The traits of fresh and frozen semen on brahman bulls

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Abstract. The present study aimed to determine quality and parameters traits of both fresh and frozen semen in 4-year old Brahman bulls. Semen was collected from 5 bulls twice a week using artificial vagina in Ungaran Artificial Insemination Center, Central Java, Indonesia. Immediately after collection, samples were evaluated for semen volume, color, odor, viscosity, mass movement, individual movement, spermatozoa concentration and live spermatozoa percentage. Sperm concentration was measured using a spectrophotometer. Good quality fresh semen was processed to be frozen semen. Parameters evaluated for frozen semen include spermatozoa individual movement, live spermatozoa, and abnormal spermatozoa percentage. The results showed that the semen volume is 6.90 ± 0.54 whereas the average of sperm concentration, individual motility and live sperm are 1754.54 ± 212.67; 65.50 ± 3.74% and 76.30 ± 2.64%, respectively. Meanwhile, for frozen semen, the average percentage of motility is 38.00 ± 2.74% while the average proportions of live sperm and abnormal sperm are respectively 45.20 ± 9.86% and 6.00 ± 2.83%. In conclusion, in average, all parameters of fresh and frozen semen of Brahman bulls are in normal range. Based on the Indonesian National Standard, frozen semen produced could be used for insemination.
Keywords: Spermatozoa, fresh semen, frozen semen, Brahman

Introduction

Brahman is a *Bos indicus* breed. Brahman bulls which were developed in Indonesia come from India. Brahman and Brahman based bulls are adapted to adverse environmental conditions of the tropics and subtropics. According to Sudarmono and Sugeng (2008) these type of bulls are suitable with Indonesian climate. They can adapt well with high temperature, feed limitation and tolerance of internal and external parasites. Moreover, they are very popular among Indonesian farmers since they are one of breeds of beef cattle raised in Indonesia. Therefore, their frozen semen is produced by all Artificial Insemination (AI) centers in Indonesia including Ungaran AI center.

Artificial insemination is a powerful biotechnology that allows producers to use superior sires, promoting faster genetic improvement and increasing profitability (Brito *et al*., 2002). The use of the best sires is often restricted by the limited number of doses of semen produced. To satisfy the high demand for semen from superior sires, the industry AI has to optimize the number of of spermatozoa per dose of semen in order to produce the maximum number of straws with a limited effect on conception rate (Mathevon *et al*., 1998).

Knowledge of fertility in bulls is an objective of great importance for the production of bovine semen, which is achieved by good analysis of the semen, among other assessments. The ideal semen analysis would be simple and effective, allowing the breeding capacity of a particular ejaculate to be predicted. A fertile ejaculate must meet certain semen parameter quality standards (Dahmani, 2011).

The objective of the present study was to determine both fresh and frozen semen traits from Brahman bulls of 4 years old at Ungaran AI Centre, Central Java, Indonesia so that the fertilizing potential of the semen samples can be determined.

Materials and Methods

Semen collection and Fresh semen analysis

Five heads Brahman bulls which are clinically health, aged 4 years old were used in the present study. The same diet and the same sexual preparation were applied to all animal tested. All ejaculates were obtained after natural ejaculation in an artificial vagina. Then, they were evaluated immediately after collection. Volume of the ejaculate was read directly from the tube after removal from the artificial vagina. Sperm concentration was measured with a spectrophotometer, which was properly calibrated. The proportion of progressively motile spermatozoa was assessed immediately following semen collection. A small drop of semen was placed on a pre-warmed slide, covered with a cover slip, and examined with a bright-field

microscope (400× magnification) with a heated stage. The proportion of spermatozoa that were progressively motile was estimated. Meanwhile, live spermatozoa was measured with eosin-nigrosin stain, mixing 10µL of semen and 10 µL of stain for 30 seconds. Spermatozoa were smeared onto a pre-warmed glass slide and air dried. The percentage of viable (unstained) sperm was determined by smear observation under microscope at 1000x magnification. All these analyses were conducted by one technician in Ungaran AI Center. After routine evaluation, only ejaculates that reached at least the minimum quality standards were processed to be frozen semen (concentration : $1000x10^6$ sperm/mL; individual progressive motility : 70%).

Frozen semen analysis and Spermatozoa motility

Straws obtained from Ungaran AI center were evaluated in the Laboratory of Reproduction and Obstetry, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta. Three traits were evaluated for frozen semen samples, namely spermatozoa motility, live spermatozoa, and abnormal spermatozoa.

Motility was assessed immediately after thawing. Semen thawing was carried out by plunging the frozen straws (0.25mL) in a 30 °C water bath for 30 seconds. After that, they were removed and dried with a paper towel, because water can be hazardous to sperm. The content within the straw were shaken toward the cotton plug end. The other end of the straw was cut off, and semen was released into a small clean disposable test tube by cutting a small opening just below the cotton plug. A small drop of semen was placed on a warmed (37ºC) slide, covered by a coverslip and examined under electrical microscope-BX51 (Olympus, Japan) with magnification 400x. The sperm motility was observed and scored from minimum five view fields and the average scores were recorded as final motility score (Ax *et al*. 2000).

Live spermatozoa and Abnormal spermatozoa

A drop (10 µL) of semen mixed with an equal drop of eosin-nigrosin stain, prepared in accordance with Barth and Oko (1989). This films were made by spreading the stained content onto clean slides and quickly dried on a hot plate (37ºC). Eosin is a differential stain, unable to pass through living cell membrane but can pass through non living ones. A background nigrosin stain made the unstained sperm heads visible. Microscope observations area were selected randomly from ten fields with total two hundred sperm cells per bull scored under microscope (1000X) for determining the incidence of live and dead spermatozoa (pink cells), expressed in percent.

The same steps as evaluation of live spermatozoa were performed to evalute sperm morphology. A total of 200 sperm cells were observed with a 1000x magnification (100x objective under oil immersion).

Statistical Analysis

The data on parameters of fresh and frozen semen are presented as mean and standard deviation.

Results and Discussion

Fresh semen

Average proportions of semen volume, sperm concentration, progressive individual motility and live sperm of fresh semen in Brahman bulls are presented in Table 1.

Table 1. Mean ± SD of parameters of fresh semen of Brahman bulls

Parameters	Brahman (mean ± SD)
Volume (mL)	6.90 ± 0.54
Sperm concentration	1754.54 ± 212.67
Individual motility (%)	65.50 ± 3.74
Live sperm (%)	76.30 ± 2.64

Volume of semen is a parameter that depends on the function of the semen vesicle and sex glands plus other factors such as age (Garner and Hafez, 2000), species, body weight and training (Hunter, 1982). Garner and Hafez (2000) stated that mature bulls produce 5.0-

8.0 mL ejaculates. Average of semen volume in this research was 6.90 ± 0.54 mL (Table 1). Directorate General of Livestock Production (2000) in Indonesia stated that the average of semen volume of bulls is 5 mL. Therefore, this volume is in a normal range. However, Sumeidiana *et al*. (2007) reported that the average of semen volume of Brahman bulls in Ungaran Artificial Insemination Center was 5.74 ± 2.38 mL and ranging 2.60-10.20 mL. Therefore, our finding was higher than theirs. Although, both of us used the same species but age of bulls used affected these results. Four years old Brahman bulls were used in this study whereas they used Brahman bulls aged 24-30 months. According to Hafez *et al*. (2000) young animals within a species will produce less semen volume.

Moreover, this result differences were also caused by the differences of body weight of bulls. The average of body weight of Brahman in recent study was 783,20 kg while Sumeidiana *et al* (2007) used bulls which have body weight average 666,33 kg (574-851 kg). Hunter (1982) claimed that an increase in body weight in livestock shows an increase in the volume of semen, which is due to an increase in the weight of accessory glands. Furthermore, Hafez *et al*. (2000) stated that lower volume of semen is produced by small animals within a species. Sperm concentration observed during the recent study (1754.54 ± 212.67 x 10^6 /mL) was far from the finding of Sumeidiana *et al*. (2007) in Brahman bulls (1547 ± 318.68 x 10^6 /mL and ranging from 1130 to 2106 million/mL. According to Hafez *et al*. (2000) young bulls have sperm concentration 2.0 x 10^8 sperm cells/mL while adult bulls have 1.8 x 10^9 sperm cells/mL semen. Also, Garner and Hafez (2000) stated that bulls ejaculate 5.0-8.0 mL semen which contains 800-2000 million sperm/mL semen. Therefore, this sperm concentration in this study was in normal range. The progressive motility observed during the present study was 65,50±3,74 %. Nevertheless, only samples which have minimal progressive motility 70% will be processed to be frozen semen. According to Grahman *et al*. (1980), sperm motility is a fairly reliable indication of the viability of both fresh and frozen semen in bulls. There are differences in motility in various reports, these could be due to variations in the judgement of motility, number of bulls studied, or difference of season in studied (Javed *et al*., 2000).

Frozen Semen

Average proportions of sperm motility, live sperm and abnormal sperm of frozen-thawed semen in Brahman bulls are presented in Table 2. The proportion of sperm motility in the fresh semen is totally different from that in the frozen-thawed semen. There is a significant decrease in sperm motility and viability in frozen-thawed semen caused by the decrease in energy sources and the accumulation of toxic waste of metabolized diluents. Furthermore, processing and storage inevitably cause changes to the sperm membranes. These changes are generally assumed to be detrimental and have been associated with a loss of motility and fertility. Moreover, Chandolia *et al*. (1999) reported that sperm cells undergo warm shock during thawing. Warm shock reduces the proportion of motile sperm cells in Brahman, Holstein, and Angus bull studied. It may be because of those reasons, the average of motility of sperm in recent study dropped from 65.50% in fresh semen to 38% after thawing. The post thawing motility (PTM) was also influenced by equilibration temperature, handling of straw (Foote and Kaproth, 2002) and liquid N_2 storage (Said *et al*., 2004) which was cited by Pratiwi *et al*. (2009).

Table 2. Mean ± SD of parameters of frozen-thawed semen of Brahman

Parameters	Brahman (mean ± SD)
Sperm motility (%)	38.00 ± 2.74
Live sperm (%)	45.20 ± 9.86
Abnormal sperm (%)	6.00 ± 2.83

In this study, sperm motility of frozen semen on Brahman bulls after semen were distributed was 38% (Table 2). It had range from 35% to 40%. Based on quality standard of frozen semen determined by Indonesian National Standard or SNI (2008), minimal PTM for

frozen semen is 40%, it is generally done 24 hours after freezing. Thus, sperm obtained in this study fullfil SNI requirement. The average PTM in this study was quite higher than PTM (30-35%) reported by Ichwandi (2004) evaluated semen of Brahman in Ungaran AI Center. However, sperm motility obtained in this study was lower compared to motility found in Brahman (40%) by Pratiwi *et al*. (2009) or (43.33±2.50%) by Kartika (2012). This differences perhaps due to thawing method and age of the bulls used. In recent research, ejaculates were obtained from 4 year old Brahman bulls and thawing was conducted at 30^{o}C for about 30 seconds whereas thawing duration done by Pratiwi *et al*. (2009) was 45 seconds and they used Brahman aged 2-3 years old. Meanwhile, Nava-Trujillo *et al*. (2011) stated that the percentage of motility in cryopreserved spermatozoa from Brahman bulls was significantly affected by bulls, ejaculate and the interaction between both variables. They found motility ranked from 23% to 56% with a mean of 33.88 ± 12.43%.

Barth and Oko (1989) reported that morphology of the sperm cells is an important criterion in evaluating quality of semen in bulls. Furthermore, Saacke (2008) stated that a high incidence of abnormal sperms has been believed to be associated with reduced fertility. In agreement with this statement, Ax *et al*. (2000) claimed that morphologic abnormalities of sperm have the greatest correlation with the fertility in livestock.

According to Arifiantini *et al*. (2010) in general, level of sperm abnormality of bulls at AI centers in Indonesia was low (3.2±1.95%). This percentage was obtained without considering the individual factors or the sperm abnormality types. In addition, Pratiwi *et al*. (2009) stated that percentage of abnormal sperms post thawing in Brahman raised in Central Java, Indonesia was 1.5% ($P<0.05$). However, we found that frozen-thawed semen in Brahman bulls have abnormal sperms (6.00±2.83 %). It is true to say that our finding was quite higher than the percentage found by Pratiwi *et al*. (2009). The proportion of sperm abnormality correlated with age of the bull. Younger bulls usually had higher sperm abnormalities than older bulls (Padrik and Jaakma, 2002).

The types of abnormal sperm cells, both primary and secondary abnormalities were observed in semen samples evaluated. Primary abnormalities are, by definition, those that originate within testes during spermatogenesis and secondary abnormalities are those that originate within epididymis (Hunter, 1982). Primary abnormal sperms were pear and tapered head while secondary abnormalities were coiled tail, bent tail, folded tail, tailless. Data about the percentage of each abnormality found is not published. Arifiantini *et al*. (2010) claimed that pear shapes were the highest incidence of primary sperm abnormality among four breeds (Brahman, Bali, Limousine, and Simmental) investigated at fourteen AI centers in Indonesia. They obtained 1.4 ± 1.6% primary sperm abnormalities in Brahman bulls observed. Moreover, Riyadhi (2010) reported that age did not influence the proportion of sperm abnormalities in Brahman.

Conclusions

In average, all parameters of fresh and frozen semen of Brahman bulls are in normal range. Based on the Indonesian National Standard frozen semen produced could be used for insemination.

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