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# FERTILITY AND FERTILE PERIOD OF DUCK EGGS AFTER ARTIFICIAL INSEMINATION WITH MUSCOVY DUCK SEMEN SUPPLEMENTED WITH VITAMIN C AND E

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## ABSTRACT

The aim of this research was to investigate the influence of duck variants and addition of vitamins into muscovy duck semen on fertility and fertile period of duck eggs after artificial insemination. Semen was collected from five muscovy ducks and divided into 3 treatment groups: without vitamin supplementation ($A_0$), supplementation of 400 µg/mL vitamin C ($A_1$), and supplementation of 80 µg/ml vitamin E ($A_2$). Each semen was inseminated into female ducks of Magelang ($B_1$) and Mojosari ($B_2$) variants. Complete Random Design was used with 3x2 factorial. The results showed that vitamins and duck variants had no significant interaction (P>0.05) with fertility and fertile period. The duck variant had no effect (P>0.05) on fertility and fertile period, while the addition of vitamins significantly affected (P<0.01) the fertility and fertile period. The addition of 400 µg/mL vitamin C increased fertility by 22.28±0.20% but reduced the fertile period by 7.8±3.5 days, whereas 80 µg/mL of vitamin E increased fertility by 11.57±2.47% but reduced fertile period by 12.3±0.9 days. It can be concluded that the addition of 400 µg/mL of vitamin C and 80 µg/mL of vitamin E in Muscovy duck semen increased fertility but shortened fertile period of duck eggs after artificial insemination.

Key words: fertile period, fertility, muscovy duck semen, vitamin

## *ABSTRAK*

*Tujuan penelitian adalah mengetahui pengaruh interaksi jenis itik dan penambahan vitamin dalam semen entok serta pengaruh masing-masing faktor terhadap fertilitas dan periode fertil telur itik melalui teknik inseminasi buatan (IB). Semen hasil koleksi dari 5 ekor entok umur 1 tahun diencerkan menggunakan ringer laktat kemudian dibagi menjadi 3 kelompok perlakuan yaitu tanpa vitamin ($A_0$), 400 µg/ml vitamin C ($A_1$) dan 80 µg/ml vitamin E ($A_2$). Masing-masing kelompok perlakuan diinseminasikan pada itik betina magelang ($B_0$) dan mojosari ($B_1$). Rancangan acak lengkap (RAL) pola faktorial 3x2 digunakan dalam penelitian ini. Hasil analisis varian menunjukan bahwa tidak terdapat interaksi antara vitamin dan jenis itik (P>0,05) terhadap fertlitas dan periode fertil. Perlakuan jenis itik tidak berpengaruh nyata (P>0,05) terhadap fertilitas dan periode fertil, sedangkan penambahan vitamin dalam semen berpengaruh sangat nyata (P<0,01) terhadap fetilitas dan periode fertil. Penambahan 400 µg/ ml vitamin C mampu meningkatkan fertilitas sebesar 22,28±0,20% tetapi menurunkan periode fertil sebesar 7,8±3,5 hari, sedangkan 80 µg/mL vitamin E mampu meningkatkan fertilitas sebasar 11,57±2,47% tetapi menurunkan periode fertil sebesar 12,3±0,9 hari. Berdasarkan hasil penelitian dapat disimpulkan bahwa penambahan vitamin C maupun vitamin E mampu meningkatkan fertilitas semen tetapi memperpendek periode fertil telur.*

*Kata kunci: periode fertil, fertilitas, semen entok, vitamin*

## INTRODUCTION

Utilization of local ducks as meat producers is still low because the weight gain is relatively small, hence there is a need for crossbreeding with quacks through artificial insemination (AI) using semen of Muscovy duck (*Cairina moschata*). Artificial insemination is preferred because male muscovy ducks body are much larger than ducks, making natural mating difficult. This effort is expected to produce breeds with higher body weight gain than local ducks. This objective can be achieved if spermatozoa motility can be maintained since semen collection. Therefore, semen needs to be mixed with diluents that support their physical and chemical needs. One commonly used of the diluents is ringer lactate which increases the semen volume and also has buffering properties to preserve the quality of spermatozoa.

Indicators of AI success can be seen from the fertility rate. According to Froman and Kirby (2008), egg fertility is influenced by the production and maturation of spermatozoa in the male reproductive tract, spermatozoa motility during copulation and the duration of spermatozoa persisting in the uterine-vaginal junction (UVJ) precisely in sperm storage tubules (SST). In addition, fertility is also influenced by passive transport of spermatozoa in reaching the oviduct above the vaginal sphincter, induction of spermatozoa against the oocyte perivitelline membrane in the acrosome reaction, spermatozoa perforation of the perivitelline membrane. The length of the fertile period in different types of birds varies according to the species.

Sperm quality can be preserved by providing antioxidants in diluents that can protect spermatozoa from being damage by free radicals. These antioxidants are in the form of vitamin C and vitamin E. Vitamin C can protect cells from oxidation reactions from free radicals that cause spermatozoa death (Kelso *et al*., 1996). According to Bebas *et al.* (2015), suplementation of vitamin C can keeps the motility and viability of pig spermatozoa during storage. Vitamin E functions as the main barrier to phospholipid peroxide in cellular membranes and subcellular spermatozoa (Surai *et al*., 1997). Addition of vitamin E to chicken semen can keeps motility, viability, and sperm normal morphology after 72 hours storage at $4^{o}$ C (Asmarawati *et al*., 2010). Until now, there has been no report of the required amount of vitamins C and E addition into

semen in order to increase the fertility and the fertile period of eggs.

## MATERIALS AND METHODS

The material used in this study were 5 male Muscovy ducks aged 1 year old, 24 female Magelang ducks, 24 female Mojosari ducks, 24 battery cages, 1 semen container (microtube), 4.8 mL of Muscovy ducks semen, 4.8 mL of solution ringer lactate, 640 µg vitamin C, 128 µg vitamin E, and 1 mL syringes.

### Semen Collection and Evaluation

Semen was collected from 5 ducks aged one year old. Semen collection was done by massaging the lower part of the pubic bone until the male responded by producing the papillae. After the papillae appeared, the lower part of the pubic bone was pressed using right and left index fingers so the that semen comes out until the ejaculation reflex disappeared.

Semen was immediately evaluated macroscopically and microscopically. Macroscopic evaluation included volume, degree of acidity (pH), consistency, and color of semen. Semen volume was measured using a measuring pipette, while semen pH was measured using a special pH indicator paper. Semen consistency was observed based on the degree of viscosity (thin, medium, or thick), while semen color of was divided into beige and milky white. Microscopic evaluation includes mass movement, concentration, motility, viability, and morphology of spermatozoa.

Spermatozoa mass movement was evaluated under a microscope at a magnification of 10x10 by observing the waves produced by the spermatozoa movement and was classified as very good (+++), good (++), and poor (+). The motility of spermatozoa was evaluated under a microscope at a magnification of 10x40 by dripping 1 gram of fresh semen and 8-10 drops of physiological NaCl. The motility value was determined based on the percentage of the number of spermatozoa that move progressively from the total number of spermatozoa that was seen on the five visual fields. Spermatozoa concentrations per ml were calculated using a Neubauer chamber at a dilution of 500 times (1 µL of semen and 499 µL of formosaline). Concentration was obtained from the number of spermatozoa in five chambers (upper right corner, lower right corner, upper left corner, lower left corner, and the middle box) multiplied by $25x10^6$. Viability of spermatozoa was evaluated by preparing a curing preparation using eosin-negrosin 2% solution at a semen and solution ratio of 1 : 2. Spermatozoa that do not absorb color were viable whereas spermatozoa that absorb color were dead. The morphological evaluation of spermatozoa was done by making a review of the same preparation as in the evaluation of spermatozoa viability. Morphology was distinguished as normal and abnormal. Percentage of viable spermatozoa and spermatozoa abnormalities were calculated based on observations in 10 visual fields or a minimum of 200 spermatozoa cells (Tabatabaei *et al*., 2009).

### Dilution and Addition of Vitamins

Semen with motility >70% were mixed with lactated Ringer's solution at a ratio of 1 : 1, and then divided into three treatment groups namely no vitamins (A0), 400 µg/mL vitamin C supplementation (A1), and 80 µg/mL vitamin E supplementation (A2).

### Artificial Insemination

Each semen treatment group was inseminated to Magelang (B0) and Mojosari (B1) ducks at a dose of 0.2 mL/duck with a spermatozoa concentration of 200 million/IB dose; each treatment unit consisted of 2 female ducks that were repeated 4 times. Artificial insemination was carried out using the intravenous method by depositing semen as deep as 3 cm.

### Fertility Evaluation and Fertile Period

Eggs were collected for 20 days and placed into a hatching machine to observe their fertility and fertile period. Fertility is the ability of spermatozoa to fertilize the ovum. Fertility is measured by calculating the percentage of fertilized eggs over the number of eggs produced. The fertile indicator was carried out by candling the eggs on the seventh day after incubation. The fertile period is the lengths of time spermatozoa survive in the female reproductive tract to fertilize the ovum. The fertile period is obtained from the average fertilized last nesting day.

### Research Design and Statistical Analysis

Laboratory research was carried out using a completely randomized design (CRD) factorial pattern of 3x2 with 6 treatment combinations and each treatment was repeated 4 times. Factor A is the addition of vitamins (A0: without vitamins, A1: 400 µg/mL vitamin C and A2: 80 µg/ml vitamin E) and factor B is the variant duck (B0: Magelang duck and B1: Mojosari duck).

The data obtained were tabulated and then analyzed using analysis of variance. If the treatment had a significant effect, it was followed by honestly significant difference test (Steel and Torrie, 1993).

## RESULTS AND DISCUSSION

### Characteristics of Muscovy Duck Semen

The characteristics of fresh semen which were a result of the average collection of 5 ducks were presented in Table 1. Based on the results of the study, the average semen volume was 0.5 ml. The volume was similar to the results of Zabiq *et al*. (2017) which stated that average semen volume was 0.69 ml. The motility of spermatozoa Muscovy duck obtained in the study was 78%. This result was consistent with Ulupi *et al.* (2015) who stated that the motility of duck spermatozoa ranges between 60-80%. These results indicated that the semen in the study was appropriate for AI because the motility requirements used in insemination is 50-80%.

The concentration of Muscovy duck spermatozoa in the study was $1.98x10^9$ cells/mL and the total

spermatozoa per ejaculate was $0.99x10^9$ cells. These results are similar to Zabiq *et al*. (2017) who found that semen contains $1.39x10^9$ spermatozoa/mL. Muscovy duck spermatozoa observed in this study had a viability of 80%, which is in accordance to Ulupi *et al.* (2015) who stated that viability of duck spermatozoa ranges between 75-90%. Spermatozoa abnormality in this study was 15% and thus appropriate for AI because according to Putranti *et al*. (2010), spermatozoa can still fertilize the ovum if the maximum of abnormality is 20%.

**Tabel 1**. Characteristic of fresh semen of muscovy duck

| Semen Characteristics | Average |
|---|---|
| Volume (mL) | 0.5 |
| Consistency | Thick |
| | Milky white |
| Mass Movement | +++ |
| Spermatozoa Consentration ($x10^9$ sel $mL^{-1}$) | 1.98 |
| Spermatozoa Mitility (%) | 78 |
| Spermatozoa Viability (%) | 80 |
| Spermatozoa Abnormality (%) | 15 |

**Tabel 2**. The fertility average (%) of muscovy spermatozoa

| Treatments | Replications | | | | Average treatment |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| $A_0B_0$ | 46.00 | 33.33 | 25.00 | 30.00 | 33.58±8.96[a] |
| $A_1B_0$ | 57.14 | 55.55 | 46.66 | 62.50 | 55.46±6.58[b] |
| $A_2B_0$ | 46.15 | 44.44 | 50.00 | 33.33 | 43.48±7.16[b] |
| $A_0B_1$ | 33.33 | 35.71 | 22.22 | 33.33 | 31.15±6.06[a] |
| $A_1B_1$ | 60.00 | 62.50 | 42.86 | 50.00 | 53.84±9.10[b] |
| $A_2B_1$ | 42.86 | 28.57 | 46.15 | 60.00 | 44.40±12.90[b] |

[a, b]Different superscripts within the same column indicate significant differences (P<0.05)

**Table 3**. Effect of addition of vitamin C and vitamin E in semen to the fertility of duck eggs

| Treatment | Fertility |
|---|---|
| $A_0$ | 32.37±7.20[a] |
| $A_1$ | 54.65±7.40[b] |
| $A_2$ | 43.94±9.67[b] |

[a, b]Different superscripts within the same column indicate very significant differences (P<0.01)

**Table 4**. Average fertile spermatozoa periods

| Treatment | Repetition | | | | Average treatment |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| $A_0B_0$ | 14 | 18 | 19 | 20 | 17.8±2.6[a] |
| $A_1B_0$ | 7 | 8 | 11 | 19 | 11.3±5.4[b] |
| $A_2B_0$ | 6 | 5 | 3 | 11 | 6.3±3.4[b] |
| $A_0B_1$ | 20 | 19 | 19 | 20 | 19.5±0.6[a] |
| $A_1B_1$ | 6 | 20 | 9 | 7 | 10.5±6.5[b] |
| $A_2B_1$ | 10 | 3 | 6 | 7 | 6.5±2.9[b] |

[a, b]Different superscripts within the same column indicate significant differences (P<0.05)

**Table 5**. Effect of addition of vitamin C and vitamin E in semen to the period of fertilization of duck eggs

| Treatment | Fertility |
|---|---|
| $A_0$ | 18.6±2.0[a] |
| $A_1$ | 10.9±5.5[b] |
| $A_2$ | 6.4±2.9[b] |

[a, b]Different superscripts within the same column indicate significant differences (P<0.05)

**Fertility of Duck Eggs Produced from AI with Semen Supplemented with Vitamin C and Vitamin E**

The average fertility of eggs collected for 20 days from each type of duck was shown in Table 2. The results showed that the lowest average fertility among the treatment groups was in no vitamin supplementation group in Mojosari ducks (31.19±6.06%) while the highest was in the supplementation of vitamin C to Magelang ducks (55.46±6.58%). These results appeared to be low because eggs were collected until the 20th day post artificial insemination.

The interaction between addition of vitamins and types of ducks showed no significant effect (P>0.05) on fertility. This was presumably because the combination of treatment between the addition of vitamins and duck variants resulted in different responses that did not have an additive effect on fertility in each variant of ducks.

Duck variant did not show a significant effect (P>0.05) on fertility. This was caused by the similarity in reproductive system between Magelang female and Mojosari ducks because the two livestock were still included under one family. Another possible reason

was the similar amount of egg production between Magelang ducks and Mojosari ducks. According to Widodo (2011), egg production difference between species had a significant effect on fertility; the higher the egg production, the lower the fertility. The fertility of *Agapornis fischeri* species was 83.33±23.57% for low production (3 items) and 49.00±14.32% for high production (≥4 items), whereas in *Agapornis roseicollis* species was 86.67±18.26% for those with low production and 53.00±13.04% for those with high production.

Vitamin supplementation had a significant effect on fertility (P<0.01). Addition of 400 µg/mL of vitamin C and 80 µg/mL of vitamin E increased fertility by 22.29% and 11.58%, respectively (Table 3). This was presumably because both vitamins are antioxidants that protect cells from the oxidation reaction of phospholipid peroxide compounds which helped maintain spermatozoa quality. Vitamin C and vitamin E as antioxidants can stop free radical chain reactions (Pavlovic *et al*., 2005).

According to Devi *et al*. (2000), in the process of cell metabolism including spermatozoa, free radicals in the form of oxygen derivatives will be produced, including single oxygen ($1O_2$), triplet oxygen ($3O_2$), superoxide anion ($.O_2^-$), hydroxyl radical (.OH) and nitric oxide ($.NO^-$) all of which are called reactive oxygen species (ROS). Single oxygen can damage the double bonds in fatty acids that can damage deoxyribonucleic acid (DNA) and protein.

Free radicals will take electrons from unsaturated fatty acids that make up the phospholipids of the plasma membrane, resulting in peroxide reaction. The effects of phospholipid peroxide on poultry spermatozoa include damaging the morphology of spermatozoa, reducing motility and causing low fertility (Long and Kramert, 2003).

Vitamin C (ascorbic acid) acts in the cytosol. Vitamin C can react immediately with superoxide anions, hydroxyl radicals, singlet oxygen and lipid peroxide. As a reducing agent, ascorbic acid will donate one electron to form a non-reactive semi-dehydroascorbate and subsequently undergo a disproportionation reaction to form an unstable dehydroascorbate. Dehydroascorbate will be degraded to form oxalic acid and treonic acid which do not harm spermatozoa, therefore helping maintain the integrity of spermatozoa cell membrane (Aurich *et al*., 1997).

Vitamin E acts in the phospholipid layer of cell membrane and serves to protect polyunsaturated fatty acids and other cell membrane components from free radical oxidation by breaking the lipid peroxide chain. Vitamin E acts by donating hydrogen ions to neutralize or reduce levels of fat peroxide (Hariyatmi, 2004).

**Period of Fertile Ducks Egg post artificial insemination with Semen which was given Vitamin C and Vitamin E**

The average fertile period of eggs collected for 20 days with the treatment of adding vitamins to different types of ducks was shown in Table 4. The results showed that the lowest average fertility period was achieved in the treatment of vitamin E in Magelang ducks (6.3±3.4 days) and the highest was in no vitamin Mojosari ducks group (19.5±0.6 days). This was presumably because semen that was not supplemented with vitamins cause lower spermatozoa motility and its energy does not run out as quickly so that spermatozoa can last longer in Sperm Storage Tubules (SST).

The interaction between the addition of vitamins and types of ducks showed no significant effect (P>0.05) on the fertile period. This is presumably because the combination of treatment between the addition of vitamins and duck variants resulted in different responses that did not have an additive effect on fertility in each variant of ducks.

The treatment of ducks had no significant effect (P>0.05) on the fertile period. This is caused by the similarity in reproductive system between Magelang female and Mojosari ducks because the two livestock are still included under one family. Spermatozoa that enter the female reproductive tract will be stored SST located in the utero-vagina junction (UVJ), the connecting channel between the uterus and vagina. There are around 25,000 SST in female ducks. Each SST can hold about 400 spermatozoa and 75% SST will contain spermatozoa that are inseminated to the hens. The length of the fertile period is largely determined by the number of spermatozoa that are lodged in SST, so that enough spermatozoa can penetrate the vitelline membrane of eggs during fertilization (Wishart, 1987).

The addition of vitamins produced a very significant effect (P<0.01) on fertile period. Addition of 400 µg/mL of vitamin C and 80 µg /mL of vitamin E each reduced the fertility period by 8 days and 13 days. (Table 5). This is thought to be caused by decreasing energy sources and higher lactic acid deposits due to the high motility of spermatozoa so that pH will drop and eventually spermatozoa will quickly die.

Vitamin C and vitamin E supplementation resulted in very significant differences (P<0.01) on fertile period. The addition of vitamin C and vitamin E in semen also shortened fertility period. This is presumably due to enhanced spermatozoa motility caused by vitamin C and vitamin E supplementation. Both of vitamins are antioxidants that protect cells from oxidation reaction of phospholipid peroxide compounds so that they can help maintain the quality of spermatozoa. The higher the motility of spermatozoa, the higher the metabolic rate of spermatozoa.

Spermatozoa move by utilizing energy from metabolism and the byproduct of the metabolism is lactic acid. Lactic acid is toxic so that it damages the function of enzymes in metabolic processes; lactic acid also decreases pH which would damage the spermatozoa cell membrane. Damage to the plasma membrane will disrupt energy supply and ultimately reduce the motility and viability of spermatozoa (Siudzinska and Lukaszewicz, 2008). The higher metabolic rate of spermatozoa will reduce the

substrates available in semen as an energy source and increases lactic acid deposits. Therefore, semen supplemented with vitamins will shorten fertility period because spermatozoa could not last longer in SST.

## CONCLUSIONS

The addition of 400 µg/mL of vitamin C and 80 µg/mL of vitamin E in Muscovy duck semen increased fertility but shortened fertile period of duck eggs after artificial insemination.

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