



Supplementation of Coenzyme Ubiquinone (COQ10) in the semen diluent of Balitbangtan's Superior Native Chicken in Terms of Spermatozoa Chromatin Damage

Siti Melana Syahrani¹, Achadiyah Rachmawati², Sri Wahjuningsih³

¹Post graduate of the Faculty Animal Science, Brawijaya University, Indonesia

^{2,3}Lecturer in Faculty of Animal Science, Brawijaya University, Indonesia

ABSTRACT: The development of technology in the field of animal husbandry is growing very rapidly, apart from ruminants from poultry livestock can also be done artificial insemination used for breeding. According to Apriyanti (2017) The success of the mating system can be seen from the success of livestock mating which is influenced by the quality of the semen produced. One of the supplementations used is coenzyme ubiquinone (CoQ10) where CoQ10 is one of the vitamins such as fat-soluble vitamin E which is endogenously present in the inner membrane of mitochondria of mammals and plants (El-Sayed, et al. 2021). This study used an experimental method or field experiment with 4 treatments and 10 replicates on 4 male KUB chickens aged 12 months and the diluent used was egg yolk lactated ringer. The variables observed in this study was chromatin damage of spermatozoa before freezing and post thawing. The results obtained in this study chromatin damage before freezing obtained the highest average value of 1.18 ± 0.58 with statistical calculations showed significantly different results ($P < 0.05$). chromatin damage after thawing back obtained the highest result of 2.80 ± 0.89 with statistical test calculations obtained results that were not significantly different ($P < 0.01$) to the different levels of Coenzyme ubiquinone in diluents with different levels.

KEYWORDS: breeding, supplementation, coenzyme ubiquinone, balitbangtan's superior native chicken, spermatozoa chromatin damage

INTRODUCTION

Sperm chromatin plays a vital role in fertilization and embryo development. Chromatin damage can have serious consequences for sperm quality and fertility. Recent studies have shown that various factors, including oxidative stress, can affect the integrity of sperm chromatin in chickens. Oxidative stress occurs when there is an imbalance between the production of free radicals and the ability of the body's antioxidant system to neutralize them (Khan et al., 2015). In addition, several studies have found a link between chromatin damage and decreased fertility, as well as abnormalities in embryo development (Sadeghi et al., 2016).

In chickens, sperm chromatin damage can be caused by various factors, including the environment and management practices. For example, a study by Al-Ghadi et al. (2020) showed that environmental factors such as high temperature can damage chromatin structure, leading to decreased sperm quality. In addition, the use of certain chemicals in feed has also been reported to contribute to this damage (Zhao et al., 2018). Knowing the factors that affect spermatozoa chromatin damage is very important to improve maintenance and reproduction techniques in chickens. This study aims to investigate spermatozoa chromatin damage in chickens, focusing on environmental and management factors that have the potential to affect spermatozoa quality.

Previous research conducted by Sharideh, et al. (2019) on 36 rooster broilers, weighing 5187.17 ± 92.99 g selected at 44 weeks of age with treatment 3 with results of P0, P1 and P2 plasma membrane integrity of 82.82% respectively; 88.06% and 90.30%. This evaluation is used to determine the effect of coenzyme ubiquinone supplementation on chromatin damage before freezing and post thawing.

Materials

The research material used was male Balitbangtan superior native chickens aged 12 months. Semen collection was carried out twice a week using the female angler method. This research design uses 4 treatment levels and 10 replicates with different concentrations of COQ10 addition. Treatment level P0 without additional COQ10; P1 diluent with additional COQ10 200 μ M/mL; P2 diluent with



additional COQ10 300 $\mu\text{M}/\text{mL}$; P3 diluent with additional COQ10 400 $\mu\text{M}/\text{mL}$. The results of the study were collected using Microsoft Excel and will be analyzed using a group randomized design.

METHODS

Preparation of Basic Diluent

The diluent materials used in the study were 90% lactated ringer and 10% egg yolk. The diluent was then centrifuged for 15 minutes at 300 rpm. The centrifuged supernatant was taken penicillin 1000 Iu/ml and streptomycin 1 mg/ml. The pH of the diluent was adjusted by the addition of tris hydroxyl aminomethan to pH 7.4.

Semen Collection, Dilution and Processing Technique

Semen was collected using the angler's hen technique which was then divided into 4 tubes, each of which was diluted according to the treatment with a ratio of semen and diluent of 1: 5. Semen that has been diluted is then put into a straw which is then cooled at 5°C for 2 hours for equilibration, before freezing the semen that has been equilibrated is stored in nitrogen vapor for 10 minutes then freezing KUB chicken spermatozoa with coenzyme ubiquinone supplementation is stored for 1x24 hours in a liquid nitrogen container which is then carried out post thawing semen evaluation.

RESULTS AND DISCUSSION

Chromatin damage is a condition where there is damage to the DNA of chicken spermatozoa which can include mutations or other structural changes. This damage can occur due to oxidative stress or a poor environment. Syaury (2014) stated that the quality of spermatozoa chromatin is one of the indicators that determines the quality of spermatozoa. Poor chromatin quality can reduce fertility and problems in embryo development. Evaluation of chromatin damage can be done by Toluidine blue staining. Research conducted by Masoudi, et al. (2019) produced the highest value of 1.34% in spermatozoa chromatin damage. In this study, the results of chromatin damage before freezing and after thawing can be seen in the table below.

Table 1. The results of chromatin damage

Cromatin Damage		
	Before Freezing	Post Thawing
P0	1,18±0,58	2,80±0,89
P1	1,14±0,44	2,14±0,78
P2	0,81±0,37	1,85±0,67
P3	0,95±0,61	1,43±0,82

Source: Primary data processed, 2024

Spermatozoa chromatin damage in this study obtained the highest average value before freezing of 1.19% at P0 or P control, this value is slightly lower than the research conducted by Masoudi, Sharafi and Pourazadi (2019) with the highest result of 1.34%. Spermatozoa chromatin damage in this study sequentially at P control was 1.19%; P1 was 1.14%, P2 was 0.80% and the last P3 was 0.95%. The difference in the value of chromatin damage can be caused by the antioxidants used where antioxidant supplementation in spermatozoa dilution can help reduce chromatin damage to spermatozoa by neutralizing free radicals, environmental conditions, and other factors environmental conditions when conducting the study where environmental conditions can affect chromatin damage caused by heat or cold stress.

Chromatin damage to spermatozoa before freezing which was tested statistically obtained significant results ($P < 0.05$) to the treatment of adding CoQ10 concentration with various levels. The significant results of chromatin damage can be caused by environmental conditions, an environment that is too hot when collecting or when the research process is carried out will cause significant chromatin damage. Krol, Zawisza-Raszka, and Pietras (2021) explain that environmental conditions can affect spermatozoa chromatin damage, the effect of heat stress that occurs on the chromatin structure of chicken spermatozoa. Based on this, it can be concluded that high temperatures can cause significant damage.



The results of chromatin damage research after thawing back obtained the highest average result of 2.80% obtained from P0 or P control. The average value of chromatin damage after thawing was 2.80%; P1 was 2.14%; P2 was 1.85%; and P3 was 2.43%. Previous research conducted by Yang, Zhang, Xu and Wu (2018) obtained the results of chromatin damage to spermatozoa with antioxidant supplementation in freezing media and obtained a value of around 30% after thawing again. Silva, Soares, Mello and Santos (2020) also conducted the same research that diluent media with different levels obtained a result of 35%, it can be concluded that this shows that the results obtained in this study are much different than the research conducted by Yang, et al. (2018) and Silva, et al. (2020).

Damage to spermatozoa chromatin after thawing back which has been tested statistically obtained insignificant results ($P > 0.01$), insignificant differences in each treatment can be caused by the quality of the diluent used, the longer the storage of the diluent used will allow increasing damage to spermatozoa chromatin after thawing back. Bozkurt, Yaman and Turan (2021) explained that the evaluation of diluent in the freezing process greatly affects the quality of spermatozoa chromatin after thawing, the length of time the diluent is used is likely to have a very significant impact on spermatozoa chromatin damage after freezing. Lee, Kim, and Kim (2019) also explained that the effect of providing different antioxidants in the diluent on freezing media on spermatozoa chromatin damage after thawing, this is in accordance with this study where the diluent with different levels in each treatment medium allows a very significant difference when thawing again.

CONCLUSION

The addition of ubiquinone to egg yolk lactated ringer diluent produced a significant difference before freezing and did not indicate a significant result. This difference may be due to the process carried out when handling semen during treatment or due to cold or heat shock.

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