Impact of Aging on Sperm Quality of Sentul Roosters

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ABSTRAK

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Tujuan dari penelitian ini adalah untuk mengetahui dampak penuaan terhadap kualitas spermatozoa pejantan Sentul. Bahan yang digunakan dalam penelitian ini adalah pejantan Sentul umur 48, 58, 68 dan 78 minggu, NaCl, eosin dan aquades. Semen dikumpulkan dengan menggunakan metode pijat abdomen. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan diulang sebanyak 5 kali. Analisa statistik menunjukkan bahwa peningkatan umur pejantan memberikan pengaruh yang sangat nyata ($p<0,01$) terhadap penurunan konsistensi semen, gerak masa, konsentrasi spermatozoa dan peningkatan molitilitas spermatozoa. Penuaan pada pejantan Sentul memberikan pengaruh nyata ($P<0,05$) terhadap volume semen tetapi tidak berdampak nyata ($p>0,05$) terhadap pH semen, spermatozoa hidup dan abnormalitas spermatozoa. Rataan volume semen yang dihasilkan dalam penelitian ini berkisar antara 0,54-0,88ml; pH semen 6,80-7,12; skor gerak masa 1,60-3,00; konsentrasi spermatozoa 2,76-4,86 x109/ml; motilitas spermatozoa 66-79%; Spermatozoa hidup 91,75-93,10% dan abnormalitas spermatozoa 1,75-2,51%. Semen dari pejantan Sentul umur 48-68 minggu memiliki konsistensi yang tebal dan berwarna putih keruh. Pada umur 78 minggu didapatkan konsistensi yang bervariasi mulai dari tebal, sedang dan cair. Warna semen pada pejantan Sentul umur 78 minggu juga bervariasi antara putih bening hingga krem. Kesimpulan dari penelitian ini penuaan yang terjadi pada pejantan Sentul menyebabkan gangguan reproduksi yang ditandai dengan rendahnya kualitas spermatozoa. Kualitas spermatozoa yang terbaik dihasilkan oleh pejantan Sentul yang berumur 58-68 minggu.

Kata Kunci: Penuaan, Pejantan Sentul, Kualitas Spermatozoa

ABSTRACT

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This study was done to determine impact of aging on sperm quality of Sentul roosters. Materials used in this study were Sentul males aged 48, 58, 68 and 78 weeks, NaCl, eosin and aquades. Semen was collected by abdominal massage method. Completely randomized design (CRD) was applied in this study with 4 treatments in 5 repetitions. Statistical analysis showed that increasing age of rooster had a very significant effect ($p<0.01$) on decreasing semen consistency, mass motility, spermatozoa concentration and increasing spermatozoa motility. Aging in Sentul roosters affected semen volume significantly ($P<0.05$) but did not significantly affect ($p>0.05$): semen pH, live spermatozoa, and spermatozoa abnormalities. Average volume of semen produced in this study ranged from 0.54-0.88ml; semen pH 6.80-7.12; mass movement score 1.60-3.00; spermatozoa concentration 2.76-4.86 x109/ml; spermatozoa motility 66-79%; Live spermatozoa 91.75-93.10%, and spermatozoa abnormalities 1.75-2.51%. Semen from Sentul males aged 48-68 weeks had a thick consistency and cloudy white in color. At the age of 78 weeks, the consistency varies from thick, medium and liquid. The color of semen in Sentul males aged 78 weeks also varied from clear white to cream. It is concluded that aging in Sentul roosters causes reproductive disorders which are characterized by low sperm quality. The best quality spermatozoa were produced by Sentul roosters aged 58-68 weeks.

Key Words: Aging, Sentul Rooster, Spermatozoa Quality

INTRODUCTION

An unavoidable and inevitable life process in living things is aging. Slowly but surely the aging process that occurs in living things has an impact on the decline in the physiological functions of the organs in the body and the ability to survive and eventually leads to death (Colloca et al. 2020; Das 2021). Aging is defined as a dynamic process related to the existence of damage that comes from within and outside the individual. The theory of aging is generally based on the process of damage that occurs in the individual. This damage is caused by the influence of age or mutations in genes due to stressors or oxidative stress triggers that cause damage to cells (Colloca et al. 2020). Damage to the physiological functions of the organs in the body due to

the aging process makes the tissue unable to maintain its structure so that it loses the ability to functioning. If this kind of damage occurs in the reproductive organs, the hormone cannot be produced optimally and even stops.

Sentul chicken is one of Indonesia's original chickens that has great potential to be developed as a producer of eggs and meat. Sentul chicken originates and grows a lot in Ciamis, West Java, Indonesia (Saleh et al. 2019). The advantages of Sentul chickens compared to other types of local chickens are fast body weight growth, disease resistance, high egg production, high fertility and high hatchability. Sentul chicken is one of superior local rooster because it is able to produce spermatozoa in good quality (Ariyanti et al. 2019).

Development of poultry industry in Indonesia requires the development of technology in the field of reproduction. Artificial insemination (AI) is a technology that is widely used to increase poultry reproduction. This technology is effective to be applied in poultry industry because one male can fertilize dozens of hens (Gao et al. 2021). The success of artificial insemination in poultry depends on the quality of spermatozoa. Artificial insemination in poultry will be successful if the spermatozoa quality is good. Spermatozoa motility plays an important role in the success of the fertilization proces. In breeding industry, it is very necessary to have a selection of roosters used for artificial insemination because the quality of semen reflects its ability to fertilize (Rochmi et al. 2019; Haryuni et al. 2021). The problem that often occurs in the breeding and hatchery industry is that the reproductive performance of roosters is not optimal. The quality of rooster spermatozoa at the age of 50-55 weeks gradually decreased. In aging roosters, there are some important changes in reproductive characteristics such as decreased spermatozoa concentration, motility and antioxidant capacity (Gao et al. 2021). Research is needed to determine impact of aging on the sperm quality of Sentul roosters.

MATERIALS AND METHODS

This study was an experimental study using 20 Sentul roosters with several age variations namely: 48, 58, 68 and 78 weeks. Completely randomized design (CRD) with 4 types of treatment and 5 replications was applied in this study. Evaluation of the semen quality of Sentul's rooster was carried out macroscopically and microscopically.

Bird management and diet

Sentul roosters were placed in cages measuring 50 x 100 x 70 cm where each box contains 1 rooster. Sentul roosters used in this study had body weights ranging from 2.10 to 2.20 kg and were in good health. Feed is given in the morning at 07.00 WIB for 40 g and at 15.00 WIB in the afternoon for 60 g. Drinking water is provided ad libitum. The composition and quality of feed from Sentul roosters is presented in Table 1.

Semen collection

Semen was collected using massage methods with direction from abdomen to the cloaca. The semen collection process was initially carried out by cleaning the cloaca by tissue sprayed with 70% alcohol (Haryuni et al. 2022). Furthermore, the semen secreted was collected in a scale tube. In this study, semen was collected every 5 days. Semen quality was evaluated macroscopically and microscopically in each treatment and the results were statistically analyzed.

Parameters measure

Semen volume

Volume of semen can be determined by measuring the semen produced by Sentul roosters. The steps taken to determine the volume were: the semen that is accommodated was put in a scale tube, the number listed on the scale tube was read and then recorded as the volume of semen.

Semen color

Color of semen can be observed in several steps as follows: semen is put in a scale tube, the color of semen is observed using the five senses and recorded (Mustaqim et al. 2021). The indicators to determine the quality of spermatozoa based on the color as cream for semen with milky white color indicating that the semen has a high concentration of spermatozoa. While clear white for semen with clear color indicating that the semen has a low concentration of spermatozoa.

Semen consistency

Steps taken to determine semen consistency are as follows: semen is put in a test tube, the test tube is tilted, the test tube is then re-enforced, the movement of semen when the test tube is tilted and erected is observed carefully and the results are recorded.

Semen pH

Steps taken to determine pH of the semen are as follows: litmus paper along with a standard to see the pH value is prepared, the tip of the litmus paper is

Table 1. Composition of the diet

Ingredient	Amount	Nutrient	Amount
Corn (%)	50.28	Metabolic energy (kcal/kg)	2,700.9
Soybean meal (%)	19.00	Crude protein (%)	17.90
Rice bran (%)	15.00	Crude fiber (%)	3.65
Meat bone meal (%)	8.00	Crude fat (%)	4.58
Grit (%)	4.90	Methionine (%)	0.40
Milestone (%)	3.20	Lysine (%)	0.92
Premix (%)	0.50	Calcium (%)	3.91
Dicalcium phospate (%)	0.30	Phosphorus total (%)	0.84
Salt (%)	0.10	Phosphor available (%)	0.50
Sodium bicarbonate (%)	0.70	Sodium (%)	0.13

Calculations using Brill Formulation Software

dipped in semen, then waited for 60 seconds, the color changes that occur on the litmus paper are observed, the colors observed are matched with standard litmus paper to determine the pH and recorded.

Spermatozoa mass movement

Mass movement of spermatozoa can be observed using a microscope at 400x or 100x magnification (Usman, Tijjani et al. 2021). The movement of spermatozoa can be assessed as very good (+++) when observed under a microscope it looks like a big thick wave, many, dark and moving actively to form a thick black cloud; good (++), when observed under a microscope it looks like the form of small waves that are thin, rare, moving slowly and seem less clear; not good (+), when observed under a microscope, only the progressive motion of the individual appears and there is no visible mass collection that forms like a wave; bad (0), when observed under a microscope, the only visible movement of individuals in small numbers and looks sluggish

Spermatozoa concentration

Spermatozoa concentrations were calculated using an erythrocyte pipette and haemocytometer using a Neubauer counting chamber. The cement was sucked using an erythrocyte pipette to a scale of 0.5 then added with 3% NaCl solution and sucked up to 1.01. The solution in the pipette is shaken to make it homogeneous with the direction of movement like forming a figure of eight for 2-3 minutes. The next step is to discard the solution as much as 3 drops so that no oxygen is included. After that, the cement was dripped into the Neubauer counting chamber and covered with a cover slip. Observations were made using a microscope at 400x magnification. Spermatozoa concentration was calculated by averaging the number of spermatozoa in 5 Neubauer counting chambers and multiplied by 10^7 per milliliter. The calculation of the number of spermatozoa is done by counting the number of spermatozoa in the counting room (Usman, Tijjani et al. 2021).

$$\textbf{Concentration} = \frac{\sum \mathbf{S(A+B+C+D+E)}}{\mathbf{5}} \mathbf{x\ 10^7}$$

where S is spermatozoa in the counting cahmber, A, B, C, D and E are haemocytometer counting chambers.

Spermatozoa motility

Evaluation of spermatozoa motility was carried out using a microscope. The first stage in this observation is to make preparations by means of which the semen to be evaluated is dropped on a glass object and closed using a cover glass and then observed under a microscope with a magnification of 100x to observe the movement of spermatozoa. Spermatozoa motility is calculated by looking at the movement of spermatozoa that move actively and progressively forward. Spermatozoa motility was observed using a microscope with a magnification of 100x (Mustaqim et al. 2021).

Sperm viability

Measurement of sperm viability was carried out by dripping semen on an object glass, then 2 drops of eosin and observed under a microscope with a magnification of 400X. Indications of live spermatozoa are colorless (transparent), dead sperm will be red. The percentage of live spermatozoa was calculated using the following formula (Najafi et al. 2019).

$$\text{Sperm Viability (\%)} = \frac{\sum \text{ST} - \sum \text{LS}}{\sum \text{ST}} \text{x } 100\%$$

where ST is spermatozoa total and LS is live spermatozoa.

Spermatozoa abnormalities

Spermatozoa abnormalities in general can be observed in terms of shape (head shape, head size, tail

shape, tail size etc.) (Mustaqim et al. 2021). Spermatozoa abnormalities were evaluated by making smear preparations. Review preparations were made using 2 glass objects. The first stage of semen is diluted using 3% NaCl with a ratio of 1:4. The second stage of diluted semen is dripped on a glass object. The next step is to take an empty glass object and the tip is touched to a glass object that has been dripped with semen and lightly rubbed and then allowed to dry. The dried smear preparations were observed under a microscope at 400x magnification. The percentage of spermatozoa abnormalities can be calculated as follows:

$$\textbf{Abnormalities}\ (\%) = \frac{\sum \textbf{ST} - \sum \textbf{AS}}{\sum \textbf{Spermatozoa total}} \textbf{x}\ \textbf{100}\%$$

where ST is spermatozoa total and AS is live abnormal spermatozoa.

Experimental design

The data from the evaluation of cement quality were tabulated and statistically analyzed using a Completely Randomized Design (CRD) with 4 treatments and 5 replications. If there is a significant or very significant different effect, it will be continued with Duncan's test.

$$\mathbf{Y_{ij}} = \mu + \boldsymbol{\delta_i} + \boldsymbol{\varepsilon_{ij}}$$

where Y_{ij} is observation value of treatment i repetition j; μ is mean, δ_i is effect of treatment I; ε_{ij} is effect of error on the i^{th} treatment and j repetition; I is 1, 2, 3, 4, 5, j is 1, 2, 3, 4, 5.

RESULTS AND DISCUSSION

Semen quality that can be observed macroscopically includes semen volume, pH, consistency, color and microscopic quality includes mass movement concentration, motility, live spermatozoa and spermatozoa abnormalities. The impact of aging on Sentul males on the quality of semen is presented in Table 2.

Aging in a biological context is a complex phenomenon that is described as a decrease in the physiological function of organs (Zia et al. 2021). Aging occurs slowly and is difficult to measure qualitatively. Aging involves degenerative and biological processes (Warraich et al. 2020). Aging have an impact on cell damage, changes in cell size and the ability to survive (Rudzińska et al. 2020). Aging has an impact on damage and loss of function in organs, tissues, cells and reproductive abilities in poultry (Tabibzadeh 2021). Aging in Sentul rooster causes the cessation of growth hormone (GH) production and increased glucocorticoid hormone production. This has an impact on low ATP production due to decreased muscle mass (Figure 1). The low production of ATP causes disturbances in energy homeostasis in the brain so that the brain and hypothalamus function decreases. Under these conditions the production of *gonadotropin releasing hormone* (GnRH) decrease. GnRH functions to stimulate the anterior pituitary to synthesize and secrete gonadodropin hormones (luteneizing hormone and follicle stimulating hormone) (Shi et al. 2019). LH (*luteneizing hormone*) will stimulate the interstitial Leidig cells in the testes to synthesize and secrete the hormone testosterone. FSH (*follicle stimulating hormone*) stimulates spermatogenesis in the seminiferous tubules of the testes. Testosterone will increase the development of male sexual organs. Low production of LH and FSH due to the aging process has an impact on spermatogenesis disorders. Based on this explanation, it can be illustrated that the impact of the aging process on the spermatogenesis process is as shown in Figure 1.

Impact of aging on the macroscopic quality of Sentul rooster semen

Volume

Semen is produced by a complex process of synergistic action between the hypothalamus, pituitary gland and testes. Semen can be produced if the hypothalamus, pituitary gland and testes can work normally (Behnamifar et al. 2021). Table 2 above shows that semen production increased with increasing age of the rooster until the rooster was 68 weeks old and semen production decreased at 78 weeks of age. The increase in semen production until the rooster is 68 weeks old is related to the development of reproductive organs. Semen production can be described as a curve where semen production will increase with increasing age of the rooster and after reaching peak production and the rooster getting older the semen production decreases gradually (Khaeruddin et al. 2019; Shi et al. 2019). Aging in Sentul roosters causes disturbances in the reproductive organs and the inability to produce reproductive hormones (Figure 1). The imbalance of reproductive hormones in the male body has an impact on the production of semen in a small volume (Adeoye et al. 2018).

pH semen

The pH obtained in this study ranged from 6.80 to 7.12. Previous research also found that the pH of semen in Sentul roosters was 6.73 (Ariyanti et al. 2019) or 7.24 (Elokil et al. 2019). These results indicate that semen of this research has a good quality. Although statistically the aging process has no effect on semen pH, Table 2 shows a decrease in semen pH. Figure 1 shows that there is an increase in glucocorticoid hormone level in old

Table 2. Average macroscopic and microscopic quality of Sentul rooster semen

Variable	Age of Sentul roosters			
	48 weeks	58 weeks	68 weeks	78 weeks
Macroscopic quality				
Volume (ml)	0.54 ± 0.13[a]	0.74 ± 0.15[b]	0.88 ± 0.19[c]	0.58 ± 0.13[a]
Average pH	7.12 ± 0.16	7.12 ± 0.27	6.80 ± 0.45	6.86 ± 0.49
Consistency	100% Thick	100% Thick	100% Thick	50% liquid, 25% medium and 25% Thick
Color	Cream	Cream	Cream	Cream, clear white
Microscopic quality				
Mass movement	3.00 ± 0.00[b]	3.00 ± 0.00[b]	3.00 ± 0.00[b]	1.60 ± 0.55[a]
Concentration (10^9/ml)	4.86 ± 0.39[b]	4.66 ± 0.39[b]	4.30 ± 0.29[b]	2.76 ± 0.66[a]
Motility (%)	79.00 ± 2.24[b]	79.00 ± 2.24[b]	79.00 ± 2.24[b]	66.00 ± 5.48[a]
Sperm viability (%)	92.20 ± 1.11	92.92 ± 0.63	91.75 ± 3.20	93.10 ± 2.89
Abnormality (%)	1.75 ± 0.42	2.51 ± 0.55	2.12 ± 0.75	1.91 ± 0.41

Different superscripts in the same row indicate significant effect (P<0.05) on semen volume and a very significant effect (p<0.01) on spermatozoa mass movement, spermatozoa conscentration and motility of spermatozoa

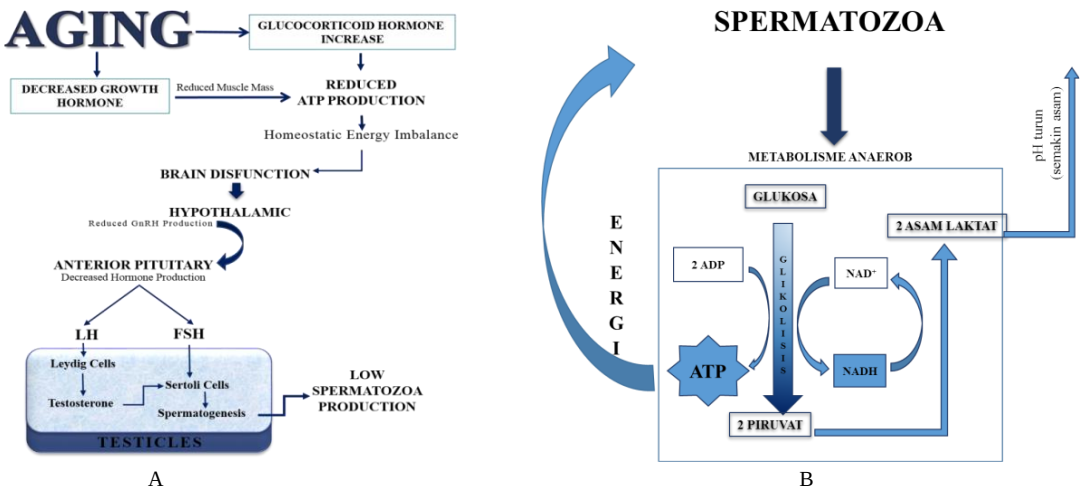

Figure 1. Impact of aging on spermatogenesis (A) and Spermatozoa anaerobic metabolism (B)

age roosters so that it has an impact on increased glucose metabolism. Spermatozoa metabolize anaerobically when they are outside the body to survive. Anaerobic metabolism produces lactic acid which will make semen more acidic. The higher metabolism causes the semen to become more acidic (Figure 2). Spermatozoa that are outside the body in an acidic state can survive only a few hours (Esguerra et al. 2020).

Consistency

Semen consistency is related to the concentration of spermatozoa. Thick semen can be used as an indicator of a high concentration of spermatozoa, however liquid semen indicates that the concentration of spermatozoa is low (Mustaqim et al. 2021). In this study, a thick consistency was found in roosters aged 48-68 weeks with 100% of the replicates showing a thick consistency. In this study, the lowest consistency was found in roosters aged 78 weeks where the consistency of the semen changed to 50% of the repeats the consistency was liquid, 25% medium and 25% thick.

Figure 1 shows that the aging is a complex metabolism that causes changes both morphologically and biochemically and has an impact on body functions that decrease irreversibly and progressively. In old rooster morphological changes occur in the testes. These morphological changes include a decrease in the volume and quantity of germ cells that have an impact on low semen quality (Gao et al. 2021). Aging causes damage to the thermostat in the body and has an impact on disruption of homeostasis in the body. Feed back system in the hypothalamus, pituitary gland and

endocrine glands work very well at a younger age (Tabibzadeh 2021).

Color

Semen color is one of the factors used as an indicator to determine the concentration. Semen that is cream color can be an indicator of a high concentration of spermatozoa. Creamy semen is normal semen and is capable of maximum fertilization (Mustaqim et al. 2021). Semen with a clear white color in a 78-week-old male indicated of low spermatozoa concentrations. Semen with a clear white color in a 78-week-old male indicated of low spermatozoa concentrations. Figure 1 shows that biologically in old roosters (78 weeks) the function of the reproductive organs decreases. Disorders that occur in the reproductive organs cause low semen quality (Inyawilert et al. 2019).

Impact of aging on the macroscopic quality of Sentul rooster semen

Mass movement

The aging process in Sentul roosters had a very significant impact ($p<0.01$) on spermatozoa mass movement. Increasing age in roosters causes an increase in polyunsaturated fatty acids in the plasma membrane and a decrease in antioxidant capacity. High lipid metabolism causes oxidative stress so that ATP production is disrupted (Gao et al. 2021). Decreased ATP production results in the inhibition of the energy source for the movement of spermatozoa (Figure 2). Energy metabolism is a key factor that supports the mass movement of spermatozoa (Qi et al. 2020). Mass movement is a wave of spermatozoa movement together in semen. Spermatozoa in a group will have a tendency to move together in the same direction. Spermatozoa are categorized as good quality if they are active. The movement of individual spermatozoa is reflected in the mass movement, the faster and more active motions of spermatozoa make the mass movement thicker and better. Spermatozoa with good quality will move actively, quickly and progressively towards the ovum. Poor mass movement was found in Sentul roosters aged 78 weeks with an average score of 1.60.

Concentration

The aging process in Sentul roosters had a very significant impact ($p<0.01$) on spermatozoa concentration. The spermatozoa concentration of Sentul roosters found was in the range of previously reported publications from 1.83-3.00 x 10^9/ml (Saleh et al. 2017). Spermatozoa concentration will decrease with increasing age of the male (Khaeruddin. & Amir 2019). The lowest concentration of spermatozoa in this study was found in roosters aged 78 weeks at 2.76x10^9/ml. This result is higher than white leghorn roosters aged 64 weeks of 2.79-2.81 x 10^9/ml.(R Richard Churchil, Lijo John 2019).

Age of the rooster plays an important role in sexual maturity and reproductive performance of the male. Reproduction can be maximized at a certain age. Aging causes degenerative changes in the hypothalamus and is followed by a failure of GnRH secretion (Shi et al. 2019). Disruption in the spermatogenesis process causes poor quality of spermatozoa and low fertility. Factors that influence spermatozoa concentration are age, feed, strain, body weight and frequency of semen collection (Esguerra et al. 2020; Usman, Tijjani et al. 2021).

Motility

Spermatozoa motility is usually used as a parameter in determining the success of the fertilization process. Spermatozoa motility has a great influence on high or low fertility rates (Rochmi et al. 2019). High fertility can be achieved when spermatozoa can move actively and progressively towards the ovum. Quantity of the motile spermatozoa can be observed from the number of active and progressive spermatozoa (Saleh & Mugiyono 2017).

Aging process in Sentul roosters had a very significant impact ($p<0.01$) on spermatozoa motility. Previous reported research found the motility of Sentul rooster spermatozoa were 68-70% (Ariyanti et al. 2019), and 83.33% (Junaedi et al. 2016). Normal spermatozoa motility in chickens was 60-80% (Sigit Mugiyono et al. 2015). The lowest motility in this study was 66% in Sentul roosters aged 68 weeks.

Spermatozoa motility is related to the availability of energy used to move towards the ovum (Rochmi 2019). Homeostatic energy disturbances that occur in old roosters (Figure 1) have an impact on lower energy supply from energy requirements. Adenosine triphosphate (ATP) is a source of energy for advancing spermatozoa resulting from anaerobic metabolic processes (Figure 2). Low ATP causes motility of spermatozoa to decrease. Aging causes a decrease in bone mineral density (BMD) which is associated with loss of trabecular and cortical bone. Another major feature of age-related bone loss is the accumulation of bone marrow fat (Romero-m et al. 2021). Another factor that affects the motility of spermatozoa are microbial contaminants, ambient temperature, cold shock, storage, oxygen and Ca ion content in semen (S. Mugiyono et al. 2015).

Sperm viability

Aging had no significant impact ($p>0.05$) on sperm viability. In previous research with Sentul rooster, it was found that the number of sperm viability were 90.44% (Asmarawati et al. 2019) and 81.72-84.37% (Ariyanti et al. 2019). In old roosters the quality of semen (viability, membrane integrity, motility and volume) produced was low (Inyawilert et al. 2019). Availability of antioxidants plays an important role in the viability of spermatozoa. In old roosters there is an increase in lipid peroxidation in the seminal plasma as a result of increased production of glucocorticoid hormones. The increase in lipid peroxidation in old roosters that is not matched by the intake of antioxidants from feed causes a decrease in the viability of spermatozoa (Hayanti et al. 2022). Viability of spermatozoa can be increased by adding antioxidants as a semen diluent. Antioxidants that can be used include vitamin C. Vitamin C can be used to protect cells from damage caused by free radicals resulting from metabolism when spermatozoa are outside the body (Esguerra et al. 2020).

Based on the report of (Nugrahini et al. 2019), the viability of spermatozoa is related to the fluid balance in the spermatozoa cell membrane. High temperatures or the presence of cold shock can cause high spermatozoa mortality. The ability of spermatozoa to survive outside the male body is influenced by temperature, light, shelf life and nutrients in semen. The acidity of the semen plays an important role in the survival of the spermatozoa (Figure 2). Semen pH that is too high or low has an impact on the death of spermatozoa (Mustaqim et al. 2021). Storage of spermatozoa in the long term causes the viability of spermatozoa to decrease (Karen et al. 2020).

Abnormality

Aging had no significant impact ($P>0.05$) on abnormalities of spermatozoa. The abnormalities of spermatozoa in this study were lower than the abnormalities of Sentul rooster spermatozoa in the previous study which reached 6.87% (Junaedi et al. 2016) and 6.79-6.82% (Ariyanti et al. 2019). Spermatozoa abnormalities have an impact on fertility. Semen has good quality if the spermatozoa abnormality is less than 20% (Saleh & Mugiyono 2017; Haryuni et al. 2021).

Spermatozoa abnormalities indicating morphological disorders of spermatozoa including a double tail, a misshapen shape, head, and crooked (Rochmi et al. 2019). Abnormalities that occur in spermatozoa in principle can be classified into 2 categories which are called primary and secondary abnormalities. Abnormalities caused by low levels of gonadotropins and testosterone are called primary abnormalities (Esguerra et al. 2020). Low levels of gonadotropins and testosterone have an impact on the disruption of the process of spermatogenesis (Figure 1) so that spermatozoa become deformed. Secondary abnormalities are influenced by environmental factors. Secondary defects can occur when spermatozoa pass through the epididymis, mishandling during seollection and damage during preparation of preparations in the laboratory (Feyisa et al. 2018).

Spermatozoa abnormalities found in this study were mostly secondary abnormalities where spermatozoa defects were found on the tail and head. Table 2 shows that in old roosters spermatoza abnormalities were higher than in young roosters (48 weeks). Spermatozoa in old roosters tend to be easily damaged due to the process of spermatogenesis that is not optimal which has an impact on the formation of spermatozoa walls that are susceptible to damage. Aging that occurs in rooster causes a decrease in the production of the LH (*luteneizing hormone*) and FSH (*follicle stimulating hormone*) (Shi et al. 2019).

CONCLUSION

Aging that occurs in Sentul roosters causes reproductive disorders which are characterized by low sperm quality. The best quality spermatozoa are produced by Sentul roosters aged 58-68 weeks.

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