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The Relationship Between Semen Production And Sexual Behavior In Madura And Bali Bulls

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ABSTRACT

The objective of this study was to examine the relationship between sexual behavior and semen production in Bali and Madura bulls. 4 Bali bulls and 4 Madura bulls were used in this observational study. Sexual behavior observation included the duration of libido, duration of ejaculation, and false mounting. Semen production variables observed were total sperm and total motile sperm. All of these observations were replicated 10 times. Results showed that there was a significant correlation ($p < 0.05$) between duration of ejaculation and total sperm in Bali bulls with the regression equation of $Y = 8874 - 302x$ and $R^2 = 0.12$ ($r = -0.34$). Bali bulls also had a significant correlation ($p < 0.05$) between duration of ejaculation and total motile sperm with the regression equation of $Y = 6218 - 215.1x$ and $R^2 = 0.11$ ($r = -0.34$). Madura bulls had a significant correlation ($p < 0.05$) between duration of ejaculation and total motile sperm per ejaculate with the regression equation of $Y = 1812 + 268x$ and $R^2 = 0.11$ ($r = 0.34$). In conclusion, Bali bulls had undesirable correlated response for the duration of ejaculation toward total sperm and total motile sperm. While Madura bulls had desirable correlated response for the duration of ejaculation toward total motile sperm.

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Introduction

Artificial insemination (AI) is one attempt to improve the livestock genetic because some selected bull can produce enough sperm to inseminate thousands of cows per year (Ax *et al.*, 2008). The success of the AI program depends on the fertility of the bull used to produce semen. Thereof, the superior bull used for the production of frozen semen must be selected through rigorous selection by considering genetic potential, disease conditions, reproductive health, libido and sexual behavior, as well as the potency for semen production (Shukla, 2011).

Several studies conducted in pasture proved that libido and mating service capacity affect conception rates and fertility in natural mating because libido showed the bulls desire to mate the cows (Abell *et al.*, 2017). Normal sexual behavior parameters can be seen from the bulls desire to mount and thrust, ability to ejaculate and attainment of erection (Hafez *et al.*,

2008). Quirino *et al.*, (2004) suggested that the bulls selection can be done earlier based on libido because it could describe the fertility of the bulls.

Sexual behavior is influenced by male genetics and environmental condition changes (Galina *et al.*, 2007, Hafez and Hafez, 2008). Dairy cattle have more active sexual behavior than beef cattle due to genetic factors (Hafez and Hafez 2008). Susilawati (2017) stated that Indonesian native cattle are known to be able to adapt in the tropics both in high temperature and humidity, able to consume low-quality feed and resistant to ectoparasites and endoparasites so can minimize the possible environmental factors which may influence the sexual behavior and semen production. Currently, there has not been enough research on sexual behavior in Indonesian native bulls which their semen was collected by using artificial vagina. Therefore, the objective of this study was to examine the relationship between sexual behavior and semen production in Bali and Madura bulls. The results of this study are expected to be a guide in the selection of superior bulls with an easy and simple method through sexual behavior.

Materials and Method

Location and Materials

This study was carried out at Singosari National Artificial Insemination Center, Malang Regency, East Java, Indonesia. A total of 8 bulls (consisted of 4 Bali bulls and 4 Madura bulls) were used in this study. Bali bulls had a mean \pm standard deviation age and body weight of 6.75 ± 0.5 years and 638 ± 58.13 kg, respectively, while Madura bulls had a mean \pm standard deviation age and body weight of 9.25 ± 1.5 years and 524.75 ± 76.86 kg, respectively. Semen collection was done twice a week by using an artificial vagina.

Research Method

The method used in this research was an observational study by examining the sexual behavior and semen production of Bali and Madura bulls. These observations were replicated 10 times.

a. Sexual Behavior

Sexual behavior observation included the duration of libido, duration of ejaculation, and false mounting were observed according to Sholikah *et al.*, (2018a) and Sholikah *et al.*, (2018b). The duration of libido was measured as the amount of time between the first contact of the bull to the teaser and its first false mounting. Duration of ejaculation was measured as the amount of time between the first contact of the bull to the teaser and its semen ejaculation to the artificial vagina. Both of duration of libido and duration of ejaculation were expressed in second. False mounting was measured as the amount of the bull mounted to the teaser but not ejaculated.

b. Semen Production

Semen production variables observed were total sperm and total motile sperm counts per ejaculate which were calculated according to Ax *et al.*, (2008), Kusumawati *et al.*, (2017) and Susilawati *et al.*, (2018). Total sperm count was calculated by formula = semen volume \times sperm concentration. Total motile sperm count was calculated by formula = semen volume \times sperm concentration \times individual motility. Both of total sperm and total motile sperm counts were expressed in 10⁶/ejaculate. Sperm concentration measured using spectrophotometer. The machine is calibrated at 550 nm. The solution used for dilution of the ejaculate is 2.9% sodium citrate and 5 mL of 10% formalin per liter then homogenized using a thermomixer. The mixture is then inserted into the cuvet, then the sperm

concentrations appear on the monitor screen. A standard curve measuring concentration versus 0.5% increments of light transmittance gives a range needed to measure concentration (Ax et al., 2008; Susilawati, 2013). Individual motility evaluation was performed to observed the progressive movement of spermatozoa in one point of view by using a 400x light magnification microscope at constantly temperature covered with a cover glass. Progressive movement is a toward movement that is the best movement for spermatozoa (Ax et al., 2008; Susilawati 2013).

Data Analysis

The differences in sexual behavior and semen production between Bali and Madura bulls were analyzed by using unpaired t-test. The relationship between sexual behavior and semen production was examined by using Pearson correlation coefficient and simple linear regression analyses.

Results and Discussion

Sexual Behavior

Libido or the desire to mate is one of the assessment parameters in the selection of superior bulls for breeding programs (Shukla, 2011). Libido can be seen based on the sexual behavior of the bulls. Table 1 shows the sexual behavior in Bali and Madura bulls.

Table 1. Sexual behavior in Bali and Madura bulls

| Sexual behavior | Bali bulls | Madura bulls | <i>p</i> |
|----------------------------------|------------------------|------------------------|----------|
| Duration of libido (second) | 58.9±36.6 ^a | 63.4±33.6 ^a | 0.78 |
| Duration of ejaculation (second) | 407±120.6 ^a | 356±87.6 ^a | 0.11 |
| False mounting | 5.15±0.94 ^b | 3.98±1.24 ^a | 0.02 |

^{ab} means in the same column without common letter are different at $p < 0.05$

Results showed that the duration of libido did not differ ($p > 0.05$) between Bali and Madura bulls. Bali bulls tended to have a more varied duration of libido (58.9±36.6 seconds) as compared to Madura bulls (63.4±33.6 seconds). Variations arise because individual bulls have diverse sexual behaviors. In this study, Bali and Madura bulls had a duration of libido close to 1 minute. The duration of libido in both of breed was slower than Ongole crossbred bulls which have been studied by Sholikah et al., (2018a) with the average duration of libido in 0.21 to 0.68 minute.

Results showed that the duration of ejaculation did not differ ($p > 0.05$) between Bali and Madura bulls. This finding may be because both of the bulls had adapted to the environment at the research site. According to Pineda (2003) season, nutrition and social interaction during pre-puberty are the major external factors which influence the sexual behavior. For that reason, the bulls needed to be adapted first before being used for the breeding program. Yanuarista (2022) stated that males with a relatively old age will trigger a slow process of courtship, this is due to a decrease in biological responses which indirectly impacts the decrease in stimulants released from the male genital organs so that the opportunity for the emergence of courtship behavior tends to be slower.

The duration of ejaculation in each bull in the study was influenced by the habits of the individual bull, the bull handling officers and also the semen collectors. The semen collector already knew the appropriate time for semen collection in each bull based on the number of false mounting, erection quality and previous experience regarding the habits of each bull. This result was in agreement with Sholikah et al., (2018b) who stated that the ejaculation duration depended on the decision of the officer who determined whether the bull was ready

or not in the semen collection process based on the penis erection. Hafez and Hafez (2008) and Pineda (2003) explained that sexual behavior was influenced by social interaction and previous experience.

False mounting is done until the bulls secreted complementary glands and the penis must be erect (hard and red), usually false mounting is done 2 to 3 times (Susilawati, 2013). The purpose of false mounting is to increase the libido (Susilawati, 2011). In this study, Bali bulls need more false mounting ($P < 0.01$) compared to Madura bulls. Bali bulls need longer sexual stimulation to reach the peak erection. Susilawati (2011) stated that one of the shortcomings of Bali bull is having a poor libido so that the sexual stimulation and preparation are needed. In addition, the observation of erect quality parameters and a fairly calm environment must also be achieved to obtain optimal semen results.

The differences in sexual behavior are influenced by many factors. Hafez and Hafez (2008) explained that the intensity of sexual behavior was influenced by genetic, hormonal-related physiology, and external factors, as well as previous experience. The use of bull teasers and artificial vagina may also affect the appearance of libido in native cattle. Palmer (2005) stated that the use of artificial vaginal was not effective in shy bulls. According to Hafez and Hafez (2008), sexual activity of bulls will increase when new receptive cows appear. The bulls which were gathered with estrus cows will have a higher libido, but the young bulls which were gathered with other bulls will develop homosexuality (Phillips 2002).

Semen Production

The semen production assessment was carried out immediately after collection which aimed to find out whether semen had good quality and is suitable for further processing or not. The evaluation included total sperm and total motile sperm counts. Table 2 shows the semen production of Bali and Madura bulls.

Table 2. Semen production in Bali and Madura bulls

| Sexual behavior | Bali bulls | Madura bulls | <i>p</i> |
|---|--------------------------------------|--------------------------------------|----------|
| Total sperm count (10^6 /ejaculate) | 6,826.18 \pm 2,084.88 ^b | 5,392.25 \pm 2,038.77 ^a | 0.008 |
| Total motile sperm count (10^6 /ejaculate) | 4,760.44 \pm 1,515.30 ^b | 3,404 \pm 1,414.20 ^a | <0.001 |

^{ab} means in the same column without common letter are different at $p < 0.01$

The differences in the breed of the bulls affected ($p < 0.01$) total sperm count. Zamuna et al., (2016) stated that sperms production varies between breeds. Bali bulls had higher total sperm count compared to Madura bulls. This finding may be because of the age of Madura bulls was older than that of Bali bulls, resulting in a decrease in semen production. This finding was in accordance with Ratnawati et al., (2018) and Susilawati et al., (2017) who reported that the older age of the bulls resulting in the lower of the semen quality.

The breeds of the bulls also affected ($p < 0.01$) total motile sperm count. Bali bulls had higher total motile sperm count compared to Madura bulls. Yates et al., (2003) explained that the breed of cattle is a significant source of variation in total ejaculation volume, initial sperm concentration and motility. Susilawati (2013) explained that the differences in the breed of cattle affected the semen quality.

Relationship Between Sexual Behavior and Total Sperm Counts

Table 3 shows the relationship between sexual behavior and total sperm count in Bali and Madura bulls. The duration of libido with the total sperm count had a negative correlation in Bali bulls while this correlation was positive in Madura bulls. The relationship between

duration of libido and total spermatozoa was not significant ($p>0.05$) in both of Bali and Madura bulls.

Table 3. Correlation between sexual behavior and total sperm count in Bali and Madura bulls

| Sexual behavior | | Total sperm count | |
|-------------------------|---------------------|-------------------|-------------------|
| | | Bali bulls | Madura bulls |
| Duration of libido | R | -0.30 | 0.02 |
| | R ² | 0.09 | 0.00 |
| | Regression equation | $y = 7244 - 427x$ | $y = 5262 + 124x$ |
| Duration of ejaculation | R | -0.34* | 0.15 |
| | R ² | 0.12 | 0.02 |
| | Regression equation | $y = 8874 - 302x$ | $y = 4230 + 196x$ |
| False mounting | R | 0.25 | -0.22 |
| | R ² | 0.06 | 0.05 |
| | Regression equation | $y = 4206 + 509x$ | $y = 6801 - 354x$ |

* Correlation is significant at the 0.05 level (2-tailed)

The duration of ejaculation and total sperm count in Bali bull had a negative significant correlation ($p<0.05$), while in Madura bull had a positive non-significant correlation. The coefficient of determination between the ejaculation duration of Bali bulls and total sperm count was 0.12, which means that the 12% total sperm count was affected by the ejaculation duration and the rest was influenced by other factors. In Bali bulls, the longer ejaculation duration could decrease the total sperm count with the form of $Y = 8874 - 302x$. Meanwhile, an insignificant correlation was found in the relationship between the duration of ejaculation and total sperm count in Madura bulls.

False mounting and total sperm count had a positive relationship in Bali bulls and a negative relationship in Madura bulls. The relationship between false mounting and total sperm count was not significant ($p>0.05$) in both breeds. This result was presumably because the Bali and Madura bulls had a higher number of false mounting because usually, the bulls had 2 to 3 times of false mounting before semen collection. Shearer and Katz (2006) described that the bulls which were given the opportunity to see mounting activities of other bulls (both homosexual or heterosexual mounting) before semen storage had greater potential to produce semen.

Relationship Between Sexual Behavior And Total Motile Sperm Count

Table 4 shows the relationship between sexual behavior and total motile sperm count. The duration of libido with total motile sperm count had a negative correlation in Bali bulls, while a positive correlation was found in Madura cattle. The correlation between duration of libido and total motile sperm count in Bali and Madura bulls was not significant ($p>0.05$).

Table 4: Correlation between sexual behavior and total motile sperm count in Bali and Madura bulls

| Sexual behavior | | Total motile sperm count | |
|-------------------------|---------------------|--------------------------|-------------------|
| | | Bali bulls | Madura bulls |
| Duration of libido | r | -0.31 | 0.12 |
| | R ² | 0.10 | 0.01 |
| | Regression equation | $y = 5074 - 320x$ | $y = 3061 + 325x$ |
| Duration of ejaculation | r | -0.34* | 0.34* |
| | R ² | 0.11 | 0.11 |
| | Regression equation | $y = 6218 - 215.1x$ | $y = 1812 + 268x$ |

| | | | |
|----------------|---------------------|---------------|---------------|
| False mounting | r | 0.24 | -0.10 |
| | R ² | 0.06 | 0.01 |
| | Regression equation | y = 2978+346x | y = 3829-107x |

* Correlation is significant at the 0.05 level (2-tailed)

The duration of ejaculation had a significant negative correlation ($p < 0.05$) with total motile sperm count in Bali bulls, while in Madura bulls had a significant positive correlation ($p < 0.05$). In this study, both of Bali and Madura bulls had a coefficient of determination (R^2) between the duration of ejaculation and total motile sperm count of 0.11. It could be interpreted that 11% total motile sperm count was influenced by the duration of ejaculation of both in Bali and Madura bulls.

A positive non-significant correlation ($p > 0.05$) between false mounting and total motile sperm count was found in Bali bulls, while a negative non-significant correlation was found in Madura bulls. These findings indicated that sexual stimulation in the form of restraint when ejaculating was not affected the total motile sperm count of Bali and Madura bulls. This study was in accordance with Philips (2002) who stated that sexual preparation or by prolonging sexual stimulation is very important to maximize total motile sperm count but this is more ineffective in the beef bulls than the dairy bulls, because not only have lower libido, the beef bulls also have a low response to sexual stimulation.

Conclusion

The conclusion of this research was Bali and Madura bulls have similar duration of libido and duration of ejaculation, however, Bali bulls have higher false mounting, total sperm and total motile sperm counts than Madura bulls. The duration of ejaculation was found to be useful in predicting the semen production. In Bali bulls, the duration of ejaculation had undesirable correlated response for total sperm and total motile sperm counts. While in Madura bulls, the duration of ejaculation had desirable correlated response for total motile sperm count.

Conflict of Interest

This research has been approved by all authors and there is no conflict of interest either in funds or other matters concerning the interests of researchers.

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