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Liquid Semen Quality of Landrace Boar in Tris-Egg Yolk Extender and Zorlesco Extender at 20 °C

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Abstract

Liquid semen quality of landrace boar during preservation needs an extender to maintain sperm from the negative effect such as loss of energy, pH change, and membrane permeability damage. To reduce the negative effects, good extenders are required. Tris-EY and zorlesco are two common extenders that used for the preservation of boar semen. This research was aimed to evaluate that ability of tris-EY extender and zorlesco extender to maintain the quality of landrace liquid semen at 20 °C. Semen was collected from 3 years of landrace boar in healthy condition, by hand glove method twice a week. A completely randomized design with 2 treatments and 7 replications were used to collect data. Semen was evaluated macroscopically and microscopically then extended with tris-EY extender and zorlesco extender. The motility and viability of sperm were evaluated every six hours. Data were analyzed by T-test, SPSS Programed. Research results showed that sperm motility of landrace semen in a tris-EY extender and zorlesco extender has no significant difference ($P > 0.05$) at 24 h evaluation, which were 41.00±2.23% in a tris-EY extender and 45.00±5.00% in zorlesco extenders. The viability of sperm also has no significant difference ($P > 0.05$), the observed values were 54.38±6.38% in a tris-EY extender and 58.32±10.35% in a zorlesco extender. It can be concluded that tris-EY extender and zorlesco extender have a similar ability in maintaining the quality of landrace boar liquid semen at 20°C.

KEYWORDS
Artificial Insemination, Liquid semen, Tris-Egg yolk, Zorlesco

INTRODUCTION

Science advances in the field of livestock reproduction technology have experienced many developments and contributed to sharing of new knowledge in terms of increasing livestock populations. Artificial insemination (AI) is the first generation of reproductive technology that has increased the productivity of livestock and improved reproduction, especially in pigs. AI is widely practiced in many districts of the country with intensive production of pigs for consuming such as in Southeast Asia. The use of AI in commercial pig herds has increased significantly in the last decade (Maes *et al*., 2011; Riesenbeck, 2011). In Indonesia, pigs are one of the livestock species that have the potential to be well developed and have a high birth rate based on the unique characteristics of reproduction as prolific livestock that can produce 6-12 piglets per birth. Landrace pigs are one of several types of pigs that have been successfully developed in Europe and America as well as Southeast Asia. East Nusa Tenggara, Indonesia as a pig farming development area has good opportunities for increasing landrace pig production. The increase in the productivity of pigs in the East Nusa Tenggara region is known from the data on the population of pigs in 2010-2011 which was 1,724,591 heads to 1,782,705 pigs (Directorate General of Indonesia Livestock, 2012).

Artificial insemination (AI) is a biological reproductive technology that has a role in preserving and increasing livestock production. Artificial insemination in landrace pigs requires good quality semen. Pig semen is generally voluminous with low concentrations, this property causes an excessive amount of semen after storage. Excess pig semen can be utilized in the future through preservation in diluent and stored at a certain temperature so that the viability and motility of sperm can be maintained for a relatively long time.

Tris extender (hydroxymethyl aminomethane) is an extender commonly used to dilute the semen of cattle, goats, sheep, but there is not much information about its use for the preservation of boar semen and its function as a buffer to prevent changes in pH due to the accumulation of lactic acid from the metabolism of sperm. A number of pH regulators, such as bicarbonate, sodium citrate, HEPES, or tris, have been added to the extender to control the pH of the medium and tris has a physiological pH representative of most living cells, is less expensive than any other buffering agents and is highly water-soluble (Gadea, 2003; Namula *et al*., 2019). Tris extender also contains citric acid which acts as a pH stabilizer and fructose as an energy source. Energy source in the form of egg yolk which is added to tris extender mainly acts as

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an energy source and protects sperm from cold shock during storage. Egg yolk is a common ingredient of mammalian semen extender to protect sperm against initial cold shock (Anzar *et al*., 2019).

Zorlesco extender is one of the commonly used diluents for the preservation of pig semen, which contains tris and amino acids in the form of cysteine (Gadea, 2003). Cysteine contained in zorlesco diluent functions in membrane stability and inhibitory capacity (Johnson *et al*., 2000). Cysteine has been shown to prevent the loss in the motility of frozen-thawed bull, ram, and goat semen and to improve viability, the chromatin structure, and membrane integrity of boar sperm during liquid preservation. Cysteine also has a cryoprotective effect on the functional integrity of exosome and mitochondria improving post thawed sperm motility (Uysal *et al*., 2007; Bansal and Bilaspuri 2011).

Based on the chemical and biochemical composition contained in the tris-egg yolk (tris-EY) extender and zorlesco extender, the two extenders were used to maintain liquid semen quality in sperm motility and sperm viability of landrace boar sperm for the preservation of boar liquid for AI purposes application.

MATERIALS AND METHODS

Samples collection

Two clinically healthy landrace boars aged 3 years, selected to the source of normal semen quality were used in this research. Boars were housed individually, maintained under natural daylight, fed with 16% of protein and 3200 kcal. Semen was collected twice a week with the glove hand method (Frangeî *et al*., 2005).

Preparation of semen extenders

Tris-EY extender based on Hu *et al*. (2006) and zorlesco extender based on Huo *et al*., (2002) was prepared. The composition of the Tris-EY extender was 1.10 g fructose, 2.42 g tris, and 1.48 g citric acid then dissolved in 100 mL of aquabidest, 20 mL of this solution was removed, and EY 20% v/v was added. The composition of the zorlesco extender was 1.15 g glucose, 0.23 g ethylenediaminetetraacetic acid, 1.17 g sodium citrate, 0.125 g sodium bicarbonate, 0.65 tris, 0.41 g citric acid, 0.01 g cysteine, then dissolved in 100 mL of aquabidest.

Evaluation of boar fresh semen quality

Semen was evaluated macroscopically and microscopically. The macroscopical analysis of semen included the color, pH, consistency, volume, and pH. Semen microscopical analysis included concentration, sperm morphology, sperm motility, and sperm viability (Althouse *et al*., 2006; Menkveld, 2010; Ducha *et al*., 2012).

Experimental design

This study used two treatments: tris-EY and zorlesco. Semen was diluted in a tris-EY extender and zorlesco extender as 2 treatments with 7 replicates.

Liquid semen processing and analysis

Semen containing sperm with more than 70% motile and 80% normal morphology were diluted with tris-EY extender and zorlesco extender, extenders are also supplemented with antibiotics (penicillin and streptomycin). Liquid semen was stored at 20°C and evaluated for sperm motility and sperm viability under a light microscope 400x magnification every six hours.

Statistical analysis

The quantitative data were presented as means ± standard deviation. Sperm motility and sperm viability were analyzed using T-Test in software program SPSS 19.00 for windows. Treatments were considered statistically different from one another at $p<0.05$.

RESULTS

The results of the study on the fresh semen characteristics of landrace boars were shown in Table 1. Fresh semen characteristics of landrace boar obtained have the normal range value of boar fresh semen.

Table 1. Fresh Semen Characteristics of Landrace Boar

Parameter	Mean ± SD
Volume (mL)	202.00±20.49
Color	White gray
Consistency	Watery
pH	6.58±0.16
Concentration (10^6/mL)	260.2±30.77
Motility (%)	78.00±2.74
Viability (%)	88.70±3.31
Abnormality (%)	4.58±1.05

Primary data of Landrace Fresh Semen (2013); SD: Standard deviation; mL: milliliter; pH: potential Hydrogen

Sperm motility is the number of sperm in straightforward movement / total number of sperm × 100%. The results of this study on sperm motility after dilution using a tris-EY extender and zorlesco extender have no different values (P>0.05) on all hours of observation (Table 2). Sperm motility after dilution or 0-hour observation in a tris-EY extender and zorlesco extender was 69.50±1.11% and 69.00±2.23% then gradually decreased to 41.00±2.23% and 45.00 ± 5.00% at 24 h of observation.

Table 2. Sperm motility in a tris-EY extender and zorlesco extender

Observation Time (Hour)	Treatments	
	Tris-EY (%)	Zorlesco (%)
0	69.50±1.11[a]	69.00±2.23[a]
6	61.00±2.23[a]	63.00±2.73[a]
12	54.50±3.70[a]	56.00±6.51[a]
18	46.00±2.23[a]	50.00±6.12[a]
24	41.00±2.23[a]	45.00±5.00[a]

Means value in each treatment with the same superscripts in the same column did not differ significantly (P > 0.05)

Sperm viability is the total of viable sperm within an ejaculate for fresh semen or semen after preservation. The value of sperm viability from the results of the study that the tris-EY extender and zorlesco extender was not different (P > 0.05) at all hours of observation (Table 3). Sperm viability after dilution or 0-hour observation in a tris-EY extender and zorlesco extender was 81.56±2.06% and 85.64±2.24% then gradually decreased to 54.38±6.83% and 58.32±10.35% at 24 h of observation.

Table 3. Sperm viability in a tris-EY extender and zorlesco extender

Observation Time (Hour)	Treatments	
	Tris-EY (%)	Zorlesco (%)
0	81.56±2.06[a]	85.64±2.24[a]
6	72.34±4.01[a]	78.20±4.51[a]
12	66.90 ± 3.98[a]	71.83±7.60[a]
18	59.44±4.51[a]	62.56±10.18[a]
24	54.38±6.83[a]	58.32±10.35[a]

Means value in each treatment with the same superscripts in the same column did not differ significantly (P > 0.05)

DISCUSSION

The preservation of boar liquid semen has many purposes in AI as a biotechnology reproductive tool. Tris-egg yolk and zorlesco as semen extenders consist of sources of energy, stabilizing pH, and maintaining membrane stability of sperm. The utility of the extender's composition is aimed at maintaining sperm motility and sperm viability during preservation and after AI. Sperm motility is an important parameter of liquid semen quality that needs to be considered for AI application, for it is related to the sperm's ability to fertilize an ovum.

The obtained results in zorlesco extenders are different from Huo *et al*. (2002), who found that zorlesco extender can maintain sperm motility of boar sperm up to 50% for 3 days of storage at 17°C and Sumarandi (2009) in liquid semen preservation using zorlesco was able to maintain sperm motility for 30 h of storage with 53.39% at 17°C. The difference in the value of sperm motility can be influenced by storage temperature factors. Another factor that affects the motility and viability of sperm is the temperature (White, 1993) then the sperm of mammals are very sensitive to temperature fluctuations (Lemma, 2011). Results of research on the use of tris-EY in the preservation of liquid semen of pigs are still scant. The results of research by Hu *et al*., (2006) on boar semen dilution using tris-EY showed that post-thawed sperm motility was 30.1%.

There was no difference in sperm motility using both tris-EY extender and Zorlesco at 0-24 h of observation, indicating that the composition of tris-EY and Zorlesco extenders contained nutrients and buffers needed by sperm as an energy source and prevented changes in pH values. Memon and Ott (1981) stated that the ideal extender has conditions, including providing an energy source (carbohydrates), containing ingredients that can protect spermatozoa against the negative effects of cooling and freezing, buffers are useful in preventing changes in pH due to the formation of lactic acid. Johnson *et al*. (2000) stated that one of the factors that determine the motility of sperm during in vitro storage is the availability of nutrients contained in the diluent. Availability of nutritional sources of the two specific diluents is fructose, lipoprotein, phospholipid, and lecithin which in tris-EY, and glucose, and cysteine in zorlesco which have the same role in maintaining the motility of sperm in anaerobic storage. The fructose contained in tris-EY is utilized by spermatozoa as an energy source in both anaerobic and aerobic conditions in the female reproductive tract (Garner and Hafez, 2000). Tambing *et al*., (2000) stated that fructose acts as an energy-producing substrate in the form of ATP which can maintain the sperm motility. The glucose contained in zorlesco is used in the process of spermatozoa metabolism by utilizing glucose through the glucose stage before becoming 6P fructose, it must be converted first to 6P glucose then to 6P fructose, and finally to fructose bisphosphate to produce ATP (Dube *et al*., 2004; Sumarandi, 2007). Lipoprotein and lecithin function to maintain and protect the integrity of the lipoprotein sheath of spermatozoa cells (Toelihere, 1993), while the phospholipids found in egg yolk function protect spermatozoa against the effects of cold shock and prevent calcium influx (White, 1993). Cysteine is a source of protein that provides a backup source for spermatozoa during storage (Johnson *et al*., 1982). Cysteine is a sulfur-containing amino acid with a thiol group is an important component of sperm nucleic acid and maintains the integrity of the DNA (Perumal *et al*., 2011).

A gradual decrease in the value of spermatozoa motility during storage at 0-24 h indicates a negative effect on the metabolic activity of spermatozoa during preservation. The resulting negative effect is related to lipid peroxidation which can reduce the motility of spermatozoa. The free radicals are known to be involved in lipid peroxidation as well as DNA and sperm membrane damages that may lead to decreased sperm motility or cell death (Uysal *et al*., 2007).

The surface of the sperm is covered by a lipoprotein membrane which when the sperm dies, the permeability of the membrane increase especially in the base of the head (Feradis, 2010) so it is easier to absorb the dye. Viable sperm cells have good membrane conditions so that the dye has difficulty penetrating the cell membrane, as a result, the sperm cells remain clear focused in the sperm head. The percentage of viable sperm (sperm head unstained) indicating living sperm and non-viable sperm (sperm head stained) indicating dead spermatozoa (Samplaski *et al*., 2015). The study results in table 3 showed no significant differences in the value of sperm viability between tris-EY extender and zorlesco extender during preservation (P > 0,05), the respective value of sperm viability in a tris-EY extender and zorlesco extenders were 81.56±2.06% and 85.64±2.24 at 0 h of observation time further 54.38±6.83 and 58.32±10.35 at the 24 h. At the end of the observation (24 h) both extenders still showed the same value of sperm viability. This explained that both extenders can maintain the viability of sperm for up to 24 h while boar semen without the addition of diluent can only last 3-4 hours (Blakely and Bade, 1992).

The gradual decrease in the sperm viability which did not differ (P > 0.05) in the two diluents indicated good effectiveness of the content of tris-EY and zorlesco, but when zorlesco extender added with bovine serum albumin (BSA) in several studies has a better ability to maintain sperm quality. EY in tris-EY extender as protein sources with the ability to be a membrane stabilizer for sperm cells preservation. BSA is a protein that reduces cell damage and cell loss and egg yolk-based extenders are often used to protect spermatozoa against cold shock during short-term storage that contain low-density lipoproteins (LDL) be required for sperm protection (Larbi *et al*., 2017; Sandal *et al*. , 2019). In addition, the two extenders also contain buffers as pH stabilizers that play a role in maintaining the viability of sperm. Zen *et al*. (2001) stated that the most important factor for sperm to prolong their viability is the availability of nutrients and acid-base balance (pH). In the present study, results of the sperm viability showed a fluctuated decrease every six hours of observation. The decrease in the value of sperm viability in both extenders was also associated with the type of respiration experienced by spermatozoa during storage. Campbell *et al*. (2002) stated that anaerobic respiration will produce compounds that can poison cells and the amount of energy will be small produced, this can reduce the motility of sperm and shorten the life span of sperm. The motility and viability of sperm are strongly influenced by the age of the spermatozoa, the availability of energy (ATP), active, biophysical, and physiological agents, suspension fluids, and the presence of respiratory stimuli or barriers Hafez and Hafez (2000).

CONCLUSION

Based on the result of this study was concluded that the tris-EY extender and zorlesco extender have the same ability to provide liquid semen quality for sperm motility and sperm viability of landrace boar during preservation for 24 h.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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