

Semen Evaluation, Preparation of Bangkok Roosters, and Insemination to Indonesian Native Hens

Asnawi¹, Maskur^{1*}, Adji Santoso Dradjat², Muhammad Muhsinin¹

¹Department of Genetics and Breeding, Faculty of Animal Sciences, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;

²Department of Animal Reproduction, Faculty of Animal Sciences, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;

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*Corresponding Author:

Maskur, Department of Genetics and Breeding, Faculty of Animal Sciences, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;
Email: maskur@unram.ac.id

Abstract: The success of artificial insemination in chickens is very dependent on sperm quality. Sperm temperature and diluent during storage can affect sperm quality. This study evaluated the quality of Bangkok chicken sperm diluted with 5% glucose and NaCl stored at 5 °C and 26 °C. The research design used was completely randomized. The data obtained were analyzed using a variance. The results showed that the motility of sperm preserved for 9 hours at 26 °C with infused NaCl and Glucose 5% differed significantly ($P < 0.05$) with motility of $50 \pm 0.0\%$ and $34 \pm 8.94\%$, respectively. While preservation under 5 °C for 9 hours with NaCl, better than Glucose 5% ($P < 0.05$) with motility of $58.00 \pm 10.95\%$ and $38.00 \pm 10.95\%$, respectively. The viability of sperm preserved at 26 °C with glucose 5% extender was better than NaCl ($P < 0.05$) with a value of $58.93 \pm 1.27\%$ and $33.43 \pm 1.27\%$, respectively. While preservation at 5 °C of viabilities of sperm under Glucose 10% and NaCl were not significantly different ($P > 0.05$) from values of $52.57 \pm 5.15\%$ and $48.14 \pm 8.09\%$, respectively. The abnormality of sperm stored at 26 °C and 5 °C for 9 hours with NaCl and Glucose 5% were not different ($P > 0.05$). Insemination using 100 million sperm with infused NaCl extender from 18 eggs produced 94.44% ($n=17$) fertility and 72.22% ($n=13$) hatchability. While insemination using infuse glucose 5% extender, 11 eggs produced 63.64% ($n=7$) fertility and 54.54% ($n=6$) hatchability. The infusing NaCl extender produced better sperm quality, fertility, and hatchability than glucose 5% at 5 °C.

Keywords: Eggs fertility, hens, insemination, rooster, semen quality.

Introduction

Chicken consumption in Indonesia, especially on Lombok Island of West Nusa Tenggara province, has continued to increase. Before the pandemic, this Island becomes a tourism destination. There is a traditional culinary of local "kampung," a chicken called "Taliwang cuisine," which is smokey grilled on a coconut hard shell, husk, and skin fire mixed with local ingredients. This Taliwang cuisine needs chicken of 600 g body weight to get the best meals. However, Kampung chicken has limitations, such as low growth rates, the flesh

relatively hard, and getting 600 g bodyweight to need 60 days of raising time, while Broiler may only need three weeks or 20 days (Asnawi 1997; Rahayu et al., 2021). As this cuisine becomes branded and the consumer increases, the farmers have difficulties supplying regularly and in uniform bird sizes.

There are large species of chicken available in Lombok Island, such as Bangkok, Cochin, and Brahma chickens, with a body weight range of 2.5 to 3.5 kg, which have the potential to improve body weight, growth rate, and meat tenderness of Kampung chicken. Recently technology for the frozen preservation

of rooster sperm has been unavailable as frozen-thawed provides low fertility (Lin et al., 2022; Th  lie et al., 2019). Sperm motility and viability were reduced by more than 50% and motility after thawing was 18,9%, whereas cooled storage produced motility of up to 85-90% (  iftci and Ayg  n, 2018; Lemoine et al., 2011; Mohammad et al., 2021).

According to another study, many cold storage extenders were investigated. The glucose addition extender considerably increased sperm motility compared to the other extenders (Kuzlu and Taskin, 2016; Taskin et al., 2022). Additional studies found that physiological NaCl is an ionic compound, and carbohydrates such as glucose may provide sperm with additional energy sources. There are infused NaCl and infused with glucose 5% available commercially, which may be easy to get and can be used to store rooster sperm.

Their artificial insemination technology is available, and it has been proven advantageous. Those advantages, such as one cockerel, could be used to inseminate the fourfold number of hens and may also ease successful cross-breeding (Karayat et al., 2016; Mohan et al., 2018). The glucose utilized to boost energy reserves as sperm were diluted with NaCl for injection to improve viability and motility (Taskin et al., 2022) may result in improved fertility and hatching. This study collected and evaluated sperm quality by physiologic NaCl and Glucose 5% extenders stored at 26   C and 5   C. Then insemination was performed every second day using physiologic NaCl and glucose 5% extenders into hens to evaluate the egg's fertility and hatchability.

Material and Methods

Total sample

Materials used in this study were one mature Bangkok rooster with a body weight of 3.04 kg. This rooster was kept in an individual pen, fed twice daily in the morning and evening with concentrate (Hi-Pro-Vite 512BG, PT Charoen Pokphand Indonesia, Surabaya), and drinking water was always available. Five kampung hens weighing 1.045 ± 0.179 g were maintained in a separate enclosure. The chickens were fed a combination of commercial concentrate (30%), corn (40%), and branded rice

(30%). This experiment was carried out on the Teaching farm of the Animal Science University of Mataram, Indonesia. The Animal Reproduction Laboratory at the University of Mataram in Mataram, Indonesia, conducted analyses on sperm samples.

Semen sampling

The Bangkok rooster's sperm was collected by placing a Millipore cover cup (Merck Millipore Ltd) on the rooster's cloaca and securing it with an elastic string through the base of both wings (Li et al., 2020). The right and left base of the wings of hens as a teaser (Bangkok rooster) was held by the operator's hands and let the rooster stand for mating, and sperm could be collected on the Millipore cover cup (Figure 1). The collected semen was then divided into four tubes; two tubes each were given an extender of NaCl and glucose 5 % infuse sterile, pyrogen-free, with osmolarity of 308 mOsm/l (produced by PT Widatra Bhakti Pandaan, Pasuruan Jawa Timur Indonesia).

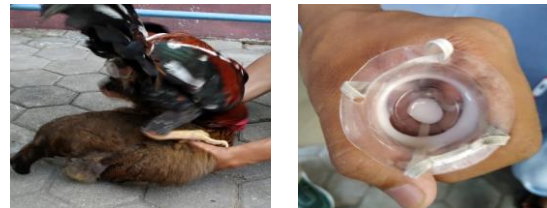


Figure 1. Sperm were collected using a hen as a teaser and a Millipore cup attached to a rooster cloaca

Semen evaluation

Semen evaluation consists of visual evaluation, such as Volume, color (Peters et al., 2008), and microscopic evaluation, such as sperm concentrations, motility (%), normality (%), and viability of sperm (Arifiantini, 2012; Ervandi et al., 2020; Hayanti et al., 2022). Macroscopic evaluation, the Volume of semen was measured by sucking on the 1 ml graded syringe. The color of semen was evaluated visually as creamy white, opaque, or between opaque and creamy white.

Microscopic evaluation

The microscopic evaluation consists of Concentration, live, normal, and motile sperm. Semen concentration was evaluated by direct cell count using standard hemocytometer counting

blood cells (Arifiantini, 2012; Peters et al., 2008). This examination was conducted by drawing sperm into a dilution pipette to the 0.05 mark and then filling the pipette to the 101 mark with a hypotonic solution. The hypotonic solution consists of 50 ml distillation water, 1 ml 2% eosin, and 1 ml 3% sodium chloride. The counting chamber was then filled and left to settle for 5 minutes. Count sperm in 5 large squares in both chambers. The Concentration of sperm was calculated by multiplying the mean count by 10^7 .

Calculation of sperm

Sperm were counted on a slide stained with nigrosine eosin to determine if they were alive or dead. Five drops of stain were added to a drop of semen in pre-warmed object glass and smeared to get thin samples on the object glass. Then allowed to air dry before being evaluated under a microscope, all sperm absorbing the stain were classified as dead. In contrast, the unstained heading of sperm was considered to live sperm within at least 200 sperm count (Arifiantini, 2012; Peters et al., 2008).

Assessment of motility

Assessment of motility was performed by dropping 5 μL on the object-glass then the percentage of progressively motile sperm or moving forward direction was evaluated. Semen was diluted until individual sperm could be observed after placing a drop on the pre-warmed slide under coverslips and examined under 400x magnification (Ervandi et al., 2020; Hayanti et al., 2022).

Insemination

Insemination using semen extended with infusing glucose 5% was performed in 5 kampung hens every second day with 100 million sperm for seven days. Eggs were collected every day beginning the second day following the first insemination. Then the hens rested for ten days, followed by insemination using semen extended with NaCl. Insemination was performed between 0.5 to 1 hour after collection, using fresh semen; volume times concentrations, motility, and normality can estimate sperm quality. Percentage of sperm life, motile, and normal = Concentration (%) x Life (%) x Normal (%) x Motile (%). The number of live, motile, and normal sperm divided by an insemination dose of 100×10^6 (Karayat et

al., 2016) would be the number of hens that can be inseminated. The volume dilution was calculated by the Volume of semen (ml) x Concentration ($\times 10^6$ /ml) x Number of live motile and normal sperm (%) divided by Insemination dose (100×10^6) x Volume per insemination (0.2 ml). Then the semen was diluted up to the volume dilution calculated. Insemination was performed by restraining and massaging hen abdomen around the vent until the cloaca protruded. They followed by inserting an ml plastic syringe at the dorsal part of the cloaca and pushing 1-2 cm deep into the vagina. Then 0.2 ml extended sperm was injected into the uterine (Karayat et al., 2016).

Egg fertility

Then eggs produced were hatched in the incubator at a temperature of 39 °C; egg fertility was examined five days following incubation by light scooping in the darkroom as embryos can be seen clearly. Hatched eggs divided by fertilized eggs evaluated hatchability. The research was designed by treatments of two extenders that were NaCl and Glucose 5 %, with two ways of preservation at 5 °C and 26 °C. The semen was evaluated for viability, normality/abnormality, and motility at 0.5 hours, 1 hour, and every 2 hours, up to 9 hours following preservation. The complete random design was used in this study, comparing two extenders, NaCl and Glucose 5 %, and two preservation at 26 °C and 5 °C, with seven replications. Data were evaluated using analysis of variance.

Parameters measured in this study were macroscopic evaluations consisting of Volume and color. Microscopic evaluation such as concentration (%), life (%), normal (%), and motile (%) sperm. The percentage of the number of life normal and motile sperm was calculated by Concentration (%) x Life (%) x Normal (%) x Motile (%) sperm. The number of insemination doses was calculated as Volume x Sperm Concentration per milliliter x Sperm Viability, Normality, and Motility divided by the number of insemination doses.

The results of insemination evaluate the egg's fertility and hatchability. The percentage of egg fertility was evaluated by the number of fertilized eggs divided by the number of incubated eggs time 100%. The percentage of hatchability was evaluated by the number of

hatched eggs divided by fertilized eggs times 100% (Tesfay et al., 2020).

Statistical analysis

Research data were analyzed using variance, and data was found to be significantly different between treatments, followed by Duncan's test (Bewick et al., 2004). Data were processed using the IBM SPSS version 21 program.

Results and Discussion

Evaluation of semen

The results of the evaluation of semen following collection were a volume of 0.53 ± 0.05 ml, a color of creamy white, a concentration of $1.252 \pm 7.92 \times 10^6$ /ml, live 90.2 ± 1.30 %, motility 89.1 ± 2.74 % with normal morphology sperm 94.6 ± 2.30 %. Sperm concentration in the present study was higher than the results of Peters et al. (2008). They found that the Concentration of sperm in 7 strains of the rooster was reported between 311 ± 0.42 and $421 \pm 1.45 \times 10^6$ /ml. However, the results of sperm concentration in the present study were lower than those of Tesfay et al. (2020), who found in Rhode Island Red and White Leghorn roosters at 5.61 ± 0.03 , and $5.04 \pm 0.03 (\times 10^9/\text{mL})$ respectively. Finally, there are large variations in semen quantity and quality in indigenous cock and exotic (Mussa et al., 2023; Tesfay et al., 2020; Usman et al., 2019).

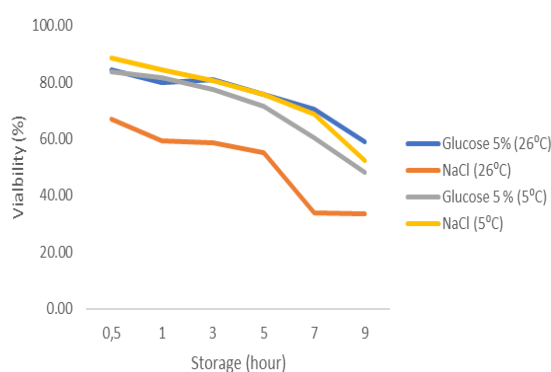


Figure 2. Viability (%) sperm were preserved for 9 hours at 26 °C and 5 °C, with extenders infusing NaCl and Glucose 5%

The results of dilution and preservation of sperm for 9 hours at 26 °C and 5 °C with extenders infusing NaCl solution and glucose 5% can be seen in Figures 2-5. Figure 2 showed that

the percentage of life sperm preserved 9 hours at 26 °C with glucose 5% ($58.93 \pm 1.27\%$) higher than NaCl extender ($33.43 \pm 1.27\%$) ($p < 0.05$). The highest liveability was under extender infuse Glucose 5% than NaCl ($p < 0.05$). It can be seen that percentage of life sperm preserved for 9 hours at 5 °C with NaCl extender was higher ($52.57 \pm 5.15\%$) than that of glucose 5% ($48.14 \pm 8.09\%$); however, they were not significantly different ($p > 0.05$). In line with these results, the best viability was found with NaCl, as reported that extender NaCl provides good liveability in low temperatures (Chankitisakul et al., 2022; Silyukova et al., 2022; Vasicek et al., 2015).

The evaluation temperature effect viability 26°C and 5°C (Figure 2) showed preservation at 26°C for 9 hours with the viability of $42.19 \pm 1.31\%$ was significant ($p < 0.05$), lower than that of 5 °C with the viability of 54.1 ± 5.78 %. This result is in line with Eslami et al., (2016) and Hayanti et al., (2022) that sperm preserved at 4°C may produce life ability better than at 27°C. Cold preservation was also reported to protect sperm viability, as metabolism can be depressed with minimum activities (Ervandi et al., 2020; Triadi et al., 2022).



Figure 3. Normality (%) sperm were preserved for 9 hours at 26 °C and 5 °C, with extenders infusing NaCl and Glucose 5%

Figure 3 showed that the percentage of normal sperm stored from 0,5 to 9 hours at 5 °C and 26 °C with NaCl and glucose 5% infuse for extender varied between 90 to 95 %, and there were not significantly different ($p > 0.05$). Morphology evaluation was commonly used to identify abnormality, but normality sperm were more appropriate for insemination. The results of this study indicate that, following preservation

for 9 hours, abnormality sperm were not different ($p>0.05$) between preservation 26 °C and 5 °C with the value of $7.42\pm 3.79\%$ and $7.27\pm 3.79\%$. It was reported that the lower abnormality of sperm was produced under a NaCl physiology extender of 4.5-5.5% (Hayanti et al., 2022). It was found in other studies that sperm kept at 4 °C for 72 hours produced abnormality of 8-12% (Petrićáková et al., 2022; Zong et al., 2023).

Figure 4 shows that sperm motilities after 5 hours of storage fluctuated. Following preservation of semen for 9 hours at 26 °C. The best motility of sperm was found with NaCl and Glucose 5% infuse different significantly ($p<0.05$) with motility of $50\pm 0.0\%$ and $34\pm$

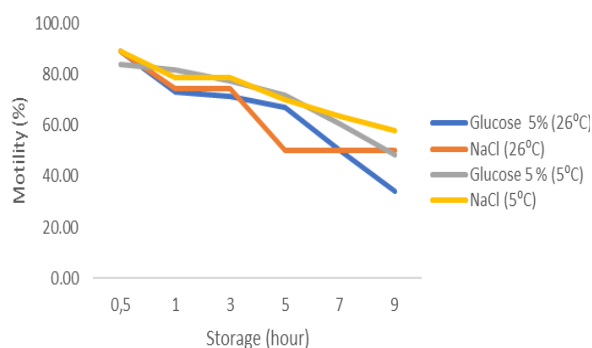


Figure 4. Motility (%) sperm were preserved for 9 hours at 26 °C and 5 °C, with extenders infusing NaCl and Glucose 5%.

Evaluation of viable, normal, and motile sperm with NaCl and glucose 5% extenders at 5 °C and 26 °C is presented in Figure 5. This Figure showed that life, normal, and motile sperm stored at 5 °C have better quality than 26 °C. It has been reported that seminal rooster plasm rich in glucose may stimulate higher sperm motility (Cordeiro et al., 2021; Santiago-Moreno and Blesbois, 2020). It was found that rooster semen had lactose content of as much as 4 mg/100 ml (Janosikova et al., 2023; Partyka and Niżański, 2022). This indicates that sugars in the plasma semen are enough to stimulate sperm motility even without addition from the extender. The results (Figure 5) also showed that viable, normal, motile sperm stored for 1 to 3 hours still 40% or more is visible for insemination.

The results of artificial insemination (Figure 6) using 100 million sperm with infusing glucose 5% extender produced 11 eggs, with fertility of 63.64% ($n=7$) and hatchability of

8.94%, respectively. It also can be seen that preserving at 5 °C motilities decreased without fluctuation. Up to 9 hours of preservation, the highest sperm motility was with NaCl than that of Glucose 5% extenders, with values of $58.00\pm 5.15\%$ and $46.00\pm 5.02\%$, respectively, values were significantly different ($p<0.05$). Considering with effect of temperature on the motility of sperm was $41.14\pm 7.11\%$ at 26 °C, significantly lower ($p<0.05$) than 47.71 ± 10.09 at 5 °C. This result is comparable to the report of (Hayanti et al., 2022) that preservation at 4 °C provides better motility than that at a room temperature of 27 °C.

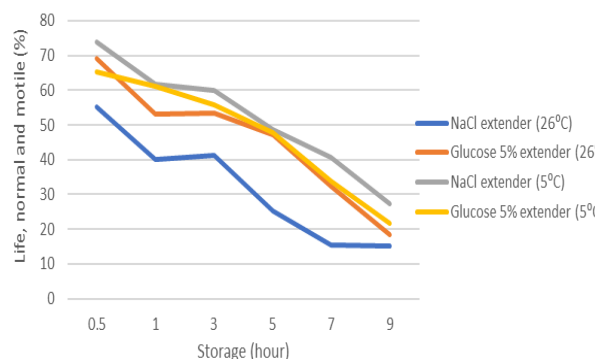


Figure 5. Life, normal and motile sperm preserved for 9 hours at 26 °C and 5 °C, with extenders of infusing NaCl and Glucose 5%.

54.54% ($n=6$). While under infuse, the NaCl extender produced 18 eggs, with a fertility of 94.44% ($n=17$) and a hatchability of 72.22% ($n=13$). Hence, a NaCl extender's insemination produced better fertility and hatchability than a glucose 5% extender. Fertility and hatchability following insemination in the present study were lower than those reported in 4 strains of chicken, which were reported between 87,8 to 94,9 and 82,1 to 92,9%, respectively (Feyisa et al., 2018). Other researchers performed insemination using 100 million sperm. Kampung chickens produce fertility lower than in the present study (Saleh et al., 2012). The egg fertility results in the present study were higher than that of Hayanti et al. (2022) and lower than those of Zong et al. (2023). Fertility and hatchability results of insemination varied considerably; the present study's results fell within the moderate range.

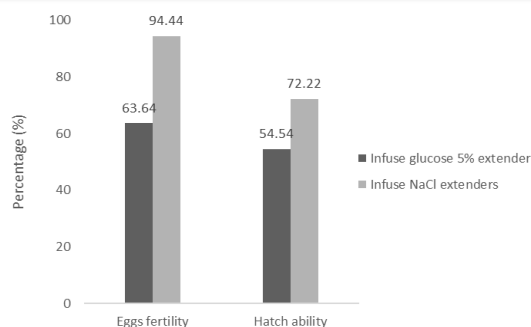


Figure 6. Fertility and hatchability of insemination with semen extended with infusing glucose 5% dan infuse NaCl

Conclusion

Extenders infusing glucose 5% did not improve sperm quality, which resulted even less than infusing NaCl. Preservation of semen at 5 °C provides better viability of sperm than those at 26 °C. Artificial insemination of 100 million sperm with infuse glucose 5% extender resulted in lower fertility and hatchability than artificial insemination with infuse NaCl extender. It seems that the seminal plasma of the rooster may have enough components. Consequently, adding glucose or other sugar is unnecessary and may even reduce sperm quality and fertility.

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