Seasonal effects on the semen production of Bali bulls (*Bos javanicus*) in West Nusa Tenggara's Lelede regional artificial insemination center

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Objective: The purpose of this research is to determine the effect of the rainy and dry seasons on the sperm qualities and frozen sperm production of Bali bulls at the Lelede Regional Artificial Insemination Center (RAIC) in West Nusa Tenggara.

Methods: A total of 1226 ejaculates were examined from five Bali bulls during a 24-month period (November 2018–October 2019), which was classified into rainy (November–April, 658 ejaculates) and dry (May–October, 568 ejaculates) seasons. Semen volume (mL), sperm velocity (1-4), motility...
(%), concentration (x106 cell/ml), pre-freezing motility (%), post-freezing motility (%), recovery rate (%), and total frozen semen production were examined as sperm quality measures.

**Results:** The results indicated that during the rainy season, ejaculates and processed sperm were more abundant than during the dry season. Additionally, the features of discarded fresh semen in the volume parameter were significantly (P<0.05) larger during the dry season than during the rainy season. The pattern in semen output rises during the dry season and decreasing during the rainy season.

**Conclusions:** It was concluded that the rainy season results in a higher number of ejaculates and total sperm production than the dry season. Meanwhile, no differences in sperm characteristics exist between the two seasons.

**Keywords:** Ejaculates; Sperm quality; Seasons; Bali bull; Semen production

**INTRODUCTION**

Bali cattle (*Bos javanicus*) is one of the Indonesian native cattle that originated from Bali Island, which equipped with high productivity and robust to a tropical climate environment. Suryanto *et al.* [1] reported that the carcass quality of carcass weight and carcass percentage in Bali bulls were 113.49±7.97 kg and 54.76±0.10%, respectively. Artificial insemination (AI) has been widely used to improve the effectiveness and efficiency of livestock production [2]. By using this technique, a superior bull can breed thousands of cows, producing progeny with high genetic potential. It demonstrated that AI is a highly compatible tool for managing breeding. However, the bull fertility becomes a critical factor in determining the technique’s success.

Combined with other reproductive technology and genetic selection, AI will be an efficient strategy in improving the productivity performance of the livestock farm industry, particularly in cattle [3]. As a tropical country, Indonesia has rainy and dry seasons. Indonesia lies in the equatorial region bounded by latitude 10°N and 10°S [4]. For beef cattle farming and breeding, the aggregation of numerous changes in environmental variables between the wet and dry seasons may have a substantial effect on physiological and reproductive processes, resulting in variances in bulls generating sperm [5].

Season significantly influences the Luteinizing Hormone (LH) secretion, the concentration of testosterone, the number of spermatozoa per ejaculate, and its freeze ability [6]. The seasonal change throughout the year might have an impact on the quality of the sperm. Snoj *et al.* [7] reported that the season has an effect on the fresh semen characteristics of *Bos taurus* breed. Furthermore, in a separate research, seasonal fluctuation in the semen freeze ability of bulls of the species *Bos taurus* and *Bos indicus* [6].

In Friesian Holstein, the short rainy season (October-November) had the best quality semen [8]. The Winter season was the most favourable season for good quality semen production, while the rainy season might be considered as intermediate between the two extremes (hot dry or summer and cold humid or winter) in Karan Fries Bulls [9].

The purpose of this research was to determine the impact of rainy and dry seasons on the characteristics of fresh semen and frozen semen production in Bali bulls raised at the Lelede Regional Artificial Insemination Center. As a result, this research was conducted at the Lelede Regional Artificial Insemination Center to determine the effects of the rainy and dry seasons on the fresh semen qualities and frozen semen output of Bali bulls.

**MATERIALS AND METHODS**

**Location and climatic condition**

This study carried out at the Lelede Regional Artificial Insemination Center (AIC) West Nusa Tenggara Province, Indonesia. This area located at 115°46’-116°20’ East longitude and 8°25’-8°55’ South latitude. The data record on climatic conditions were obtained from the Meteorology, Climatology, and Geophysics Council of Lombok Barat,
with rainy seasons ranging November to April and dry seasons ranging May to October.

The average rainfall in this place is about 51-300 mm/month with air temperature of about 23.6-33.0°C, relative humidity about 57-100%, and sunshine duration 3.96-9.78 hours/month.

Animals

A total of 1,226 records data of fresh semen quality traits from 2018 to 2019 were used in this study. The data was collected from five LSPro certified Bali bulls (12 years). At the Regional Artificial Insemination Center (RAIC) in Lelede, the bulls were reared in a similar manner. All bulls were kept separately and were fed and cared for in a similar/equal manner on a regular basis management. The ration composition for each bull consisted of King grass (50 kg/day/head), concentrate with a protein content of 16% (4 kg/day/head), and adlibitum water.

Semen collection and evaluation procedure

Bali bulls sperm were collected twice weekly at 8:00 a.m. using an artificial vagina (Kruuse, model 340284, Denmark) [10]. The volume of semen was determined directly using a scaled vial [11], and the pH was determined using pH indicator paper. The concentration of sperm was determined using the SDM 6 photometer (Minitube, Germany). The total number of sperm was determined using the calculation volume x sperm concentration [9]. Sperm motility in fresh semen was evaluated under a light microscope (200x magnification) before, after freezing and thawing [12]. The recovery rate was estimated using the following formula: (sperm motility post-thawing / sperm motility of fresh sperm) x 100% [13]. We discarded the frozen sperm with a post-thawing motility of less than 40% and a recovery rate of less than 50%, as defined by the Indonesian National Standard, SNI 4869-1:2017 [14].

Statistical analysis

Minitab 18.1 (Minitab for Windows) was used to perform the statistical analysis (United States Minitab) A t-test was used to determine the effect of the season on the sperm quality of Bali bulls. Data are expressed as the mean ± standard error. P<0.05 was used as the level of significance. Only qualified semen (total of 1,048 ejaculates) was included in the statistical analysis. Meanwhile the discarded semen was separated, then counted and analyzed descriptively.

RESULTS

The quality of fresh sperm must be evaluated in order to ascertain the quality of spermatozoa produced by each individual bulls. Fresh sperm that meets the criteria can be processed to frozen sperm for artificial insemination. Throughout the observation period, ejaculates were evaluated for semen quality using a standard procedure and SNI 4869-1-2017. The semen characteristics are shown in Table 1, for the discarded semen in rainy and dry seasons are shown in Table 2.

The results showed that 1226 ejaculates, in the rainy season was 53.76% (n=658), and in the dry season was 46.32% (n=568) ejaculates respectively. The approval rate of fresh semen in the rainy and dry seasons was 89.05% and 84.15%, respectively (Table 3). It is demonstrated that seasonal variation in Bali cattle semen production has an effect on the dose of frozen semen produced. The rainy season produced a lower dose than the dry

| Parameter                          | Season (Mean±SEM) | P value |
|-----------------------------------|-------------------|---------|
| semen volume (mL)                 | Rainy             | Dry     |        |
|                                   | 3.91±0.38         | 4.32±0.37 | 0.09  |
| sperm velocity (scoring 1-4)      | 3.63±0.05         | 3.58±0.10 | 0.33  |
| sperm motility of fresh semen (%) | 69.27±0.44        | 69.10±0.68 | 0.64  |
| sperm concentration (x10⁶)        | 1111.11±89.33     | 1135.40±56.20 | 0.58  |
| sperm motility before-freezing (%)| 52.78±0.74        | 52.99±0.65 | 0.60  |
| recovery rate (%)                 | 57.74±0.37        | 57.88±0.57 | 0.63  |

*=Within a row, means differ significantly at P<0.05 |https://jurnal.uns.ac.id/lar/index
season in this regard. Figure 1 depicts the sperm production of a Bali bull during the rainy and dry seasons. Semen production was decreased during the rainy season and increased during the dry season.

![Sperm production graph](image)

**Figure 1.** Semen production of Bali bulls on rainy and dry seasons

**DISCUSSION**

The results showed the effect of the rainy season and dry season on the semen characteristics of Bali bulls, where the mean of semen velocity, sperm motility, sperm concentration, sperm motility before-freezing, and the recovery rate was similar between both seasons. In general, the semen quality of the Bali bull met the standard bull semen quality. According to the Indonesian National Standard [14], the bovine sperm used for artificial insemination should have a post-thawing motility of at least 40% and a recovery rate of 50%.

There was no significant difference between the characteristics of fresh semen in both seasons P>0.05, the semen volume seemed to tend to be higher in the dry season although there was no significant difference between the rainy and dry seasons.

The semen volume in this study was lower than the study reported [15-17] measured the semen volume of Bali cattle at different age groups at BIB Singosari, namely 4 and 7 years old, obtained their respective volumes: 4.55 ± 0.91 ml/ejaculate and 5.18 ± 1.58 ml/ejaculate is lower than semen volume of Bali bull at AIC Baturiti during the rainy and dry seasons was 5.98 ± 1.35 ml/ejaculate and 6.06 ± 1.56 ml/ejaculate respectively. Bhakat et al. [9] reported that the higher the level of rainfall impact to the lower the semen volume, whereas the lower the level of rainfall affected to higher semen volume.

The present analysis found no significant seasonal effect on the semen quality of Bali bulls, which is consistent with previous results. Similarly, [18] reported no noticeable seasonal variation in the semen quality of Karan Fries crossbred bulls throughout the winter and summer months. By contrast, [19] found that season had a substantial influence

| Table 2. Discarded semen characteristic in rainy and dry season |
|-------------------|----------------|----------------|
| **Semen Characteristic** | **Rainy** | **Dry** |
| Semen volume (mL) | 2.74±1.23 | 3.44±1.53* |
| pH | 6.2 | 6.2 |
| Consistency | Aqueous | Aqueous |
| Sperm velocity (scoring 1-4) | 2.12±0.54 | 2.12±0.48 |
| Sperm moving mass (scoring 1-3) | 1.38±0.69 | 1.35±0.63 |
| Sperm motility of fresh semen (%) | 46.88±10.97 | 45.17±9.86 |
| Sperm concentration (x10⁶) | 772.40±553.47 | 771.72±479.90 |

*Within a row, means with bearing different superscripts differ significantly at p<0.05

| Table 3. Number of ejaculate and semen production in rainy and dry seasons (n=1226 ejaculation) |
|-------------------|----------------|----------------|
| **Item** | **Rainy** | **Dry** |
| Total ejaculate, n (%) | 658 (53.67) | 568 (46.32) |
| Approved ejaculate, n (%) | 586 (89.05) | 478 (84.15) |
| Discarded ejaculate, n (%) | 77 (10.95) | 85 (15.85) |
| Total frozen semen (doses) | 54.406 | 48.710 |
| Average frozen semen (doses) | 13.439±2992 | 10.970±4724 |
on spermatozoa motility but not on semen volume or sperm concentration in Ongole crossbreeds. According to [20], seasons had an effect on all semen parameters in Holstein cattle (semen volume, spermatozoa concentration, total number of spermatozoa, and spermatozoa motility). Prior to this, [7] reported that in the location near to the equator, there is a little fluctuation in day duration between the seasons, resulting in an apparent seasonal influence on the semen quality of bulls that was not identified.

According to a previous study [21], seasonal variations in androgen hormone synthesis may lead to varying spermatogenesis capabilities manifested in sperm production. As a consequence, it has been hypothesized that the rainy season reduces sperm production because it suppresses spermatogenesis. The seasonal change in androgen production remains unsolved.

This result might be because the bull was previously characterized as a non-seasonal rearing breed [22], meaning that seasonal variation had no influence on quality of the sperm. In tropical environments, the lack of significant temperature variations between seasons and between day and night may potentially contribute for the lack of a seasonal influence on sperm quality in this study.

During the rainy season, 10.95% of fresh ejaculates were discovered to have impaired sperm motility, necessitating their removal. On the other hand, during the dry season, 15.85% of fresh ejaculate was rejected owing to poor sperm motility, and the remainder due to low sperm concentration in comparison to the Indonesian National Standard (SNI 4869-1:2017).

In compared to previous studies, this study found a very small quantity of wasted sperm from both breeds. According to [23], 34.97% of breeding bulls' semen was discarded. Another study [6] discovered that wasted sperm in bulls used for artificial insemination ranged between 48 and 59.4% respectively of total discarded sperm.

Seasonal changes affect rainfall, temperature, air humidity, and wind speed, reducing feed and water availability, increasing nutrient loss due to increased temperature and decreased appetite, and causing dynamic stress that can impair spermatozoa quality [24]. Prastowo et al. [22] reported temperature, humidity, photoperiod, feed composition, and management are only a few of the possible causes of the seasonal influence on semen quality. Bull sperm quality declines during the rainy season due to a lack of sun exposure and exercise [17]. In contrast, [22] reported that during the rainy season, all sperm quality (semen volume, spermatozoa concentration, total number of spermatozoa, and sperm motility) were significantly higher than during the dry season.

Frozen semen production that can be produced every day individual bull depending on the total concentration spermatozoa. Total concentration of spermatozoa in rainy season seems have an impact on the average frozen semen production. Aisah et al. [17], explained that the relationship between volume and semen concentration with frozen semen production was positively correlated. The higher the volume of semen and the concentration of spermatozoa, the higher the production frozen semen produced.

**CONCLUSION**

In comparison to the dry season, the rainy season results in a higher number of ejaculate and total sperm production. In addition, there are no differences in sperm characteristics between two seasons.

**CONFLICT OF INTEREST**

The authors state that they have no financial ties to any of the financial institutions described in the publication.

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