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Research Article

Fertility Rate, Fertility Period, and DOC Sex Ratio of Kokok Balenggek Chicken after Artificial Insemination

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Abstract | This study aims to evaluate the characteristics of fresh semen of the Kokok Balenggek chicken (KBC) as well as fertility, fertile period, and sex ratio as a result of artificial insemination with variations in spermatozoa concentrations. Semen from 12 KBC was collected and evaluated to determine their characteristics (motility, viability, concentration, and abnormalities). Semen showing motility >70% was diluted into 500µl of Ringer's lactate solution with various concentrations of spermatozoa, namely 50×10^6, 100×10^6, and 150×10^6. Fertile period and fertility were evaluated by inseminating spermatozoa in hens. Eggs were collected two days after insemination and observed for embryo development using the candling method. Observation of fresh semen of the KBC showed that macroscopically the semen of the KBC was white, thick, and had a volume of 415 ± 218.08 µl. While the results of the microscopic evaluation showed that the semen quality of the KBC was a positive mean mass movement of 2 (++), spermatozoa motility was 79.71 ± 2.91 %, spermatozoa concentration was 2432.34 ± 668.08×106, spermatozoa viability was 93.06 ± 5.61 % and spermatozoa abnormality was 15.67 ±7.07 %. Insemination using a concentration of 150×10^6 spermatozoa significantly ($P<0.05$) increased fertility (50.87±4.92 %) and fertile period (6.05±4.75 days) and did not affect the sex ratio. Combination research on spermatozoa concentrations and insemination time intervals needs to be carried out to optimize fertility and the fertile period of spermatozoa in KBC.

Keywords | KBC, Artificial insemination, Fertility, Fertile period, Sex ratio.

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INTRODUCTION

Indonesia is a country that has a very high diversity of local chickens. In addition to producing meat and eggs, Indonesia local chickens are also often used as fighting chickens, ornamental chickens, superior breed chickens, and singing chickens with a different crowing sound from other chickens. There are several types of singing chickens that have long been developed and are popular with the public, such as Pelung Chicken in West Java, Bekisar Chicken in East Java, Ketawa Chicken in South Sulawesi and Kokok Balenggek Chicken (KBC) in West Sumatra. KBC is a germplasm that must be preserved, this chicken is a cross between the Red Junglefowl (*Gallus gallus*) and the local chicken (Rusfidra, 2004). The KBC population is suspected of continuing to decline. The latest report by Abbas et al. (2014) stated that the adult KBC population counted 189 and 271 young male chickens in the in-situ area and the highest population was in Kenagarian Rangkiang Luluih, Tigo Lurah District, Solok Regency, West Sumatra. Mukhdi et al. (2011) noted that the migration of KBC out of the area reached 30 individuals every month and was one of the reasons for the decline in the KBC population. Therefore, conservation is important to in-

crease productivity and population.

Artificial insemination (AI) is one of the most widely used assisted reproductive technologies (ART) in livestock and aims to increase the population and genetic quality of livestock (Arifiantini, 2012). The advantages obtained after carrying out AI are the efficiency of using males for the mating process and can increase in fertility. The low KBC population in Indonesia makes the AI application essential to do. However, approximately only 1% of spermatozoa from a dose of AI will enter the sperm storage tubular (SST) at the uterovaginal junction. At the same time, the rest are squeezed out of the vagina (Steele and Wishart, 1996), so it is essential to do a study to find the correct dose to be inseminated. The number of sperm in the SST will be sufficient for fertilization for approximately two weeks. Chicken sperm production according to Garner and Hafez (2000) is around 0.2 ml to 0.5 ml with an average of 0.25 ml per ejaculation. The small volume of chicken sperm must be dosed correctly so that not a lot of sperm is wasted. The dose of AI in chickens using fresh sperm varied widely between $70x10^6$/ml (Cerolini et al., 1997) to $200x10^6$/ml (Blesbois et al., 2005). The AI dose in chickens according to Brillard (2003) is $150x10^6$/ml with a frequency of once a week. Castillo et al. (2010) also conducted a spermatozoa fertility test on breeding chicks using AI at 4-day intervals. Blanco et al. (2009) performed AI on storks with a dose of $15x10^6$ motile spermatozoa with a frequency of 3 times or more per week. This business produces fertility of 80%. Another study by Gumułka and Kapkowska (2005) used a dose of $125x10^6$/0.06 ml and AI was done 12 to 13 hours after sunrise. AI success indicators can be seen in the fertility rate by looking at the number of fertilized eggs. According to Darmana and Sitanggang (2003), fertilization is the success of one spermatozoa cell to fertilize one egg, which then forms a zygote that will develop into an embryo. The above studies have not specifically reported the effect of the sperm concentration of artificially inseminated on the fertilization rate of KBC spermatozoa. In addition, the fertile period and sex ratio were also measured in this study.

MATERIALS AND METHODS

Ethic statements

This experiment was conducted according to the Guideline for ethics study of experimental animals based on the law of Republic of Indonesia number 18 of 2009 about Animal Livestock and animal husbandry.

Source of Semen and Eggs

Semen comes from 12 male Kokok Balenggek Chicken (KBC) aged ± 1-1.5 years. Each of them collected semen 2 times a week. Roosters are placed in individual cages and fed as much as 100g/head/day which is given in the morning and evening. A fertility test was carried out using 60 female KBC aged ± 1-1.5 years.

Evaluation of Fresh Semen of Kokok Balenggek Chicken (KBC)

Semen collection was repeated 3 times using the abdominal massage method and was collected using a 1.5 mL microtube. Semen quality includes macroscopic; volume, pH, color, and consistency. Microscopic quality evaluated mass movement, sperm motility, viability, morphology, and sperm concentration. The evaluation procedure refers to Arifiantini (2012) which is adapted for poultry semen. Sperm motility was evaluated by diluting semen with Ringer's lactate solution, homogenized, and viewed using a microscope. Sperm motility was assessed from five visual fields and values were expressed in percent. Sperm morphology and viability were stained with eosin nigrosin. Sperm concentration was calculated using a Neubauer chamber. Microscopic evaluation using an Olympus microscope (CX 23).

Artificial insemination

The results of calculating the volume of each diluent are then mixed in the tube with the sperm, which has been divided according to the number of treatments. There were three treatments with AI doses, namely $50x10^6$/0.5 ml (dose 1), $100x10^6$/0.5 ml (dose 2), and $150x10^6$/0.5 ml (dose 3). The diluent used was Ringer's Lactate solution. Hens are Inseminated shortly after the sperm is diluted. AI is carried out in the afternoon at around 16.00 WIB using sperm from the shelter just before.

The hens used were two years old with an average weight of 1.5 - 2 kg. Hens are placed in individual cages. Insemination is carried out by the intra-vaginal method (sperm is injected into the vagina with a depth of 2 to 4 cm). Each hen was inseminated once with 15 replicates (individual) for each treatment (45 hens used). Insemination is done once to find out how long it takes for the spermatozoa to fertilize the egg in the hen's reproductive tract (Fertil Period). Implementing AI involves cleaning the dirt that sticks to the cloaca and its surroundings using cleaning wipes. The part of the chicken's body below the cloaca is pressed until the reproductive tract (left) and feces can be seen (right). The diluted sperm is sucked up with a syringe without a needle as much as 0.1 ml and then inserted into the female reproductive organ. In the same way, AI was carried out at different treatment doses.

Egg Collection and Fertility Egg Examination

The eggs used as hatching eggs are produced within 15 days from the second day of the first AI. A good hatching egg has the following requirements: oval, not deformed,

and has a shell that is not too thick or thin. Eggs are stored for five days in the storage room before being put in the incubator. The incubator used is a manual incubator type C 100 with a capacity of 100 eggs. The inside of the incubator is sprayed with disinfectant the day before the eggs are placed in the incubator to maintain their sterility. The eggs are arranged on an egg-holding rack with an inclined position of approximately 60°, and the blunt part is at the top. Eggs are put in bulk in the incubator. The temperature of the incubator is set in the range of 38-39°C with a humidity of 45-60%. The eggs put in the hatching machine are marked to make it easier to turn the eggs so that they are evenly distributed. Egg turning is done from the 4th day of incubation to the 18th day, at least thrice in 24 hours. On the 10th day, candling was carried out to determine egg fertility. The fertile period of spermatozoa is the length of time spermatozoa are in the female reproductive tract and can still fertilize an ovum in one artificial insemination. This parameter was calculated from the second day after insemination until the eggs were not fertile, observed over 14 days. Hatching eggs not hatched after 22 days are broken up to observe their fertility. The number of infertile, fertile, and dead eggs was calculated for each treatment. Then the results obtained are calculated by the formula so that the fertility percentage can be known.

DOC Sex Ratio

The sex of the DOC can be identified based on the feathers on the wings. Male DOCs have wing feathers that are the same length, while female DOCs have wing feathers that are not the same length (Iswati et al., 2020). The sex ratio is known by counting the number of male DOC and female DOC from 1 female KBC.

Data Analysis

This study used a completely randomized design (CRD). All data were transformed using a variance. If there is a difference between the treatments, it is continued with the Least Significant Different multiple comparison tests and Duncan's test using a significance level of 95%. The data were then analyzed descriptively.

RESULT AND DISCUSSION

Fresh Semen Quality of Kokok Balenggek Chicken (KBC)

The quality of fresh semen of the Kokok Balenggek chickens (KBC) observed in this study included macroscopic qualities, namely volume, color, pH, odor, and consistency, and microscopic qualities, namely mass movement, motility, concentration, viability, and abnormality. The average volume of semen of KBC is 415 µL, pH 7.46, has a characteristic odor, and has a thick consistency (Table 1).

The results of the microscopic evaluation of the semen of the KBC: the wave motion showed a positive average of 2 (++), 79.71% semen motility, 2432×10^6 concentration, 93.06% viability, and 15.67% abnormality.

The male-female Kokok Balenggek Chicken (KBC) ratio in West Sumatra is still relatively low, namely 1:1.7 (Rusfidra et al., 2015). This makes male KBC require good fertilization ability to increase the population, so it is essential to evaluate the quality and quantity of fresh KBC semen (Table 1). Based on a search conducted by Rusfidra (2006), there are at least three types of singing chickens in Indonesia: the KBC chicken, the Pelung chicken, and the Bekisar chicken, which are descended from the Green Forest chicken. In this study, the average semen volume was higher, namely 0.415 mL/ejaculate, when compared to the Pelung chicken reported by Junaedi & Husnaeni (2019), which was 0.23 mL/ejaculate and lower than the evaluation results reported by Kusuma et al. (2018) which is as much as 0.46 mL. Furthermore, the results of this study were also higher when compared to the semen of the Green Forest chicken, which was 0.15 ± 0.05 mL/ejaculate (Andaruisworo and Yuniati, 2021). Semen volume variations can vary depending on the nation, type, storage method (Hijriyanto et al., 2017), age, body size, nutrition, and environmental temperature (Almahdi et al., 2014).

Table 1: Fresh semen quality of the Kokok Balenggek chicken

Parameter	Average
Volume (µL)	415 ± 218.08
Color	White
pH	7.46 ± 0.53
Consistency	Thick
Concentration (10^6)	2432.34 ± 668.08
Wave motion	2.29 ± 0.76
Sperm Motility (%)	79.71 ± 2.91
Sperm Viability (%)	93.06 ± 5.61
Sperm Abnormality (%)	15.67 ± 7.07

Furthermore, the observations in this study found that the color and consistency of semen in KBC were classified as usual, white and thick. The consistency and color of KBC semen in this study tended to be the same as the Pelung chicken (Junaedi & Husnaeni, 2019) but slightly different from the Green Jungle chicken. In contrast, Andaruisworo & Yuniati (2021) reported that the semen of the Green Jungle chicken was white. Color and consistency illustrate the concentration of spermatozoa (Almahdi et al., 2014). Semen with color white milk it means that semen contain of a lot of spermatozoa and have good quality. According to Forman et al. (2013) chicken semen generally white to yellow. Meanwhile, according Sturkie (2012), macroscopic,

Table 2: Effect of spermatozoa concentration on fertility rate, hatchability, and sex ratio of Kokok Balenggek chickens.

Parameter	Sperm Concentration		
	50×10^6	100×10^6	150×10^6
Fertility rate (%)	38.76±3.85[a]	41.67±4.27[b]	50.87±4.92[c]
Fertility period (day)	4.8±3.49[a]	5±4.38[a]	6.05±4.75[b]
Hatchability (%)	77.78±2.51[a]	72.92±2.08[ab]	66.67±2.79[b]
Sex Ratio			
Male DOC (%)	50±4.47[a]	58.33±5.01[b]	58.33±4.92[b]
Female DOC (%)	50±4.47[a]	41.67±5.01[b]	41.67±4.92[b]

different letters [a], [b], and [c] in the same row indicate differences in each treatment group in the concentration of spermatozoa ($P<0.05$).

semen quality chicken and not bright white opaque, while semen had much lower concentrations of sperm mixed with clear liquid, white light and watery.

This study found that the average concentration of spermatozoa in KBC was 2432 million/ejaculate. This result is higher when compared to the concentration of spermatozoa in the Pelung chicken, which is 5043 million/mL or 1160/ejaculate (Junaedi & Husnaeni, 2019), and higher when compared to the Green Forest chicken, which is 898 million/mL or 61.96 million/ejaculation (Bebas and Laksmi, 2013). The concentration of spermatozoa plays an essential role in the reproductive success of poultry. Wishart & Staines (1999) said that less than 2% of the spermatozoa enter the sperm storage (SST) in the uterovaginal junction canal, while the rest will be pressed out of the vagina so that high concentrations will increase the number of spermatozoa that will enter the SST. Meanwhile, Arifiantini (2012) explained that the success of AI is closely related to the fertility of spermatozoa, one of which is influenced by the concentration of spermatozoa.

The KBC pH value obtained in this study tends to be the same, namely, 7.46 when compared to the Pelung chicken, pH 7.2 (Kusuma et al., 2018) and the Green Forest chicken, which is pH 7 (Andaruisworo & Yuniati, 2021). The degree of acidity (pH) is essential to know because it will affect the viability of spermatozoa, where the viability of spermatozoa will decrease as the pH decreases. Differences in the degree of acidity can be influenced by individual differences, motility, and metabolism of spermatozoa (Mphaphathi et al., 2016).

The average motility of KBC spermatozoa shown in this study was 79.71%. This result is lower when compared to the results reported by Junaedi & Husnaeni (2019) on Pelung chickens, namely 84.69%, and Kusuma et al. (2018), namely 86%. However, it is higher when compared to the Green Forest chicken, which is 45% (Andaruisworo & Yuniati, 2021). It is essential to know the motility of spermatozoa, considering that motility will significantly affect the speed of spermatozoa in the female reproductive tract so that it can increase fertilization rates (Danang et al., 2012). The viability of KBC fresh semen in this study was 93.06%. This result is lower when compared to the Pelung chicken, which is 89.17% (Junaedi & Husnaeni, 2019) and higher when compared to the Green Forest chicken, which is 30% (Andaruisworo & Yuniati, 2021). Viability and abnormality are needed in assessing the quality of spermatozoa because of their role in the success of fertilization, especially in determining the success of insemination. Solihati et al. (2006) said that the minimum standard required for viability is ≥ 45%, with motility ≥ 40% to support the success of insemination in chickens.

Fertility rate and fertility period of KBC Spermatozoa

The fertile period was calculated from the eggs collected on the 2nd day after the AI until the 19th day. Fertile eggs were observed using the candling method on the 7th day. On the seventh day, the embryo has passed its critical stage and the blood vessels are visible. The fertile period was observed in 60 chickens which were successfully fertilized with 20 chickens each per treatment. Fertilized eggs in inseminated chickens used a concentration of 150×10^6 spermatozoa more than in inseminated chickens with 100×10^6 and 50×10^6 spermatozoa (Table 2). The number of eggs fertilized by spermatozoa with concentrations of 50×10^6 was 50.87±4.92%, while the number of eggs fertilized by spermatozoa with concentrations of 100×10^6 and 100×10^6 respectively was 41.67±4.27% and 38.76±3.85%. The results of the fertility test in the group of chickens with a concentration of 50×10^6 spermatozoa were good because the fertility of spermatozoa from fresh semen of chickens was around 44%-66% (Asmarawati et al., 2013) or 62%-77% (Long and Kramer, 2003).

Table 2 shows that the spermatozoa of the KBC which were diluted using a concentration of 150×10^6 spermatozoa had a longer fertile period, namely 6.05±4.75 days, while the spermatozoa which were diluted using a concentration of spermatozoa 50×10^6 and 100×10^6 respectively only reached 4.8 ± 3.49 and 5±4.38 days. The analysis of variance (Table 2) showed that variations in spermatozoa

concentrations significantly affected the fertility of KBC. The reason for this is the sufficient concentration of spermatozoa that can enter the tubular sperm storage (SST) to fertilize further the ovum, namely at a concentration of 150 million spermatozoa. This can be compared with the average fertility between 100 million spermatozoa and 50 million spermatozoa concentrations. Thus, the more concentrations used, the higher the fertility produced.

The difference between the fertile period and the number of eggs collected is due to the pause day in chickens which causes the chickens to temporarily stop laying eggs during the laying period. In addition, the fertile period and fertility of spermatozoa in chickens can be influenced by many factors. The quality of the inseminated spermatozoa and the number of sperm storage tubules (SST) at the utero vaginal junction (UVJ) can be factors for successful fertilization. Bakst et al. (2010); Birkhead and Brillard (2007) concluded that the main causes of fertilization failure in poultry are the ability of spermatozoa to reach the SST, failure of spermatozoa to enter or leave the SST, the ability of spermatozoa to reach a fertilization site in the infundibulum and the ability of spermatozoa to penetrate the ovum. This could be caused by the quality of the inseminated spermatozoa. In addition, the number of SSTs also correlates with the fertile period and duration of fertilization (Bakst et al., 2010). It was further explained that the number of SSTs determines the storage capacity of spermatozoa in the female reproductive tract. In addition, the concentration of spermatozoa also determines the success of fertilization (Asmarawati et al., 2013). A high number of spermatozoa has high success in achieving SST because during the journey towards SST spermatozoa undergo selection in the female reproductive tract.

This study also measured the egg hatchability of the KBC, although so far there has not been found a link between sperm concentration and egg hatchability. Studies on the hatchability of inseminated eggs are more concerned with the frequency of insemination than the volume or concentration of inseminated spermatozoa. However, this also results in increased labor costs and inefficiencies in terms of time (Hughes, 1978). Further studies need to be carried out to increase the fertility and hatchability of the KBC eggs produced by artificial insemination (Hughes, 1978).

In general, the theoretical sex ratio of offspring in poultry is 50:50 (1:1). In this study, the male:female DOC ratios were 50:50 (50×10^6), 58:42 (100×10^6), and 58:42 (150×10^6) respectively. These results are expected to provide an overview for breeders to determine the appropriate concentration of spermatozoa before insemination. The determination of the sex of Kokok Balenggek chicks has never been reported before. In this study, the determination of the type of Kokok Balenggek chicken during DOC was based on the shape of the feathers (Figure 1). Meanwhile, in Arabic chickens, sex determination was not carried out when the DOC hatched because the newly hatched Arabic chickens were almost monomorphic so that males and females could not be distinguished and according to previous studies, sexing uses feather sexing only on newly hatched Arabic chicks has an accuracy of 80.47% (Iswati et al., 2020).

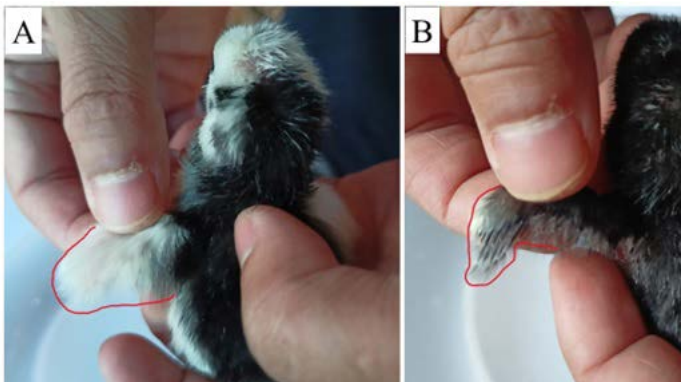

Figure 1: DOC of the Kokok Balenggek chicken; A. Male; B. Female

CONCLUSION

Using a concentration of 150×10^6 spermatozoa can significantly increase fertility and the fertile period of KBC. At the same time, higher hatchability was produced in the insemination treatment with a concentration of 50×10^6 spermatozoa. The sexing results showed that DOC males were more inseminated using 100×10^6 and 150×10^6 spermatozoa concentrations.

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CONFLICT OF INTEREST

All authors declared that there is no conflict of interest.

NOVELTY STATEMENT

This present study highlights the reproductive characteristics of the Kokok Balenggek chicken based on the results of artificial insemination with variations in spermatozoa concentrations.

AUTHORS CONTRIBUTION

The study was carried out by J, A, HG, RSW, and FAC, and all authors contributed equally. In addition, R and KS made essential contributions to the organization and analysis of the data for this work. All authors above consented to be held responsible for all parts of the work and participated in its preparation, drafting, and revision. They also provided final permission for the version that was published.

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