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Influences of bull age and season on sperm motility, sperm concentration, and ejaculate volume of Ongole Grade cattle in Singosari National Artificial Insemination Center

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ABSTRAK

Sapi Peranakan Ongol (PO) merupakan sapi lokal Indonesia. Penelitian ini bertujuan untuk menganalisis pengaruh umur pejantan dan musim terhadap kualitas semen secara makroskopis (motilitas sperma dan konsentrasi sperma) dan mikroskopis (volume ejakulasi semen) sapi PO untuk mendukung kebijakan dan manajemen dalam peningkatan produksi semen. Data dikumpulkan dari 533 ejakulasi pada 10 ekor pejantan berumur 3, 4, dan 5 tahun di Balai Besar Inseminasi Buatan (BBIB) Singosari. Sapi pejantan berumur 4 tahun memiliki motilitas sperma dan volume ejakulasi tertinggi. Volume ejakulasi terendah dilaporkan pada sapi PO berumur 3 tahun. Motilitas dan konsentrasi sperma pada musim hujan lebih tinggi (P<0,01)dari musim kemarau. Kesimpulannya, pemilihan umur pejantan dan musim seharusnya dipertimbangkan oleh Balai Besar Inseminasi Buatan (BBIB) Singosari ketika mengkoleksi semen, terutama pada sapi PO pejantan berumur 3 tahun dan pada musim kemarau.

Kata kunci : pejantan, sapi Peranakan Ongol, motilitas sperma, konsentrasi sperma, volume

ABSTRACT

Ongole Grade cattle is one of local cattle in Indonesia. This study was aimed to analyze the effect of bull age and season on semen quality of Ongole Grade cattles macroscopically (sperm motility, and sperm concentration) and microscopically (semen ejaculate volume) to support the policy and management on improvement of semen production. Data were collected from 533 ejaculates of 10 bulls aged 3, 4, and 5 yr at Singosari National Artificial Insemination Center (SNAIC). The 4-year-old bulls had highest sperm motiliy and semen ejaculate volume. The lowest ejaculate volume was reported in Ongole Grade cattles aged 3 yr. Sperm motility and concentration were significantly higher (P<0.01) in wet season than dry season. In conclusion, the bull age and season should be considered by Singosari National Artificial Insemination Center when collecting the semen, especially on bulls aged 3 yr old and in dry season.

Keywords: bull, Ongole Grade cattle, sperm motility, sperm concentration, volume

INTRODUCTION

In 2012, Ongole Grade cattle was officially declared by the government as one of local cattle in Indonesia and known as Peranakan Ongole (PO) cattle with development region center in East Java Province (Kementan, 2012). PO cattle is a crossbred cattle between Sumba Ongole cattle (originated from Indian Brahman cattle, and was well adapted and developed in Sumba Island, a region with dry climate of the Eastern Part of Indonesia) and Java cattle (Suyadi *et al.,* 2014) during Holand colonialization period around 1930s. Furthermore, Ongole Grade cattles have good meat quality, well disease resistance, and good reproductive performance (Astuti, 2004; Rohyan *et al.,* 2016; Sumadi *et al.,* 2017).

Artificial insemination is one of method used to increase the productivity and population. The success of artificial insemination is affected by the semen quality (Oliveira *et al.,* 2012). The high quality semen has an important role in improving reproductive efficiency. The semen quality is influenced by the genetic and environmental factors. In genetic factor, the semen quality could be detemined based on the repeatability coefficient (Sitanggang, 2018). The environmental factors that affect semen quality are bull age, collection interval, collection frequency, and season (Fuerst-Waltl *et al.,* 2006; Fiaz *et al.*, 2010; Bhakat *et al.*, 2011; Boujenane *et al.*, 2013; Snoj *et al.*, 2013). However, there was no information about the interaction effect of age and season on Ongole Grade cattle in previous study. This information will help researcher to provide the best strategy and policy of breeding management at Singosari National Artificial Insemination Center (SNAIC), especially in semen production.

Recently, the studies on genetic and breeding of Ongole Grade cattle were already explored such as genetic relationship, estimated breeding value, heritability of body size, and genetic diversity using DNA or blood protein (Hartatik *et al.*, 2018; Paputungan *et al.*, 2015; Sumadi *et al.*, 2017; Sutiyono *et al.*, 2018). Meanwhile, the studies of environmental factors that affect on Ongole Grade performance were rarely investigated. This research was designed to explore the effect of bull age and season on sperm motility, sperm concentration, and semen ejaculate volume of Ongole Grade cattle to support breeding management in Singosari National Artificial Insemination Center.

MATERIALS AND METHODS

Data Collection and Animal Management

This study was conducted in 2017-2018. A total of 533 ejaculates from 10 Ongole Grade bulls were collected at Singosari National Artificial Insemination Center, Malang, Indonesia. Data of semen production of Ongole Grade bulls were collected from the record book including sperm motility, sperm concentration, and ejaculate volume. The bulls were categorized into years of age ranging from 3, 4, and 5 yr. Data were categorized according to season of semen collection : wet season (from October to March) and dry season (from April to September). The monthly climate data were obtained from Bereau Meteorological, Climatological, and Geophysical Agency including the temperature and humidity. Bulls were fed and mantained under similar management and feeding system. Bulls were housed in a barn individually. Animal care procedures were approved by The Animal Care and Use Committee of Universitas Brawijaya (No.1156-KEP-UB).

Semen Collection

Semen was collected three times a week at Singosari National Artificial Insemination Center. Prior to semen collection, the bulls were sexually stimulated using a teaser bull. Each bull was handled by an experienced barn technician. The technician allowed the bulls to false mount three times before collecting semen using artificial vagina. The semen volume was gravimetrically determined using the scale of tube installed on artificial vagina. Sperm concentration was analyzed by taking 35 µL semen diluted in 3.5 mL of 0.9% NaCl solution. Sperm concentration of diluted semen was calculated using photometer SDM 6 (Minitube, Germany). Sperm motility was examined by diluting 0.1 µL semen in 0.1 µL prewarmed (37^{o}C) diluent containing Tris. Sperm motility was microscopically evaluated using 200x magnifications. Sperm motility was calculated as a percentage of the total sperm population including motile and nonmotile.

Data Analysis

Sperm motility, sperm concentration, and semen ejaculate volume were analyzed using General Linear Model procedure in SPSS ver. 26.0. The bull age and season were included as fixed effects. Differences among means were determined by F-tests using type-III sum of

square. The Duncan test were used to analyze pairwise comparisons between means. The statistical model used in this research followed the formula of Steel and Torrie (1993):

$$Y_{ijk} = \mu + A_i + S_j + (AS)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} : semen volume, sperm concentration, and sperm motility

μ : overall mean

A_i : effect of bull age

S_j : effect of season

$(AS)_{ij}$: interaction between bull age and season

ε_{ijk} : random error

RESULTS AND DISCUSSION

Semen quality is an important traits for supporting the success of artificial insemination program. Semen quality was affected by some factors such as environment, physiological status, management, and genetics. In this study, the sperm motility, concentration and volume of Ongole Grade bulls were 67.43%, 1167.13 x10^6/mL and 5.32 mL respectively (Table 1). This result was similar with the semen quality of Bali bulls in SNAIC where the bulls have >70% motility, 1100 billion/mL concentration, and 5-6 mL volume (Bayu and Isnaini, 2020). The average sperm motility of PO bulls was less than Indonesia National Standard (70%). Even though the sperm motility was lower, SNAIC only used the semen with minimum 70% motility for frozen semen straws processing.

Effect of Bull Age on Semen Production and Sperm Motility

Sperm motility is one of important parameters to know the ability of spermatozoa during fertilization. In this study, the bull age significantly affected sperm motility (P<0.01), sperm concentration (P<0.01), and semen ejaculate volume (P<0.01) (Table 2). It was similar with the previous study on Ongole Grade cattle (Sitanggang, 2018), Holstein bulls (Boujenane *et al.*, 2013), and Sahiwal bulls (Bhakat *et al.,* 2011). The highest sperm motility was collected on the 4-year-old Ongole Grade bulls. Sperm motility increased from 3 to 4-year-old bulls and decreased on 5-year-old bulls. This pattern was also reported in previous study that sperm motility of Holstein bulls increased from 2 to 4 yr of age and started to decrease at 5 yr of age (Argiris *et al.*, 2018). Generally, the sperm motility of 4-year-old Ongole Grade bulls in recent study was higher than Bali bulls, yet lower than Holstein Friesian bulls. The Bali and Holstein Friesian bulls aged 4 yr had 62.5% and more 80% sperm motility, respectively (Nugraha *et al.*, 2019; Murphy *et al.*, 2018).

The fluctuation of semen quality occured on sperm concentration in this study. Sperm concentration declined on the bulls aged 4 yr and inclined on the bulls aged 5 yr. Sperm concentration of Ongole Grade bulls aged 4 yr old was the lowest. The different results were previously reported; sperm concentration decreased with age on Bali and Crossbred Jersey bulls (Isnaini *et al.*, 2018; Gopinathan *et al.*, 2018). The sperm concentration in Ongole Grade cattle was approximately 1,083 – 1,220 x10^6/mL. It was lower than sperm concentration of Holstein Frisian bulls which was more than 3,110 x10^6/mL (D'Andre *et al.*, 2017).

The current study showed that semen ejaculate volume on bulls aged 3 yr was the lowest among the age of categories. It might due to the lack of energy required for semen production. On the younger bulls, more energy was needed for the body on testicular growth. The older mature bulls had greater ejaculate volume due to full grown testicles. Furthermore, it was caused by the libido of older bulls was better than young bulls (Bhakat *et al.*, 2011). Semen ejaculate

Table 1. The Semen Quality of Ongole Grade Cattle in 2017-2018

Parameters	N	Minimum	Mean	Maximum	Standar Deviation
Sperm motility (%)	533	50	67.43	75	5.68
Sperm concentration (x10^6/mL)	533	162	1167.13	2211	363.33
Ejaculate volume (mL)	533	0.5	5.32	13	1.93

Table 2. The Effect of Bull Age on Sperm Motility, Sperm Concentration and Ejaculate Volume

Parameters	Bull Age		
	3 yr (n=167)	4 yr (n=231)	5 yr (n=135)
Sperm motility (%)	67.43 ± 0.57[b]	69.24 ± 0.73[c]	63.96 ± 0.68[a]
Sperm concentration (x10^6/mL)	1220.3 ± 9.34[b]	1083.1 ± 7.32[a]	1208.2 ± 15.26[b]
Ejaculate volume (mL)	3.96 ± 0.58[a]	6.00 ± 0.72[b]	5.67 ± 0.74[b]

Data are presented as mean ± standard error
Means in the same row with different superscript differs significantly (P<0.05)

volume of Ongole Grade bulls increased with age. This increase was affected by physiological changes (activity of the hypothalamic–pituitary–testicular axis) including an increase in body mass and the development of testis and accessory glands that consequently leads to an increase in semen production postpuberty and during sexual maturation. (Almquist, 1978; Balić *et al.*, 2012).

The present study showed that the highest semen ejaculate volume was in 4 and 5-year-old bulls. Sitanggang (2018) reported that the peak of semen ejaculate volume on Ongole Grade bulls was from 3 to 5 yr of age. Moreover, Fuerst-Waltl *et al*. (2006) described that the peak of semen ejaculate volume on Simmental bulls was from 4 to 6 yr of age. However, peak of semen ejaculate volume could be achieved at different ages in different breeds (Snoj *et al.,* 2013).

Effect of Season on Semen Production and Sperm Motility

There was no interaction between bull age and season on sperm motility, sperm concentration, and semen ejaculate volume (P>0.05). However, there was an effect of season on sperm motility (P<0.05) and sperm concentration (P<0.01). A significant diffence was not found on semen ejaculate volume amoung the groups of season (P>0.05) (Table 3). Menegassi *et al*. (2015) reported that variation in semen characteristic was mainly associated with compromised scrotal thermoregulation and heat dissipation mechanism.

In this study, the lowest sperm motility of Ongole Grade bulls was collected in dry season. It might due to the heat stress occurred during dry season. High temperature in dry season can increase testicular temperatures, metabolic rate, and oxygen requirements. The testical tissue will become hypoxic if the metabolism is not followed by enhanced blood flows. This condition results in excessive reactive oxygen species (ROS) production, lipid peroxidation, oxidative stress, and decline in sperm motility (Gadea *et al*., 2004).

Sperm concentration of Ongole Grade bulls collected in wet season were higher than dry season in present study. The result showed that the sperm concentration in dry and wet season were 1,129 x10^6/mL and 1,204 x10^6/mL, respectively. Sitanggang (2018) reported that sperm concentration of Ongole Grade cattle was approximately 1,355-1,400 x10^6/mL in one year. Sperm concentration of Bali cattle was lower than the current study which was approximately 957-1,159 x10^6/mL (Aisah *et al*., 2017). The effect of season in semen quality was affected by some factors including temperature, humidity, photoperiod, feed composition, and management (Boujenane *et al*., 2013).

The significant difference of semen ejaculate volume was not found in this study. However, semen ejaculate volume in wet season was higher than dry season descriptively. This result was similar with previous study (Aisah *et al*., 2017; Sitanggang, 2018). The different result was shown in another study that the increase in rainfall intensity was associated with decreasing semen ejaculate volume (Khairi, 2016). In one year, the variation of semen ejaculate volume were detected approximately 5.25-5.33 mL. Garner *et al.*, (2008) described that the collected semen volume was commonly 5-8 mL.

The monthly evaluation of PO bulls semen showed that there was fluctuative semen quality on sperm motility, concentration, and volume in October 2017- September 2018 (Table 4). There was only significantly difference on ejaculate

Table 3. The Effect of Season on Sperm Motility, Sperm Concentration and Ejaculate Volume

Parameters	Season	
	Wet (n=199)	Dry (n=334)
Sperm motility (%)	68.27 ± 0.60^{b}	66.78 ± 0.76^{a}
Sperm concentration (x10^{6}/mL)	1204.6 ± 9.40^{b}	1129.9 ± 9.60^{a}
Ejaculate volume (mL)	5.33 ± 0.90	5.25 ± 0.80

Data are presented as mean ± standard error
Means in the same row with different superscript differs significantly (P<0.05)

Table 4. The Monthly Evaluation of Sperm Motility, Sperm Concentration and Ejaculate Volume in Ongole Grade Cattles in 2017-2018

Month	Parameters		
	Sperm motility (%)	Sperm concentration (x10^{6}/mL)	Ejaculate volume (mL)
October	66.25 ± 4.65	1280.8 ± 374.32	8.18 ± 1.66^{e}
November	69.09 ± 4.91	1191.9 ± 253.93	7.91 ± 0.84^{e}
December	67.27 ± 5.18	1138.4 ± 430.28	7.36 ± 1.93^{e}
January	69.23 ± 5.57	1203.7 ± 392.09	5.46 ± 2.27^{bcd}
February	68.30 ± 4.70	1290.4 ± 416.07	4.39 ± 1.47^{a}
March	68.19 ± 4.92	1140.6 ± 362.59	4.58 ± 1.61^{ab}
April	67.34 ± 5.63	1110.7 ± 346.75	5.33 ± 2.02^{abcd}
May	66.96 ± 5.84	1090.2 ± 363.91	5.68 ± 1.67^{cd}
June	67.33 ± 4.87	1179.7 ± 338.32	6.03 ± 1.84^{d}
July	66.83 ± 6.44	1106.6 ± 344.99	4.84 ± 2.09^{abc}
August	66.67 ± 7.11	1168.4 ± 337.78	5.26 ± 1.57^{abcd}
September	66.25 ± 5.61	1238.6 ± 352.57	5.11 ± 1.19^{abcd}

Means in the same column with different superscript differs significantly (P<0.05)

volume among the months. Higher ejaculate volume was collected in October-December which this periode was in wet season. The ejaculate volume gradually decrease started from the beginning of wet season (October) to the beginning of dry season (April). This pattern was similar with monthly semen characteristics on Holstein bulls in Iraq (Al-Badry, 2013). This result hopefully could give proper information for the SNAIC to improve their management, especially in transition of season.

CONCLUSION

The 4-year-old bulls had higher sperm motiliy and semen ejaculate volume than 3 and 5-year-old bulls. Ongole Grade bulls had higher semen quality in wet season than dry season. The monthly semen characteristics of Ongole Grade bulls were fluctuative. This study suggests that the

challenges of semen collection on Ongole Grade bulls are occurred on 3-year-old bulls and in dry season.

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