

The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures

Lucky Diba Gitayana¹, Suzanita Utama^{2*}, Tjuk Imam Restiadi², Suherni Susilowati²,
Nusdianto Triakoso³, Dwi Wijayanti^{4,5}

¹ Kalimutu Veterinary Clinic, Denpasar 80113, Bali, Indonesia

² Division of Veterinary Reproduction, ³ Division of Veterinary Clinic,
Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia

⁴ Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of
Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China

⁵ Department of Animal Science, Perjuangan University of Tasikmalaya,
Tasikmalaya, 46115, West Java, Indonesia

* Corresponding author, e-mail: suzanitautama@hotmail.com

Open access under CC BY – SA license, DOI: [10.20473/ovz.v12i2.2023.81-89](https://doi.org/10.20473/ovz.v12i2.2023.81-89)

Received December 24 2022, Revised July 19 2023, Accepted August 7 2023

Published online August 2023

ABSTRACT

The purpose of this study was to determine the motility and viability of kampung rooster (*Gallus gallus domesticus*) spermatozoa in 0.9% Sodium chloride (NaCl) solution as an extender with the addition of egg yolk at different concentrations at cool temperature (5°C). This study was performed using two 1.5 years old healthy local roosters. Semen was collected through massage in the morning. The pooled semen sample was divided into four treatment groups. In the T0 group the semen was diluted in 0.9% NaCl, while in the T1, T2 and T3 groups the semen was diluted in 0.9% NaCl added with 5, 10 and 15% egg yolk. The results showed that the motility and viability of spermatozoa decreased when stored at cool temperatures for ten hours ($p < 0.05$). Semen of roosters stored at 5°C in saline solution without the addition of egg yolks showed the lowest motility and viability of spermatozoa ($p < 0.05$). The addition of egg yolk into the saline extender increased the motility and viability of spermatozoa. Concentration of 15% egg yolk in saline solution resulted in the highest spermatozoa motility and viability when stored for up to 8 hours ($p < 0.05$). However, motility and viability of spermatozoa at 10 hours of storage were not significantly different ($p > 0.05$) with the addition of 10% and 15% egg yolks. Therefore, it could be concluded that the addition of 15% egg yolk into a saline solution as an extender could maintain the motility and viability of kampung rooster spermatozoa when stored at 5°C for 10 hours.

Keywords: egg yolk, kampung rooster, physiological NaCl, sperm motility, sperm viability

INTRODUCTION

Kampung chickens (*Gallus gallus domesticus*) are very valuable germplasm for Indonesia (Sartika *et al.*, 2023) and for people's

lives of in rural areas as a source of meat, eggs, and additional income (Setyanovina *et al.*, 2021). Kampung chickens are more profitable than purebred chicken because they are more resistant to diseases, have high adaptability to the

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.

environment, and are relatively easy to raise. While the weaknesses of kampung chickens are slow growth, low productivity, slow sexual maturity, hatching characteristics, long egg-laying intervals due to broodstock, and low genetic quality. In addition, kampung chickens are more expensive than other poultry because high demand is not matched by high production (Pettersson *et al.*, 2016).

Kampung chickens are reared in a free-range system, with natural mating. Selective mating between roosters and hens, resulted in a limited number of mated hens, thereby, the spermatozoa of roosters are not utilized optimally (Hidayat and Asmarasari, 2015). The artificial insemination technique allowed one male ejaculate to fertilize 24-40 females (Silyukova *et al.*, 2022). By using superior roosters, artificial insemination technology can increase the population, productivity and genetic quality of kampung chickens. The success of artificial insemination in chickens depends on several factors, including the quality of the inseminated semen. Frozen or liquid spermatozoa can be used for artificial insemination (Yaman *et al.*, 2022).

One of the determining factors for the success of artificial insemination in kampung chickens is the quality of semen. Rooster semen quality was determined based on macroscopic (semen volume, color, and pH), and microscopic (sperm motility, viability, and concentration) evaluation (Mussa *et al.*, 2023). Semen must be mixed with an extender that ensures its physiological and chemical needs and stored at certain temperatures and conditions that maintain the life of the spermatozoa for the desired time. Extenders are very important to maintain sperm quality during storage and ensure their fertility for artificial insemination (Chankitisakul *et al.*, 2022). Semen extender solutions must be isotonic with body fluids, including seminal cells and seminal plasma. Saline solution meets the requirements for an isotonic solution but does not contain food sources or substances that protect against oxidative stress (Bustani and Baiee, 2021). Egg yolk contains 0.2 – 1.0% glucose, 68% low-

density lipoproteins, 16% high-density lipoproteins, 10% lecithin, and others (Réhault-Godbert *et al.*, 2019). Lipoprotein and lecithin help to maintain and protect the integrity of the spermatozoa lipoprotein sheath (Yendraliza *et al.*, 2019). Saline solution and egg yolk are easy to obtain, so it is hoped that they can be used for artificial insemination in kampung roosters. Studies on simple extenders for preserving kampung rooster semen are needed so that they can be easily applied by rural communities. Storage at 5 °C is expected to inhibit the metabolic rate, so that they can live longer (Salmah, 2014). Therefore, this study aims to determine the percentage of egg yolk in saline solution to extend the storage time of kampung rooster spermatozoa at cool temperatures based on the motility and viability of spermatozoa.

MATERIALS AND METHODS

This research proposal and procedures were reviewed and approved by the review board. This study was an experimental study with a completely randomized design. Kampung rooster ejaculates were divided into four volumes equally for T0, T1, T2 and T3 groups. In the control (T0) group, fresh semen was diluted in physiological (0.9%) NaCl; while in T1, T2, T3 groups fresh semen was diluted in physiological NaCl contained 5, 10, and 15% (v/v) egg yolk. each with five replicates.

Rooster semen collection

Semen samples were collected from two healthy male kampung roosters aged 1.5 years with a body weight of approximately 2.3 kg. Semen was collected twice a week in the morning at 8 am to obtain five ejaculates from each rooster. Prior to collection, the rooster's cloaca must be cleaned to ensure that the semen was not contaminated with faeces and feathers. Semen collection was performed by abdominal massage according to the Burrows and Quinn method, a modified non-invasive method for spermatozoa collection from roosters (Getachew, 2016). Semen collection was performed by two individuals, with one holding

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.

and massaging the rooster while the other collected semen. The rooster was sandwiched between the arms and body of the first person, with one hand used to massage and the other hand holding the two legs of the rooster. The massage was done on the lower part of the pubic bone from front to back, lifting the rooster with pressure on the end of the phallus. The massage was done quickly and regularly 5-7 times until the rooster responded and protruded the papilla. When the papillae came out, the bottom of the pubis was pressed with the index finger and thumb which made the cock stretch its feathers upwards, then the bottom of the rooster was pressed to make the papillae released semen. After ejaculation, the thick white semen that was released was immediately collected by the second person into a microfuge tube.

The fresh semen was examined macroscopically (pH, color, thickness) and microscopically (examination of concentration with a spectrophotometer, motility, and viability of the spermatozoa).

Sperm concentration

Spermatozoa concentration was measured using a Turner Model 330 Spectrophotometer (GiMiTEC, USA). The spectrophotometer was turned on for 10 minutes, the wavelength was set at 546 nm, and then 4 mL of physiological NaCl was put into the cuvette and measured on the spectrophotometer. The cuvette was taken, and 40 μ L of semen were added and stirred gently until homogeneous. The cuvette was put back into the spectrophotometer then the result was printed on paper (Yin *et al.*, 2019).

Extender

Fresh chicken eggs for laboratory use (CV. Redjo, Surabaya) were cleaned with 70% alcohol cotton. Egg shell was cracked and the entire egg white and yolk coated with vitelline membrane were moved out. Egg yolk was transferred onto a filter paper, and then poured into a measuring cylinder without the vitelline membrane. Four test tubes were prepared for T0, T1, T2, and T3 groups, each filled with 0, 0.5, 1, and 1.5 mL of egg yolk, respectively, and made

up to a volume of 10 mL with physiological saline solution. Penicillin 1000 IU and Steptomycin 1 mg per mL of extender were added to the semen extender to suppress bacterial growth. Each ejaculate obtained was divided equally into four parts and diluted in the extender according to the group with a ratio (v/v) of one part of semen and ten parts of the extender. The extended semen was homogenized, spermatozoa motility and viability were examined (pre-cooled), then stored in a refrigerator at 5°C (Pitaloka *et al.*, 2023). Spermatozoa motility and viability were then observed every two hours for up to ten hours.

Macroscopic evaluation

The volume of each ejaculate was measured using a graduated microcentrifuge tube used for semen collection. Evaluation of semen viscosity was carried out by tilting the microcentrifuge tube. Thick semen is characterized by the flow of semen going down slowly and leaving mark on the tube wall. On the other hand, dilute semen showed that semen flows down quickly without leaving mark on the tube wall. Semen color was observed visually, and semen pH was determined using pH indicator paper stick (Yaman *et al.*, 2022).

Spermatozoa motility

Sperm motility of fresh and diluted semen was assessed using the same method. Semen in sample microtube was gently stirred with a small bored pasteur pipette; a drop of the mixture and a drop of physiological saline solution were mixed homogeneously on an object glass, and covered with a cover glass. The percentage of spermatozoa with progressive movement was examined under a light microscope (Nikon Eclipse E100 LED) at 400x magnification in five fields of view at room temperature (Sari *et al.*, 2023).

Spermatozoa viability

Semen in sample tube was first homogenized, then one drop of each semen sample was mixed with one drop of eosin-negrosin on an object glass; mixture was thinly

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.

smear and fixed over a Bunsen flame. Observations were made under a microscope (Nikon Eclipse E100 LED) with 400x magnification. Live spermatozoa showed translucent (clear) head, while dead spermatozoa showed purplish red head (Sari et al., 2023).

Data analysis

Analysis using the Kolmogorov-Smirnov test showed that data were normally distributed (homogeneous) ($p > 0.05$). ANOVA results showed a significant difference ($p > 0.05$), and Duncan's New Multiple Range Test was carried out. All statistical tests were performed with a confidence level of 95% using SPSS for Windows version 23.0 software.

RESULTS

Table 1 Characteristic of kampung rooster fresh semen

macroscopic	
volume (mL)	0.64 ± 0.26
color	creamy white
consistency	thick
pH	7
microscopic	
mass movement	+++
concentration (million/mL)	1272.00 ± 262.91
viability (%)	95.20 ± 0.45
progressive motility (%)	87.60 ± 1.67

Characteristics of kampung rooster fresh semen, including macroscopic (volume, color, consistency, and pH) and microscopic (mass movement, individual movement, concentration, viability, and motility of spermatozoa), are shown in Table 1.

Spermatozoa motility (Table 2) and viability (Table 3) decreased ($p < 0.05$) up to 10 hours in the two-hour evaluations, with spermatozoa motility exceeding 40% in all cases. Rooster semen stored at 5°C in physiological saline solution without the addition of egg yolk showed the lowest spermatozoa motility and viability ($p < 0.05$). The addition of egg yolk to the saline solution increased spermatozoa motility and viability. Concentration of 15% egg yolk in saline extender solution resulted in the highest spermatozoa motility and viability when stored for up to 8 hours ($p < 0.05$). However, spermatozoa motility and viability at 10 hours of storage did not show a significant difference ($p > 0.05$) between egg yolk concentrations of 10 and 15% in saline extender.

Table 2 Spermatozoa motility (% , means ± SD) of kampung rooster in physiological saline solution with various concentrations of egg yolk stored at 5°C

	T0	T1	T2	T3
0 hours	81.20 ± 1.79 ^a	83.80 ± 0.84 ^b	85.80 ± 1.64 ^b	88.00 ± 2.00 ^c
2 hours	74.80 ± 1.48 ^a	78.20 ± 1.92 ^b	79.00 ± 3.54 ^b	84.20 ± 1.00 ^c
4 hours	68.00 ± 2.12 ^a	71.00 ± 1.41 ^b	74.60 ± 1.67 ^c	81.00 ± 0.71 ^d
6 hours	56.00 ± 1.22 ^a	64.60 ± 0.55 ^b	67.20 ± 0.45 ^c	73.00 ± 1.00 ^d
8 hours	51.20 ± 1.10 ^a	55.20 ± 1.30 ^b	59.60 ± 1.34 ^c	64.00 ± 1.73 ^d
10 hours	42.80 ± 1.48 ^a	45.20 ± 1.64 ^b	50.40 ± 1.67 ^c	52.60 ± 1.82 ^c

Different superscripts in the same row were significantly different ($p < 0.05$); T0: fresh semen was diluted in physiological NaCl; T1, T2, T3: fresh semen was diluted in physiological NaCl contained 5, 10, and 15% (v/v) egg yolk, respectively.

Table 3 Spermatozoa viability (% , means ± SD) of kampung rooster in physiological saline solution

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.

with various concentrations of egg yolk stored at 5°C

	T0	T1	T2	T3
0 hours	87.40 ± 1.82 ^a	89.80 ± 0.84 ^b	91.80 ± 1.64 ^b	94.00 ± 2.00 ^c
2 hours	81.80 ± 1.48 ^a	85.20 ± 1.92 ^b	88.00 ± 1.58 ^c	92.20 ± 1.30 ^d
4 hours	76.20 ± 1.79 ^a	79.00 ± 1.41 ^b	82.60 ± 1.67 ^c	89.00 ± 0.71 ^d
6 hours	64.60 ± 1.14 ^a	72.60 ± 0.55 ^b	74.20 ± 2.59 ^b	85.20 ± 0.84 ^c
8 hours	61.20 ± 1.10 ^a	65.20 ± 1.30 ^b	69.60 ± 1.34 ^c	74.00 ± 1.73 ^d
10 hours	54.20 ± 1.48 ^a	57.20 ± 1.64 ^b	62.40 ± 1.67 ^c	64.60 ± 1.82 ^c

Different superscripts in the same row were significantly different ($p < 0.05$); T0: fresh semen was diluted in physiological NaCl; T1, T2, T3: fresh semen was diluted in physiological NaCl contained 5, 10, and 15% (v/v) egg yolk, respectively.

DISCUSSION

Kampung rooster fresh semen was creamy white in color, had a thick consistency, and a pH of 7 (1-14 scale). Semen pH was affected by the temperature of the cage environment, contamination of germ, and the number of dead spermatozoa in the semen, which triggers the formation of ammonia (Abioja *et al.*, 2023). Semen volume of kampung rooster collected in this study was 0.64 ± 0.26 mL. Semen volume variation can be affected by breed (Hambu *et al.*, 2016; Mussa *et al.*, 2023), semen collection frequency and seasonal variations (Pimprasert *et al.*, 2023), age (Shanmugam *et al.*, 2014), degree of stimulation, quality of the feed given and the health status of the rooster (Mussa *et al.*, 2023).

Routinely, the ejaculate of roosters for artificial insemination is examined for volume, spermatozoa concentration, motility, and viability (Silyukova *et al.*, 2022). The concentration of spermatozoa in the fresh semen of kampung rooster in this study was 1272.00 ± 262.91 million per mL of ejaculate. Semen concentration varies and is influenced by the frequency of semen collection, libido, diet, temperature, and season (Mustaqim *et al.*, 2021). Volume, color, consistency, pH, and mass movement were generally consistent with those of Pitaloka *et al.* (2023). However, the spermatozoa concentration (1272.00 ± 262.91 million/mL) and spermatozoa viability (95.20 ± 0.45) were higher than those of Pitaloka *et al.* (2023) (1068 ± 360.99 million/mL and $88.80 \pm 2.28\%$, respectively).

Duration of storage

Spermatozoa motility and viability decreased when stored at cool temperatures for ten hours ($p < 0.05$). Motility, viability, and fertilization ability of avian spermatozoa are affected by in vitro storage conditions, including storage temperature and extenders (Sarkar, 2020). The use of a simple extender that is easy to obtain is useful in practical application in small-scale chicken breeding. However, the extender must maintain the viability and fertilization ability of the spermatozoa (Chankitisakul *et al.*, 2022). Storage at 5 °C can extend the shelf life of semen without significantly changing the quality of chicken spermatozoa (Blank *et al.*, 2021; Azzam *et al.*, 2022). The quality of spermatozoa decreased with increasing storage time. However, spermatozoa motility, viability, and mitochondrial function can be maintained with the right extenders (Kheawkanha *et al.*, 2023).

Several studies have reported the use of egg yolk as an additive in chicken semen extenders. The addition of 15-25% egg yolk to skim milk, maintained semen quality for up to three hours at 5°C (Saleh *et al.*, 2021). The addition of egg yolk to skim milk extender maintained the motility, viability, and abnormal rooster spermatozoa stored at 5°C for nine hours (Yuniar *et al.*, 2021). Coconut water egg-yolk glucose extender maintained progressive motility and viability of kampung rooster spermatozoa stored at 5°C for 72 hours (Khaeruddin and Amir, 2019).

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.

Spermatozoa motility

The control group with physiological NaCl extender showed the lowest results in this study. The buffer content in the saline solution has no effect on the optimal motility of spermatozoa at cool temperatures (5°C), but it can still be used for artificial insemination. In the group that used saline solution buffer added with 5% and 10% egg yolk, the motility was lower than in the 15% group. The results of observations in the group with 5% egg yolk were lower than in the group with 10% egg yolk, but both of them can still be used for artificial insemination. The addition of 5% and 10% egg yolk failed to provide optimal spermatozoa motility at cool temperatures (5°C). The group with the extender added with 15% egg yolk was added had the highest percentage of spermatozoa motility compared to the other groups. This showed that the higher the concentration of egg yolk, the higher the spermatozoa motility. High motility of spermatozoa increases the chances of fertilization because only motile spermatozoa qualify for fertilizing an egg (Chankitisakul *et al.*, 2022). Spermatozoa that move forward (progressive) are the main criteria for calculation, while spermatozoa that move around, move in place, or do not move are not counted. In this motility assessment, the number of progressively moving spermatozoa was compared to the number of all spermatozoa examined (Parker *et al.*, 2000).

Spermatozoa storage time also affects spermatozoa motility. The decrease in spermatozoa motility is consistent with storage duration of (Vašíček and Chrenek, 2013; Vašíček *et al.*, 2015). This was because storage was carried out at cool temperature (5°C), which allows the metabolism of spermatozoa to continue (Blank *et al.*, 2021). Egg yolk contains energy sources in the form of fructose and glucose (Réhault-Godbert *et al.*, 2019). Egg yolk serves as a nutrient medium, energy source, and extracellular protection for spermatozoa from cold shock during freezing, as a protective agent, which provides a buffering effect on spermatozoa (Santiago-Moreno *et al.*, 2012). Egg yolk contains components in the form of

lipoprotein and lecithin, which can maintain and protect the integrity of the lipoprotein sheath of spermatozoa (Ola *et al.*, 2020). The Low-Density Lipoprotein fraction can protect spermatozoa from cold shock (Moussa *et al.*, 2002; Bustani and Baiee, 2021).

Spermatozoa viability

The control group with saline solution showed the lowest spermatozoa viability. The buffer content in the saline solution failed to maintain the optimal viability of rooster spermatozoa at chilled temperatures (5°C). The addition of egg yolk to the buffered saline solution increased the viability of kampung rooster spermatozoa. In the groups with 5% and 10% egg yolk, the viability was lower than in the group with 15% egg yolk. The viability of the group with the addition of 5% egg yolk was still lower than the group with the addition of 10% egg yolk. The addition of 5% and 10% egg yolks could not provide an optimal effect on spermatozoa at cool temperatures (5°C).

The group with the addition of 15% egg yolk showed the highest viability compared to the control group (without egg yolk) and the group with the addition of 5% and 10% egg yolk. Egg yolk has a good source of energy to provide a suitable environment for spermatozoa and protect the membrane so that membrane permeability is maintained (Rochmi and Sofyan, 2019). In this study, the viability of spermatozoa of kampung roosters extended in saline solution with 15% egg yolk ($64.60 \pm 1.82\%$) was higher than that reported by Azzam *et al.* (2022) (viability was $60.60 \pm 1.81\%$ after 10 hours storage at 5°C), in which semen was extended in 5% Dextrose Ringer's extender with the addition of 10% egg yolk.

CONCLUSION

The addition of egg yolk at a concentration of 5 - 15% in saline solution extender maintained the motility and viability of kampung rooster spermatozoa. The best motility and viability were achieved by the addition of 15% egg yolk.

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.

ACKNOWLEDGEMENT

Thanks to Lucky Novelia and Yolanda Gilang Puspita for their technical support for this study.

AUTHOR'S CONTRIBUTIONS

Lucky Diba Gitayana (LDG), Nurdianto Triakoso (NT), Tjuk Imam Restiadi (TIR), Suherni Susilowati (SS), Suzanita Utama (SU), Dwi Wijayanti (DW).

LDG: compiling ideas, designing frameworks, data acquisition, and drafting manuscripts. SU and NT: data analysis and interpretation, supervision, and manuscript drafts. TIR, SS, and DW: read and critically revised manuscripts for intellectual content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no competing interests regarding this study

FUNDING INFORMATION

This research was fully funded by the authors

REFERENCES

- Abioja MO, Apuu S, Daramola JO, Wheto M, Akinjute OF. 2023. Semen quality and sperm characteristics in broiler breeder cockerels fed vitamin E during hot season. *Acta Sci - Anim Sci.* 45: 1-9; e56848.
- Azzam AH, Raharjo D, Hernawati T, Madyawati SP, Utomo B. 2022. Combination of 5% Dextrose Ringer's solution and egg yolk extender maintained the motility and viability of kampung rooster spermatozoa in chilled temperature. *Ovozoa: J Anim Reprod.* 11: 109-14.
- Blank MH, Ruivo LP, Novaes GA, Lemos EC, Losano JDA, Siqueira AFP, Pereira RJG. 2021. Assessing different liquid-storage temperatures for rooster spermatozoa. *Anim Reprod Sci.* 233: 106845. Advance online publication.
- Bustani GS, Baiee FH. 2021. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Vet World* 14: 1220-33.
- Chankitisakul V, Boonkum W, Kaewkanha T, Pimprasert M, Ratchamak R, Authaida S, Thananurak P. 2022. Fertilizing ability and survivability of rooster spermatozoa diluted with a novel semen extender supplemented with serine for practical use on smallholder farms. *Poult Sci.* 101: 102188.
- Getachew T. 2016. A review article of artificial insemination in poultry. *World Vet J.* 6: 25-33.
- Hambu EK, Arifiantini RI, Purwantara B, Darwati S. 2016. Raw semen characteristics of three different Indonesian local roosters. *Anim Prod.* 18: 165-72.
- Hidayat C, Asmarasari SA. 2015. Kampung chicken production in Indonesia: A review. *J Peternakan Indonesia* 17: 1-11.
- Khaeruddin K, Amir M. 2019. The effect of the combination of glucose concentration with the type of extenders on the quality of kampung rooster spermatozoa during storage. *Chalaza J Anim Husb.* 4: 36-43.
- Kheawkanha T, Chankitisakul V, Thananurak P, Pimprasert M, Boonkum W, Vongpralub T. 2023. Solid storage supplemented with serine of rooster semen enhances higher spermatozoa quality and fertility potential during storage at 5°C for up to 120 h. *Poult Sci.* 102: 102648.
- Moussa M, Marinet V, Trimeche A. 2002. Low density lipoproteins extracted from hen egg yolk by an easy method: Cryoprotective effect on frozen-thawed bull semen. *Theriogenology* 57: 1695-1706.
- Mussa NJ, Boonkum W, Chankitisakul V. 2023. Semen quality traits of two Thai kampung chickens producing a high and a low of semen volumes. *Vet Sci.* 10: 73.
- Mustaqim M, Zulkifli Z, Jannah CR, Salsabila S. 2021. The supplementation of fermented feed (*Indigofera Sp* and *Eurycoma longifolia*) leaves on Arabic chicken semen. *Sust Environ Agric Sci.* 5: 130-5
- Ola SI, Faleye OO, Adeyemi AA, Adeyosoye OI. 2020. Evaluation of egg yolk plasma as replacement for whole egg yolk in chicken

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction.* 12: 81-89.

- semen extender. *J Poul Res.* 17: 96-101.
- Parker HM, Yeatman JB, Schultz CD, Zumwalt CD, McDaniel CD. 2000. Use of a spermatozoa analyzer for evaluating broiler breeder males. 2. Selection of young broiler breeder roosters for the spermatozoa quality index increases fertile egg production. *Poult Sci.* 79: 771-7.
- Pettersson I, Freire R, Nicol C. 2016. Factors affecting ranging behaviour in commercial free-range hens. *World's Poult Sci J.* 72: 137-50.
- Pimprasert M, Kheawkanha T, Boonkum W, Chankitisakul V. 2023. Influence of semen collection frequency and seasonal variations on fresh and frozen semen quality in Thai kampung roosters. *Animals (Basel)* 13: 573.
- Pitaloka DG, Hariadi M, Hestianah EP, Akeju SI, Susilowati S, Utomo B, Hernawati T. 2023. The effect of addition of melon (*Cucumis melo* L.) flesh juice into Lactated Ringer's-egg yolk extender on spermatozoa plasma membrane integrity and spermatozoa morphological abnormalities of semen of kampung rooster. *Ovozoa: J Anim Reprod.* 12: 50-9.
- Réhault-Godbert S, Guyot N, Nys Y. 2019. The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. *Nutrients* 11: 684.
- Rochmi SE, Sofyan MS. 2019. A diluent containing coconut water, fructose, and chicken egg yolk increases rooster spermatozoa quality at 5°C. *Vet World* 12: 1116-20.
- Saleh DM, Sumaryadi MY, Nugroho AP, Hidayah CN. 2021. Effect of the addition of egg yolk to skim milk extender and storage time on the motility and fertility of kampung rooster spermatozoa. *Proceedings of the International Conference on Improving Tropical Animal Production for Food Security (ITAPS 2021)*.
- Santiago-Moreno J, Castaño C, Toledano-Díaz A, Coloma MA, López-Sebastián A, Prieto MT, Campo JL. 2012. Cryoprotective and contraceptive properties of egg yolk as an additive in rooster spermatozoa diluents. *Cryobiology* 65: 230-4.
- Sari NM, Yuliani GA, Yimer N, Hernawati T, Herupradoto EBA, Hidayatik N. 2023. Reddragon (*Hylocereus polyrhizus*) fruit peel extract increased the motility and viability of spermatozoa of hypercholesterolemic rats (*Rattus norvegicus*). *Ovozoa: J Anim Reprod.* 12: 33-41.
- Sarkar PK. 2020. Motility, viability and fertilizing ability of avian spermatozoa stored under *in vitro* conditions. *Rev Agric Sci.* 8: 15-27.
- Sartika T, Saputra F, Takahashi H. 2022. Genetic diversity of eight kampung Indonesian chicken breeds on microsatellite markers. *HAYATI J Biosci.* 30: 122-30.
- Setyanovina SS, Suryantini A, Masyhuri M. 2021. Characteristics and preferences of chicken meat consumers before and during Covid-19 pandemic in Sleman regency. *Agro Ekonomi* 32: 12-25.
- Shanmugam M, Vinoth A, Rajaravindra KS, Rajkumar U. 2014. Evaluation of semen quality in roosters of different age during hot climatic condition. *Anim Reprod Sci.* 145: 81-5.
- Silyukova Y, Fedorova E, Stanishevskaya O. 2022. Influence of technological stages of preparation of rooster semen for short-term and long-term storage on its quality characteristics. *Curr Issues Mol Biol.* 44: 5531-42.
- Vašiček J, Chrenek P. 2013. Effect of storage temperature on the motility characteristics of rooster spermatozoa. *J Microbiol Biotechnol Food Sci.* 2: 1685-91.
- Vašiček J, Kuzelova L, Kulikova B, Chrenek P. 2015. Effect of diluent and storage time on spermatozoa characteristics of rooster insemination doses. *Avian Biol Res.* 8: 41-6.
- Yaman MA, Reza MA, Abdullah AN, Usman Y, Koesmara H. 2022. Sperm quality of hybrid chicken affected by propolis, honey, or royal jelly as organic diluent materials and storage periods during sperm preservation. *Adv Biol Sci Res.* 20: 206-12.

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction.* 12: 81-89.

- Yendraliza Y, Hendriyanto Y, Mucra DA, Zumarni Z, Rodiallah M. 2019. The recovery rate of Simental spermatozoa frozen of post thawing by using tris dilution with different egg yolks. IOP Conf. Series: Earth and Environmental Science 391: 1-6.
- Yin H, Fang L, Qin C, Zhang S. 2019. Estimation of the genetic parameters for semen traits in Chinese Holstein bulls. BMC Genet. 20: 51.
- Yuniar T, Saleh D, Mugiyono S. 2021. The effect of addition of egg yolk to skim milk diluent and storage time at 5°C on spermatozoa quality of Pelung rooster. ANGON: J Anim Sci Technol. 3: 29-46.

How to cite this article: Gitayana LD, Triakoso N, Restiadi TI, Susilowati S, Utama S, Wijayanti D. 2023. The addition of egg yolk to the physiological saline extender improved the motility and viability of kampung rooster spermatozoa at cool temperatures. *Ovozoa: Journal of Animal Reproduction*. 12: 81-89.
