

# Effect of the Addition of Egg Yolk to Skim Milk Extender and Storage Time on the Motility and Fertility of Kampung Rooster Spermatozoa

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## ABSTRACT

The aim of this study was to evaluate the egg yolk addition and storage time on the motility and fertility of Kampung rooster spermatozoa. A completely randomized design with a 3x2 factorial pattern was used in this study. E factor is the addition of egg yolk into skim milk. (E1 = skim milk + egg yolk 15%, E2 = skim milk + egg yolk 20%, E3 = skim milk + egg yolk 25%) and S factor is storage time (S1 = 60 minutes, S2 = 180 minutes), at 5 °C. Pooled 10 kampung rooster semen was allocated into six experimental groups. Motility assessment in each treatment group was carried out five times. Fertility assessment was obtained by inseminating ten hens of Isa Brown (n=60) for each treatment for seven days egg collection. There is no interaction effect between storage time and egg yolk addition ( $P>0.05$ ) on the motility and fertility of kampung rooster sperm. The mean percentage motility and fertility of kampung rooster sperm in extender containing different egg yolk were ( $72.5\pm 1.73$ ;  $67.5\pm 1.73$ ;  $65.83\pm 1.81\%$ ,  $P>0.05$ ), and ( $94.13\pm 9.35$ ;  $88.64\pm 8.97$  and  $91.61\pm 11.26$ ;  $P>0.05$ ), respectively. The average sperm motility and Fertility in a different storage time were ( $70.83\pm 3.53\%$  and  $67.78\pm 3.47\%$ ,  $P>0.05$ ) and ( $91.06\pm 5.77\%$  and  $91.86\pm 3.26\%$ ,  $P>0.05$ ), respectively. It can be concluded that the addition of 15, 20 and 25 % egg yolk to skim milk stored at 5°C refrigerator temperature for up to three hours can be used to dilute Kampung rooster semen.

**Keywords:** egg yolk, skim milk, motility, fertility, Kampung rooster spermatozoa.

## 1. INTRODUCTION

The application of artificial insemination using liquid semen to chickens has been successful, with a high fertility rate, the same as the fertility resulting from natural mating, and currently insemination has been widely practiced, especially in poultry industries, while the use of frozen semen has not. In order to make good frozen semen, several steps must be passed; at least the first step is to find the type of diluent and how to store it properly.

Diluents are chemical medium used to extend semen, maximize the number of hens inseminated. A lot of factors play a role in maintaining the quality of semen during storage. Storage time, aeration, and maintain temperature play a major role [1] [2]. Many

extenders are available for chicken semen commercially. Several researchers have compared the compositions of various extender and their fertilization results [3] [4]. The conclusion is that there is no standard poultry extender, and results are also varied widely.

Extenders that have been proven to be good and have been widely produced in the manufacture of frozen semen in cattle and buffalo semen are Tris-egg yolk and skim milk-egg yolk [5] [6]. The use of standard diluents (skim milk and 20 % of egg yolk) in chicken semen gave a good result on the fertility [7]. However, [8] and [9] stated that egg yolk is good for maintaining the quality of spermatozoa especially during cooling,

freezing and thawing, but causes a contraceptive effect in the reproductive tract of the hens.

The objective of this research was to evaluate the effect of adding of 15, 20 and 25 % egg yolk to skim milk and storage time (1 and 3 h) on the kampung rooster sperm motility and the spermatozoa fertility potential.

**2. MATERIALS AND METHODS**

**2.1. Birds, semen collection and semen evaluation**

Twelve healthy adult Kampung roosters and forty commercial laying hens were selected and maintained in individual cages. The roosters were fed daily of 130gram commercial layer ration while the hens were given a daily allowance of 120 grams commercial layer ration. Clean drinking water was provided to rooster and hens at all times.

Semen samples from the roosters were collected by the massage method [10]. Pooled semen of roosters was diluted using different semen diluents i.e. skim milk+egg yolk15%, skim milk+ egg yolk 20% and skim milk+ egg yolk 25% (1:3) stored at refrigerator temperature (5°C) for one and three hours, respectively. At these time points, motility evaluation was undertaken from the semen samples under an Olympus CX 23 microscope. Thereafter the rest of the semen samples by using a tuberculin syringe containing the doses of (100x10<sup>6</sup> spermatozoa/0.1 ml) were immediately inseminated into the well everted cloaca (to the depth ±

3 cm or as close as possible to the uterus-vagina junction).

**2.2. Collection of data**

Motility assessment of the six treatments was obtained by examining under a microscope (10 x 10 and 10 x 40) repeated 5 times. Only spermatozoa that moved forward were recorded. For fertility assessment, semen from the 6 treatments was inseminated each into 10 hens (N=60). The eggs were collected from day two until day eight after the female was inseminated once intravaginally, then the eggs were put into the incubator, on day 6 the eggs were candling. Only eggs with embryos were recorded (% fertile/incubated eggs).

**2.3. Statistical analysis**

Data gathered from this experiment were subjected to two-way analysis of variance for 3x2 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].

**3. RESULTS AND DISCUSSION**

Examination using an Olympus cx23 microscope did not show significant differences in sperm motility and fertility in the treatment of adding egg yolk to skim milk and storage time (Table 1).

The motility parameter (progressive movement) was not significantly (P> 0.05), higher in spermatozoa

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

Extenders	Storage time 1 h		Storage time 3 h	
	Motility	Fertility	Motility	Fertility
Skim milk+ Egg yolk15%	73.33±2.88	93.75±12.50	71.67±2.88	94.50±6.90
Skim milk+ Egg yolk20%	68.33±2.88	84.43±6.21	66.67±2.88	92.86±10.10
Skim milk+ Egg yolk25%	66.67±2.88	95.00±10.0	65.00±2.55	88.21±12.85

No significant interaction (P>0.05) between level egg yolk and storage time

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

Variable	Skim Milk + Egg Yolk		
	15	20	25
Motility	72.5±1.73	67.5±1.73	65.83±1.81
Fertility	94.13±9.35	88.64±8.97	91.61±11.26

The treatments indicate no significant differences between groups (P>0.05) by LSD

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

Variable	Storage Time (H)	
	1	3
Motility	70.83±3.53	67.78±3.47
Fertility	91.06±6.9	91.86±3.26

The storage time indicate no significant differences between groups (P>0.05) by LSD

diluted in skim milk + 15 per cent egg yolk than spermatozoa that were diluted with egg yolk 20 and 25 per cent, storage at 4-5 during this experiment (Table 2). The storage time value also was not significantly different ( $P > 0.05$ , Table 3). This result (motility) was much different from the results of the research [12] which observed that the motility of chicken spermatozoa without the addition of egg yolk to skim milk was around 82 per cent, stored at 4°C for 4 hours. This is also consistent with the finding of [13] that the use of egg yolks from various types of poultry (purebred, kampung chickens, ducks and quails) with a concentration of 10-15 per cent can maintain the quality of Kampung rooster spermatozoa, also [14] [7] stated that the adding egg yolk 10 - 20% to skim milk extender, stored at a temperature of 5°C for up to 40 minutes still produces good quality chicken spermatozoa, which is still suitable for insemination. The storage time at 5°C has a significant influence on the motility of guinea fowl's sperm [15]. We consider that the low motility of these spermatozoa is due to the thicker composition of the yolk, which causes the movement of spermatozoa to become heavier, slower. By slowing down the movement of spermatozoa, it is possible to save energy so that the spermatozoa can enter the female reproductive tract to fertilize an egg when inseminated.

The fertility rates of this study, chicken semen which was diluted with skim milk + egg yolk of 15, 20 and 25 percent, the results were almost the same, i.e.  $94.13 \pm 9.35$   $88.64 \pm 8.97$  and  $91.61 \pm 11.26\%$  ( $p > 0.05$ ), respectively. The results of this study resulted in a slightly higher fertility rate than the author's previous study using standard diluents [7]. The results of this study were not in line with the results of research from many authors such as [8]; [9]. However, they have not been able to explain the mechanism by which the decreased fertilization is caused by the yolk, whether the result of the direct harmful effect on spermatozoa. They further stated that the decrease in fertility only occurred at the use of the egg yolk level of 15 per cent. It is interesting to note that in [9] study the use of egg yolk of less than 7.5 per cent had no negative effect on fertilization.

#### 4. CONCLUSION

The use of skim milk with chicken egg yolk concentration of 15, 20 and 25 per cent and stored at 5°C for 1-3 hours can maintain the quality of kampung rooster spermatozoa based on the motility and fertility. Which means that the addition of chicken egg yolk to skim milk as a diluent does not have a contraceptive effect nor has harmful effect on the oviduct tissue.

#### REFERENCES

- [1] P. R. Dumpala, H. M. Parker, and C. D. McDaniel, "The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen," *Int. J. Poult. Sci.*, vol. 5, no. 9, pp. 838–845, 2006, doi: 10.3923/ijps.2006.838.845.
- [2] A. Siudzińska and E. Łukaszewicz, "Effect of semen extenders and storage time on sperm morphology of four chicken breeds," *J. Appl. Poult. Res.*, vol. 17, no. 1, pp. 101–108, 2008, doi: 10.3382/japr.2007-00048.
- [3] B. Howarth, "Comparison of diluents for holding cock semen six hours at 41 C.," *Poultry science*, vol. 62, no. 6. pp. 1084–1087, 1983, doi: 10.3382/ps.0621084.
- [4] Development of diluents for domestic fowl semen\_ *World's Poultry Science Journal\_ Vol 48, No 2.*
- [5] S. S. Layek, T. K. Mohanty, A. Kumaresan, and J. E. Parks, "Cryopreservation of bull semen: Evolution from egg yolk based to soybean-based extenders," *Animal Reproduction Science*, vol. 172. pp. 1–9, 2016, doi: 10.1016/j.anireprosci.2016.04.013.
- [6] N. Raheja, S. Choudhary, S. Grewal, N. Sharma, and N. Kumar, "A review on semen extenders and additives used in cattle and buffalo bull semen preservation," *J. Entomol. Zool. Stud.*, vol. 6, no. 3, pp. 239–245, 2018.
- [7] D. M. Saleh, M. Y. Sumaryadi, F. Peternakan, and U. Purwokerto, "Penggunaan pengencer standar pada semen ayam kampung the use of standard diluents in kampung rooster semen," pp. 539–544, 2020.
- [8] J. Santiago-Moreno et al., "Cryoprotective and contraceptive properties of egg yolk as an additive in rooster sperm diluents," *Cryobiology*, vol. 65, no. 3. pp. 230–234, 2012, doi: 10.1016/j.cryobiol.2012.06.008.
- [9] F. M. K. Abouelezz et al., "Sperm-egg penetration assay assessment of the contraceptive effects of glycerol and egg yolk in rooster sperm diluents," *Theriogenology*, vol. 83, no. 9, pp. 1541–1547, 2015, doi: 10.1016/j.theriogenology.2015.02.002.
- [10] W. H. Burrows and J. P. Quinn, "The Collection of Spermatozoa from the Domestic Fowl and Turkey," *Poultry Science*, vol. 16, no. 1. pp. 19–24, 1937, doi: 10.3382/ps.0160019.

- [11] S. J. Brett, "Steel R: -," vol. 2, no. 2. pp. 117–121, 2007.
- [12] T. A. AL- Saeedi, A. A. I. AL-Juaifar, and A. H. J. Al-mahmoud, "The Effect of Different Extenders on Some Fertility Properties of Roosters Semen," *Int. J. Poult. Sci.*, vol. 18, no. 11, pp. 504–507, 2019, doi: 10.3923/ijps.2019.504.507.
- [13] K. Khairuddin, M. E. Kurniawan, and S. Soman, "Cryopreservation of Kampung Rooster Semen Using Egg Yolk Diluent from Four Types of Poultry with Different Concentrations," *J. Kedokt. Hewan - Indones. J. Vet. Sci.*, vol. 13, no. 3, pp. 82–87, 2019, doi: 10.21157/j.ked.hewan.v13i3.14892.
- [14] B. I. Santoso, D. M. Saleh, and S. Mugiyono, "Pengaruh Level Kuning Telur Pada Pengencer Susu Skim Dan Lama Waktu Penyimpanan Terhadap Motilitas Dan Abnormalitas Spermatozoa Ayam Kampung," *J. Anim. Sci. Technol.*, vol. 2, no. 1, pp. 1–11, 2020.
- [15] G. H. Hudson, A. V. Omprakash, and K. Premavalli, "Effect of semen diluents and dilution rates on motility of guinea fowl spermatozoa under short-term storage," *Indian Vet. J.*, vol. 93, no. 12, pp. 13–15, 2016.