Motility, Viability and Fertility of Indonesian Native Rooster Spermatozoa in Skim-Milk Based Semen Extender

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ABSTRACT

The purpose of this study was to determine the motility and fertility of kampung rooster spermatozoa diluted in skim-milk based semen diluent. Semen that was collected from 12 kampung roosters, collected in one tube, then divided into six treatment groups, namely P0 = semen + skim milk extender; P1= semen + skimmed milk extender + 50 mM Glucose; P2= semen + skimmed milk extender + Ringer's Lactate (4:1); P3= semen + skimmed milk extender + Ringer Lactate + 50 mM Glucose; P4= semen + skim milk extender + Physiological saline (4:1), and P5= semen + skim milk extender + Physiological saline (4:1) + Glucose 50 mM. All treated semen samples were stored at a refrigeration temperature of 3–5°C for 1 hour. Motility and viability were then observed under a microscope. This process was repeated four times. Subsequently, a single insemination was performed in the afternoon for each treatment group, with 10 Isa Brown hens aged 50 weeks assigned per group (n=60). This study used a completely randomized design (CRD). The results showed that the average of motility (%) of each treatment was: 81.25± 2.50; 83.75±4.79; 85.00±4.10; 86.25±2.50; 86.25±2.50 and 87.50±2.89. Average of viability was: 86.25±2.63; 88.75±4.11; 90.00±3.70; 90.25±6.80; 91.00±2.70 and 92.25±2.22. Average of fertility (%) was: 83.33±6.60; 85.10±8.32; 85.41±8.32; 92.85±6.58; 84.78±6.83 and 86.67±6.78. It can be concluded that the six extenders under study did not have significant effects on motility, viability, or fertility, indicating that all six skim-milk based extenders are suitable for use as extenders for fresh Kampung rooster semen.

Keywords: Indonesian native rooster, spermatozoa, motility, viability, fertility, skim milk, semen extender

INTRODUCTION

Chicken semen is very concentrated, viscous, containing billions of spermatozoa per ml. Therefore, it is necessary to do dilution before insemination (Donoghue and Wishart, 2000). Semen dilution plays a role in increasing or maintaining semen quality and also increases semen volume (Roiter and Konopleva, 2000). Many fresh semen diluents are commonly used in artificial insemination (AI) in chicken with high fertility rates. These diluents include NaCl, Ringer Lactate, coconut water (Odrada *et al*., 2023), skim milk (Saleh et al, 2020), to complex, commercial diluents such as Beltsville poultry semen extender (BPSE), EK and Lake (Bootwalla and Miles, 2007; Sarkar 2020; Mohan et al., 2018).

The use of skim milk-based semen diluent has been tested and applied as a diluent for fresh semen and frozen semen of cattle and frozen semen of buffaloes and has been produced commercially, and has been used widely in the world (Raheja et al., 2018; Bustani and Baiee, 2021). However, the application of AI in chickens is still using liquid semen. In order to lead to the production of frozen chicken semen, until now there is still a lot of research being done. Tahseen et al (2019) reported that chicken semen in skimmed milk diluent-maintained motility for up to 8 hours. Saleh et al. (2020; 2021; 2022) reported that the use of skim milk as a diluent for fresh chicken semen resulted in high fertility. Kim et al (2003) reported that skim milk diluent + glucose, stored at 5oC for 6 hours, resulted in high fertility (90.77 percent).

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This study was conducted to examine the use of several diluents -based on skim milk on the motility, viability and fertility of Kampung rooster spermatozoa.

MATERIALS AND METHODS

This research was conducted at the experimental farm of the Faculty of Animal Science, Unsoed, Purwokerto, Indonesia. A total of 12 Indonesian native roosters aged around 1 year purchased from the nearby market. All the roosters were housed individually with a cage size of 60 x 60 x 70 cm. Each rooster was given commercial feed 150 g/head/day, and drinking water was given ad libitum. About 1 week before the treatment started, all the roosters were trained to accommodate their semen. Semen collection by massaging from the back to the tail/ abdominal massage (Silyukova *et al.*, 2022).

Semen was collected in one tube, homogenized, then divided into six treatment groups. The six diluent treatment groups used: P0=semen+ skim milk diluent; P1 = semen + skimmed milk diluent + Glucose 50 mM; P2 = semen + skimmed milk diluent + Lactate Ringer (4:1); and P3 = semen + skimmed milk diluent + Ringer lactate (4:1) + Glucose 50 mM; P4 = semen + skim milk + physiological NaCl (4:1) and P5 = semen + skim milk + physiological NaCl (4:1) + 50 mM glucose. The collected pooled semen was added to each of the six treatment groups.

Skim milk diluent:

1. Skim milk: 10 g of skim milk is diluted with 100 ml of distilled water, then heated at a temperature of 92-95 ºC for 10 minutes. After cooling, then filtered using filter paper, the diluent is ready for use.
2. Skimmed milk + Glucose 50 mM: Skimmed milk diluent (10 ml) +0.09 g glucose.
3. Skimmed Milk + Ringer Lactate (4:1): Skimmed Milk Diluent (80%) + Ringer Lactate (20%).
4. Skim milk + Lactate Ringer (4:1): Skim milk thinner (80%) + Lactate Ringer (20%).
5. Skimmed Milk + Ringer Lactate (4:1): Skimmed milk thinner (80%) + Ringer Lactate (20%)
6. Skim milk + Ringer's lactate + 50 mM Glucose: Skim milk diluent (10 ml) + 0.09 g glucose, = 50 mM
7. Skim milk + physiological NaCl (4:1): Skim milk diluent (8 ml) + 2 ml physiological NaCl.
8. Skim milk + physiological NaCl (4:1) + 50 mM Glucose: Skimmed milk diluent (8 ml) + 2 ml physiological NaCl + 0.09 g glucose.

Evaluation of semen: sperm motility and viability were estimated according to Lake and Stewart (1978). Progressive motility according to (Bakst and Long, 2014) as follows: 5 µL of the semen sample was mixed with 100 µL of 0.9% sodium chloride and examined under a microscope at 400× magnification. A total of 200 sperm were counted across at least 5 fields of view to obtain the final result. Progressive motility was expressed as the percentage of motile sperm.

Sperm viability was evaluated using the eosin-nigrosin staining technique following Tvrdá *et al.*, (2023). A 10 µL drop of fresh semen was placed on a slide, followed by 20 µL of eosin-nigrosin stain, and the mixture was gently blended. The slide was then allowed to air dry for several minutes. At least 200 sperm were counted under a compound microscope at 400× magnification to calculate the percentage of live sperm. Sperm that absorbed the stain were considered dead, while those that remained unstained were regarded as live, and the results were expressed as percentages.

Evaluation of fertility: the number of fertile eggs divided by the total number of incubated eggs multiplied by 100 percent. Motility and fertility data were analyzed using Analysis of Variance (Steel and Torrie, 1993). Each treatment group was inseminated into 10 productive laying hens (around 50 weeks old). Egg collection started on the second day after insemination until the 7th day.

Insemination was carried out once, in the afternoon, intravaginally, with a dose of 100 million spermatozoa/0.1 ml. Every four days the collected eggs that have been labeled and cleaned are put into an incubator that has been prepared for incubation. Fertility data was obtained from the results of candling which was carried out on the sixth day.

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RESULTS AND DISCUSSION

The mean and its standard deviation of motility, viability and fertility are presented in Table 1.

Table 1. Mean and its standard deviation of motility, viability and fertility of Kampung rooster spermatozoa

Diluent/ Extender	Motility^{ns}	Viability^{ns}	Fertility^{ns}
Skim milk	81.25± 2.50	86.25±2.63	83.33±6.6
Skim milk+Glucose 50 mM	83.75±4.79	88.75±4.11	85.10±8.32
Skim milk+Ringer Lactate (4:1)	85.00±4.10	90.00±3.70	85.41±8.32
Skim milk+Lactated Ringer (4:1) + Glucose 50 mM	86.25±2.50	90.25±6.80	92.85±6.58
Skim milk+Physiological Saline (4:1)	86.25±2.50	91.00±2.70	84.78±6.83
Skim milk+Physiological Saline (4:1) + Glucose 50 mM	87.50±2.89	92.25±2.22	86.67±6.78

ns: Non-significant result based on Analysis of Variance

Motility of Indonesian Native Rooster Spermatozoa

The results of the analysis showed that the average motility (%) for each treatment ranged from: 81.25 ± 2.50 to 87.50 ± 2.89; P>0.05; Mean viability (%) ranged from 86.25±2.63 to 92.25±2.22 and Mean fertility (%) ranged from 83.33±6.6 to 92.85±6.58; P>0.05).

This indicates that the addition of Ringer's lactate, physiological NaCl and glucose to the skim milk diluent showed good spermatozoa motility in each diluent treatment, although in ANOVA the motility value did not show a significant difference (P> 0.05). Even so, the average motility in the skim milk + NaCl physiologically increased, and the increase in the average motility was slightly higher than the skim milk + glucose treatment.

The results of this study, in particular, the average motility value of Indonesian native rooster spermatozoa was higher than the results of previous research (Saleh et al., 2020; 2022). In this case the difference is the addition of physiological NaCl and Lactate Ringer and the addition of Glucose. It seems that the mixture of skimmed milk added with physiological NaCl (4:1), Ringer's lactate (4:1) and the addition of 50 mM Glucose tended to increase the motility rate. This is probably because the more complete the diluent composition, the more complete the nutrients needed by spermatozoa, which results in increased spermatozoa motility.

The mean value of motility in skim milk diluent + Ringer's Lactate (4:1) was higher than the average motility of spermatozoa skim milk diluent. It is possible that the mixture of skimmed milk (80%) + Ringer Lactate (20%) has a balanced electrolyte ratio, close to the electrolyte conditions in semen plasma which causes the diluent to be isotonic and also a better buffer than skim milk diluent alone. The mean motility values in skim milk diluent + physiological NaCl (4:1) and 50 mM glucose were higher than the average spermatozoa motility values in other skim milk diluents. It is possible that in addition to isotonic conditions and good buffers, this glucose works as an energy reserve for spermatozoa, especially during storage. The main energy for spermatozoa is in the form of ATP which is obtained from glucose through glycolysis and oxidative phosphorylation in the tail of spermatozoa (Setiawan et al., 2020; 2021).

Viability of Indonesian Native Rooster Spermatozoa

The mean value of viability of each treatment was also relatively similar (P> 0.05) in each diluent treatment, although the viability of semen diluted with skim milk + physiological NaCl (4:1) was higher than that of semen diluted with skim milk, skimmed milk. + Glucose 50 mM, skimmed milk +Ringer lactate. It is suspected that the diluent composition of skimmed milk + physiological NaCl (4:1) + 50 mM glucose makes the spermatozoa cell membrane more resistant during storage.

Fertility of Indonesian Native Rooster Spermatozoa

As shown in Table 1. Mean (%) fertility ranged from 83 to 92%, P>0.05 The percentage of fertility sequentially from low to higher values of each treatment, namely: Skim milk treatment, Skim milk +

physiological NaCl treatment (4:1), skimmed milk+ Glucose 50 mM, skimmed milk + Ringer Lactate (4:1) and skimmed milk + Riger lactate (4:1) + Glucose 50 mM.

Fertility data on this skim milk diluent resulted in a higher fertility rate than the results of previous studies, 72.88 ± 8.70% vs. 83.33 ± 6.6% (Saleh et al., 2021). This difference is possible due to the different time of the research implementation, the possibility of different environmental conditions and other factors. The use of the six skimmed milk diluents was relatively the same in producing the level of fertility (P>0.05). It can be presumed that the diluent is suitable in facilitating spermatozoa life during storage. The use of skim milk diluent + Ringer lactate (4:1) + 50 mM Glucose resulted in the highest fertility rate compared to the fertility values of the other five diluent treatments, around 92 percent. It is possible that this diluent contains optimum glucose to maintain the life of spermatozoa. The results of this study agree with the results of the research of Setiawan et al (2020; 2021) which revealed that glucose and pyruvate can effectively assist flagella motility and production of ATP with the principal function being mitochondrial oxidation; Nevertheless, glucose is primarily responsible for sperm to fertilize the eggs.

CONCLUSION

It can be concluded that the six extenders under study did not have significant effects on based on the motility, viability and fertility. It's implying that the the six skim-milk based extender (skimmed milk, skimmed milk + 50 mM glucose, skimmed milk + Ringer's lactate (4:1) and skimmed milk + Ringer's lactate + 50 mM glucose, skimmed milk + NaCl (4 :1), and skim milk + NaCl (4:1) + Glucose 50 mM) in this study are suitable to be used as extender for fresh Indonesian native rooster semen.

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