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Original Article

Cryopreservation of Simmental cattle semen with egg yolk from different avian species and level glycerol of different in tris diluent

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Abstract

Objective: This research was conducted to determine the effect of egg yolk from different avian species and the level glycerol of different in maintaining sperm quality in Simmental cattle in tris diluent.
Methods: Semen comes from Simmental (n=3) kept at the Tuah Sakato Artificial Insemination Center (BIB), Payakumbuh, West Sumatra. The diluent used in this research was tris egg yolk derived from three types of egg yolk (Factor A; the egg yolks used to come from ducks, quails and chickens) and different doses of glycerol (Factor B; 6, 6.5 and 7%). The research method used was a Randomized Block Design (RBD) with 2 factors. The shelter sperm is a group. The variables observed were the percentage of motility, abnormality, percentage of live sperm and intact plasma membrane of Simmental cattle sperm after thawing.
Results: The results showed no interaction between glycerol levels with types of egg yolk in tris extender of Simmental sperm quality. The tris diluent with duck egg yolk was better than chicken egg yolk which was equivalent which was able to motility up to 52.67%, percentage of live sperm was 70.78, abnormal sperm was only 14.44% and IPM was up to 42.44%. The dose of glycerol 6 ml is better than 6.5 ml and 7 ml resulting in motility up to 52.44%, live percentage reaching 70.22%, abnormal sperm at 14.33% and IPM up to 43%.
Conclusion: The use of duck egg yolk in tris extender and the addition of 6% glycerol resulted in better sperm quality in Simmental cattle with high motility, percentage of live sperm, IPM and lower abnormal sperm.

Keywords: Abnormalities; Motility; Percentage live; Plasma membrane intact

INTRODUCTION

The success rate of artificial insemination (AI) is expected to increase the efficiency of livestock production. This is marked by the increasing population of cattle in Indonesia. The success of AI is determined by the type of diluent and the breed of cattle used. Simmental cattle are exotic cattle that are in great demand by rural communities because they have a larger frame compared to local cattle.

Dilution is a critical stage of sperm survival. So that efforts to find diluents that suit each sperm are still being carried out. Osmotic pressure and electrolyte balance of sperm are maintained with tris buffer. Egg yolk is usually used to protect sperm from cold shock. This is

due to the presence of lipoprotein and lecithin in egg yolks. Ducha *et al.* [1] used 20% egg yolk in mammalian sperm diluent. Magistri *et al.* [2] stated that each poultry egg yolk has different cholesterol levels. Polat *et al.* [3] have started a study using 5 types of egg yolks from poultry in the dilution of bovine semen. The use of duck and quail egg yolks has never been used on Simmental cattle sperm. Glycerol is a cellular cryoprotectant that functions to prevent the formation of ice crystals during freezing, but at certain levels, it can also be toxic to sperm [4].

Several reports are contradictory on the use of glycerol levels in various diluents and sperm quality in livestock. Intellect *et al.* [5] stated that a level of 5% glycerol produced good sperm quality, in contrast to Setiono *et al.* [6] who stated that a glycerol level of 6% produced the best sperm quality in Brahman cattle. Ariantie *et al.* [7] stated that each cryoprotectant will give a different effect in each diluent.

The objective of this study was to determine the effect of egg yolk from different avian species and the level glycerol of different in maintaining sperm quality in Simmental cattle in tris diluent.

MATERIALS AND METHODS

Materials

Semen is collected from Simmental bull (n=3) originating from BIB Tuah Sakato, Payakumbuh, West Sumatra. Simmental bull is 7±1.20 years old and weighs 700±2.20 kg. Cattle are raised according to the standard operating procedures of BIB Tuah Sakato. The egg yolks used consist of duck, quail, and chicken egg yolks which are obtained from the commercial market. Sperm collection was carried out once a week for 10 weeks. Semen was collected using an artificial vagina (IVM, France) at 42°C from a Simmental bull. The sperm motility used in this study was more than 70%.

Diluent material

All chemicals used in this study were from Sigma (St. Louis, MO, USA). Tris diluent is prepared by mixing 3.64 g tris (hydroxymethyl-aminomethane), 1.70 g citric acid, and 1.25 g fructose. Then dissolved in 100 ml of aquabidest. 80 ml tris buffer plus 20 ml egg yolk, homogenized and centrifuged at 3000 rpm for 10 minutes. Then added the antibiotic Benzylpenicillin (1000 IU/ml, Pharmacia, Belgium), streptomycin sulfate (1000 μg/ml, Pharmacia, Belgium) and then added glycerol (Merck, Germany).

The semen was divided into 3 parts and each was diluted with egg yolk tris diluent. The concentration of sperm in each milliliter of semen is 100 million cells per ml (25 million cells/straw). Before packaging, glycerol (6, 6.5 and 7%) was added. Then packed into mini straw. After the semen is packed, it is equilibrated at 5°C for 5 hours using an automatic machine. Next, the straws were put into a container containing liquid nitrogen for 24 hours.

Semen quality test

Frozen straw was thawed again for 30 seconds in water at a temperature of 37°C. The two ends of the straw plugs were cut and inserted into the microtube. Sperm were evaluated to determine motility, percentage live, abnormality, and intact plasma membrane (IPM) values.

Motility

Spermatozoa motility was tested by dripping semen on a clean glass object and covered with a covered glass. Spermatozoa were observed under a light microscope with a magnification of 10×45 (450x). Next, the forward movements of the spermatozoa are calculated and divided by the number of sperm counted (200 individuals) multiplied by 100% [5].

Abnormality

Abnormal sperm were observed by making a smear preparation on a glass object from one drop of spermatozoa mixed with one drop of eosin-nigrosin. Observations were made under a microscope with a magnification of 10×45. Abnormal spermatozoa were counted and divided by the number of sperm counted up to 200 cells multiplied by 100% [5].

Percentage of live sperm

Live percentages were performed by differential staining. One drop of spermatozoa

Table 1. Average percentage of sperm motility Simmental cattle after thawing

Treatment	Glycerol percentage (B)			Average
Egg type (A)	B1 (6%)	B2 (6.5%)	B3(7%)	
A1 (KTAB)	51.33 ± 1.15	50.33 ± 2.08	48.33 ± 1.53	50.00^{ab} ± 1.94
A2 (KTI)	53.67 ± 2.08	52.67 ± 0.58	51.67 ± 0.58	52.67^{b} ± 1.41
A3 (KTP)	52.33 ± 0.58	50.33 ± 0.58	49.00 ± 1.00	50.56 ^{a} ± 1.59
Average	52.44 ^{a} ± 1.59	51.11 ^{b} ± 1.62	49.67^{c} ± 1.80	

^{a,b,c} The same letter superscript in the column and row shows a very significant effect ($p<0.01$); KTAB is chicken egg yolk; KTI is duck egg yolk; KTP is quail egg yolk.

is placed on a glass object and one drop of eosin is added. Then mixed evenly using a sterile stem glass. Before being observed under a microscope with a magnification of 45×10, the preparations were dried over a flame. Live sperm are marked with a clear color and dead sperm are marked with red. The percentage of viable spermatozoa is calculated by adding up the viable spermatozoa divided by the total of all calculated spermatozoa multiplied by 100% [5].

Intact plasma membrane (IPM)

An intact plasma membrane test was performed using a hypoosmotic sewer solution (HOS) test [8]. HOS solution was prepared by mixing 2.70 g of fructose, and 0.47 g of sodium citrate with 100 ml of aquabidest and homogenized. Furthermore, 9.90 ml of HOS solution was mixed with 0.10 ml of semen and incubated for 1 hour at 37°C in a water bath. Then evaluated under a microscope with a magnification of 10×45. Sperm with intact membranes are marked with a bulging or coiled tail. The IPM percentage is calculated from the total sperm with intact membranes divided by the total calculated spermatozoa multiplied by 100%.

Data analysis

Data were analyzed using a two-factor with Randomized Block Design (RBD). The first factor is the type of egg yolk (chicken, duck, quail) in tris diluent, the second factor is the level of glycerol usage (6%, 6.5%, and 7%). Differences in the mean values of the treatment results were further tested with Duncan's test. Data is processed using Microsoft excel 2013.

RESULTS

Motility

The average sperm motility of Simmental cattle was significantly different at $p>0.01$ at different egg yolks and glycerol levels (Table 1). Glycerol level of 6.5% with duck egg yolks results in higher motility.

Percentage of live sperm

The average percentage of live sperm in Simmental cattle differed significantly on the origin of the egg yolk used and the level of glycerol used. The 6% glycerol level resulted in a higher life percentage value than the 6.5% and 7% levels (Table 2). The use of duck egg yolk tris resulted in a higher percentage of live sperm of Simmental cattle than the use of chicken and quail egg yolk tris.

Sperm abnormalities

The mean sperm abnormality after thawing was significantly different between the uses of chicken, duck and quail egg yolks

Table 2. Average percentage of live sperm in Simmental cattle after thawing

Diluent type (A)	Glycerol percentage (B)			Average
	B1 (6%)	B2 (6.5%)	B3(7%)	
A1 (KTAB)	68.00 ± 0.00	67.00 ± 1.00	66.00 ± 1.00	67.00^{ab} ± 1.12
A2 (KTI)	72.00 ± 1.00	70.33 ± 0.58	70.00 ± 1.00	70.78^{b} ± 1.20
A3 (KTP)	70.67 ± 0.58	69.67 ± 0.58	68.67 ± 0.58	69.67^{c} ± 1.00
Average	70.22^{a} ± 1.86	69.00^{b} ± 1.66	68.22^{c} ± 1.92	

^{a,b,c} The same letter superscript in the column and row shows a very significant effect ($p<0.01$); KTAB is chicken egg yolk; KTI is duck egg yolk; KTP is quail egg yolk.

Table 3. Average percentage of semen abnormalities in simmental cattle after thawing

Diluent type (A)	Glycerol percentage (B)			Average
	B1 (6%)	B2 (6.5%)	B3(7%)	
A1 (KTAB)	15.00 ± 1.00	17.00 ± 1.00	16.00 ± 1.00	16.00 [ab] ± 1.22
A2 (KTI)	13.33 ± 1.15	14.67 ± 0.58	15.33 ± 0.58	14.44 [b] ± 1.13
A3 (KTP)	14.67 ± 0.58	14.67 ± 0.58	15.00 ± 1.00	14.78 [b] ± 0.67
Average	14.33 [ab] ± 1.12	15.44 [b] ± 1.33	15.44 [b] ± 0.88	

[a,b,c] The same letter superscript in the column and row shows a very significant effect (p<0.01); KTAB is chicken egg yolk; KTI is duck egg yolk; KTP is quail egg yolk.

Table 4. Average Simmental cattle sperm intact plasma membrane after thawing

Diluent type (A)	Glycerol percentage (B)			Average
	B1 (6%)	B2 (6.5%)	B3(7%)	
A1 (KTAB)	68.00 ± 0.00	67.00 ± 1.00	66.00 ± 1.00	67.00[a.] ± 1.12
A2 (KTI)	72.00 ± 1.00	70.33 ± 0.58	70.00 ± 1.00	70.78[b] ± 1.20
A3 (KTP)	70.67 ± 0.58	69.67 ± 0.58	68.67 ± 0.58	69.67[c] ± 1.00
Average	70.22[a] ± 1.86	69.00[b] ± 1.66	68.22[c] ± 1.92	

[a,b,c] The same letter superscript in the column and row shows a very significant effect (p<0.01); KTAB is chicken egg yolk; KTI is duck egg yolk; KTP is quail egg yolk.

(p<0.01). Different glycerol levels produced different sperm abnormalities (p<0.01). Duck egg yolks and 6% glycerol levels resulted in lower sperm abnormalities than chicken, quail egg yolks and 6.5% and 7% glycerol levels (Table 3).

Intact plasma membrane (IPM) of sperm

The mean sperm IPM of Simmental cattle was significantly different in the difference in egg yolk and glycerol levels (p<0.01). the use of duck egg yolks and a glycerol level of 6% resulted in a higher IPM value than the use of chicken, quail egg yolks and a glycerol level of 6.5% and 7% (Table 4).

DISCUSSION

The decrease in temperature during freezing causes changes in the structure and permeability of the plasma membrane. This can be seen from the decrease in sperm motility from 70 to 50.56% after thawing. Hirwa *et al.*. [9] stated that the process of dilution and freezing of sperm greatly affects sperm motility. The sperm motility value of Simmental cattle in this study was higher than the sperm motility of Harar cattle after thawing (49.6%) [10] and bovine sperm motility in Ethiopia [11]. This difference is due to differences in age, type of livestock and environment as well as different methods of diluting semen. The sperm abnormality and intact plasma membrane of Simmental cattle in this study were different from that of beef and dairy cattle in the study of Morel *et al.* [12]. However, the intact plasma membrane values in this study were not much different from the intact plasma membrane values of Pasundan cattle [13]. The type of diluent and dilution method as well as sperm concentration in the study led to differences in abnormal values and intact plasma membranes between cattle.

This change in membrane function causes a decrease in motility function, and sperm viability. The level of tolerance to changes in the membrane phase will affect the survival of spermatozoa during freezing [14]. So that the presence of tris fructose diluent with egg yolk gives strength to sperm to survive during the freezing and thawing process. This can be seen from the motility percentage, sperm survival percentage, IPM percentage and sperm abnormality percentage in Simmental cattle in this study. Differences in egg yolk produce different qualities of motility, percentage of life and IPM. Duck egg yolks have higher cholesterol levels than chicken and quail egg yolks [2], so the low density protein content is also greater. Perumal *et al.* [15] stated that LDL can attach to the surface of the plasma membrane of spermatozoa to replace the phospholipids lost and damaged during clotting. Added by Akhter *et al.* [16] that the disintegrated LDL fraction releasing phospholipids will form a protective layer for

the spermatozoa plasma membrane and prevent phospholipid efflux. However, based on Indonesian national standards, the use of different egg yolks is still included in the SNI quality category [17]. So that the addition of egg yolks in the tris diluent can use these three egg yolks according to the availability of egg yolks in the semen dilution. Unlike the results of Polat *et al.* [3] who recommended pigeon egg yolk in tris diluent after a chicken. This difference is due to the different types and diluents used.

Cell physiological changes occur during the cooling process. As the temperature drops during the freezing process, the size of the ice crystals increases, causing the solute in the extracellular fluid to shift to the liquid fraction. High ion concentrations in the extracellular fluid can damage the structure and stability of the plasma membrane [18]. The presence of glycerol in the diluent can prevent cell dehydration because glycerol will prevent the collection of H2O molecules to prevent ice crystals. The interaction of glycerol and phospholipids can prevent membrane dehydration by replacing bound water released from the cell membrane [19]. From this study it appears that although glycerol has a good effect in preventing the formation of ice crystals, glycerol has a usage threshold. This can be seen when using glycerol at levels of 6.5% and 7% there is a decrease in motility, percentage of life and sperm membrane integrity of Simmental cattle. Although based on the 2017 SNI for cow sperm, glycerol levels of 6.5% and 7% can still be used, in terms of efficiency, the use of ingredients using levels higher than 6% is not efficient. Unlike Herbowo *et al.* [20] who recommended 7% use of glycerol in the freezing of buffalo semen and was the same as the study by Setiono *et al.* [6] who recommended the use of 6% glycerol in freezing Brahman cattle. This is thought to be due to differences in the type of sperm and the diluent used.

CONCLUSION

The use of duck egg yolk in tris diluent and the addition of 6% glycerol resulted in high motility, live sperm percentage, increased IPM percentage and lower sperm abnormalities.

CONFLICT OF INTEREST

All authors in this study have no conflict of interest.

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