa under the phase of contrast microscopy (×1000 magnifications; oil immersion). Abnormal sperm was examined using eosin-nigrosin stain. A 10 µL drop of diluted semen was placed on a slide. A 40 µL drop of eosin-nigrosin was added and smeared on the slide which was dried quickly at 37 °C. Microscope views were selected randomly from ten fields for a total of 200 cells (Ax *et al.*, 2000).

2.6 Plasma membrane integrity (PMI)

Sperm plasma membrane integrity was determined using a hypo-osmotic swelling (HOS) assay (Jayendra *et al.*, 1984). The HOS solution consisted of 0.73 g sodium citrate and 1.35 g fructose dissolved in 100 mL distilled water (osmotic pressure: −190 mOsmol/Kg). To assess the sperm

tail plasma membrane integrity, 50 µL of semen was mixed with 500 µL of the HOS solution and incubated for 30 min at 37 °C before examination with a phase contrast microscope (×400, Olympus BX20, Tokyo, Japan).

2.7 Recovery rate (RR)

The percentage of successful spermatozoa that recovered from the total freezing process was determined by comparing the percentage of motile spermatozoa after thawing to the fresh semen (Hafez & Hafez, 2016).

2.8 Pregnancy rate (PR)

The number of pregnant cattle was divided by the number of cattle in the AI and multiplied by 100% (Hafez & Hafez, 2016).

2.9 Non-return rate (NRR)

The NRR is the percentage of cattle that does not estrus between 30 and 60 days after being mated divided by the total number of cattle that were mated.

2.10 Data analysis

A completely randomized block design in two factors with 3 extenders × 3 equilibration times, with 12 replications per experimental unit was used. The results are presented as mean±standard deviation. The effects of the extender and equilibration time were evaluated by ANOVA with the means compared by Duncan's test at a 5% level. All the statistical analyses were performed using SAS software (version 9.0, SAS Institute Inc., USA). The differences were considered significant at $P<0.05$.

3. Results and Discussion

3.1 Fresh semen quality of Bali cattle

The average evaluation of fresh semen of Bali cattle can be seen in Table 1. The average volume obtained during the study was 9 mL. The color of the Bali bull semen was cream and the smell was normal with a distinctive fishy smell. The consistency of semen in this study was vicious with concentrations of 1000 million to 2000 million or more cells per mL. The pH of the fresh semen was 6.8 and it had good mass motion (++).

The volume of the Bali bull semen in this study was higher (9 mL) than Aceh bull (2.80–4.50 mL) (Zulyzaini, Dasrul, Wahyuni, Akmal, & Abdullah, 2016). Bali bull aged 3.5 years had a reported mean volume of 4.5±2.3 mL (Ratnawati, Affandhy, Pratiwi, & Prihandini, 2008). However, in this study the volume was lower than Bali bull in Udayana (12±1,269 mL) (Setyani, Sarini, & Lanang Oka, 2017). These differences may be due to the differences in species, age, body weight, and interval of shelter (Hafez & Hafez, 2013). The semen pH (6.8) of the Bali bull in this study was relatively similar to Aceh bull (6.8) (Zulyzaini *et al.*, 2016) and FH bull (6.5 to 7.0) (Arifiantini *et al.*, 2005).

Table 1. Average evaluation of fresh semen of Bali bull in Pekanbaru.

Characteristics	Average
Macroscopic Evaluation of Cement	
Volume	9±2.5 mL
pH	6.8±1.2
Colour	Cream
Consistency	Viscous
Microscopic Evaluation of Spermatozoa	
Concentration	1600±1.5
Mass motion	++
Individual motion	2
Motility (%)	80,00±1.5
Viability (%)	90,00±2.7
Abnormality (%)	3,00±1.5
PMI (%)	81,00±2.5

Bali bull sperm concentration (1600×10^6/mL) in this study was higher than the concentration of Aceh bull ($1194.00\pm52.25\times10^6$/mL) (Zulyzaini *et al.*, 2016), Limousin ($1153.64\pm127.50\times10^6$/mL) (Wiratri *et al.*, 2014), and Simmental ($1129.75\pm180.99\times10^6$ sperm/mL) (Sukmawati, Arifiantini, & Purwantara, 2014). The differences in spermatozoa concentrations were due to genetic quality in the bulls (Hafez & Hafez, 2016).

Sperm motility (80%) in this study was higher than the Bali Bull in Indonesia (74.50±3.69%) (Dewi, Ondho, & Kurnianto, 2012), and Limousin bull (67.56±1.46%) (Lestari, Saleh, & Maidaswar, 2013). This was due to differences in feed, shelter frequency, technique, and maintenance management (Hafez & Hafez, 2016).

The percentage of live Bali bull sperm (90%) in this study was higher than the Aceh bull (70%) (Zulyzaini *et al.*, 2016) and the Bali bull in the Station research of Semarang (88.03±3.07%) (Ratnawati *et al.*, 2008). On the other hand, it was relatively similar to the percentage of Limousin bull live sperm (94.08%) (Sukmawati *et al.*, 2014). The percentage of abnormal sperm (3%) in this study was relatively similar to Bali bull in Indonesia (6.56±3.05%) (Ratnawati *et al.*, 2008) and the Aceh bull (Zulyzaini *et al.*, 2016).

3.2 Quality of Bali bull sperm after thawing

The post-thaw sperm quality of the Bali bull in the 3 types of extenders and 3 different equilibration times were quite significant in motility values, plasma integrity membrane, and RR values ($P<0.01$), while the values of abnormality sperm were not significant. The results were similar to Limousin bull (Wiratri *et al.*, 2014), but different from FH bull (Afriantini & Purwantara, 2010). This difference is likely due to the chemical content of several different types of extenders. However, this could happen due to differences in extender density, viscosity or even the presence of large particles (Anzar, Kroetsch, & Boswall, 2011). Celeghini *et al.* (2008) reported that during prolonged equilibration, sensitive sperm undergo membrane and axonemal changes and lose their ability to move in a straight line, which results in a decrease in some kinematic parameters such as linearity and straightness during the freezing and thawing processes.

3.3 Percentage of viability

The average viability rate of Bali bull sperm was higher in the use of TEY (76.67%) and AND® (74.4%) compared to the TSY extender (71.34%) with a 4-h equilibration time (Figure 1). This was probably because the TEY and TSY extenders have a high lactose content that accelerates the metabolism of spermatozoa. The build-up of lactic acid due to sperm metabolism causes dead spermatozoa. Furthermore, the fat in skim milk also inhibits sperm motion (Suharyati & Hartono, 2011). The results of this study agreed with the results of research conducted by Shah, Andrabi, and Qureshi (2016) on Nili-Ravi buffalo semen with a 4-h equilibration time. However, Arifiantini, Yusuf, and Graha (2005) reported that the Triladyl® extender was the best for FH bull sperm.

3.4 Motility

Different types of extenders and equilibration times have a significant effect on Bali bull sperm motility after thawing, where the type of extender interacts with the equilibration time to give post-thaw sperm motility values (Figure 2). The TEY extender with the 4-h equilibration time gave a higher motility value (66.66%) compared to the AND® (63.67%) and TSY (60.66%). This was probably caused by TEY having lecithin and lipoproteins that are capable of maintaining motility. The structure of the lipoproteins in TEY is similar to the structure of the plasma membrane and it could protect the spermatozoa (Gotham & Mayes, 2009). The TEY was able to protect spermatozoa from cold shock (Alves *et al.*, 2013). The motility of spermatozoa depends on the energy source of the mitochondrial metabolism derived from fructose in the diluent. At low temperatures, metabolism of the spermatozoa will run slowly so as to save the use of energy sources. The fructose present in the extender can be a source of energy for sperm (Schorin *et al.*, 2012) and become the main source of energy for the motility of sperm (Stefanov, Anev, & Abadjieva, 2015).

Sperm motility of the Bali bull was higher than the Limousin bull (36%–55%) (Wiratri *et al.*, 2014) and lower than the FH bull (73%) (Afriantini & Purwantara, 2010). This difference is likely due to the chemical content of several different types of extenders.

3.5 Abnormality of spermatozoa

The average abnormality of the Bali bull sperm was not significantly different amoung the three types of extenders but abnormality was significantly different at 4- and 6-h

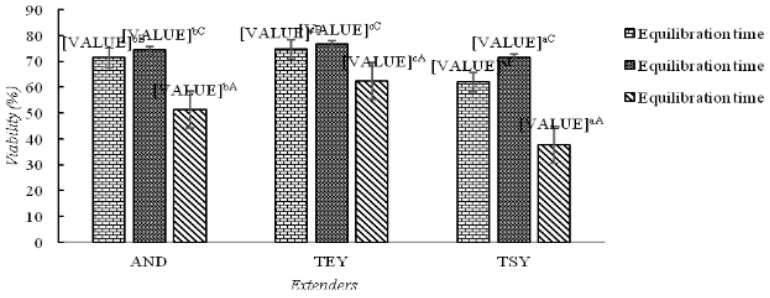

Figure 1. Effect of different extenders and equilibration time on viability sperm parameters for the Bali bull semen samples thawed at 37 °C. Data are means±SD. $^{a, b, c}$; Values in the same row with different superscripts are significantly different (P<0.05) and ^{A,B,C} Values in the same column with different superscripts are significantly different (P<0.05). AND®: Andromed, TEY: Tris-citric egg yolk, TSY: Tris-skim milk-egg yolk.

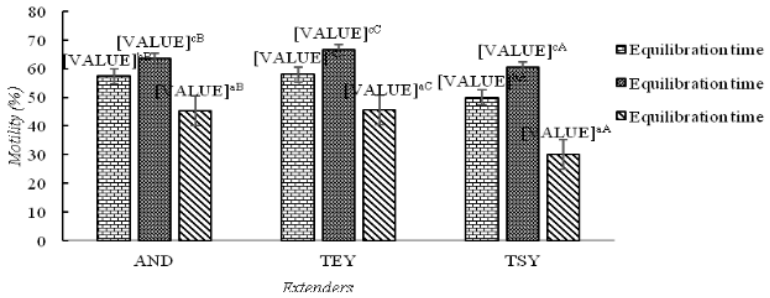

Figure 2. Effect of different extenders and equilibration time on motility sperm parameters for the Bali bull semen samples thawed at 37 °C. Data are means±SD. $^{a, b, c}$; Values in the same row with different superscript different significantly (P<0.05) and ^{A,B,C} Values in the same column with different superscript different significantly (P<0.05). AND®: Andromed, TEY: Tris-citric egg yolk, TSY: Tris-skim milk-egg yolk.

equilibration (Figure 3). However, the percentage of abnormality was still within the normal range. Ax *et al*. (2000) pointed out that the requirement for abnormal frozen spermatozoa is 10%−20%. The percentage of abnormalities was lower than the Holstein Bull of Iraq (15.94% up to 20.91%) which used the TEY extender (Hussain, Shahad, & Al-Badry, 2016). However, if it is more than 25%, it will have a negative effect on fertility (Parera, Prihatini, Souhoka, & Rizal, 2009). Gordon (2017) suggested that the longer the storage time, the higher the abnormality will be.

3.6 Plasma membrane integrity (PMI)

Different types of extenders and equilibration times have a significant effect on Bali bull sperm on the PMI after thawing. The type of extender interacts with the equilibration time in giving the post-thaw sperm PMI value (Figure 4). The AND® and TEY extenders with the 4-h equilibration time gave higher PMI values of 72.33% and 73.33%, respectively, compared to the TSY (69.33%). This difference was caused because the AND® and TEY extenders contain active constituents of egg yolk, low-density lipoprotein (LDL) fraction which is responsible for protecting sperm cells from the cold shock (Manjunath, 2012). Since LDL adheres to the cell membrane during the cryopreservation, it helps to restore the loss of phospholipids and apparently induces a temporary change in its composition which consequently prevents rupture of the plasma membrane (Wahjuningsih, Hermanto, Nuryadi, & Bhontoro, 2012).

The value of the Bali bull PMI was different from the results obtained by Febretrisiana *et al*. (2016) in Boer goat which was 79% preserved at 5 °C with Triladyl® extender and Rizal (2009) in the Bali bull where the PMI was 51.60%. The differences in PMI values were likely due to the TEY requiring sufficient time to enter the cell membrane and keep the fluid balance of the cells stable. Therefore, it did not damage the spermatozoa or the substrate and electrolytes in the cell (Ariantie, Yusuf, Sajuthi, & Arifiantiny, 2014). The egg yolk protects and maintains the integrity of the spermatozoa sheath due to the lecithin and lipoproteins (Manjunath, 2012). Furthermore, Hayati (2011) added that the high value of membrane integrity obtained in Bali bull semen plasma was due to the ability of egg yolk to protect the plasma membrane better, so that only a few plasma membranes were susceptible to lipid peroxidation.

3.7 Recovery rate (RR)

Different types of extenders and equilibration times have a significant effect on Bali bull sperm RR after thawing, where the type extender interacts with the equilibration time to give the post-thaw sperm RR value (Figure 5). The TEY and AND® extenders at the 4-h equilibration time gave higher RR values of 82.50% and 78.75%, respectively, compared to the TSY (75.00%). This was probably due to the basic ingredients of TEY which are thought to be better able to protect the sperm from the bad effects of freezing because the base material, buffers, and sugars are used differently

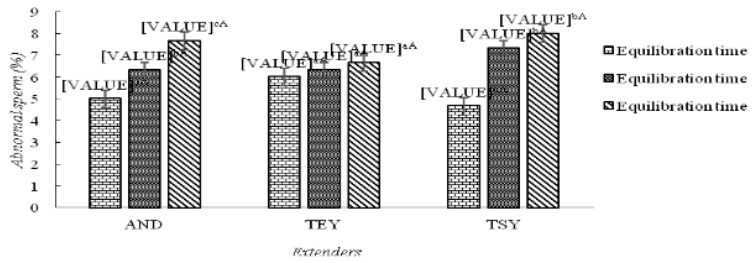

Figure 3. Effect of different extenders and equilibration time on abnormal sperm parameters for the Bali bull semen samples thawed at 37 °C. Data are means±SD. $^{a, b, c}$; Values in the same row with different superscript different significantly (P<0.05) and ^{A,B,C} Values in the same column with different superscript different significantly (P<0.05). AND®: Andromed, TEY: Tris-citric egg yolk, TSY: Tris-skim milk-egg yolk.

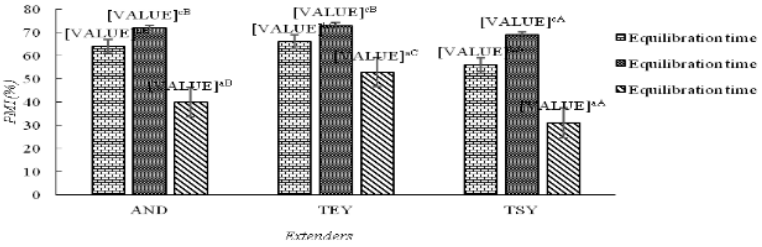

Figure 4. Effect of different extenders and equilibration time on Plasma Membrane Integrity (PMI) sperm parameters for the Bali bull semen samples thawed at 37 °C. Data are means±SD. $^{a, b, c}$; Values in the same row with different superscript different significantly (P<0.05) and ^{A,B,C} Values in the same column with different superscript different significantly (P<0.05). AND®: Andromed, TEY: Tris-citric egg yolk, TSY: Tris-skim milk-egg yolk.

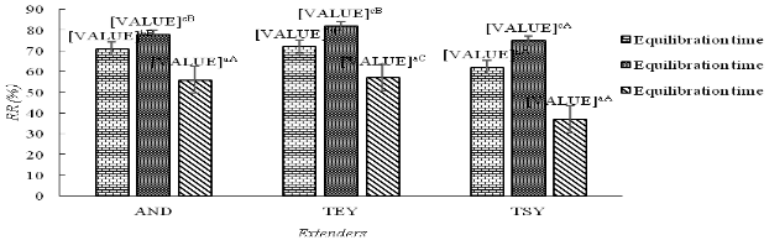

Figure 5. Effect of different extenders and equilibration time on Recovery Rate (RR) sperm parameters for the Bali bull semen samples thawed at 37 °C. Data are means±SD. ^{a,b,c} ; Values in the same row with different superscript different significantly ($P<0.05$) and ^{A,B,C} Values in the same column with different superscript different significantly ($P<0.05$). AND®: Andromed, TEY: Tris-citric egg yolk, TSY: Tris-skim milk-egg yolk.

(Arifiantini *et al*., 2005). Cell damage during clotting and thawing is due to lipid peroxidation of the spermatozoa that results in decreased survival (Alvarez & Storey, 1982). Damage to the spermatozoa cell membrane occurs in freezing and thawing between the temperatures of −15 °C to −60 °C, but it does not occur during dispersal in the liquid nitrogen (Park & Graham, 1993). The average value of the Bali bull RR sperm with TSY was lower than the Bali bull RR sperm that was extended with either TEY or AND®. This was possibly caused by damage to the plasma membranes of spermatozoa due to lipid peroxides. In accordance with Maxwell and Watson (1996) the spermatozoa membranes contain a large amount of unsaturated fat that is particularly susceptible to lipid peroxidation reactions.

3.8 Pregnancy rate (PR) based on non-return rate (NRR) value

The percentage of Bali-cow pregnancy is influenced by many things such as the fertility of the cows and the quality of the sperm used. However, the observation on day 21 indicated that 90%, 100%, 100% of the inseminated cows with semen that used the AND®, TEY, and TSY extenders, respectively, did not show signs of estrus on day 21 after insemination (Table 2). The high NRR values were caused because all of the acceptor cows were in the luteal phase. We can conclude that the synchronization hormone worked well. Other than that, the Bali cattle were reported to be superior to other breeds in fertility and conception rate (Toelihere, 2002).

A pregnancy test was also performed on day 60 following insemination using rectal palpation methods instead of the visual estrus detection method. The results showed that the PRs in the inseminated cows with semen that used the AND®, TEY, and TSY extenders were 88.9%, 90.0%, and 70.0%, respectively (Table 2). There was a decrease in the number of cows that did not show signs of estrus on day 21 following insemination with the number of cows that were pregnant using rectal palpation. Jainudeen, Wahid, & Hafez (2000) described the main cause of pregnancy failure in cattle was embryonic death followed by placentation, male factor, fetal death, lethal gene, and ovum transport.

The PR was higher than sheep in Bangladesh (Rekha, Zohara, Bari, & Alam, 2016) and buffalo in Pati, Indonesia (Rizal & Riyadhi, 2016). This difference may be caused by different values of PMI, RR, and motility of the semen used. The PR is also determined by the type and age of the livestock (Hafez & Hafez, 2016). A high PR in this research probably was due to timely insemination because the signs of estrus in the treatments were very clear. Therefore, the inseminator knew the appropriate time for the AI.

Table 2. Effects of different extenders with 4 h equilibration time on non-return rate (NRR) and pregnancy rate (PR) for 60 Bali cows.

Extenders (equilibration 4 h)	Variables	
	NRR	Pregnancy Rate
AND (20 n)	90% (18/20)	88.9% (16/18)
TEY (20 n)	100%	90.0% (18/20)
TSY (20 n)	100%	70.0% (14/20)

4. Conclusions

The TEY, AND®, and TSY extenders at the 4-h equilibration time produced sperm motilities of 66.66%, 63.67%, and 60.66%, abnormalities of 6.33%, 6.33%, and 7.33%, PMI values of 73%, 72%, and 69%, RRs of 82%, 78%, and 75%, PRs of 90%, 88.9%, and 70%, and NRRs of 100%, 90%, and 100%, respectively.

Acknowledgements

The authors would like to thank Balitbang Bengkalis for the financial support and for providing the research location. The authors also thank the Team of BIBD Tenayan Raya especially Mr. Fauzun Rais, Dedy Junianto, Berliana, and Sanif Tandi for their kind assistance throughout the experiment. There is no conflict of interest in this study.

References

Alves, H. M., Oliveira, I. R., Castelo, T. S., Lima, G. L., Souza, A. L., Moreira, A. L., Silva, A. R. (2013). Comparison of different glycerol and egg yolk concentrations added to tris-based extender for the collared peccaries (*Tayassu tajacu*) semen freezing. *Reproduction in Domestic Animals, 48*, 506-511. doi: 10.1111/rda.12115

Anzar, M., Kroetsch, T., & Boswall, L. (2011). Cryopreservation of bull semen shipped overnight and its effect on post-thaw sperm motility, plasma mem-

brane integrity, mitochondrial membrane potential and normal acrosomes. *Animal Reproduction Science, 126*(1-2), 23-31. doi:10.1016/j.anireprosci. 2011.04.018

Arifiantini, I., Yusuf, T. L., & Graha, N. (2005). Longivitas dan recovery rate Pasca Thawing Semen Beku Sapi Fresian Holstein Menggunakan Bahan Pengencer Yang Berbeda. *Buletin Peternakan*, *29*(2), 53–61. doi:10.21059/buletinpeternak.v29i2.1163

Arifiantini, R. I., & Purwantara, B. (2010). Motility and viability of Friesian Holstein spermatozoa in three different extender preserved at 5°C. *Journal of the Indonesian Tropical Animal Agriculture*, *35*, 222-226. doi:10.14710/jitaa.35.4.222-226

Ariantie, O. S., Yusuf, T. L., Sajuthi, D., & Arifiantiny, R. I. (2014). Kualitas semen cair kambing Peranakan Etawah dalam modifikasi pengencer tris dengan trehalosa dan rafinosa. *Jurnal Veteriner, 15*, 11-22. Retrieved from https://ojs.unud.ac.id/index.php/jvet/article/view/8780

Ax, R., Dally, M., Didion, B., Lenz, R., Love, C., Varner, D., . . . Bellin, M. (2008). Semen evaluation in reproduction in farm animal (7th ed.), Edited By Hafez, E. S. E. 365-370. SC: Reproductive Health Kiawah Island.

Celeghini, E. C., de Arruda, R. P., de Andrade, A. F., Nascimento, J., Raphael, C. F., & Rodrigues, P. H. (2008). Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. *Animal Reproduction Science, 104*(2-4), 119–131. doi:10.1016/j.anireprosci.2007.02.001

Dewi, A. S., Ondho, Y. S., & Kurnianto, E. (2012). Kualitas semen berdasarkan umur sapi jantan jawa. *Journal of Animal Agriculture*, *1*(2), 126-133.

Febretrisiana, A., Anwar, & Sinulingga, S. (2016). Relationship of sxtender and packaging system and the length of preservation and the quality of child semen of boer goat. *Indonesian Journal of Animal and Veterinary Science*, *2*(1), 49-54. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20173030825

Gotham, K. M., & Mayes, P. A. (2009). Pengangkutan dan penyimpanan lipid. In R. K. Murray, D. K. Granner, & V. W. Rodwell (Eds.), *Harper's illustrated biochemistry* (27th ed., pp. 225-238). Jakarta, Indonesia: EGC.

Gordon, I. (2017). Reproductive technologies in farm animals (2nd ed.). Retrieved from https://Books.Google.Co.Id/Books?Isbn=178064602X

Hafez, E. S. E., & Hafez, B. (2016). Reproduction in farm animals (7th ed.). Maryland, MD: Lippincott William and Wilkins. doi:10.1002/9781119265306

Hayati. (2011). *Spermatologi*. Surabaya, Indonesia:Pusat Penerbit dan Percetakan Universitas Air Langga Press.

Hussain, S. O., Shahad, H. K., & Al-Badry, K. I. (2016). Effect of dilution, cooling and freezing on physical and biochemical properties of semen for Holstein Bull Born in Iraq. *Advances Journal in Animal and Veterinary Sciences*, *4*(11), 575-579. doi:10.14737/journal.aavs/2016/4.11.575.579

Jainudeen, M. R., Wahid, H., & Hafez, E. S. E. (2000). Ovulation induction, embryo production and transfer. 2000. In E. S. E. Hafez & B. Hafez (eds.), *Reproduction in farm animal* (7th ed., pp; 405-431). Philadelphia,PA: Lippincott Williams and Wilkins.

Jeyendran, R. S., Van der Ven, H. H., Perez-Pelaez, M., Crabo, B. G., & Zaneveld, L. J. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *Reproduction*, *70*(1), 219-228. doi:10.1530/jrf.0.0700219

Komariah, I., Arifiantini, & Nugraha, F. W. (2013). Kaji Banding Kualitas Spermatozoa Sapi Simmental, Limousin, dan Friesian Holstein terhadap Proses Pembekuan. Buletin Peternakan. *Bulletin of Animal Science*, *37*(3), 143-147. doi:10.21059/buletinpeternak.v37i3.3078

Kulaksız, R., Çebi, Ç., Akçay, E., & Daşkın, A. (2010). The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen. *Small Ruminant Research, 88*(1), 12-15. doi:10.1016/j.smallrumres.2009.11.014

Lestari, D. M., Saleh., & Maidaswar. (2013). Profil of fresh semen quality of liousin bulls with different age in the centere of artificial insemination in Lembang West Java. *Jurnal Peternakan*, *1*(3), 1165-1172.

Manjunath, P. (2012). New insight into the understanding of the mechanism of sperm protection by extender components. *Animal Reproduction, 9*(4), 809-815. Retrieved from http://www.cbra.org.br/portal/downloads/publicacoes/animalreproduction/issues/download/v9n4/p809-815.pdf

Parera, F., Prihatini, Z., Souhoka, D. F., & Rizal, M. (2009). Utilization of carrot extract as an alternative diluent in spermatozoa epididymis of Bali cattle. *Journal of the Indonesian Tropical Animal Agriculture*, *34*(1). Retrieved from http://eprints.undip.ac.id/16472/

Paulenz, Soderquist, H., Perez-pe, L.R., & Berg, K.A. (2002). Effect of different extenders and storage temperatures on sperm viability of liquid ram. *Theriogenology*, *57*, 823-836. Retrieved from http://www.biozar.es/BIOZAR/ARTICULOS_files/2002%20Effectdiffexte%20THERIO%20.pdf

Ratnawati, D., Affandhy, L., Pratiwi, W.C., & Prihandini, P.W. (2008). The effects of traditional supplements on quality of cement of Bali cattle. *Veterinaria Journal,* 116-121. [Indonesian]. Retrieved from http://peternakan.litbang.pertanian.go.id/fullteks/semnas/pro08-18.pdf

Rekha, A., Zohara, B. F., Bari, F.Y., & Alam, M. G. S. (2016). Comparisons of commercial Triladyl and locally manufactured extenders for the chilling of semen and their effects on pregnancy rates after transcervical AI in Bangladeshi Indigenous (Ovis aries) sheep. *Animal Reproduction*, *13*(4), 735-742. doi: 10.21451/1984-3143-AR733

Rizal, M. (2009). Vitality of spermatozoa caudal epididymis Bali cattle preserved at temperature 3-5^{O}c in Tris extender with different lactose concentration *Jurnal Ilmu Ternak Veteriner*, *14*(2), 142-149. Retrieved from http://medpub.litbang.pertanian.go.id/index.php/jitv/article/view/355/364

Rizal, M., & Riyadhi, M. (2016). Fertility of swamp buffalo semen (bubalus bubalis carabanensis) diluted with sugar palm juice extender. *Jurnal Veteriner*, *17*(3), 457-467. doi:10.19087/jveteriner.2016.17.3.457

Shah, S. A. H., Andrabi, S. M. H., & Qureshi, I. Z. (2016). Effect of equilibration times, freezing, and thawing rates on post-thaw quality of buffalo (*Bubalus bubalis*) bull spermatozoa. *Journal of Andrology*, *4*(5), 972-976. doi:10.1111/andr.12214

Setyani, N. M. P., Sarini, N. P., & Lanang Oka, I. G. (2017). Heterogenity of bali bull semen quantity and quality in balai inseminasi buatan d baturiti, tabanan. *Jurnal Peternakan Tropika*, *5*(1), 91-104.
https://ojs.unud.ac.id/index.php/tropika/article/view/29466/0

Schorin., Marilyn, D., Sollid., Kris, R. D., Edge., Marianne, M. S., Bouchoux., & Ann, M.S.W. (2012). The science of sugar part 1: A closer look at sugars. *Nutrition Today, 3*, 96-101. doi:10.1097/NT.0b013e3182435de8

Stefanov, R. G., Anev, G., & Abadjieva, D. V. (2015). Effect of different extenders and storage periods on motility and fertility of ram sperm. *Macedonian Veterinary Review*, *38*, 85-89. doi: 10.14432/j.macvetrev.2014.12.036

Susilawati, T., Wahyudi, F. E., Anggraeni, I., Isnaini, N., & Muhammad Nur Ihsan, M. N. (2016). Penggantian Bovine Serum Albumin Pada Pengencer Cep-2 Dengan Serum Darah Sapi Dan Putih Telur Terhadap Kualitas Semen Cair Sapi Limousin Selama Pendinginan. *Jurnal Unsyiah*, *10*(2), 98-102. Retrieved from jurnal.unsyiah.ac.id/JKH/article/download/5025/4259

Suharyati, S., & Hartono, M. (2011). Preservation and cryopreservation of Limousin cattle cement in various diluents. *Journal of Veterinary Medicine, 5*(2), 53-58.

Sukmawati, E., Arifiantini, R. I., & Purwantara, B. (2014). Freezing capacity of sperm on various type of superior bulls. *Indonesian Journal of Animal and Veterinary Sciences*, *19*(3), 168-175. doi:10.14334/jitv.v19i3.1079

Sukhato, P., Thongsodseang, S., Utha, A., & Songsasen, N. (2001). Effects of cooling and warming conditions on post-thawed motility and fertility of cryopreserved buffalo spermatozoa. *Animal Reproduction Science, 67*, 69-77. Retrieved from https://pdfs.semanticscholar.org/a812/be1f1aa22f56f79358e7baa80999fd668704.pdf

Toelihere, M. (2002). Increasing the success rate and adoption of artificial insemination for genetic improvement of Bali cattle. *Workshop on strategies to improve Bali cattle in Eastern Indonesia*. Udayana Eco Lodge Denpasar Bali 4-7 February 2002.

Tsutsui, T., Tezuka, T. Y., Mikasa, Sugisawa, H., Kirihara, N., Hori, T., & Kawakami, E. (2003). Artificial insemination with canine semen sored at a low temperature. *Journal of Veterinary Medicine Sciences, 65*(3), 307-312. doi:10.1292/jvms.65.307

Watson, P. F. (1995). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. *Reproduction and Fertilization Developtment*, *7*(4), 871-891. doi:10.1071/RD9950871

Wahjuningsih, S., Hermanto., Nuryadi, A. B., & Bhontoro. (2012). Effect of sperm concentration and Length of storage at 5^0C motility of goat spermatozoa. *World Academy of Science Engineering and Technology*, *66*.

White, I. G. (1993). Lipid and calcium uptake of sperm in relation to cold shock and preservation a review. *Journal of Reproduction Fertility and Development, 5*, 639-658. doi:10.1071/RD9930639

Wiratri, V. D. B., Susilawati, T., & Wahjuningsih, S. (2014). Quality semen cattle limousin in different controllers during cooling. *Jurnal Ternak Tropika*, *15*(1), 13-20. Retrieved from http://ternaktropika.ub.ac.id/index.php/tropika/article/view/193/191

Sarastina, T. (2007). Analisa Beberapa Parameter Motilitas Spermatozoa pada Berbagai Bangsa Sapi Menggunakan Computer Assisted Semen Analysis (CASA). *Jurnal Ternak Tropika, 6*(2), 1-12. Retrieved from http://ternaktropika.ub.ac.id/index.php/tropika/article/viewFile/146/154

Yendraliza. (2014). Reproduksi Ternak Kerbau. Retrieved from https://books.google.co.id/books/about/Reproduksi_ternak_kerbau.html?id=sJZTAQAACAAJ&redir_esc=y

Zulyazaini., Dasrul., Wahyuni, S., Akmal, M., & Abdullah, M. A. N. (2016). The characteristics of semen and chemical composition of the seminal plasma of aceh cattle maintaned in BIBD Saree Aceh Besar. *Jurnal Agripet*, 16(2), 121-130. Retrieved from http://jurnal.unsyiah.ac.id/agripet/article/view/5803/5130

Quality of Bali bull cryopreserved sperm using different extenders and equilibration times on pregnancy rate of Bali cows

ORIGINALITY REPORT

5%	5%	4%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	pubs.rsc.org Internet Source	1%
2	Shah, S. A. H., S. M. H. Andrabi, and I. Z. Qureshi. "Effect of equilibration times, freezing, and thawing rates on post-thaw quality of buffalo (Bubalus bubalis) bull spermatozoa", Andrology, 2016. Publication	1%
3	www.scribd.com Internet Source	1%
4	www.macvetrev.mk Internet Source	1%
5	www.rohh.ugent.be Internet Source	1%
6	ijas.ir Internet Source	1%

Exclude quotes	Off	Exclude matches	< 1%
Exclude bibliography	On		