Characteristics of frozen-thawed semen on Simmental and Limousin bulls in Ungaran, Indonesia

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Abstract. The present research aimed to study the characteristics of frozen-thawed semen in beef bulls ex-import in Ungaran, Indonesia. 5 heads Simmental and 5 heads Limousin of 5.5 years old were used in this research. The research was done in Ungaran Artificial Insemination Center, Central Java, Indonesia and Laboratory of Reproduction and Obstetry, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia. Frozen semen was obtained from this AI center. Results of the research indicated that the average individual motility of Simmental and Limousin is 39.00±5.48% and 36.00±2.24%. The average percentage of live spermatozoa is 54.00±10.75% for Simmental and 48.20±9.78% for Limousin while the average proportion of abnormal spermatozoa is 9.60±3.36% and 6.80±4.15% for both Simmental and Limousin, respectively. On average, Simmental frozen-thawed semen have the higher mean proportion of sperm motility, live sperm, and abnormal sperm than that of Limousin frozen-thawed semen.

Key words: Spermatozoa, frozen semen, Simmental, Limousin.

Introduction

Artificial insemination (AI) is the single most important technique used for the genetic improvement of cattle. This is possible because a few highly selected bulls produce enough spermatozoa to inseminate thousands of cows per year. The goal of the AI field services is to maximize the number of viable offspring per breeding animal per unit time. This can be achieved by inseminating cows with sufficient progressively motile spermatozoa from a given ejaculate without reducing their fertilizing capacity. Thus, the small number of frozen-thawed spermatozoa in each insemination dose must be of very high quality to ensure acceptable pregnancy rates (Brinsko and Varner, 1993).

Success with AI is dependent on proper preparation of cryopreserved semen, proper handling of frozen semen, maintenance of an unbroken chain of cryogenic temperature (less than −130°C), healthy cows, detection of estrus or an effective synchronization program, and correctly following procedures for straw thawing and AI (Kaproth and Foote, 2011).

Ungaran AI Center is one of AI centers in Indonesia that has major task to provide qualified bull frozen semen (Simmental, Limousin, Brahman, F.H and Brangus). Bulls are selected based on their pedigree or the progeny test. Another method of bull selection is by using breeding soundness evaluation (BSE) which is a technique to identify individual problems affecting the bull fertility. In general, BSE consists of three parts; physical evaluation (evaluation on external genital organs and rectal exploration), measurements of scrotal circumference and semen analysis (Alexander, 2008).

Frozen semen of Simmental and Limousin is quite popular among Indonesian farmers since both of them are some of breeds of beef cattle raised in Indonesia. In general, they come from Australia and arrived in Indonesia at the age of 1 year. Information about quality of frozen-thawed semen from young beef bulls ex-import (1-3 years old) have been well documented, but data published about their frozen semen quality after adaptating more than 3 years in Indonesia is limited. The aim of the present investigations was to characterize the sexual and semen characteristics from ex-import bulls (Simmental and Limousin) of 5.5 years old in Ungaran, Central Java, Indonesia.

Materials and Methods

Clinical examination of bulls

Five heads Simmental and five heads Limousin were examined for clinical examination. The bulls were 5.5 years old. The scrotum of bulls was inspected with respect to size, symmetry and any visible skin diseases. The bulls were weighted routinely once a month by digital scales-FX1 (Iconix, New Zaeland) and scrotal circumferences were measured by tape (Coulter et al. 1976) before semen collection.

Semen collection

Semen was collected of clinically healthy bulls using artificial vagina. Good quality semen was processed to be frozen semen.

Frozen semen analysis

Frozen semen samples obtained from the Ungaran Artificial Insemination Centre was evaluated for spermatozoa motility, live spermatozoa, and abnormal spermatozoa in the Laboratory of Reproduction and Obstetry, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta.

Spermatozoa motility

Motility was assessed immediately after thawing. Straws (0.25 mL) were thawed in a 30ºC water bath; after 30 seconds, straws 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

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 (400X) for determining the incidence of live and dead spermatozoa (pink cells), expressed in percent.

Abnormal spermatozoa

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 body weight, size of scrotal circumference of bulls and their frozen semen are presented as mean and standard deviation for each group.

Results and Discussion

The average of body weight and scrotal circumference of both Simmental and Limousin bulls are presented in the Table 1. The bulls were 5.5 years old. Simmental weighed between 971 and 1173 kg and their scrotal circumference varied from 40.0 to 45.0 cm. The average of Simmental body weight is 1071.60±101.49 kg while that of their scrotal circumference is 42.60±2.30 cm. Limousin have body weight between 914 and 1109 kg and their scrotal circumference varied from 30.0 to 38.0 cm. The average of Limousin body weight and scrotal circumference is 1010.80±97.14 kg and 33.20±3.11 cm, respectively. The size difference of these scrotal circumferences may be influenced by breed and body weight. The effect of breed (Coulter and Keller, 1982; Latimer et al. 1982), testes weight (Coulter and Keller, 1982), age (Madrid et al. 1988) on scrotal circumference has been well documented.

Table 1. Mean ± SD of body weight, and scrotal circumference of Simmental and Limousin bulls.

Parameters	Simmental (mean±SD)	Limousin (mean±SD)
Body weight (kg)	1071.60±101.49	1010.80±97.14
Scrotal circumference (cm)	42.60±2.30	33.20±3.11

Spermatozoa motility

The goal of semen analysis is to determine the fertilizing potential of the semen sample (whether it be fresh, cooled or frozen-thawed), using a rapid, inexpensive procedure (Mocé and Graham, 2008). In a succesful AI organization, it is important to obtain high fertility from every sample.

Motility is a common feature of spermatozoa throughout the animal kingdom. For species with internal fertilisation, motility is important for sperm transport within the reproductive tract and for egg penetration (Holt and Van Look, 2004).

In case of bulls, sperm motility is a fairly reliable indication of the viability of fresh and frozen semen (Grahman et al. 1980). Sperm motility has an important role in fertility because during copulation, cervical mucus represents a barrier which allows only migration of progressively motile spermatozoa with normal morphology and high nuclear stability (Rodriquez-Martinez et al. 1997).

The average percentage of spermatozoa motility, live spermatozoa, and abnormal spermatozoa of both Simmental and Limousin are presented in Table 2. Simmental bulls have the mean percentage of sperm motility 39.00±5.48% while Limousin ones have 36.00±2.24%. On average, Simmental bulls have the higher mean proportion of sperm motility than that of Limousin. Donors have the same age (5 years old), therefore this is presumably influence of age of spermatozoa, sperm maturity, seminal plasma and sperm membran integrity (Ax et al. 2000). Pratiwi et al (2009) reported that post thawing motility of Limousin of 2-3 years old in Blora, Central Java, Indonesia, is 41.5%. These Limousin frozen semen were thawed for 45 seconds in a 25-30ºC water bath.

Table 2. Mean ± SD of sperm motility, live sperm and abnormal sperm in Simmental and Limousin frozen-thawed semen

Parameters	Simmental (mean±SD)	Limousin (mean±SD)
Sperm motility (%)	39.00±5.48	36.00±2.24
Live sperm (%)	54.00±10.75	48.20±9.78
Abnormal sperm (%)	9.60±3.36	6.80±4.15

Ax et al. (2000) stated that there are some endogenous and exogenous factors that influence sperm motility. Endogenous factors are age of donor, age of spermatozoa in epididymis, sperm maturity (morphology, physiology, and biochemics) and sperm membran integrity. Exogenous factors are biophysical and physiological factors of semen (hydrodynamics, viscosity, osmolality, pH, temperature, and ionic composition), suspending fluids (epididymal fluid, seminal plasma, vaginal pool, cervical mucus, uterine and oviduct fluid), inorganics ions (Cu, Zn, Cd, Mn, Hg) hormones, and environmental pollutants.

Live spermatozoa

Percentage of live spermatozoa in each ejaculate is an indication of the quality of semen (Bearden and Fuquay, 1984). Fertilizing ability does not rely solely on motility and abnormal sperm parameter. It has been studied in ram that the total number of live sperm per insemination is more important than the percent abnormal sperm. The inability of a

single sperm to penetrate the zona pellucida of the ova is believed to be one of the limitating factors in semen fertility (Ax et al. 2000).

The percentage of live sperm in these semen samples is higher than that of motil sperm. This is in accordance with the statement of Bearden and Fuquay (1997). It is happened because there are a number of live sperm cells that are immotile (Campbell et al. 2003). The mean proportion of live spermatozoa in Simmental is higher (54,00±10,75) compared to that of live sperm of Limousin (48,20±9,78) (Table 2). By using eosin-nigrosin stain, the live sperm cells can be evaluated easily since they will not absorb stain, while the dead sperm will absorb stain so that they become pink cells since permeability of sperm membran decreases.

Abnormal spermatozoa

Every semen sample contains some abnormal sperm cells. Morphologic abnormalities of sperm have the greatest relationship to fertility of livestock. Morphologic abnormalities are categorized as primary, secondary, or tertiary. Primary abnormalities are associated with sperm heads and the acrosome; secondary abnormalities refer to the presence of a droplet on the midpiece of the tail; and tertiary abnormalities refer to other tail defects (Ax et al. 2000).

According to Hunter (1982), types of sperm abnormalities can be grouped into primary and secondary abnormalities. Primary abnormality occurs due to abnormalities in the seminiferous tubules, testicular disorders or phase of sperm maturation in the epididymis is not perfect while the secondary abnormalities occur after sperm cells leave the tubular epithelial cells. According to Field and Taylor (2003) primary abnormality is considered permanent and more severe, whereas the secondary abnormal is temporary.

Table 2 shows that Limousin bulls have the lower mean percentage (6.80±4.15) of abnormal sperm than that of Simmental (9.60±3.36). Some forms of primary and tertiary abnormalities were found in both Limousin and Simmental semen samples evaluated. Primary abnormalities found are microcephalic and macrocephalic whereas tertiary abnormalities (according to Ax et al. 2000) found are coiled tail, bent tail, folded tail, and tailless. Barth and Oko (1989) stated that the incidence of microcephalic was less than 1%. Microcephalic and macrocephalic abnormality of bull sperm was highly associated with the genetics. Both of these abnormalities occur due to the lack or excess of nuclear chromatin, contributing to the nuclear chromatin formation. Ax et al (2000) reported that these primary abnormalities occur during the development of spermatozoa in the seminiferous tubules while tertiary abnormalities occur due to improper handling of semen after semen were ejaculated, during storage and thawing of semen.

Conclusions

On average, Simmental frozen-thawed semen have the higher mean proportion of sperm motility, live sperm, and abnormal sperm than that of Limousin frozen-thawed semen.

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References

Alexander J.H. 2008. Bull breeding soundness evaluation : A practitioner's perspective. Theriogenology, 70 : 469-472.

Ax R.L., Dally M., Didion B.A., Lenz R.W., Love C.C., Varner D.D., Hafez B., Bellin, M.E. 2000. Semen evaluation. In : Hafez, B. and E.S.E. Hafez (eds), Reproduction in farm animals, 7th edition, pp 365-375. Lippincott Williams & Wilkins, Philadelphia, USA.

Barth A.D, Oko R.J. 1989. Abnormal morphology of bovine spermatozoa. pp 8-18. Iowa State University Press, Ames, Iowa.

Bearden H.J., Fuquay J.W. 1984. Applied animal reproduction, 2nd edition. Prentice-Hall., New Jersey, USA.

Bearden H.J., Fuquay J.W. 1997. Semen collection. In : Bearden, H.J. and J.W. Fuquay (eds), Applied animal reproduction, 4th edition, pp 147-157. Prentice-Hall Incorporation, New Jersey, USA.

Brinsko S.P., Varner D.D. 1993. Artificial insemination. In: McKinnon, A.O. and J.L. Voss (eds), Equine reproduction, pp 790-797. Lea and Febriger, Philadelphia, USA.

Campbell J.R., Campbell K.L., Kenealy, M.D. 2003. Animal Sciences, 4th edition. Mc Graw-Hill, New York, USA.

Coulter G.H., Rounsaville T.R., Foote R.H. 1976. Heritability of testicular size and consistency in Holstein bulls. J. Anim. Sci , 43: 9-12.

Coulter G.H., Keller D.G. 1982. Scrotal circumference of young beef bulls, relationship to paired testes weight, effect of breed, and predictability. Can. J. Anim. Sci, 62 : 133-139.

Grahman E.F., Schmehl M.K.L., Nelson D.S. 1980. Problem with laboratory assays. Proceedings of the 8th Technical Conference on Artificial Insemination and Reproduction, N A A B. pp 1-8.

Holt W.V., Van Look K.J.W. 2004. Concepts in sperm heterogenity, sperm selection and sperm competition as biological foundations for laboratory tests of semen quality. Review. Reproduction, 127 : 527-535.

Hunter R.H.F. 1982. Reproduction in farm animals, 1st edition. Longman, London.

Kaproth M.T., Foote, R.H. 2011. Reproduction, events and management. Mating management: Artificial insemination, utilization. Encyclopedia of Dairy Sciences, 2nd edition, pp 467-474.

Latimer F.G., Wilson L.L., Cain M.F. 1982. Scrotal measurements in beef bulls: Heritability estimates, breed and test station effects. J. Anim. Sci, 54 : 473-479.

Madrid N., Ott R.S., Veeramachaneni, D.N.R., Parrett D.F., Williams C.L. 1988. Scrotal circumference, seminal characteristics and testicular lesions of yearling angus bulls. Am. Vet. Res, 49 : 579-585.

Mocé E., Graham J.K. 2008. In vitro evaluation of sperm quality. Animal Reproduction Science, 105 : 104-118.

Pratiwi W.C., Affandhy L., Ratnawati D. 2009. Effects of thawing on frozen semen quality of Limousin and Brahman bulls. Animal Production, 11 (1) : 48-52.

Rodriguez-Martinez H., Larson B., Pertoft H. 1997. Evaluation of sperm damage and techniques for sperm clean-up. Reprod. Fertil. Dev. 9: 297-308.