Seasonal Variations in Semen Quality of Pasundan Bulls in their Native Tropical Environment

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Abstract: The success of artificial insemination highly depends on the semen quality, in which it may significantly associate with the environmental factor. However, the information regarding the seasonal effects on semen quality of Pasundan bulls is still scarce. This study aimed to examine seasonal variation in semen quality of Pasundan bulls in their native environment. A total of 203 ejaculates produced by two Pasundan bulls aged at four years old were used in this study. The bulls were kept under standard management conditions in Lembang Artificial Insemination Center. The results showed that mean values of semen volume, sperm concentration, total sperm number, individual sperm motility, post-thawing sperm motility and frozen semen production of Pasundan bulls were 5.7 mL, 1.17 billion/ml, 6.57 billion/ejaculate, 62.1, 40.6 and 272% doses/ejaculate, respectively. Semen volume, individual sperm motility and post-thawing sperm motility were not affected by different seasons (P>0.05). Whereas, the significant reduction of sperm concentration (P<0.05), total sperm number (P<0.01) and frozen semen production (P<0.01) were observed in the dry season as compared to the rainy season. The minor change in ambient temperature, maximum ambient temperature, relative humidity and temperature-humidity index were detected between seasons. On the other hand, about 52% greater sunshine duration was detected in the dry season compared to the rainy season. In conclusion, the semen quality of Pasundan bulls is reduced during the dry season, which is considerably associated with the increase in sunshine duration.

Keywords: Artificial Insemination, Heat Stress, Indonesian Native Cattle, Semen Cryopreservation, Sunshine Duration

Introduction

Beef is a nutrient-dense food that provides essential amino acids, vitamins, minerals and bioactive components for humans (Mwangi et al., 2019). Beef consumption is highly crucial for humans to support optimal physiological development and intellectual performances (Ribas‐Agustí et al., 2019). Recently, beef demand in Indonesia is increased year by year. In 2009, beef consumption by Indonesian society was 0.31 kg/capita/year, while in 2018, the beef consumption was increased up to 0.47 kg/capita/year (DGLAH, 2014; 2018). It could be calculated that there was a 51.61% increase in beef demand during 2009-2018. Additionally, (CDISA, 2017) predicted that national beef demand in Indonesia in 2022 would increase by 8.15% as compared to the beef demand in 2018. However, the high Indonesian national beef demand is still cannot be fulfilled by the productivity of local cattle. As stated by (Agus and Widi, 2018), about 55% of beef demand is still supplied from imported cattle. This condition mostly due to the relatively low population of local cattle so that they could not produce enough beef for society. Hence, the use of biotechnology becomes a crucial strategy to increase the local cattle population.

Artificial Insemination (AI) currently becomes the most accepted reproductive biotechnology applied in the livestock. The procedure of AI includes three main steps, namely semen collection from the male sire, semen processing and semen insemination into the female reproductive tract (Mohammed, 2018). The beneficial effect of AI, including improve fertility, increase the
genetic potential of offspring, as well as reduce disease transmission among livestock, in which those benefits could, in turn, ultimately affect economic profit for farmers (Moore and Hasler, 2017). Additionally, the goal of this reproductive technology is to generate more offspring with a higher growth rate so that they could produce higher meat production.

The success of AI highly depends on the semen quality, in which it may significantly associate with the environmental factor. In the temperate region, photoperiod (time interval between sunset and sunrise) is the most responsible factor affecting seasonal variation in semen quality (Snoj et al., 2013). While in the tropical region, since the photoperiod is almost similar throughout the year, other factors such as temperature and relative humidity probably become two significant contributors in the semen quality variation (de Castro et al., 2017). In more recent, sunshine duration, which defined as the duration of direct sunshine exposure to the land surface, also reported as one factor which could influence semen quality (Golher et al., 2018). All of these-mentioned factors should be discussed to provide in-depth knowledge of seasonal effects on semen quality.

Pasundan cattle is officially registered as one of the Indonesian-origin breeds (Ministry of Agricultural Decree Number: 1051/Kpts/RI/SR.10/2014). These cattle were originally the result of crossbreeding between Bali cattle (Bos javanicus) and Ongole and Madura cattle (Bos indicus), which was conducted in 1904 (Said et al., 2017). The distribution of this cattle mainly in the West Java area, including Ciamis, Cianjur, Garut, Indramayu, Kuningan, Majalengka, Pangandaran, Purwakarta, Sukabumi, Sumedang and Tasikmalaya Districts (Sulasmi et al., 2017). Pasundan cattle had a solid reddish-brown coat with the presence of black color on the hoof, switch of the tail, eyelid, muzzle and horn, as well as whitish color on mouth lash and light brown color on legs (stocking) and rump patch (Said et al., 2017). Moreover, it was also reported that adult male Pasundan cattle had the average body length, withers height, chest girth and scrotal circumference of 129, 129, 159 and 18 cm respectively. On the other hand, female adult Pasundan cattle had 116, 123 and 140 cm, respectively (Said et al., 2017). The carcass yield of Pasundan cattle was approximately 53% (Sulasmi et al., 2017). According to (Sutarno and Setyawan, 2015), Pasundan cattle is easy to adapt to extreme weather and has high resistant to tropical diseases. Additionally, (Setiawati et al., 2018) reported that Pasundan cows had sexual maturity, age at first mating, gestation length, age at first calving and calving interval of 21, 23, 9-10, 33 and 14 months, respectively. However, the information regarding the seasonal effects on semen quality of Pasundan bulls is still scarce. For that reason, this study was conducted to clarify the effect of the rainy and dry seasons on semen quality of Pasundan bulls in their native tropical environment.

Materials and Methods

Study Area

This study was conducted at the Lembang AI center (Bandung, West Java Province, Indonesia). This area was located at 6° south latitude and 107° east longitude. The altitude of this area was 1,241 m above mean sea level.

Animals and Experimental Design

For the purpose of the study, 2 Pasundan bulls (4 years old) were used as AI donors. The bulls were reared under standard management practice in the Lembang AI center. Each bull was kept in an open-sided cage with a size of 4×2.5×2 m (length, width and height, respectively). The feed consisted of 1 kg hay of Africa grass, 50 kg fresh forage of elephant grass, 4 kg concentrate feed and 0.5 kg fresh sprout of mung bean. The total digestible nutrient and crude protein contents of the concentrate feed were 65 and 16%, respectively. The drinking water was provided ad libitum. The semen was collected from each bull using an artificial vagina (Kruuse, model 340284, Denmark). The semen collection was done for two years from 2017 to 2018, resulting in 89 ejaculates in the rainy season and 114 ejaculates in the dry season.

Semen Quality Evaluation

The semen quality was measured directly upon collection, including semen volume, sperm concentration, total sperm number, individual sperm motility, post-thawing sperm motility and frozen semen production. The semen volume was measured using a scaled vial (Isnaini et al., 2019a). Sperm concentration was measured using the spectrophotometric method (Isnaini et al., 2019a). The total sperm number was calculated by semen volume x sperm concentration (Isnaini et al., 2019a). The individual sperm motility and post-thawing sperm motility were assessed under a light microscope at 400 × magnification (Susilawati et al., 2018). Frozen semen production was calculated according to the Indonesian National Standard (SNI 4869-1:2008, 2008) with the formula of total sperm number/25 million sperm.

Climate Factors

The climate factors, including mean ambient temperature, maximum ambient temperature, relative humidity and sunshine duration, were collected from the local climatological office near the bull station. Temperature-humidity index was calculated by 0.8x mean ambient temperature + (relative humidity/100) × (mean ambient temperature-14.4) +46.4.

Data Analysis

The statistical procedure was performed using SPSS software for Windows, version 13.0 (SPSS Inc., Chicago,
Results and Discussion

The mean values of semen volume, sperm concentration, total sperm number, individual sperm motility, post-thawing sperm motility and frozen semen production of Pasundan bulls were 5.70 mL, 1.17 billion/ml, 6.57 billion/ejaculate, 62.10, 40.60 and 272% doses/ejaculate, respectively. Table 1 shows that the semen volume, individual sperm motility and post-thawing sperm motility were not affected (P>0.05) by different seasons. On the other hand, the significant change in sperm concentration (P = 0.049), total sperm number (P = 0.006) and frozen semen production (P = 0.005) were observed during the rainy and dry seasons. In the dry season, Pasundan bulls had 8.13, 14.03 and 14.24% lower sperm concentration, total sperm number and frozen semen production, respectively, as compared to the rainy season.

The minor changes in mean ambient temperature, maximum ambient temperature, relative humidity and temperature-humidity index were detected in this study (Table 2). The variation coefficient of monthly mean values of mean ambient temperature, maximum ambient temperature, relative humidity and temperature-humidity index were 1.60, 2.16, 5.78, 0.93% respectively. On the other hand, sunshine duration was found to be varied between seasons, with about 52% greater sunshine duration was detected in the dry season compared to the rainy season.

The results of the semen quality of Pasundan bulls obtained in this study were slightly higher as compared to other Indonesian native cattle. Previously, (Isnaini et al., 2019b) found that Bali bulls had semen volume, sperm concentration, total sperm number, individual sperm motility, post-thawing sperm motility and frozen semen production of 4.81 mL, 1.11 billion/ml, 5.37 billion/ejaculate, 68.8, 43.9 and 215% doses/ejaculate.

Nugraha et al. (2019) reported that semen volume, individual motility and sperm concentration of Bali bulls were 4.90 mL, 62.5 and 1.07% billion/ml. In another study, (Yekti et al., 2018) showed that Madura bulls had semen volume and sperm concentration of 4.57 mL and 1.07 billion/ml, respectively. Additionally, frozen semen production of Pasundan bulls in this study was comparable to that of Simmental bulls reared in tropical climate conditions, which had frozen semen production of 277 doses/ejaculate (Isnaini et al., 2019b).

In this study, it was found that sperm concentration, total sperm number and frozen semen production were altered during different seasons. In agreement with this finding, (Prastowo et al., 2018) also reported that the season could alter semen quality of Bali bulls.

Similarly, it was also found that the semen quality of Ongole grade cattle was changed during rainy and dry seasons (Suretno et al., 2018; Isnaini et al., 2019c). In another study, (Nichi et al., 2006) also found that the season could alter semen quality of Simmental bulls in tropics. Landeta-Hernández et al. (2020) also reported that the seasons significantly affect semen quality of Bos indicus, Bos taurus, Crossbred Bos taurus x Bos indicus and tropically adapted Bos taurus composites bulls. Nongbua et al. (2020) also detected the variation of semen quality of Bos indicus bulls among different seasons in Thailand.

Table 1: Effects of season on semen quality of Pasundan bulls

| Parameters                          | Rainy               | Dry                  | P-value |
|------------------------------------|---------------------|----------------------|---------|
| Semen volume (ml/ejaculate)        | 5.8±0.17 (89) a     | 5.57±0.17 (114) a    | 0.218   |
| Sperm concentration (billion/ml)   | 1.23±0.04 (89) b    | 1.13±0.03 (114) b    | <0.05   |
| Total sperm number (billion/ejaculate) | 7.13±0.29 (89) b    | 6.13±0.22 (114) b    | <0.01   |
| Individual sperm motility (%)      | 62±1.61 (89) a      | 62.2±1.28 (114) a    | 0.911   |
| Post-thawing sperm motility (%)    | 40.4±0.18 (60) a    | 40.8±0.24 (74) a     | 0.203   |
| Frozen semen production (doses/ejaculate) | 295±12.2 (60) b     | 253±8.59 (74) a      | <0.01   |

Values are expressed as means ± SE followed by the number of ejaculates in parenthesis * Means with uncommon superscripts within the same row differ significantly

Table 2: Summary of environmental conditions during the study

| Parameters                          | Rainy               | Dry                  |
|------------------------------------|---------------------|----------------------|
| Mean ambient temperature (°C)      | 23.6 (23.2-24.3)    | 23.5 (22.9-24)       |
| Maximum ambient temperature (°C)   | 29.1 (27.8-29.6)    | 29.6 (28.7-30.5)     |
| Relative humidity (%)              | 79.3 (74.4-81.9)    | 73.4 (67.2-78.8)     |
| Temperature-humidity index         | 72.6 (71.8-73.1)    | 71.9 (70.4-73)       |
| Sunshine duration (minutes)        | 265 (172-318)       | 389 (274-498)        |

Values are expressed as means followed by minimum and maximum values in parenthesis
In the previous studies, it was reported that the change in semen quality was much associated with mean ambient temperature, maximum ambient temperature, relative humidity and temperature-humidity index (Bhakat et al., 2011; Chacur et al., 2013; Valeanu et al., 2015; Sabés-Alsina et al., 2017; Sabés-Alsina et al., 2019). However, in this study, those climate factors were almost equal during rainy and dry seasons. For that reason, it could be stated that the change in semen quality in the present study was not as a reflection of those factors.

The marked variation on sunshine duration probably more relevant to explain the reduction of semen quality of Pasundan bulls found in this study. This result was corroborated by (Perumal et al., 2017), who also found that the semen quality was decreased when collected at the season which had higher sunshine duration. The high sunshine duration had an indirect effect on semen quality through the elevation of solar radiation. In this study, Pasundan bulls were kept in the open-sided cage so that the solar radiation may reach them, thus inducing heat stress (Herbut et al., 2018). Additionally, Pasundan bulls used in this study had a black coat color. The body coat color of Pasundan bulls could change from solid red to black. This phenomenon was related to the production of androgen hormone when Pasundan bulls reaching sexual maturity (Arifin et al., 2019; Wulandari et al., 2019). Body coat color is one of the critical aspects that determine the susceptibility of the individual livestock to heat stress. The individual livestock with dark or black coat color tended to be more impacted by heat stress (Brown-Brandl, 2013). In a study by (Hillman et al., 2005), Angus cattle, which had black hair coat color, had the highest solar absorption (81%) as compared to MARC III, MARC I and Charolais cattle, which had dark red, tan and white hair coat color with solar absorption rate of 67, 45 and 38%, respectively. Van laer et al. (2014) also noticed that the livestock with dark coat color had lower heat tolerance due to the higher absorption rate of radiation. Therefore, it could be speculated that the black coat color of Pasundan bulls could elevate the indirect effect of solar radiation on heat stress. The existence of heat stress in bulls could deteriorate the spermatogenic cycle (Krishnani et al., 2017), which then could decrease sperm production, thus reducing sperm concentration. The decrease in sperm concentration was then also impacted on the decrease in total sperm number and frozen semen production.

Conclusion

It could be concluded that there are seasonal variations on the semen quality of Pasundan bulls in their native tropical environment. The semen quality of Pasundan bulls is notably reduced during the dry season, which is considerably associated with the higher sunshine duration at this time point. However, the reduction of semen quality is still tolerable and for that reason, the semen collection of Pasundan bulls is accepted to be done both in rainy and dry seasons.

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Author’s Contributions

Nurul Isnaini: Conceptualization, research planning, data interpretation and manuscript preparation.

Tri Harsi: Conceptualization, research planning and data collection.

Anggit Damaratri Lapoliwa, Muhammad Mas‘ud Chabiburochman and Abimahradimas Dwi Amarsyah: Data collection, data analysis and manuscript preparation.

Ethics

The authors declare that there are no ethical issues may arise after the publication of this study.

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