Age-Dependent Changes in Fresh Semen Quality of Swamp Buffalo (*Bubalus bubalis*)

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Abstract. The purpose of this study was to evaluate the fresh semen quality of swamp buffalo raised in Lembang Artificial Insemination Center with respect to different age. The semen was collected from one swamp buffalo bull at three different stages (5, 6, and 7 years old). The evaluation of semen quality was conducted directly upon collection. Semen color, consistency, and mass sperm motility were analyzed descriptively, while semen volume, pH, sperm concentration and individual sperm motility were analyzed using analysis of variance followed by least significant difference test. The results showed that the dominant physical characteristics of swamp buffalo’s semen were milky white color with moderate consistency and fast mass sperm motility (++). There was a highly significant effect (P<0.01) of age on semen volume, sperm concentration, and individual sperm motility, whereas semen pH was not affected (P>0.05) by age. Swamp buffalo had higher (P<0.01) semen volume and sperm concentration at 7 years old, but with lower (P<0.01) individual sperm motility as compared to 5 and 6 years old. In brief, this study reveals that semen production of swamp buffalo is dependent of age with 7 years old become the most superior age.

1. Introduction

Recently, the demand for meat consumption as a source of animal protein is increasing along with the increase of population as well as the increase in public awareness toward the importance of balanced nutrition. The Indonesian beef consumption was increased by 6.6% from 2.25 kg per capita in 2015 become 2.39 kg per capita in 2017 [1]. However, the increase in beef production from 2013 to 2017 was only 1.54% per year [1]. These data indicate that the beef cattle production is not sufficient to fulfill the demand for meat consumption and is still far from the target of national meat self-sufficiency program.

Swamp buffalo is one type of meat-producing livestock, which is very adaptive to the humid tropical climate conditions [2] so that it is very potential to be reared in Indonesia to support national meat self-sufficiency program. In addition, swamp buffalo can digest crude fiber better than cattle so can be more efficient in utilizing feed for growth. Whereas, buffalo farming has several disadvantages such as low reproductive performance, limited number of superior sire, difficult in estrus detection, as well as long pregnancy period. The buffalo population in 2000 was 2.41 million head [3], while in 2018 became 1.36 million head [4] so that the buffalo population had decreased by 43.56%. Therefore, efforts should be made to increase the buffalo population, particularly by optimizing the application of reproductive technology.
Artificial insemination is a reproductive technology, which has proven to be effective and can be widely applied in the livestock sector [5]. One important factor that determines the success of artificial insemination is semen quality. Some aspects that may affect the semen quality including the age of bull, body weight, genetic traits, ejaculation frequency, feed, temperature, and season [6]. In this study, the effect of age on fresh semen quality of swamp buffalo was investigated.

2. Materials and methods

The bull station was located in Lembang Artificial Insemination Center (Bandung, Indonesia). The coordinate position of this site is 6°S latitude and 107°E longitude with the altitude of 1,241 m above mean sea level. One swamp buffalo bull was used as a semen donor in this study. The bull was kept in an individual flock with a size of 4 x 2.5 m of length and width, respectively. Throughout the study period, the bull was fed 65 kg/day elephant grass, 4 kg/day concentrate feed, 1 kg/day hay, and 0.5 kg/day mungbean sprout with ad libitum access to drinking water.

The semen was collected using artificial vagina at three different stages (5, 6, and 7 years old). The evaluation of semen quality was conducted directly upon collection. Semen color was measured by visual observation and defined as creamy, milky, and abnormal. Semen consistency also measured by visual observation and divided into three categories namely watery, moderate, and dense. Semen volume was measured using a scaled collection tube. Semen pH was measured using pH meter. Sperm concentration was measured using spectrophotometric method. Individual and mass sperm motility were assessed under a light microscope with 400 and 100 x magnification, respectively. Mass sperm motility was defined into three categories, namely slow (+) when no waves were seen, but progressively active individual movements, fast (+++) when there were small, thin, sparse and unclear waves, and very fast (++++) when there were large, numerous, dark, thick and actively moving waves [7].

Data of semen color, consistency, and mass sperm motility were presented as percentage value and analyzed descriptively. While data of semen volume, pH, sperm concentration and individual sperm motility were presented as mean±standard deviation and analyzed using analysis of variance. When analysis of variance detected significant or highly significant differences (P<0.05 or P<0.01, respectively), the data were further analyzed using least significant difference test.

Table 1. Effects of age on semen color, consistency, and mass sperm motility of swamp buffalo bull

| Parameters                  | Age of bull          |
|-----------------------------|----------------------|
|                             | 5 years old (n=50)   | 6 years old (n=23) | 7 years old (n=58) |
| Semen color (%)             |                      |
| Creamy                      | 32.00                | 13.04              | 43.10              |
| Milky white                 | 68.00                | 86.96              | 55.17              |
| Abnormal                    | 0.00                 | 0.00               | 1.72               |
| Semen consistency (%)       |                      |
| Watery                      | 30.00                | 39.13              | 37.93              |
| Moderate                    | 70.00                | 60.87              | 60.64              |
| Dense                       | 0.00                 | 0.00               | 1.73               |
| Mass sperm motility (%)     |                      |
| Slow (+)                    | 6.00                 | 8.70               | 20.69              |
| Fast (+++)                  | 94.00                | 91.30              | 79.31              |
| Very fast (++++)           | 0.00                 | 0.00               | 0.00               |

3. Results and discussion

Table 1 shows that the dominant semen color of swamp buffalo was milky white, which was followed by creamy. No abnormal color was recorded, except at 7 years old (1.72%). Moderate consistency of swamp buffalo’s semen was accounted for more than 60% at all stages, while dense semen was only recorded at 7 years old (1.73%). Most of the observed semen sample had fast (+++) mass sperm motility and no semen sample had very fast (++++) mass sperm motility. Previously, Koonjaenak et al. [8] also reported that the semen of swamp buffalo raised in Thailand had milky to creamy color. Similarly, Kiani
et al. [9] also found that buffalo bull had milky white and creamy white semen. In another study, Amin et al. [10] also found that swamp buffalo had milky white color with slow (+) to fast (++) mass sperm motility.

### Table 2. Effects of age on semen volume, pH, sperm concentration, and individual sperm motility of swamp buffalo bull

| Parameters                              | Age of bull         |
|-----------------------------------------|---------------------|
|                                         | 5 years old (n=50)  | 6 years old (n=23) | 7 years old (n=58) |
| Semen volume (ml)                       | 2.83±0.76<sup>b</sup> | 2.49±0.55<sup>a</sup> | 3.53±0.98<sup>c</sup> |
| Semen pH                                | 6.69±0.16           | 6.69±0.11           | 6.65±0.13           |
| Sperm concentration (10<sup>6</sup>)     | 918.00±233.14<sup>a</sup> | 866.96±242.19<sup>a</sup> | 1,059.98±321.65<sup>b</sup> |
| Individual sperm motility (%)           | 69.50±3.68<sup>b</sup> | 70.00±0.00<sup>b</sup> | 66.29±9.01<sup>a</sup> |

<sup>a-c</sup> Different superscript indicates a highly significant difference (P<0.01)

As can be seen in Table 2, there was a highly significant effect (P<0.01) of age on semen volume, sperm concentration, and individual sperm motility, while semen pH was not affected (P>0.05) by different age. Swamp buffalo had higher (P<0.01) semen volume and sperm concentration at 7 years old, but with lower (P<0.01) individual sperm motility as compared to 5 and 6 years old. However, it should be noted that the reduction rate of individual sperm motility was only 3.2 to 3.7%, which was not crucially important. In line with this finding, Ahmed et al. [11] also observed a higher semen volume of buffalo bull at an older age. Javed et al. [12] also recorded a significant age effect on sperm concentration of buffalo bulls. According to Pant et al. [13], the age of buffalo bull had a positive association with scrotal circumference and testicular volume, which in turn may increase sperm production capacity thus increasing semen volume and sperm concentration. It could be concluded that under the condition of this study, the semen production of swamp buffalo is dependent of age with 7 years old become the most superior age.

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