Effect of Number of Spermatozoa, Oviduct Condition and Timing of Artificial Insemination on Fertility and Fertile Period of Kampung Rooster Spermatozoa

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Abstract. This study was carried out to determine the optimum fertility and fertile period using the number of spermatozoa, oviduct condition and timing of insemination of native rooster spermatozoa. Ninety six commercial Isa brown pullets and nine kampung roosters were used in this study in a 3×2×2 factorial arrangement with one bird in a cage constituting a unit. The factor levels were the number of spermatozoa (50, 100 and 150 million/0.1 ml), oviduct condition (hard-shelled eggs and free hard-shelled eggs), and timing of artificial insemination (in the morning, at 7 AM and in the afternoon, at 4 PM). The results showed that among the treatments there was no significant interaction to fertility and fertile period. Insemination with 50 million sperm number seemed to be the same result with the other 2 treatments. Oviduct condition had a highly significant difference on fertility and fertile period percentage, and timing of insemination did not differ between morning and afternoon. In conclusion, the only oviduct condition (free hard-shelled eggs) was the best results for insemination in terms of fertility and fertile period of native roosters. It is recommended that for the maximum fertility and fertile period, hens should be inseminated with 50 million spermatozoa, free of hard-shelled eggs and insemination performed in the morning or in the afternoon.

Keywords: timing of artificial insemination, fertility, fertile period, semen dose, oviduct condition

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Abstrak. Penelitian bertujuan untuk mengetahui tingkat fertilitas dan periode fertil yang optimum dari spermatozoa ayam kampung dengan perlakuan beberapa jumlah spermatozoa, kondisi uterus dan waktu inseminasi. Sembilan puluh enam ayam betina Isa Brown dan sembilan ekor ayam kampung jantan digunakan dalam penelitian ini dengan pola faktorial 3x2x2. Faktor perlakuan jumlah spermatozoa (50, 100 dan 150 juta per 0,1 ml), kondisi uterus (ada telur kerabang keras dan tidak ada telur) di uterus, dan waktu inseminasi (pagi hari pukul 07.00 dan sore hari pukul 16.00). Hasil penelitian menunjukkan bahwa tidak ada pengaruh interaksi antar perlakuan. Inseminasi dengan jumlah spermatozoa 50 juta hasilnya hampir sama dengan perlakuan jumlah spermatozoa 100 dan 150 juta. Kondisi uterus memiliki perbedaan yang sangat nyata terhadap persentase fertilitas dan periode fertil, dan waktu inseminasi tidak menunjukkan perbedaan terhadap persentase fertilitas dan periode fertil. Kesimpulannya adalah hanya kondisi uterus (uterus tanpa telur kerabang keras) yang menghasilkan fertilitas dan periode fertil paling baik. Saran untuk menghasilkan fertilitas dan periode fertil yang maksimum, maka ayam betina sebaiknya di inseminasi cukup dengan dosis 50 juta spermatozoa, sewaktu tidak ada telur berkerabang keras di uterus, dan waktu pelaksanaan inseminasi bisa pagi hari ataupun sore hari.

Kata kunci: waktu inseminasi, fertilitas, masa subur, dosis semen, kondisi uterus

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Introduction

It is no doubt that artificial insemination in poultry is more beneficial when compared to natural mating (Penfold et al*.,* 2000; Brillard, 2003). Some of the advantages of artificial insemination include increased mating ratio, the use of older males from outstanding performers, being able to use an injured bird and for cross breeding (Martin, 2004; Saleh and Sugiyatno, 2006). To achieve the optimum success of artificial insemination, then several

factors need to be considered, such as: sperm quality and quantity, sperm dosage, depth of insemination, frequency and timing of artificial insemination (Lake, 1978; Van Krey and Siegel, 1980; King et al., 2002).

Period of artificial insemination in a given poultry breeder house should not be done in 1-3 hours around the laying time for maximum insemination results (Brillard, 2003). However, Saleh and Sugiyatno (2006) have shown that spermatozoa deposited 1-2 hours just after oviposition, and insemination was done in the afternoon (at 16.00 h, Indonesian time) gave a maximum fertility results.Insemination timeaffects fertility in Shikabrown breeder hens and this was probably mediated by meteorological factors. They concluded that for better fertility of Shikabrown hens, insemination should be carried out at 10:00 h, Nigeria time (Obidi et al., 2008).The sperm numbers affect the fertility of eggs. The maximum fertility and hatchability were achieved by inseminating hens with at least 100 million spermatozoa (Mauldin, 2009; Tabatabaei, 2010).

The environment in the oviduct is most favorable for sperm cells when the hen is in good laying condition, and at this time the oviduct is most receptive to semen just after oviposistion. Moreover, they recommended that for the best fertility hens, insemination should be performed at time of day (morning and afternoon) when most hens are without hard-shelled eggs in the shell gland (Lake and Stewart, 1978).

The objective of this study was to contribute further knowledge regarding the number of spermatozoa, oviduct condition and timing of insemination for maximum fertility and fertile period in Nativechickens.

Materials and Methods

Location. This study was carried out at the experimental farm and laboratory of physiology and reproduction of Jenderal Soedirman University Purwokerto, Indonesia.

Experimental animals and semen collection Ninety six commercial Isa brown pullets about 35 weeks old were randomly housed in cages, one bird per cage, in a unit of 3×2×2 factorial arrangement with one bird in a cage. The factor levels were the number of spermatozoa (50, 100 and 150 million/0.1 ml), oviduct condition namely hard-shelled eggs and non hard-shelled eggs), and timing of artificial insemination (7 AM and 4 PM). Semen was extracted by massage technique from nine local roosters. Both pullets and cocks were fed on commercial layer ration (18% crude protein and 11.10 MJ ME/kg, 110 g/chicken/day and had water available in ad libitum).

Semen dilution and insemination. An average of 0.32 ml semen per kampung rooster was pooled and a portion was diluted to the required ratios with Ringer solution, then divided in 3 parts in which every 0.1 ml of diluted semen contained 50, 100 and 150 million spermatozoa, respectively. Two groups of pullets containing hard-shelled eggs and without hard-shelled eggs were inseminated at around 7.00 h and 4.00 h local time. Inseminations were completed within 30 minutes from the semen collection time.

Semen quality tests. Sperm motility in diluted samples was subjectively rated on a scale of 0 (no motility) to 100 (vigorous motility) on examination under a light microscope, both before and after insemination. Calculation of spermatozoa in all samples were done with an Albert Sass haemocytometer.

Egg collection and incubation. Collection of eggs was started around 48 h after the first insemination and daily to day 21. Eggs were properly marked, stored at room temperature (about 25°C). Every 5 days, the selected eggs were incubated for 7 days followed by candling to assess fertility and fertile period. Eggs

containing embryos, alive or dead, were regarded as fertile. Candle fertility was computed as the ratio of the number of fertile eggs to the total number of egg sets, expressed as a percentage.

Data analysis. Untransformed percentage data (fertility and fertile period) were analyzed, since preliminary analysis did not indicate any need for transformation. Data were subjected to a three factor analysis of variance using Steel and Torrie (1994).

Results and Discussion

Effect of semen dosage on percent fertility and fertile period

The results of the fertility and fertile period in days following single inseminations of the different doses of semen are presented in Table 1. There is indication that fertility increased along with the increasing number of spermatozoa. The highest fertility (56.89±4.83%) was obtained from a 150 dose of semen. However, this fertility value was not significantly different from the other two semen dosages.

Contrary to the results, Tabatabaei (2010) and Brillard and McDaniel (1986) reported that the maximum fertility of eggs was achieved with the use of 100 and 200 million spermatozoa, respectively, not 50 million spermatozoa. However, this result was in agreement with the work of Kim et al. (1974) and Sexton (1977) which used sperm concentration of 50 – 100 million showing adequacy for good fertility in chickens and turkeys.

Mean fertile period in different semen dosage is shown in Table 1. The duration of fertile period ranged between 13.98–15.54 days. The higher the number of spermatozoa inseminated the longer the fertile period. However, statistically the treatment dose was not significantly different (P>0.05). The results of this study were not much different from the those of Lake (1978), using 100 million chicken spermatozoa per insemination and long fertile period ranged from 12 and 14 days. Wishart (1987) did not find that the number of spermatozoa in a series of eggs decreased until the 11^{th} or 12^{th} day after insemination, after which number fell to zero by the 15^{th} day. Furthermore, Wishart (1987); Brillard and Antoni, (1990) stated that the length of fertile period in chickens is determined by the number of spermatozoa that enter the SST (sperm storage tubules).

Effect of oviduct condition on percent fertility and fertile period

Average percentage fertility in hens with hard-shelled eggs and non hard shelled eggs when inseminated is listed in Table 1. The highest fertility value (59.56%) was noted in the group of hens inseminated non hard-shelled eggs, and showed significant difference (P<0.01) from the group of hens inseminated containing hard shell eggs (46.09%). The result was consistent with the results of research conducted by Brate and Ibe (1989), Saleh and Sugiyatno (2006), Obidi et al. (2008), Tabatabaei et al. (2010) that insemination should be done when condition of hen's oviduct was of no hard-shelled eggs.

Mean fertile period presented in Table 1 showed that oviduct condition in hens had a highly significant (P<0.01) effect on fertile period. The highest fertile period was obtained when the uterine of hens was free of hard-shelled eggs. These results were in agreement with Saleh and Sugiyatno (2006); Wishart (1987); and Obidi et al. (2008) that sperm numbers may affect the in vivo storage of spermatozoa, subsequently the fertile period. Brillard (2003), Donoghue and Wishart (2000) stated that mechanism of sperm storage and slow release assured a succession of fertilized eggs in the absence of repeated copulation or artificial insemination.

Table 1.Percentage fertility and fertile period from each treatment factor (mean±sd)

Factors		Fertility (%)	Fertile period (day)
Semen dose	50 million	49.86±4.17	13.98±1.22
	100 million	54.45±3.49	14.24±0.88
	150 million	56.89±4.83	15.54±1.30
Oviduct condition	No hard-shelled eggs	59.56±2.49[a]	16.10±0.69[a]
	Hard-shelled eggs	46.09±2.70[b]	12.43±0.67[b]
Insemination time	07.00 AM	55.56±4.77	14.74±1.00
	04.00 PM	56.87±3.22	15.15±0.64

Values bearing different superscript at the same column differ significantly (P<0.01)

Effect of timing of insemination on percent fertility and fertile period

Table 1 shows the difference of fertility rate between treatment groups inseminated in the morning and in the afternoon (55.56±4.77 and 56.87±3.22%), respectively was not significant (P>0.05). In this study, fertility in eggs laid by hens inseminated in the morning was almost the same with those in the afternoon. A study by Saleh and Sugiyatno (2006) reported that the maximum fertility of eggs was achieved when hens were inseminated in the afternoon, when the most oviducts were free of hard-shelled eggs. Obidi et al. (2008) reported that timing of artificial insemination influenced fertility in Shikabrown breeder hens and this was probably mediated by meteorological factors. In this study, the hens were both inseminated in the morning and in the afternoon when their uterine were absolutely free of hard-shell eggs.

Mean fertile period as shown in Table 1, hens were inseminated in the morning and in the afternoon were 14.74±1.00 and 15.15±0.64 d, respectively (P>0.05). Brillard (2003) stated that the length of fertile period depends on the sperm storage in the tubules at the utero-vaginal junction where the spermatozoa are released for movement toward the infundibulum for ova fertilization.

Conclusion

The results of this study concluded that insemination with 50 million sperm, no hard-shelled eggs in the morning or afternoon would be adequate for achieving optimum fertility and fertile period.

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