MOTILITY AND FERTILIZING ABILITY OF KAMPUNG ROOSTER SPERM DILUTED WITH SKIM MILK +GLUCOSE+ DMA EXTENDER STORED AT 5ºC FOR UP TO 6 HOURS

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Abstract. Semen extenders are crucial for maintaining sperm quality during storage and ensuring the success of fertilization after insemination. The aim of this research is to test the effects of a skim milk-based extender for rooster semen supplemented with glucose and DMA on sperm motility and fertilization ability after being stored in a refrigerator at a temperature of 5°C for up to 6 hours. In this study, to observe the quality of spermatozoa and fertility diluted with skim milk + 50 mM Glucose + (0.5 and 10%) DMA at a storage temperature of 5°C and storage duration (0, 3 dan 6 h).The results show that there is no interaction effect from the type of diluent (addition of DMA: (0, 5, and 10%) and storage duration (0, 3, and 6 hours) on the motility and fertility of Kampung rooster spermatozoa.Skim milk extender plus DMA (0, 5, and 10%) was not significant ($P>0.05$) in improving the quality of rooster semen (motility and fertility) during storage for up to 6 hours. Sperm diluted with skim milk + Glucose + DMA (0.5% and 10%) stored at 5°C for 3 hours resulted in significantly higher motility and fertility values ($P<0.01$) compared to the motility and fertility values stored for 6 hours. In conclusion, the extender of skim milk + 50 mM glucose and + (5 and 10 percent DMA) stored at 5°C for 3 hours, positively affects the motility and fertility of Kampung rooster spermatozoa.

Keywords: dimethylacetamide, spermatozoa, rooster, motility, fertility

A. Introduction

The requirement for the distribution of high-quality sperm is highlighted by the poultry industry's growing use of artificial insemination, or AI. According to [1], evaluating the semen quality characteristics of poultry birds is a good way to gauge their capability for reproduction and has been shown to be a significant factor in determining egg hatchability and fertility. However, hatchability and fertility are the main factors that determine a hatchery's profitability; for this reason, a safe and appropriate methodology for handling rooster spermatozoa must be established.

Proper storage of poultry semen is required for the poultry business to benefit from modern AI procedures. It should be noted that in one collection of Rooster sperm has low volume, 0.2 ml, dense or viscous, and highly concentrated (3-4 billion spermatozoa/ml), and gradually losses whitin an hour, hence clean sperm extension with an appropriate diluent is necessary before AI and storage.

In order to prolong semen, preserve spermatozoa viability in vitro, and increase the number of hens that can be inseminated, diluents are buffered salt solutions. The biological makeup of chicken seminal plasma is the basis for the selection of semen diluents [2].

The quality of semen is maintained throughout storage for a variety of reasons. The diluents utilized, for instance, in semen extension and parameters for storage, including duration, aeration, and holding temperature is a significant factor [3].

There are numerous diluents for poultry semen on the market, including both commercially accessible items and formulas that have been published. Numerous researchers have examined the ingredients in different diluents and compiled fertility data from different trials [4]. These reviews make clear that there is no standard diluent for poultry sperm and that studies vary widely in terms of experimental design, number of spermatozoa inseminated, length of fertility analyzed, vaginal depth and frequency of AI, and time of insemination. As a result, it can be challenging to determine the relative advantages of various diluents [5]. Similarly, because to variations in experimental design, it is challenging to evaluate results regarding the long-term liquid storage of poultry semen described in the literature [5]. Within an hour of collection, sperm motility and the capacity to fertilize undiluted fresh fowl semen kept in vitro typically decline [6]. Consequently, to prevent a decline in sperm quality when storing poultry semen, the kind of diluent and storage temperature are crucial [3].

Skim milk, which is commonly used as a diluent for bull semen, can also be used for diluting chicken semen [7]. The addition of 50 mM glucose to skim milk can improve the quality of fresh spermatozoa in terms of motility and fertility [8].

Glucose is expected to function as extracellular cryoprotectant to protect spermatozoa cell membranes from the effects of cold shock due to storage of spermatozoa at low temperatures and as an energy source for the metabolism of spermatozoa during storage.

Permeant CPAs (P-CPAs) dimethylacetamide (DMA) is used to protect the cell membrane integrity and intracellular environment to reduce or avoid the damage to the structures and properties of membrane lipids, proteins, and nucleic acids. [9], [10].

The results of several researchers revealed that skim milk can be used as a diluent. The author revealed that skim milk diluent, plus 50 mM glucose produces quite high chicken egg fertility. In this study the author wants to know: the use of skim milk + glucose, plus Dimethylacetamide, as a cryoprotectant, and stored in a refrigerator at a temperature of 5 C for up to 6 hours can extend the storage period of chicken semen, which is suitable for use in insemination in chickens.

B. Methods

Bird Management and Semen Collection. The current study was conducted at the Animal Science Department's Experimental Research Station at Jenderal Soedirman University in Purwokerto, Indonesia. A total of twelve mature Kampung roosters (Gallus gallus domesticus) were kept at the Poultry Unit of Experimental Farm, Animal Science, Jenderal Soedirman University, fed a laying mash diet with 17% crude protein and 2700 kcl ME per kg of feed, starting at 50 weeks of age. There was water accessible all day.

Semen collection. After a few weeks of acclimatization, birds were trained to gather semen, which they subsequently did twice a week in the afternoon, [19] had earlier described the semen collection procedure. Semen was carefully filtered to remove any cloacae products, and yellow or atypical ejaculates were disposed of in a methodical manner. On each collection day, only ejaculates with mass motility of at least 70% were pooled. Using a Neubauer hemacytometer, the concentration of sperm cells in the semen was measured. Then, the semen was extended at a ratio of one semen to two extenders using skim milk plus glucose and DMA, and split into three sections. The first section was expanded using a control extender without the use of DMA (E1). However, the 2nd, 3rd parts were supplemented with 5 % (E2), 10 % (E3) DMA, respectively. Semen extender supplemented with different DMA levels was stored in refrigerator at 5°C and evaluated after 0, 3 and 6 h.

Semen evaluation included Motility. Semen evaluation:The Progressive sperm motility (PSM) was assessed according to [7] at a high power magnification (1000 x).

Fertility trial: Artificial insemination was performed using syringes (1.0 ml) for the deposition of the semen according to [7] to assess fertility rate. Hens from the same local breed were divided into 9 groups (10 hens for each treatment), including semen supplemented with: 0, 5 and 10 % of DMA level at 3 storage periods).

Total of 425 eggs, 9 from each group were collected after 1day post-insemination for a week (d2-6), and incubated to evaluate the fertility rate (FR). Eggs were candled to identify the fertile eggs according to the method of [7] as the following: FR = Fertilized eggs/ incubated eggs x100.

Statistical analyses. Data gathered from this experiment were subjected to two-way analysis of variance for 3x3 factorial designs, repeated 5 times. Observed results for motility were evaluated statistically using two-way ANOVA, and the fertility data were evaluated using one-way ANOVA [11].

C. Results And Discussion

The result showed the effect of different extenders after six hour of semen collection (Table 1). In Table 1, statistically, there is no interaction effect (P>0.05) between the diluent with 3 levels of DMA and 3 storage times on the motility and fertility of village chicken spermatozoa. Table 1 shows that the motility and fertility of village chicken spermatozoa diluted with skim milk + 50 mM Glucose with 3 levels of DMA stored at a refrigerator temperature of 5°C for up to 6 hours. The motility and fertility values diluted with 3 diluents showed no significant difference (P >0.05), although the motility values increased numerically with the addition of DMA (Tabel 2).

At storage time 0 (T0), the total motility and fertilization of the fertilized eggs appeared high in all diluted semen when stored at a refrigerator temperature of 5°C. The motility and fertilization values appeared to decrease with the increasing storage time. At 0 and 3 hours of storage, motility and fertility statistically did not show significant differences (P>0.05), but the motility and fertility values at 6 hours of storage were the lowest (P<0.01) compared to semen stored at 0 and 3 hours (Table 3).

Table 1. Average percentage motility and fertility ±STD in a different extenders and storage time

Extenders	Storage Time 0h		Storage Time 3h		Storage Time 6h	
	Motility	Fertility	Motility	Fertility	Motility	Fertility
SM+ 0% DMA	80.00±5.00	85.70±8.46	78.33±5.00	83.33±10.11	70.00±4.03	80.00±4.92
SM+ 5% DMA	83.33±2.88	100.00±10.11	83.33±2.88	85.70±7.50	71.66±16.73	66.66±5.06
SM+10% DMA	86.33±2.88	85.00±7.70	85.00±2.88	100.00±10.00	73.33±16.73	66.66±8.43

Table 2. Average percentage motility and fertility ±STD in a different extenders

Variable	Skim Milk+0% DMA	Skim Milk+5% DMA	Skim Milk + 10% DMA
Motility	76.11 ±0.94a	79.44±1.28a	81.55±0.94a
Fertility	83.01 ±0.90a	84.12±0,67a	83.89±0.22a

The mean motility and fertility (%) at the DMA of 0, 5, 10 were; (P>0,05); and (P>0,05), respectively

Table 3. Average percentage motility and fertility ±STD In a different storage time

Variable	Storage Time 0h	Storage Time 3h	Storage Time 6 H
Motility	83.22±1,11a	82.22±1,48a	71.66±2.60b
Fertility	90.23±10.00a	89.67±9.67a	71.11 ±5.07b

D. DISCUSSION

The goal of this study is to find a skim milk-based semen extender that is suitable for use by farmers in the field and can preserve the motility and fertilizing ability of chicken semen for up to six hours. Our results show that there is no interaction effect ($P>0.05$) between the extender added with DMA and storage time on spermatozoa motility and fertilization ability. Skim milk extender + 50 mM Glucose and added DMA improved spermatozoa quality during storage up to 6 hours at 5°C, although statistically, it did not show a significant difference.

The fertility value of semen diluted with skim milk + DMA is slightly higher than the fertility of spermatozoa without the addition of DMA. Dilution of chicken semen immediately after collection is important to maintain semen quality (motility, viability); otherwise, spermatozoa will die due to dehydration [12]. However, this procedure increases the respiration rate due to the high amount of nutrients in the cement slurry [13]. Therefore, the decrease in metabolism by storing it at low temperatures.

Without compromising the quality of the semen, it is necessary for the storage of semen. Even though low refrigerator temperatures are recommended for storage up to 24 hours, farmers in the field do not need to bring a refrigerator; it is sufficient to bring ice crystals stored in the semen storage box. As reported by other researchers, the quality of semen stored at a temperature of 2-5°C is better than semen stored at a temperature above 10°C. In this study, semen diluted with skim milk + DMA showed better spermatozoa motility than spermatozoa motility without the addition of DMA. It is suspected that storage at a temperature of 2-5°C can cause cold shock to spermatozoa. To address this, the role of cryoprotectants becomes necessary.

The motility and fertility values in this study indicate that motility and fertility are good up to 3 hours, not significantly different from zero storage. Meanwhile, at 6 hours of storage, motility and fertility decrease, showing a significant difference compared to 3 hours of storage.

The usual osmolarity range for semen additives should be between 320 and 450 milliosmoles, and their pH should be neutral. 7.4–6.8. To further prolong the life of spermatozoa during storage, the diluent must also contain energy sources like glucose [14];[20]. According to [15], semen motility that is treated with skim milk at 5 ^{o}C for around 4 hours is better than that of semen treated with tris.

The low levels of cholesterol, phospholipid, and protein phospholipid [16] in the sperm may be the reason of this. Liquidity of membranes is influenced by fatty acids [17]. Lower percentages of plasma membranes with higher cholesterol and fatty acid levels make the membranes more resilient to oxidative damage, which enhances protection. As well as enhancing sperm motility and viability, milk casein reduces lipid damage to cell membranes [18].

E. Conclusion

The use of skim milk extender + 50 mM Glucose and the addition of DMA up to 10% stored at 5°C can maintain its quality (producing high motility and fertility comparable to the use of fresh semen) for up to 3 hours of storage.

F. Acknowledgement

We expressed our gratitude to the Rector of Jenderal Soedirman University, the Head of the Institute for Research and Community Service, and the Dean of the Faculty of Animal Husbandry for providing the opportunity to carry out activities in Professorial Research (FTGB). Thanks also goes the Head of the Experimental Farm of the Faculty of Animal Science Unsoed and its staffs for their cooperation in the implementation of this research.

G. References

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