INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0031/2024/32–6–618–622
DOI: 10.17957/IJAB/15.2244
http://www.fspublishers.org

Full Length Article

Supplementation of CoQ10 on LREY Diluent in Gaga Chicken Semen After Cryopreservation

Sri Wahjuningsih[1*], Ani Atul Arif[1], Ardyah Ramadhina Irsanti Putri[1], Khaeruddin[2] and Siti Melana Syahrani[3]
[1]*Department of Animal Reproduction and Genetic, Faculty of Animal Science, University of Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia*
[2]*Student of Doctoral program in Animal Science, Faculty of Animal Science, University of Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia*
[3]*Student of Magister program in Animal Science, Faculty of Animal Science, University of Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia*
[*]For correspondence: yuning@ub.ac.id
*Received 17 January 2024; Accepted 21 August 2024; Published 07 November 2024*_____

Abstract

Coenzyme Q10 (CoQ10), a potential of antioxidant, has been hypothesized to mitigate *oxidative stress* and improve sperm quality after freezing. By understanding the impact of CoQ10 in the LREY diluent, the study aims to provide insights into potential improvements in cryopreservation techniques, which are crucial for poultry breeding and genetic conservation efforts. The study aims to determine the CoQ10 in the Lactate ringer egg yolk (LREY) diluent on the quality of the chicken's sperm after freezing. The samples were divided into four different treatment groups, namely, containing samples of semen + LREY (P0), semen + LREY+ CoQ10 100 μM (P1), semen + LREY+ CoQ10 200 μM (P2) and (P3) semen + LREY+ CoQ10 300 μM. The variables include sperm motility, sperm viability, sperm abnormality, sperm plasma membrane integrity and sperm DNA damage. The data were analyzed using the one-way ANOVA and continued with the Duncan test. The results showed no significant influence on the treatment of P0, P1, P2 and P3 on sperm motility, sperm viability, sperm abnormality, sperm plasma membrane integrity of gaga chicken semen during freezing ($P > 0.01$). However, there was a significant effect on sperm DNA damage ($P < 0.01$). The results are expected to contribute to the development of more effective sperm preservation methods, thereby supporting the poultry industry's productivity and genetic diversity.

Keyword: Gaga chicken; Cryopreservation; CoQ10; Sperm DNA damaged

Introduction

Cryopreservation of Gaga chicken semen has become one of the most important aspects of keeping and preserving local animals (Khaeruddin *et al.* 2022). The critical factor that determines the success of cryopreservation of Gaga chicken semen is the quality of semen. In an effort to maintain the integrity and quality of frozen semen, semen diluents play a central role in determining the success of the freezing and storage processes. One of the major challenges faced in the maintenance of frozen semen is susceptibility to oxidative stress, which can damage the cell structure and DNA of sperm (Getachew 2016). Therefore, innovation in diluent formulation is needed to increase sperm resistance to adverse conditions.

Coenzyme Q10 (CoQ10) is an antioxidant compound that is naturally present in the cells of the body, including sperm. Its ability to protect cells from oxidative damage makes it a potential candidate for improving semen quality (Yang *et al.* 2021). In the context of semen diluents, CoQ10 supplementation can be a promising strategy to cope with oxygen stress that may occur during the freezing process (Xie *et al.* 2020). The importance of adding CoQ10 enzymes to frozen semen is because during sperm cryopreservation it can increase free radical production, which causes oxidative damage. CoQ10 helps reduce the production of these free radicals, protects the integrity of sperm, and increases the chances of successful fertilization (Salvio *et al.* 2021).

Previous research has shown that CoQ10 can play a key role in protecting sperm cells from oxidative stress, improving motility, and improving DNA integrity. However, research on the influence of CoQ10 supplementation in Lactate Ringer-Egg Yolk (LREY) on the quality of frozen semen and fertility of Gaga chicken sperm has not been done. Therefore, this study aims to fill this gap in knowledge by evaluating the effects of CoQ10 supplementation in LREY diluents on the quality of frozen semen and its potential in improving the fertility of Gaga chicken spermatozoa.

To cite this paper: Wahjuningsih S, AA Arif, ARI Putri, Khaeruddin, SM Syahrani (2024). Supplementation of CoQ10 on LREY diluent in gaga chicken semen after cryopreservation. *Intl J Agric Biol* 32:618–622

Materials and Methods

Animals and approval from ethics commission

The University of Brawijaya's Research Ethics Commission has approved all methods used in this study including the use of animals, and their certificate number: 135-KEP-UB-2023.

Semen collection

The research used semen from 10 male Gaga chickens 12 months old, with healthy, normal reproductive organs. Semen was taken out by using *massage* techniques (Wahjuningsih *et al.* 2024). Gaga chickens were kept in different cages with feed and water. They were given 100 g of commercial feed per day. *Ad libitum* water was provided for drinking. Semen was separated into nine tubes and each tube had its sperm concentration diluted based on treatment (minimum of sperm concentration 100 x 10^6 in 0.25 mL of straw; IMV, France).

Extender preparation

The extender used was 90% Ringer Lactate (PT Widatra Bakti) and 10% egg yolk (Junaedi *et al.* 2016). It was centrifuged (Hettich Mikro 200, Germany) for 15 min at a speed of 3000 x G. The supernatant was taken and added 7% DMSO or dimethyl sulfoxide (Merck, KgaA,64721 Darmstadt, Germany), 1000 IU/mL penicillin (PT Meiji, Indonesia) and 1 mg/mL streptomycin (PT Meiji, Indonesia). The pH of the diluent was adjusted with the addition of tris (hydroxymethyl) aminomethane (Merck, KgaA,64721 Darmstadt, Germany) up to pH 7.4. Treatments in this research include:

P0 = LREY diluent (Egg Yolk Lactate Ringer) or without CoQ10
P1 = LREY diluent + CoQ10 100 μM
P2 = LREY diluent + CoQ10 200 μM
P3 = LREY diluent + CoQ10 300 μM

Equilibration and freezing

The semen accordingly treated was loaded into 0.25 mL straw then equilibrated at a temperature of 5°C for 1 h of equilibration. It was pre-freezing for 10 min by placing the straw on a liquid nitrogen surface a distance of 3 cm (Madeddu *et al.* 2016) and then put into a liquid nitrogen container (-196°C) for 24 h for cryopreservation (Khairuddin *et al.* 2019).

Parameter semen of evaluation

The evaluation of frozen semen (more than 24 h) after thawing was done by placing frozen semen in a water bath. Percentage of sperm motility, percentage of sperm viability, PMI, and sperm DNA damage were evaluated after thawing.

Sperm motility: Sperm motility analysis was classified as a progressive motility. After thawing, 10 μL of frozen semen was examined under a binocular microscope (Olympus CX-23, Japan) with a 400X magnification. The number of progressive motile spermatozoa from one field of view was counted and reported as a percentage (Abdel-Khalek *et al.* 2018).

Sperm viability: The sperm viability was done by the *eosin-nigrosin* (Merck, KgaA,64721 Darmstadt, Germany) colouring method. One drop of diluted frozen semen after thawing, placed on the object glass, then added with one drop of *eosin-nigrosin* (Merck, KgaA,64721 Darmstadt, Germany) dye (1:3) and then homogenized. Then the preparation is made by pressing and pushing the object glass to form a 45° angle and dried. Then observe with a magnification of 400X- microscope in ten fields of view or 200 cells of sperm. The dead sperm will absorb red matter because the permeability of the cell wall has been weakened while the living sperm would look transparent (Silyukova *et al.* 2022).

Sperm plasma membrane integrity (PMI): Evaluation of sperm PMI using hypoosmotic swelling test solution (HOS). 10 μL semen diluted in hypoosmotic solution (0.9 g of fructose and 0.49 g of sodium citrate (Merck, KgaA,64721 Darmstadt, Germany) dissolved in 100 μL of distillation water) kept at a temperature of 37°C for 30 min, 200 of cell spermatozoa were evaluated which indication for intact was swelling in the tail of spermatozoa, and if not swelling in the tail of spermatozoa so damaged plasma membrane (Mehdipour *et al.* 2018; Najafi *et al.* 2022).

Sperm DNA damaged: Observation of Sperm DNA damage was used with *Toluidine blue* colouring. The semen was checked on the glass of the object and irrigated. The semen was fixed for 30 min using an ethanol solution of 96% acetone (1:1) at a temperature of 4°C. The preparation was then air-dried and hydrolysed in 0.1 N HCL for 5 min at 4°C. The preparation was washed with running water 3 times (each 2 min) and dried in the air. A drop of the *toluidine blue* solution was placed on the glass of the object for 10 min at room temperature, then washed with purified water and air-dried. The sample was observed using a light microscope with a magnification of 400X. The sperm head that has chromatin integrity will be either bright or bright blue, while the damaged of chromatin integrity would be old blue or purple (Fig. 1; Kim *et al.* 2013; Rui *et al.* 2017).

Data analysis

The all data were analyzed using a one-way analysis of variance (ANOVA), if the F-value was significant ($P < 0.05$) then it was continued with the Duncan multiple range test. Statistical analysis using the SPSS application version 29.0 and presented as mean ± standard error (SE).

Results

The quality of fresh semen gaga chicken in this study included

a) the evaluation of macroscopic among others: volume, colour, pH and consistency; b) microscopic among others: mass activity, sperm motility, sperm viability, sperm abnormality, sperm plasma membrane integrity, sperm DNA damage and sperm concentration. The characteristic of fresh gaga chicken semen in the study was good. The results of the macroscopic and microscopic evaluation of fresh gaga chicken semen showed good quality for freezing. The characteristics of fresh Gaga chicken semen can be seen in Table 1.

Table 1: The characteristic of fresh Gaga chicken semen

Variable	Mean ± SE
Volume (mL)	0.15 ± 0.02
Colour	creamy
pH	8.33 ± 0.08
Consistency	watery
Mass activity	++/+++
Sperm Motility (%)	77.67 ± 0.67
Sperm Viability (%)	98.59 ± 0.25
Sperm abnormality (%)	6.63 ± 0.95
Sperm plasma membrane integrity (%)	98.86 ± 0.21
Sperm DNA damaged (%)	0.6 ± 0.08
Sperm concentration (x10^6 sperm/mL)	3.72 ± 0.31

Discussion

The percentage of sperm motility is the most important variable in determining semen quality. The quality of semen includes sperm viability indicators can be demonstrated through the evaluation of sperm motility (Getachew 2016). The sperm motility is one of the important measures that indicate the sperm's ability to fertilize the egg in the fertilization process. Good sperm quality is indicated by the high level of progressive motility that will determine the speed it reaches the fertilization site. The results of analysis of variance showed that the treatment of different concentrations of CoQ10 during freezing have no significant influence ($P > 0.05$) on the motility of gaga chicken semen (Table 2). The study showed that there were no significant differences between treatments ($P > 0.05$). The study was lower than the motility in previous research reports, that was chicken frozen semen motility after thawing has 38.6% using Beltsville Poultry Semen Extender (BPSE) with hyaluronic acid added (Lotfi *et al.* 2017), 37.22% in chicken semen with egg yellow milk ringer with DMSO cryoprotectant (Junaedi *et al.* 2016) and 35.8% using L-carnitine (Fattah *et al.* 2017).

Average sperm viability of Gaga chicken semen post thawing with different concentration CoQ10 in LREY shows ranges between 48.62 ± 5.50 to 57.11 ± 3.97. The study showed that there were no significant differences between treatments ($P > 0.05$). That was because it protects sperm plasma membranes against the internal and external electrolyte-electrolyte balance of spermatozoa, so that the sperm metabolism process was uninterrupted so that it can maintain sperm survival. This result was higher than compared to Mosca *et al.* (2016) in Lohman chickens, for DMA (Dimethyl-Acetamide) produced live spermatozoa which were 46.9%.

Average percentage of plasma membrane integrity of Gaga chicken semen post thawing was presented in Table 2. Chicken Gaga spermatozoa after cryopreservation with different concentration CoQ10 in LREY showed that there were no significant differences between treatments ($P > 0.05$). Semen freezing can result in physical stress, often called cold shock, which affects structural and biochemical damage, thereby affecting cell function and ultimately causing cell death (Khan *et al.* 2021). Cold shock causes changes in the *phospholipids* that make up the plasma membrane during the transition phase from the liquid to the

Table 2: The quality of microscopic Gaga chicken semen post thawing

Variable	Treatment	Post thawing
Sperm Motility (%)	P0	21.30 ± 0.76
	P1	21.40 ± 0.83
	P2	21.40 ± 0.83
	P3	22.40 ± 1.08
Sperm Viability (%)	P0	48.62 ± 5.50
	P1	54.47 ± 4.35
	P2	55.99 ± 3.50
	P3	57.11 ± 3.97
Sperm Abnormality (%)	P0	43.37 ± 1.34
	P1	39.40 ± 1.88
	P2	37.95 ± 1.42
	P3	37.97 ± 2.23
Sperm Plasma Membrane Integrity (%)	P0	58.36 ± 3.02
	P1	61.83 ± 2.78
	P2	62.35 ± 2.67
	P3	62.96 ± 2.60
Sperm DNA damaged (%)	P0	2.72 ± 0.30^{a}
	P1	2.30 ± 0.24^{ab}
	P2	1.94 ± 0.18^{b}
	P3	1.63 ± 0.21^{b}

^{a,ab,b} Different superscripts in the same row and column showed significant difference ($P < 0.01$)

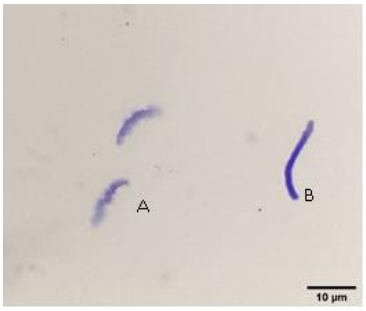

Fig. 1: Sperm DNA damage assessed by toluidine blue staining. **A**: The sperm head that has good chromatin integrity (bright or bright blue). **B**: The sperm head that has DNA damaged (dark blue)

freezing phase (Ghetler *et al.* 2005), changes in plasma lipid chain and protein membrane arrangements that can result in leakage or decreased selectivity. The plasma membrane will lose its selective permeability and affect many cellular components such as lipids, proteins and ions it releases (Blesbois 2012). Cold shock can also predict the dislocation of plasma membrane proteins such as glucose transporter groups, which have a role to play in the transport of hexose

through plasmatic membranes (Kokk *et al.* 2005).

Average percentage of sperm DNA damaged of Gaga chicken semen post thawing with different concentration CoQ10 in LREY showed that there were significant differences between treatments ($P < 0.01$). Sperm DNA damage can be one of the main causes of infertility in sperm. Some previous studies have shown that high levels of oxidative stress in spermatozoa can result in DNA damage, which in turn affects the ability of sperm for fertilization. CoQ10 is known to be a powerful antioxidant, and the results of this study show that concentration of CoQ10 at a level of 100 or 200 μM can reduce oxidative stress in gaga chicken semen in cryopreservation. Alahmar (2019) studied the effects of CoQ10 supplementation 1 x 200 mg/day for 3 months in men with oligoasthenoteratzoospermia, the results of studies show that CoQ10 supplementation can significantly improve sperm quality and decrease sperm DNA fragmentation damage ($P < 0.001$). CoQ10 works by stopping or reducing damage caused by free radicals, which can damage the structure of DNA. Free radicals can appear as a result of the oxidative reactions that can occur during the storage of frozen cement and CoQ10 helps to neutralize this free radical.

Conclusion

The conclusion in this study showed no significant influence on the treatment of P0, P1, P2, P3 on sperm motility, sperm viability, sperm abnormality, sperm plasma membrane integrity of gaga chicken semen during freezing. However, there was a significant effect on sperm DNA damage. Based on the results of the study it can be concluded that the addition of CoQ10 in the LREY diluent of 100 or 200 μM can reduce high post thawing sperm DNA damage.

Acknowledgments

The authors would like to thank the Ministry of Research Technology and Higher Education of the Republic of Indonesia for having granted this project through “the Grant for Strengthening Research Ecosystem for Professors”, University of Brawijaya, under the research contract No: 1759.1.2/UN10.C20/2023.

Author Contributions

SW, KH, and ARIP conceptualized and designed the experiment. SW and AAA wrote literature search manuscript drafts. SW and AAA edited and revised the manuscript. KH, ARIP, SMS helped with interpretation of analytical statistics. All authors were critically read, reviewed and approved the final manuscript.

Conflicts of Interest

All authors declare that they have no conflict of interests.

Data Availability

Data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Ethics Approval

The research has been approved by the Research Ethics Committee of University of Brawijaya under the approval number 135-KEP-UB-2023.

Funding Source

This research was funded by the Grant for Strengthening Research Ecosystem for Professors, University of Brawijaya, under the research contract No: 1759.1.2/UN10.C20/2023.

References

Abdel-Khalek AKES, AM Sakr, WM Nagy, HS Abou-Serie, EZ Eliraqy (2018). Role of propolis ethanolic extract as an antioxidant supplement in tris-extender to enhance semen quality of egyptian buffaloes during cryopreservation. *Adv Anim Vet Sci* 4:527–534

Alahmar AT (2019). The impact of two doses of coenzyme Q10 on semen parameters and antioxidant status in men with idiopathic oligoasthenoteratozoospermia. *Clin Exp Reprod Med* 46:112–118

Blesbois E (2012). Biological features of the avian male gamete and their application to biotechnology of conservation. *J Poult Sci* 49:141–149

Fattah A, M Sharafi, R Masoudi, A Shahverdi, V Esmaeili, A Najafi (2017). L-Carnitine in rooster semen cryopreservation: Flow cytometric, biochemical and motion findings for frozen-thawed sperm. *Cryobiology* 74:148–153

Getachew T (2016). A review article of artificial insemination in poultry. *World Vet J* 6:25–33

Ghetler Y, S Yavin, R Shalgi, A Arav (2005). The effect of chilling on membrane lipid phase transition in human oocytes and zygotes. *Human Reprod* 20:3385–3389

Junaedi J, RI Arifiantini, C Sumantri, A Gunawan (2016). The use of dimethyl sulfoxide as cryoprotective agent for native chicken frozen semen. *J Vet* 17:300–308

Khaeruddin, S Wahjuningsih, G Ciptadi, M Yusuf, Hermawansyah, Sahiruddin (2022). Cryopreservation of Gaga’ chicken semen from South Sulawesi, Indonesia with the addition of L-carnitine, hyaluronic acid, sucrose and their combination in diluent. *Biodiversitas* 23:3297–3302

Khairuddin K, ME Kurniawan, S Soman (2019). Cryopreservation of Kampung rooster semen using egg yolk diluent from four types of poultry with different concentrations. *Indo J Vet Sci* 13:82–87

Khan IM, Z Cao, H Liu, A Khan, SU Rahman, MZ Khan, A Sathanawongs, Y Zhang (2021). Impact of cryopreservation on spermatozoa freeze-thawed traits and relevance OMICS to assess sperm cryo-tolerance in farm animals. *Front Vet Sci* 8:609180

Kim HS, MJ Kang, SA Kim, SK Oh, H Kim, SY Ku, SH Kim, SY Moon, YM Choi (2013). The utility of sperm DNA damage assay using toluidine blue and aniline blue staining in routine semen analysis. *Clin Exp Reprod Med* 40:23–28

Kokk K, E Verajankorva, M Laato, XK Wu, H Tapfer, P Pollanen (2005). Expression of insulin receptor substrates 1-3, glucose transporters Glut-1-4, signal regulatory protein 1 alpha, phosphatidylinositol 3-kinase and protein kinase B at the protein level in the human testis. *Anat Sci Intl* 80:91–96

Lotfi S, M Mehri, M Sharafi, R Masoudi (2017). Hyaluronic acid improves frozen-thawed sperm quality and fertility potential in rooster. *Anim Reprod Sci* 184:204–210

Madeddu M, F Mosca, A Abdel Sayed, L Zaniboni, MG Mangiagalli, E Colombo, S Cerolini (2016). Effect of cooling rate on the survival of cryopreserved rooster sperm: Comparison of different distances in the vapor above the surface of the liquid nitrogen. *Anim Reprod Sci* 171:58–64

Mehdipour M, HD Kia, G Moghaddam, H Hamishehkar (2018). Effect of egg yolk plasma and soybean lecithin on rooster frozen-thawed sperm quality and fertility. *Theriogenology* 116:89–94

Mosca F, M Madeddu, A AbdelSayed, L Zaniboni, N Iaffaldano, S Cerolini (2016). Combined effect of permeant and non-permeant cryoprotectants on the quality of frozen/thawed chicken sperm. *Cryobiology* 73:343–347

Najafi A, H Daghigh-Kia, M Mehdipour, H Mohammadi, H Hamishehkar (2022). Comparing the effect of rooster semen extender supplemented with gamma-oryzanol and its nano form on post-thaw sperm quality and fertility. *Poult Sci* 101:101637

Rui BR, DS Angrimani, JDA Losano, L de Cássia Bicudo, M Nichi, RJ Pereira (2017). Validation of simple and cost-effective stains to assess acrosomal status, DNA damage and mitochondrial activity in rooster spermatozoa. *Anim Reprod Sci* 187:133–140

Salvio G, M Cutini, A Ciarloni, L Giovannini, M Perrone, G Balercia (2021). Coenzyme Q10 and male infertility: A systematic review. *Antioxidants* 10:874

Silyukova Y, E Fedorova, O Stanishevskaya (2022). Influence of technological stages of preparation of rooster semen for short-term and long-term storage on its quality characteristics. *Curr Issue Mol Biol* 44:5531–5542

Wahjuningsih S, AA Arif, Khaerudin, H Pratiwi, ARI Putri (2024). The effects of equilibration time and post-thawing temperatures in cryopreservation of gaga chicken semen. *Adv Anim Vet Sci* 12:807–814

Xie T, C Wang, Y Jin, Q Meng, Q Liu, J Wu (2020). CoenzymeQ10-induced activation of AMPK-YAP-OPA1 pathway alleviates atherosclerosis by improving mitochondrial function, inhibiting oxidative stress and promoting energy metabolism. *Front Pharmacol* 11:1034

Yang S, B Fan, X Chen, Z Meng (2021). Supplementation of the freezing medium with Coenzyme Q10 attenuates oxidative stress and improves function of frozen-thawed giant grouper (*Epinephelus lanceolatus*) spermatozoa. *Theriogenology* 175:77–82