Individual Variance Component of Fresh Semen Quality in Bali Cattle (Bos javanicus) Bull

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Abstract. Semen quality is an important factor influencing the success of a cattle breeding program, therefore an effort to continuously evaluate semen quality is needed. Indonesia has Bali cattle; it is indigenous, tropically adapted, robust, and has high fertility. Bali cattle need to be developed into meat producer by selecting best traits from bulls and disseminate their sperm through artificial insemination program. To obtain the desired improvement, semen quality became one of the keys to ensure. This study aimed to determine the factor(s) affecting fresh semen quality of Bali cattle bull. In total, 742 ejaculates were collected from nine bulls in 2016-2017 over a 12-month period. Semen was collected twice a week, followed with semen quality evaluation as semen volume (ml), sperm concentration ($x10^6$/ml), sperm motility (%), and pH. A linear model was built to obtain the significant fixed factor of season and/or age affecting sperm quality followed by mixed model procedure including individual bulls as random effect to estimate the variance components. The result showed that season didn’t give any effect ($p>0.05$) in all fresh semen quality observed, while there was a significant effect of age ($p<0.05$) on volume, sperm concentration and pH. There is no interaction ($p>0.05$) between season and age in this study. The variance component of individual bulls contributed 71.15, 67.92, 48.22, and 11.76% of the total variance of semen volume, sperm concentration, sperm motility, and pH respectively. This study shows that there is a wide variation of semen quality resulted due to the variation between individual of the Bali cattle bull, which mirroring the diverse of Bali cattle genetic. In bull’s selection as semen source, careful selection and the application of genetic standard need to be concerned.

Keywords: semen quality, Bali cattle bull, individual variance component
1. Introduction

Bali cattle is an Indonesian endogenous animal, known by its superiority to be adapted in tropical area, robust, high fertility and able to survive in limited feed quality [1] [2]. This cattle has genetic potential to be developed as meat produced by good strategic breeding program for example crossbreeding [3], due to its still has large room for genetic improvement [1]. To overcome that aims, a breeding strategy to disseminate the genetic material especially from male side is needed by carefully applying reproductive biotechnology namely Artificial Insemination (AI) program.

It is generally known, sperm produced from spermatogenesis and carry genetic material [4]. If the sperm came from high genomic selected bull, disseminating its sperm is equal to spreading best selected genetic material. However, semen quality must be considered and need to be continuously evaluated to have good result in the breeding program. Hence, semen quality have high correlation with fertility [5–9], therefore ensuring semen in high quality level would maintain and improve the success of AI application as well as its efficiency.

Sperm production and its quality belong to factors need to be considered in animal breeding program and AI industry. In fresh, semen quality was reported to be affected by several factors such as age [10], season [11] [12], temperature [13], interval collection [14], individual variation [15], and genetic [16]. For that, this study aims to evaluate the factor(s) associated with fresh semen quality especially the individual variation of Bali cattle bull. Moreover, the outcome of this study could be an input to improve and maximizing the sperm harvest for sustainable semen production in AI center by ensuring and managing factor(s) related to semen quality.

2. Methodology

Semen samples collection and bulls rearing

This study was conducted at AI Centre Singosari Malang Indonesia (7.8377° S, 112.6464° E) in 2016-2017 over a 12-months period. As humid tropical country, Indonesia has rainy and dry season that may influence the semen quality, therefore we also consider season as the factor. Rainy season was occurred in November – May and dry season at June – October according to the climatology rainfall data from nearby weather station. Season was categorized as rainy when rainfall was > 100 mm, while dry was less than 100 mm.

Semen samples were collected from 4 y.o. (n=5) and 7 y.o. (n=4) bulls using artificial vagina. Prior semen collection 3-5 false mounting were performed to sexually stimulate the bull for optimal semen harvest [17]. Semen was collected two times a week and only one ejaculate per collection time. During the study, bulls were reared in individual pen with two times feeding (morning and evening) and free access to mineral block and water.

Semen quality

Semen qualities were evaluated as macroscopic and microscopic. For macroscopic evaluation, semen volume (ml) was determined by directly from semen collection tube soon after collection. Sperm concentration (×10^6 cell/ml), sperm motility (%) and semen acidity (pH) were observed as microscopic data. Sperm concentration was determined using spectrophotometer (Minitube SDM6). Briefly, 0.035 ml semen samples were mixed with 3.5 ml of 0.9% NaCl followed with 5-7 seconds homogenization, then the mixture was transferred to the cuvette for measurement. The sperm motility was subjectively scored (0-100%) of progressive motile using microscope (Olympus BX53) at magnification 400x. The semen pH was evaluated by immersing pH meter probe in tube contained semen sample.
Data analysis
Statistical summary of sperm quality. In the present study, season and age were selected as factors which affecting semen quality. During observation period, all the acceptable ejaculates were evaluated on its quality, then the data were tabulated as mean ± standard deviation. Following that, mean of group comparison between season (rainy vs. dry), age (4 vs. 7 y.o.) and both interaction (rainy_4 vs. rainy_7; dry_4 vs. dry 7) were done by t-test at α = 5%.

Variance component analysis of sperm quality on the individual bull. A linear model was built as preliminary analysis to check the significance of season, age and the interaction between age and season as factor(s) affecting the semen quality. Later, we employed a mixed model procedure including the significant fixed effect(s) and individual bulls as random effect to estimate the variance components as in the following equation:

\[ y = Xb + Ia + e \]

where \( y \) is a vector of observed variables, \( b \) is a vector of fixed effects accounted for age and season and \( X \) is the incidence matrix corresponded to the fixed effects. \( I \) is an identity matrix corresponding to the random individual effect \((a)\) where \( a|I, \sigma_a^2 \sim MVN(0, \sigma_a^2) \). Random residual vector is \( e \) with \( e|I, \sigma_e^2 \sim MVN(0, \sigma_e^2) \). The equation was solved with maximum likelihood estimator in R programming language [18].

3. Result and Discussion
In total, 742 ejaculates were collected from nine bulls during 12 months observation period. The result showed that season didn’t give any effect (p>0.05) in all fresh semen quality observed, while there was a significant effect of age (p<0.05) on volume, sperm concentration and pH. We also found, there is no interaction (p>0.05) between season and age in this study (Table 1).

Age, reported to have significant effect to semen volume [10], motility and sperm concentration [19]. In the present study, we found bulls at 7 y.o. produce more (p<0.05) sperm compared to 4 y.o. (Table 1). According to the earlier study, the semen volume was increased in line with bull age [20], due age has positive correlation with scrotal circumference and daily sperm production capacity [21] [22]. Pervious study confirmed the result by found a positive correlation between semen volume (r=0.63) and sperm concentration (r=0.60) with scrotal circumference [23]. Testis with larger scrotal circumference shows ability to produce more number of spermatozoa during spermatogenesis [24], because its has more number of seminiferous tubules. In this regard, scrotal circumference could be a useful indicator of potential sperm output and may serve as an important criterion for selecting bulls as AI sires [22].

The semen pH in this study shows differences between age group (p<0.05). Semen pH is influenced by accessory gland secretion and feed composition [25]. This could be the indicator of sperm metabolism, because acid condition will reduce sperm motility and further sperm fertility [26] [27]. Age also influencing sperm freezeability during cryopreservation. Earlier report said that genetic, age, breed and individual factors influence sperm viability with thermal shock [28]. Previous finding in our house demonstrated that younger Bali bulls have lower decrease in sperm quality of fresh to chill or frozen stage during cryopreservation [29]. As generally known, cryopreservation has number of potential stress damaging plasma membrane such as temperature variation, osmotic and toxic stress, and the formation of ice in the extracellular environment [30].
Table 1. Summary statistics of sperm quality

| Variable       | Mean ± sd | Volume (ml) | Concentration (x10^6/ml) | Motility (%) | pH       |
|----------------|-----------|-------------|--------------------------|--------------|----------|
|                |           | 5.33±1.27   | 1128.77±182.59           | 67.16±6.57   | 6.50±0.05|
| Season         | Dry       | 5.11±1.21   | 1028.90±171.96           | 64.78±8.20   | 6.47±0.06|
|                | Rain      | 5.62±1.31b  | 1113.32±228.57a          | 67.46±6.08   | 6.50±0.06b|
| Age (year)     | 4         | 4.87±1.07a  | 1036.26±126.77b          | 64.42±8.48   | 6.47±0.06a|
|                | 7         | 5.62±1.31b  | 1113.32±228.57a          | 67.46±6.08   | 6.50±0.06b|
| Season*Age     | Dry_4     | 5.05±1.08   | 1108.44±104.18           | 66.24±8.01   | 6.50±0.06|
|                | Dry_7     | 5.69±1.41a  | 1154.07±259.26           | 68.30±4.05   | 6.57±0.05|
|                | Rainy_4   | 4.75±1.06   | 984.70±116.87            | 63.11±8.67   | 6.45±0.06|
|                | Rainy_7   | 5.56±1.26   | 1084.14±212.14           | 66.86±7.20   | 6.50±0.06|

Table 2. Variance components of Bali cattle’s semen quality

| Variable       | Residual variance | Individual variance |
|----------------|-------------------|---------------------|
|                | Absolute Percentage | Absolute Percentage |
| Volume (ml)    | 0.39 ± 0.63       | 1.27±1.13           | 71.15              |
| Concentration (x10^6/ml) | 11991.00 ± 109.50 | 2538±159.30         | 67.92              |
| Motility (%)   | 32.43 ± 5.69      | 30.20±5.49          | 48.22              |
| pH             | 0.0003 ± 0.05     | 0.00004±0.02        | 11.76              |

Based on the observed variables, individual variation contributes around and/or more than 50% to the semen quality. We found the variance component of individual bulls contributed 71.15, 67.92, 48.22, and 11.76% of the total variance of semen volume, sperm concentration, sperm motility, and pH respectively (Table 2). All the non-genetic factor(s) in this study, age and season, have been corrected. Since the bulls were the same breed, reared under the same environment, same feed, thus the only different or variation presumably due to genetic variation between the bull. In the simple way, we could say that variation among bulls has large influence on the variation of semen quality. This result is in agreement with pervious study which reported individual genetic, management and environment as factors contribute to semen production [16] [31].

According to the sperm production factors, presumably the differences is caused by the difference capacity between bull in spermatogenesis. Foote in his study [32] stated that the major contributor to variation in semen quality is the environment. Environmental effects may be temporary or permanent. Permanent effects occurring during prenatal and prepubertal periods and temporary or permanent factors acting after spermatogenesis is initiated can alter semen quality. To the best of our knowledge, bull’s selection for sperm source were performed at smallholder farm, then selected and reared in the AI station after puberty. Since we do not know the environment condition during prepubertal bulls are grown, this is likely the plausible explanation of variation come from. Difference prepubertal environment could lead to the growth difference of testicular and reproductive accessory gland, resulted in difference semen production capacity.
Secondly, in the molecular perspective, the variation of genes controlling sperm production could explain the difference sperm production capacity between bull. Difference expression of genes lead to the difference sperm production. For example, the difference of FSHR expression is associated with spermatogenic capacity in the testis. Expression of Aqp8 as well as other FSH-stimulated genes is highly dependent on the hormone action during puberty [33]. The genotype variation of FSH beta-subunit gene reported to be associated with the sperm abnormality during spermatogenesis [34].

In bull’s selection as semen source, careful selection and the application of genetic standard need to be concerned. By intense selection accompanied with maintain the environment, bull individual variation could be reduced. All in all, the semen production could be fulfilling the genetic demand for AI program as well as keep it fertility in high level.

4. Conclusions

This study shows that there is a wide variation of semen quality resulted due to variation between individual Bali cattle bull in AI center. It is mirroring the diverse of Bali cattle genetic, which still have a lot of possibility to be improved. Especially in bull’s selection for AI center purpose, careful selection and the application of genetic standard need to be concerned in order to maximizing sperm production by considering its capability and limitation of bull reproductive capacity.

Acknowledgement

The authors extending a sincere gratitude to Universitas Sebelas Maret for funding this study. Thousand thanks also goes to AI centre Singosari, Malang, Indonesia for their permission to perform this study.

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