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BANGKOK CHICKEN SPERM QUALITY DURING STORAGE USING KA-EN 3B® EGG YOLK DILUENT SUPPLEMENTED WITH RESVERATROL

Abdul Hakim Fattah[1*], Khaeruddin[1], Lusi Yunita Sasa[1], and Rachmat Budianto[1]
[1]Animal Husbandry Study Program, Faculty of Agriculture, Universitas Muhammadiyah of Sinjai, Sinjai Utara, South Sulawesi, Indonesia
*Corresponding author: hakimabdulfattah@gmail.com

ABSTRACT

The objective of this study was to determine the effect of resveratrol supplementation in KA-EN 3B®-egg yolk dilution on sperm quality of Bangkok chickens. The semen was collected from 2 adult male Bangkok chickens with an age of ± 12 months and a body weight of ± 2.5 kg. The collected semen was divided into 5 treatments with 5 replicates each. The treatments used were KA-EN 3B®-egg yolk (KEY), KEY + 0.02% resveratrol, KEY + 0.04% resveratrol, KEY + 0.06% resveratrol, and KEY + 0.08% resveratrol. The parameters observed were sperm motility, viability, and abnormalities during 24 hours, 48 hours, and 72 hours of storage in the refrigerator. The results showed that resveratrol supplementation in the diluent had no effect (P>0.05) on sperm motility, viability, and abnormalities during storage. This study concluded that supplementation of resveratrol in KA-EN 3B®-egg yolk diluent did not affect the quality of Bangkok chicken sperm during storage.

Key words: Bangkok chicken, diluent, resveratrol, spermatozoa

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh suplementasi resveratrol dalam pengencer KA-EN 3B®-kuning telur terhadap kualitas sperma ayam Bangkok. Semen diinokulasi dari ayam Bangkok jantan dewasa yang berumur ± 12 bulan dengan bobot badan 2,5 kg. *Penelitian ini menggunakan rancangan acak lengkap dengan lima taraf perlakuan dan lima kali ulangan. Perlakuan yang digunakan adalah pengencer KA-EN 3B®-kuning telur (KKT), KKT + 0,02 % resveratrol, KKT + 0,04 % resveratrol, KKT + 0,06 % resveratrol dan KKT + 0,08% resveratrol. Parameter yang diamati meliputi motilitas, viabilitas, dan abnormalitas sperma selama penyimpanan 24, 48, dan 72 jam pada refrigerator. Hasil penelitian menunjukkan suplementasi resveratrol dalam pengencer tidak memengaruhi (P>0,05) motilitas, viabilitas, dan abnormalitas sperma selama penyimpanan. Disimpulkan bahwa suplementasi resveratrol dalam pengencer KA-EN 3B®-kuning telur tidak memengaruhi kualitas sperma ayam Bangkok selama penyimpanan.*

Kata kunci: ayam Bangkok, pengencer, resveratrol, sperma

INTRODUCTION

Bangkok chickens are reared in Indonesia as a fighting chock and have a sturdy body, taller and heavier body weight than free range chicken. Population Bangkok chicken as one of the local chicken germplasm needs to be improved with utilize the artificial insemination (AI) technology. Utilization of AI technology is expected not only to increase population but also the genetic quality of Indonesian local chicken with utilizes a superior Bangkok rooster through a crossbreeding program. The quality of sperm that is inseminated is one possible factor influencing the success of AI. Semen storage before insemination can cause a change in sperm quality thus requires the precise diluent. Sperm quality examination in poultry has a significant relationship with the fertilization ability (Iswati *et al*. 2017).

Commercial fluid such as ringer lactate and physiological NaCl has been used as chicken semen diluent. Other commercial fluids such as KA-EN 3B® have possibility to be more potential because it contains dextrose which has been reported can maintain the chicken Bangkok sperm quality during storage at temperature of 5° C (Kudratullah and Sudrajat 2021). Combination of infusion fluid with egg yolk has also been proven could maintain the sperm quality during storage. Egg yolk is a diluent containing Low Density Lipoprotein (LDL) organic that is known capable of protecting sperm during storage (Manjunath 2012). Permatasari *et al.* (2013) also stated that egg yolk has components in the form of lipoproteins and lecithin which can defend and protect sperm from cold stress.

Chicken sperm cell membrane according to Cerolini *et al*. (2006) contains double unsaturated fatty acids that facilitate the lipid peroxidation. Lipid peroxidation causes morphological damage and decrease sperm motility and fertility (Long and Kramer 2003). Antioxidants in material diluent are used to prevent the peroxidation effect and resveratrol is one of antioxidant that can be supplemented in diluent. Resveratrol is a polyphenol naturally detected in 70 species of plants especially the in the grape bark and seeds (Salehi *et al*. 2018). Resveratrol is very strong antioxidant (Yulyani *et al.* 2017) and has succeeded used for protecting cells from stress caused by the oxidation of hydrogen peroxide (Salehi *et al*. 2018).

Tvrdá *et al.* (2015) reported that the addition of resveratrol to the culture medium can be beneficial for the stimulation whole activity of cows sperm and protection against the development of oxidative stress *in vitro*. Ahmed *et al.* (2020) observed that the addition of resveratrol in diluent repair the sperm quality and fertility ability, as well as reduce DNA fragmentation and lipid peroxidation in sperm buffalo during cryopreservation. The research of Rezaie *et al.*

(2021) found that the addition of resveratrol in Beltsville poultry semen extender (BPSE) was capable in increasing the function of chicken sperm after freezing. This research was carried out to determine the effect of resveratrol supplementation in KA-EN 3B® diluent egg yolk on the quality of Bangkok chicken sperm.

MATERIALS AND METHODS

The study uses a completely randomized design with 5 treatments and 5 repetitions. The semen was collected from 2 adult male Bangkok chickens with an average age of ± 12 months and a body weight of ± 2.5 kg. The chicken was kept in individual-sized cages 40 x 50 x 70 cm. Chickens were given *a* complete ration of 150 g/chicken and water was given *ad libitum*.

Research Procedure

Diluent base used in this research was the fluid infusion of KA-EN 3B® and egg yolk. The composition of KA-EN 3B® (PT Otsuka, Indonesia) is anhydrous dextrose 13.5 g, sodium chloride 0.875 g, potassium chloride 0.75 g, sodium lactate 1.1 g in 500 mL sterile water with osmolarity 290 mOsm/L. 10% of egg yolk purebred chicken mixed with 90% KA-EN 3B® and centrifuged at a speed of 2000 rpm for 30 minutes. The supernatant was taken and added with 0.2% glucose (Merck, Germany), 1000 IU/mL penicillin (PT Meiji, Indonesia), and 1 mg/mL streptomycin (PT Meiji, Indonesia). Supernatant was divided in five tubes and added resveratrol (Healthy Care, Australia) according to the treatment. Treatments used were KA-EN 3B® diluent-egg yolk (KEY), KEY + 0.02% resveratrol, KEY + 0.04% resveratrol, KEY + 0.06% resveratrol, and KEY + 0.08% resveratrol.

Semen was collected with massage technique and evaluated microscopically and macroscopically, then tranfered to 5 tubes with ratio diluent and semen was 10:1 (Winarto and Isnaini 2008; Iskandari *et al*. 2020). Semen liquid was furthermore kept in the refrigerator for 72 hours. Observation of sperm quality was performed at 24, 48, and 72 hours.

Research Variables

Sperm motility

Motility observed using light microscope (Boeco, Germany) with magnification 40X. A drop of semen was placed on a object glass and covered with cover glass, and the speed of movement sperm was evaluated (Arifiantini 2012).

Sperm viability

Viability observed based on Arifiantini (2012). Sperm was stained with eosin nigrosin and observed under a microscope with a magnification objective of 40X on 10 fields of view. Dead sperm absorb color whereas sperm life is not colored. Sperm viability was calculated using the following formula:

$$\text{Viability (\%)} = \frac{\text{no of live sperm}}{\text{Total sperm}}\ x\ 100$$

Sperm abnormality

Sperm abnormality was calculated as follows:

$$\text{Abnormality (\%)} = \frac{\text{no of abnormal sperm}}{\text{total sperm}}\ x\ 100$$

Analysis Data

Data were analyzed by one way analysis of variance (ANOVA) using the software Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

The Bangkok chicken sperm motility during storage is presented in Figure 1. The addition of resveratrol in

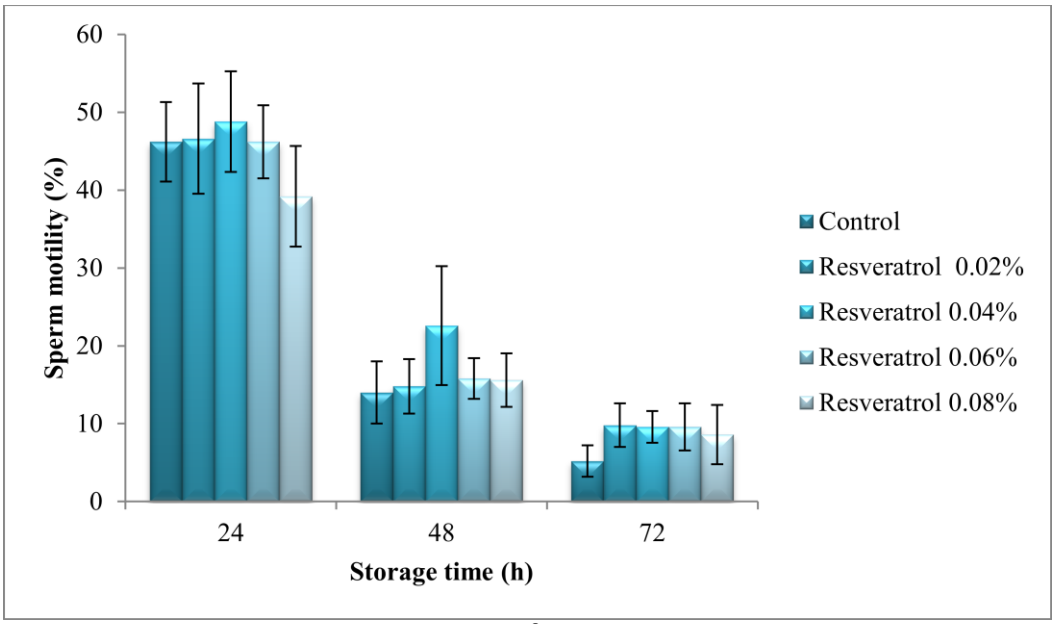

Figure1. Sperm motility of Bangkok chicken in diluent KA-EN 3B ® egg yolk supplemented with different concentration of resveratrol

the diluent did not influence sperm motility (P>0.05) of Bangkok chicken at 24, 48, and 72 hours of storage. These results were in accordance with the report of Rezaie *et al.* (2021) that the addition of 0.01 µM resveratrol did not affect total sperm motility and the addition of 0.01 and 0.1 µM resveratrol did not affect chicken sperm motility parameters after thawing.

Sperm motility during 24-hours storage was within the range of 39.2-48.8%. Similarly, Pratiwi *et al.* (2019) found that the use of ringer's lactate egg yolk astaxanthin as a diluent for sperm Menawang chicken resulted 49% sperm motility. Kudratullah and Sudrajat (2021) also reported that sperm motility of Bangkok chickens tended to decrease rapidly reaching 40% at 9 hours of storage using dextrose diluent. In contrast, Haq *et al.* (2020) observed that sperm motility in Cemani chickens reached 75% using phosphate egg yolk diluent.

Sperm motility at 48-hours storage ranged between 14-22.6%. This result was lower than Nugroho and Saleh (2016) finding which obtained above 30% sperm motility in local chickens using ringer's lactate egg white diluent. The motility of sperm at 72-hours storage was in the range of 5.2-9.8% which was lower than those reported in local chicken sperm diluted in coconut water egg yolk glucose ranged between 23 to24.6% (Khaeruddin *et al*. 2019). Hidayat *et al.* (2021) reported sperm motility of Pelung chicken was 6.25% in ringer's lactate egg yolk diluent added with Sodium Dodecyl Sulphate (SDS) and vitamin E.

The differences in sperm motility in various studies might be caused by many factors. According to Sarkar (2020), there were some possible factors that influence the motility, viability, and fertility of poultry sperm in storage such as temperature storage, diluent pH, osmolarity, level dilution, and seminal plasma. Feyisa *et al.* (2018) found that sperm motility was different among chicken breeds.

The sperm motility of Bangkok chickens decreased after 72 hours of storage. This might cause sperm damage during storage. According to Aramli *et al.* (2013), storage can increase and decrease the oxidative stress rate of Adenosine Triphosphate (ATP). The ATP originates from a part of the middle sperm and plays a role in the movement (motility) of sperm (Bennison *et al*. 2016). In vitro sperm storage formed superoxide mitochondria (O_2^-) which causes lipid peroxidation and the production of reactive aldehyde electrophilic. These aldehydes bind the functional proteins resulting in motility sperm disappearance (Gibb and Aitken 2016). Resveratrol antioxidants in this study were assumed not capable of preventing the effect of lipid peroxidation due to not producing a significant influence on sperm motility.

The results of sperm viability in Bangkok chickens during storage are shown in Figure 2. The addition of resveratrol in diluent had no effect (P>0.05) on sperm viability in Bangkok chicken at 24, 48, and 72 hours of storage. Resveratrol supplementation in this research did not produce a difference in sperm viability. Rezaie *et al.* (2021) also found that there was no difference in sperm viability after thawing in control treatment with the addition of 0.01 µM resveratrol. Similarly, Gopi *et al*. (2020) observed that there was no difference in sperm viability stored for 24-48 hours between control with additional antioxidant polyphenols 50 ppm and 100 ppm.

Sperm viability in 24-hours storage was in the range of 87.89-91.79%. This result was higher than reported by Hidayat *et al.* (2021) around 74.63% in Pelung chicken sperm diluted with ringer's lactate eggs yolk added with SDS and vitamin E. Ulus *et al.* (2019) also reported that sperm viability in local chicken was 70.96% in NaCl and tris egg yolk diluent. Low viability (40.4%) was reported by Fitriyah *et al.* (2019) on Magon chickens sperm using ringer's lactate diluent.

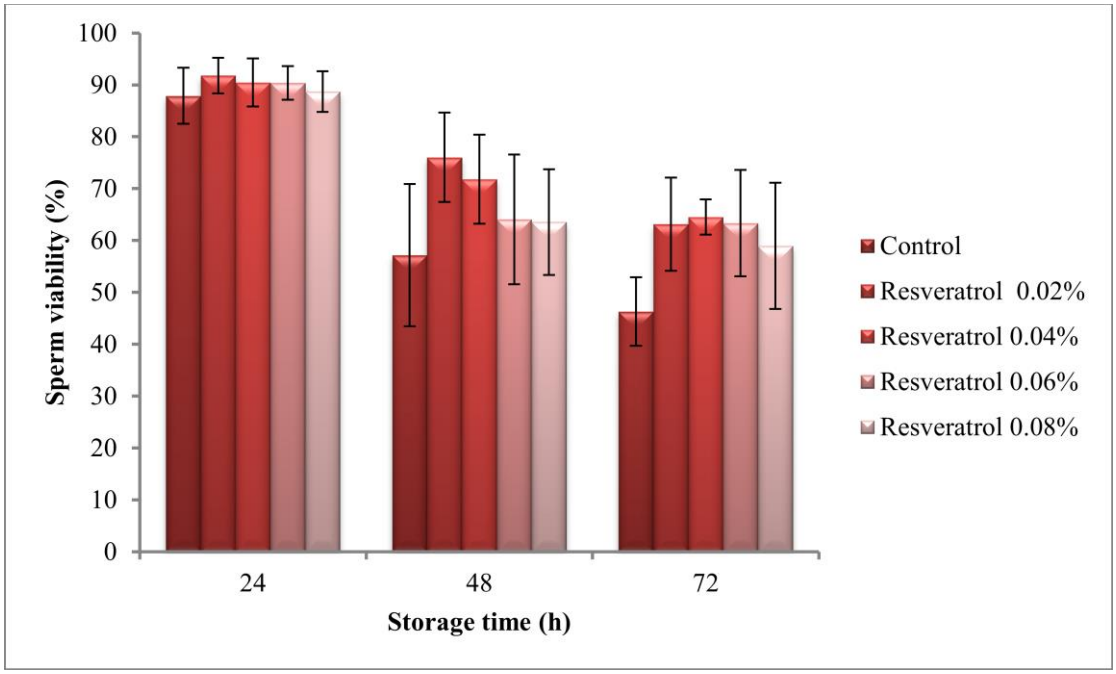

Figure 2.Sperm viability in Bangkok chicken in diluent KA-EN 3B® the egg yolk resveratrol supplementation

Sperm viability for 48-hours storage was in the range of 57.16-76.03%. This result was relatively similar to that reported by Haq *et al.* (2020) namely 65% of Cemani chicken sperm diluted with egg yolk phosphate diluent. This result was higher from Hidayat *et al.* (2021) finding which was 52.1% in Pelung sperm chicken diluted in ringer's lactate-egg yolk added with SDS and vitamin E.

Decreasing viability during storage might cause damage to sperm. According to Rizkallah *et al.* (2022), storage of sperm at 5° C or 15° C cause sub-lethal damage to sperm agglutination of membrane proteins integral, change protein function, and loss of function selective semi-permeable from cell membrane.

The result of sperm viability in this study was higher from sperm motility. This is in line with Sarkar (2020) that the high sperm viability usually found at low temperature (around 4° C) because the low temperature may decrease metabolism activity which can extend the life of sperm during storage *in vitro.* According to Gibb and Aitken (2016), cooling sperm at a temperature between 4-10° C intends to limit the rate of metabolism during storage. High sperm motility occurs at a range of 20-37° C, if temperature storage is lower or higher than that, they will produce low sperm motility (Sarkar 2020).

The results of the abnormality of sperm Bangkok chicken during storage can be seen in Figure 3 and Figure 4. The addition of resveratrol in diluent did not influence (P>0.05) on abnormalities in sperm Bangkok chicken at 24, 48, and 72 hours of storage. A common sperm abnormality found in this research was tail and

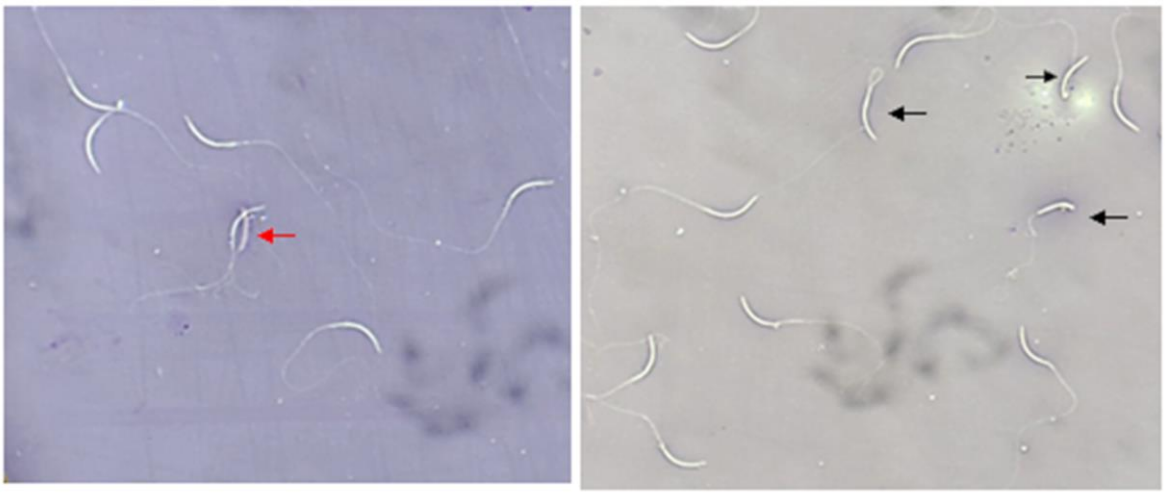

Figure 3. The examination of Bangkok chicken sperm with eosin-nigrosine staining (magnification 40 x 16). The red arrow indicates dead sperm (absorbs color) and the black arrow indicates abnormal sperm

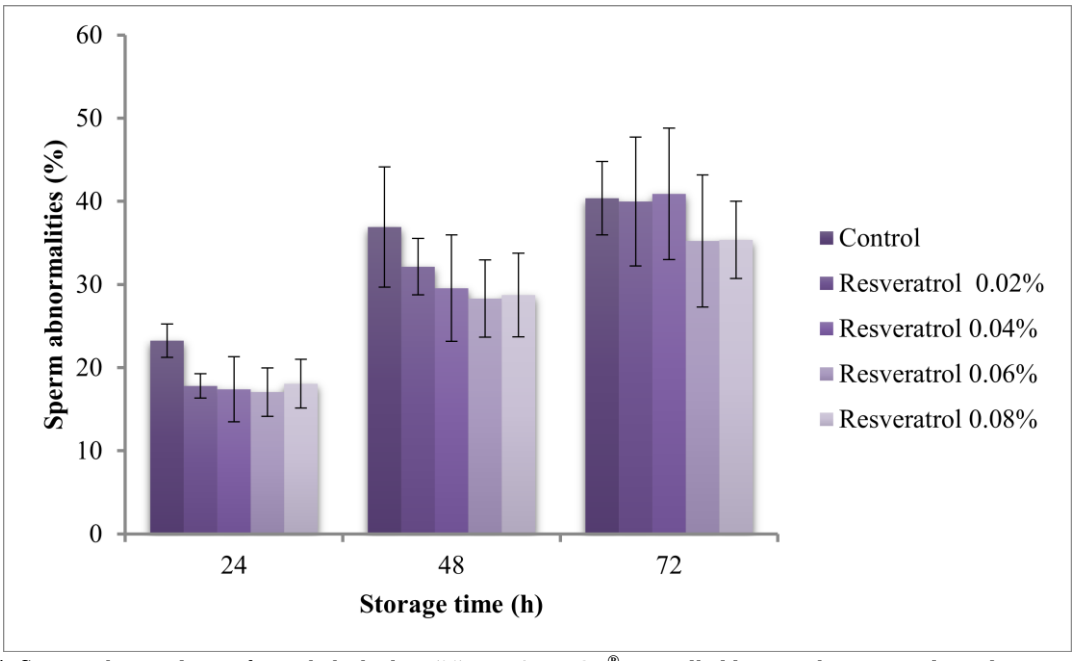

Figure 4. Sperm abnormalities of Bangkok chicken (%) in KA-EN 3B® egg yolk diluent with resveratrol supplementation

mid piece abnormality which was classified as a secondary abnormality. Tail abnormality is associated with low-motility sperm (Feyisa *et al*. 2018).

The abnormality of sperm obtained from 24-hours storage was in the range of 17.07-23.25%. This result was lower than that reported by Siudzińska and Łukaszewicz (2008) namely 33.15% in sperm chicken *Black Minorca* using Lake diluent. However, the present finding was higher than Nugroho and Saleh (2016) who reported that the sperm abnormality of local chickens in ringer's lactate white egg diluent was <12%. Sperm abnormality at 48 hours of storage was increased in the range of 28.31-36.92% and continues to increase up to 72 hours of storage ranged 35.24-40.9 % (Figure 4). Siudzińska and Łukaszewicz (2008) stated that during the storage there will be a decreasing amount of normal sperm and the quantity of abnormal sperm was influenced by type diluent used.

Resveratrol supplementation in this research did not affect the percentage of abnormal sperm. This result differed from Rezaie *et al.* (2021) who observed that the addition of 0.1 µM resveratrol can increase membrane integrity, activity of mitochondria, and malondialdehyde concentration of sperm chicken after freezing. This observation was probably due to the difference concentration of resveratrol used. However, Gopi *et al.* (2020) reported that no difference in inhibition of lipid peroxidation in chicken spermatozoa stored 24 hours between control group and the group added with 50 ppm and 100 ppm of antioxidant polyphenols.

CONCLUSION

Resveratrol supplementation at concentration of 0.02-0.08 % in KA-EN 3B® diluent yellow egg did not influence the quality of Bangkok chicken sperm during storage at low temperature.

REFERENCES

Ahmed H, Jahan S, Ullah H, Ullah F, Salman MM. 2020. The addition of resveratrol in tris citric acid extender ameliorates post-thaw quality parameters, antioxidant enzymes levels, and fertilizing capability of buffalo (*Bubalus bubalis*) bull spermatozoa. *Theriogenology*, 152:106-113.

Aramli MS, Kalbassi MR, Nazari RM, Aramli S. 2013. Effects of short-term storage on the motility, oxidative stress, and ATP content of Persian sturgeon (*Acipenser persicus*) sperm. *Animal Reproduction Science*, 143(1-4):112-117.

Arifiantini RI. 2012. *Teknik Koleksi dan Evaluasi Semen pada Hewan*. IPB Press, Bogor.

Bennison C, Hemmings N, Brookes L, Slate J, Birkhead T. 2016. Sperm morphology, adenosine triphosphate (ATP) concentration and swimming velocity: Unexpected relationships in a passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, 283(1837), Doi:10.1098/rspb.2016.1558.

Cerolini S, Zainiboni L, Maldjian A, Gliozzi T. 2006. Effect of docosahexaenoicacid and α-tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation. *Theriogenology*, 66:877-886.

Feyisa SG, Park YH, Kim YM, Lee BR, Jung KM, Choi SB, Cho CY, Han JY. 2018. Morphological defects of sperm and their association with motility, fertility, and hatchability in four Korean native chicken breeds. *Asian-Australasian Journal of Animal Science*, 31(8):1160-1168.

Fitriyah F, Humaidah N, Suryanto D. 2019. Pengaruh lama penyimpanan semen dalam pengencer ringer's lactat yang disimpan pada suhu 4º C terhadap kualitas spermatozoa ayam Magon. *Jurnal Rekasatwa Peternakan*, 1(1):28-37.

Gibb Z, Aitken RJ. 2016. The impact of sperm metabolism during in vitro storage: The stallion as a model. *BioMed Research International*, Doi:10.1155/2016/9380609.

Gopi M, Beulah PV, Prabakar G, Mohan J. 2020. Polyphenols as an antioxidant agent improves chicken sperm qualities during cold storage. *The Journal of Animal & Plant Sciences*, 30(6):1653-1658.

Haq NI, Bebas W, Laksmi NDI. 2020. Daya hidup dan motilitas spermatozoa ayam cemani dalam pengencer kuning telur fosfat pada penyimpanan 4º C. *Indonesia Medicus Veterinus*, 9(5):672-682.

Hidayat N, Hidayah CN, Nugroho AP. 2021. Honey supplementation in lactate ringer-egg yolk extender on quality of pelung chicken spermatozoa post-chilling. *Jurnal Kedokteran Hewan*, 15(1):7-10.

Iskandari NN, Madyawati SP, Wibawati PA, Suprayogi TW, Prastiya RA, Agustono B. 2020. Perbandingan pengencer tris kuning telur dan susu skim kuning telur terhadap persentase motilitas, viabilitas dan integritas membran plasma spermatozoa kambing Sapera pada penyimpanan suhu 5º C. *Jurnal Medik Veteriner*, 3(2):196-202.

Iswati I, Isnaini N, Susilawati T.2017. Fertilitas spermatozoa ayam buras dengan penambahan antioksidan glutathione dalam pengencer ringer's selama simpan dingin. *Jurnal Ilmu-Ilmu Peternakan*, 27(1):107-115.

Khaeruddin K, Hidayat A, Syamsuryadi, B. 2019. Preservasi semen ayam menggunakan pengencer air kelapa hijau dengan berbagai tingkat kematangan. *Agrominansia*, 4(2):109-119.

Kudratullah K, Sudrajat AS. 2021. Motilitas, viabilitas, dan morfologi spermatozoa ayam Bangkok dengan pengencer dextrose dan NaCl fisiologis 10% pada penyimpanan suhu 5º C dan 26º C. *Indonesian Journal of Applied Science and Technology*, 2(1):1-9.

Long JA, Kramer M. 2003. Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored Turkey semen. *Poultry Science*, 82:1802-1807.

Manjunath P. 2012. New insight into the understanding of the mechanism of sperm protection by extender components. *Animal Reproduction*, 9(4):809-815.

Nugroho AP, Saleh DM. 2016. Motilitas dan abnormalitas spermatozoa ayam kampung dengan pengencer ringer laktat-putih telur dan lama simpan pada suhu 5º C selama 48 jam. *Acta Veterinaria Indonesiana*, 4(1):35-41.

Permatasari WD, Setiatin ET, Samsudewa D. 2013. Studi tentang pengencer kuning telur dan pengaruhnya terhadap kualitas semen beku sapi Jawa Brebes. *Animal Agriculture Journal*, 2(1):143-151.

Pratiwi N, Yusuf TL, Arifiantini I, Sumantri C. 2019. Kualitas spermatozoa dalam modifikasi pengencer ringer laktat kuning telur dengan tambahan astaxanthin dan glutathione pada tiga jenis ayam lokal. *Acta Veterinaria Indonesiana*, 7(1):46-54.

Rezaie FS, Hezavehei M, Sharafi M, Shahverdi A. 2021. Improving the post-thaw quality of rooster semen using the extender supplemented with resveratrol. *Poultry Science*, 100(9):Doi:10.1016/j.psj.2021.101290.

Rizkallah N, Chambers CG, Graaf SPD, Rickard JP. 2022. Factors affecting the survival of ram spermatozoa during liquid storage and options for improvement. *Animals*, 12:244-251.

Salehi B, Mishra A, Nigam M, Sener B. 2018. Resveratrol: A double-edged sword in health benefits. *Biomedicines*, 6(3):91-98.

Sarkar PK. 2020. Motility, viability and fertilizing ability of avian sperm stored under in vitro conditions. *Reviews in Agricultural Science*, 8:15-27.

Siudzińska A, Łukaszewicz E. 2008. Effect of semen extenders and storage time on sperm morphology of four chicken breeds. *Journal of Applied Poultry Research*, 17(1):101-108.

Tvrdá E, Lukáč N, Lukáčová J, Hashim F, Massányi P. 2015. In vitro supplementation of resveratrol to bovine spermatozoa: effects on motility, viability and superoxide production. *The*

Journal of Microbiology, Biotechnology and Food Sciences, 4(4):336-341.

Ulus E, Kusumawati ED, Krisnaningsih, ATN. 2019. Pengaruh pengencer dan lama simpan semen ayam kampung pada suhu ruang terhadap motilitas dan viabilitas spermatozoa. *Jurnal Sains Peternakan*, 7(1):29-40.

Winarto A, Isnaini N. 2008. Pengaruh tingkat pengenceran terhadap kualitas spermatozoa kambing PE setelah penyimpanan pada suhu kamar. *Jurnal Ternak Tropika*, 9(2):72-80.

Yulyani Y, Aman IGM, Pangkahila W, Siswanto FM. 2017. Pemberian resveratrol oral mencegah peningkatan F2-isoprostan urin tikus wistar (*Rattus norvegicus*) jantan yang terpapar tartrazine. *Jurnal Biomedik*, 9(1):24-29.