Fertility duration of commercial laying hen inseminated with native chicken semen

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Abstract. Duration of fertility is one of the most influential factors to define the accurate frequency of artificial insemination to produce crossbred native chicken. This research conducted to determine the fertility duration of Commercial Laying Hen which inseminated using Indonesian Native Chicken Semen. Semen was collected from three rooster, 12-month-old, of native chicken, pooled and evaluated before dilution using physiological saline 0.85% NaCl solution. Eight laying hens were inseminated at dose 75 x 10⁶ per 0.1 mL for each, followed with eggs collection from 2nd day until 16th day after insemination then incubated for 21 days. The result showed that eggs fertility remained high for the first week and dropped during the second week after insemination. The average duration of fertility in laying hens inseminated was 6.5 days and the average percentage of egg fertility was 49.1 ± 18.9%.

1. Introduction

Artificial insemination (AI) is the manual transfer of semen into the female’s vagina. Basically, it is consisting of two steps procedure: first, collecting semen from the male and second, inseminating the semen into the female [1]. Artificial insemination in poultry is more beneficial when compared to natural mating [2]. Some of the advantages of artificial insemination in the poultry are: 1). increased mating ratio: normally one rooster can mate to six to ten hens, however with artificial insemination this ratio could be increased fourfold; 2). Older males which having outstanding performance can be used for several generations, whereas under natural mating their useful life is limited; 3). Valuable rooster having the leg injury can still be used for artificial insemination; 4). Elimination of preferential mating: when there is poor fertility caused by preferential mating, it can be eliminated; 5). Successful cross breeding: although cross breeding is very successful under natural conditions, but sometimes there is a kind of color discrimination as some hens will not mate with a male of a different color unless they have been reared together[3].

Fertile period is a certain period when hens can produce fertile eggs after inseminated or copulated [4]. Spermatozoa that enter the reproductive tract of female after natural mating or Artificial Insemination (AI) will enter into Sperm Storage Tubules (SST) at Utero Vaginal Junction (UVJ) and will release for fertilization during the fertile period [5]. Spermatozoa in the SST can survive for several days until within a few weeks [6]. Some researchers have found that chicken sperm can live in the female reproductive tract for several weeks, but eggs fertility will be decreasing after a few weeks also. Insemination in chickens requires supporting knowledge about the right frequency and dosage to maintain the egg fertility for practical and economical purposes. The objective of this study was to...
determine the fertile period after single AI dose in hens. Increasing AI administration for chicken rearing is expected to reduce rooster maintenance costs with reduce the number of males compare to in natural mating.

2. Material and methods

2.1. Materials
Semen was obtained from three rooster native chicken aged 56 weeks which were reared in individual cages. Eight Lohmann Brown layer hens aged 50 weeks used for artificial insemination (AI), were reared in individual cages.

2.2. Methods
2.2.1. Semen collecting and assessment. Semen was collected according to Burrows and Quinn method [7]. Rooster semen collection was initiated by stimulating the copulatory organ (phallus) to protrude with massaging the abdomen and the back part of male over the testes. This is followed quickly by pushing the tail forward with one hand and, at the same time, using the thumb and forefinger of the same hand to apply pressure in the area to collect semen from the ducts of this organ. The semen was collected using tube. Fresh semen from 3 males was pooled and assessed for semen quality, including volume, color, odor, consistency, motility, concentration, viability, and abnormality. Collected semen then diluted with physiological saline NaCl solution 0.85%.

2.2.2. Artificial insemination. The AI was performed using intravaginal method. For insemination, when holding the hen upright, pressure was applied to the abdomen around the vent, particularly on the left side. This causes the cloaca to evert and the oviduct to protrude, so that a syringe or plastic straw can be inserted (2.5 - 4 cm) into the oviduct and the appropriate amount of semen delivered. As the semen is expelled by the inseminator, pressure around the vent is released, which assists the hen in retaining sperm in the vagina or oviduct. The administrated dose for AI is single dose 75 x 10^6 per hen at 2.00 to 3.00 PM or 4 to 3 hours before dark. Eggs were collected from 2nd to 16th days after artificial insemination. The Egg fertility was determined by candling at 7th days after the incubation started.

3. Results and discussion
Pooled fresh semen characteristic of native chicken roosters was presented in Table 1 below. The results showed that volume of fresh semen was 0.2 mL; white creamy color; viscous; pH 8.5; concentration 6x10^9/mL; motility 75%; viability 90.44% and abnormality 5.03%. This result was normal comparing to other study which semen volume ranges from 0.1 to 0.9 ml [8]; viscous; white creamy in color [9]; pH between 7.1 to 7.25 [10]; concentrations between 3.10^9 to 7.10^9 spermatozoa/mL (Hafez, 2000); viabilities with average 96.64% and abnormalities around 8% [11].

A typical pattern of laying fertilized and unfertilized eggs after a single AI of hens with around 75x10^6 million sperm is shown in Table 2. The eggs fertility remained high for the first week and dropped during the second week after insemination. The average duration of fertility in laying hens inseminated at dose 75x10^6 per mL per hen was 6.5 days. The average percentage of egg fertility was 49.1 ± 18.9%. According to previous study [4], during a given post insemination interval, hens will lay different numbers of eggs, as a series of fertilized and then unfertilized eggs; although the switch from fertilized to unfertilized may be staggered.
Table 1. Pooled fresh semen characteristic from native chicken roosters

| Semen Parameter | Result |
|-----------------|--------|
| Volume (mL)     | 0.2    |
| Colour          | White creamy |
| Odor            | Specific |
| Consistency     | Viscous |
| pH              | 8.5    |
| Motility (%)    | 75     |
| Concentration (x 10^9 spermatozoa/mL) | 6 |
| Viability (%)   | 90.44  |
| Abnormality (%) | 5.03   |

In avian species, specialized simple tubular invaginations referred to as SST are found in the oviduct. Because of the presence of this structure, once ejaculated semen have entered the female reproductive tract, they can survive up to 2–15 weeks in domestic birds, including chickens, turkeys, quails and ducks, depending on the species in contrast to the relatively short life span of mammalian spermatozoa (i.e., several days). SSTs are located in the lamina propria of mucosal folds in UVJ and in the infundibulum, although the primary storage site for sperm is the SSTs in the UVJ [12]. Sperms are transported to the infundibulum, which is the site of fertilization and also serves as a secondary sperm storage site [13]. One study also reported that the biological basis of sustained fertility in chicken and turkey hens is their capacity for sperm to reside in the SSTs of the UVJ, and the differences in the duration of fertility between domestic fowl (2 to 3 weeks) and turkeys (10 to 15 weeks) are, in part, related to their respective numbers of SSTs (the mean numbers of SSTs for chickens and turkeys are 4,893 and 30,566, respectively) [14].

Table 2. A typical pattern of laying fertilized and unfertilized eggs after a single AI of hens

| Days after AI | Eggs fertility on days after AI | Percentage of eggs fertility on interval days 2nd-16th after AI |
|---------------|--------------------------------|------------------------------------------------------------------|
| 2             | x x x √ x √ - x x x x x x x x | 13.3%                                                            |
| 3             | x x x √ x √ x x x x x x x | 28.5%                                                            |
| 4             | x x x √ x x x x x x x x | 50%                                                              |
| 5             | x x x √ x x x x x x x x | 50%                                                              |
| 6             | x x x √ x x x x x x x x | 61.5%                                                             |
| 7             | x x x √ x x x x x x x x | 66.7%                                                             |
| 8             | x x x √ x x x x x x x x | 64.3%                                                             |
| Average of eggs fertility | 49.1±18.9%                                                             |

√ = fertilized egg, x = unfertilized egg

The fertile period or fertility of a single hen can be described in terms of interval until last fertilized egg is laid, until the first unfertilized egg is laid, or as the mean of these parameters [4]. There is considerable variation between hens in their response to AI. Little is known of the mechanisms of cellular interaction between avian sperm and the epithelial or fluid environment within different regions of the oviduct. However, research over the past 10 years has provided insights into the number of spermatozoa at different oviductal locations, how these change with time, and how they relate to the probability of a fertilized egg being laid [14]. Less than 2% of an inseminated dose of 100 to 200 million spermatozoa are found in the uterovaginal (SSTs) of either turkeys or chickens [12]. Less than 0.02% of the inseminated dose reaches the infundibulum in chickens or turkeys [13]. Sperm in the infundibulum may enter the infundibular SST, but this number is low following intravaginal insemination [14].
In the absence of an infundibular egg, some sperm undoubtedly pass through into the body cavity and others may be engulfed by macrophage. However, significant numbers are trapped in the forming outer perivitelline layer of the egg. The initial event of fertilization in birds is the fixation of numerous sperm on the IPVL that surrounds the oocyte at ovulation [15]. Sperm encounter the egg in the infundibulum within approximately 15 min of ovulation. They interact with the inner perivitelline layer (IPVL), which is the outer investment of the egg at this time, release their acrosomal enzymes, and hydrolyze a small hole in the IPVL (IPVL hole), through which they pass to reach the oocyte [12]. The fixation is followed by the acrosome reaction (AR), an exocytosis process involving the fusion between the sperm plasma membrane and the outer acrosomal membrane. Many sperm may undergo AR and then penetrate the bird’s oocyte after the fusion between the inner membrane of the acrosome and the plasma membrane of the oocyte [15]. On the other hand, hen age factor has an influence on the fertility of eggs and there is a general tendency of fertility to decline with age [16].

4. Conclusion
In this study, we reported our understanding of fertility period for single dose of AI. The average duration of fertility in laying hens inseminated at dose $75 \times 10^6$ per mL per hen was 6.5 days. In chickens, due to the lower spermatozoon concentration and shorter duration of fertility, 0.1 mL of undiluted pooled semen, at intervals of 7 days, is required. For maximal fertility, inseminations may be started before the initial oviposition.

References
[1] Quinn J P and Burrows W A 1936 J. Hered. 27 31–7
[2] Brillard J P 2003 World Poult. Sci. J. 59 441-6
[3] Kharayat N S, Chaudhary G R, Katiyar R, Balmurugan B, Patel M, Uniyal S, Raza M, Mishra G K 2016 RRJoVST 5 1–5
[4] Wishart G J and Staines H J 1999 Poult. Sci. 78 428–36
[5] Gumulka M and Kapkowska W 2005 Anim. Reprod. Sci. 90 135–48
[6] Das S C, Isobe N and Yoshimura Y 2008 Am. J. Reprod. Immunol. 60 477–81
[7] Burrows W A and Quinn J P 1937 Poult. Sci. 16 19–24
[8] Etches R J 1996 Reproduction in Poultrey (UK: University Press Cambridge)
[9] Utomo S 1997 Pengaruh penggantian plasma seminal dengan larutan Beltsville Poultry Semen Extender (BPSE) pada penyimpanan 4 °C selama 24 jam terhadap fertilitas ayam kampung Master Thesis Faculty of animal science (Yogyakarta: Universitas Gadjah Mada)
[10] Tri-Yuwanta, Kustono and Wibowo C H 1998 Bulletin Peternakan 22 64–72
[11] Hafez E S E 2000 Reproduction in farm animals 7th edition (Philadelphia: Lea & Febiger)
[12] Sasamani T M, Matsuzaki S, Mizushima and Hiyama G 2013 J. Reprod. Dev. 59 334–8
[13] Bakst M R 1981 1981 J. Reprod. Fertil. 62 159–64
[14] Bakst M R, Wishart G and Brillard J P 1994 Poult. Sci. Rev. 5 117–43
[15] Blesbois E 2012 J. Poult. Sci. 49 141–9
[16] King’ori, A M 2011 Int. J. Poult. Sci. 10 483–92