EFFECTS OF FREEZING ON SPERM VELOCITY IN FOUR DIFFERENT AGE GROUPS OF JERMASIA BUCK

N.H. Hashida*, M.N.K. Nor Ashikin1, M.Z. Khaironizam and R.B. Abdullah

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur.

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ABSTRACT

The present study was undertaken to investigate the effects of freezing on sperm velocity in four different age groups (young: <1 year; mature: e<1.0-2.5 years; old: e<2.5-4.5 years and very old: e<4.5 years) of Jermasia buck. Tris Citric Acid Egg Yolk Extender (TCAYE) was added to semen samples and cryopreserved using a programmable freezer prior to plunging into liquid nitrogen (-196°C). The fresh extended and frozen-thawed semen were subjected to computer automated semen analyzer (CASA) for sperm velocity evaluation. Fresh extended sperm in mature age group showed significantly highest values for average path velocity (VAP) 76.73±1.24µm/s, straight line velocity (VSL) 55.30±1.05µm/s, curvilinear velocity (VCL) 144.31±2.38µm/s, amplitude of lateral head displacement (ALH) 6.59±0.10µm and beat cross frequency (BCF) 39.59±0.36Hz as compared to young, old and very old age groups of buck (P<0.05). Significantly (P<0.05) higher values for VAP, VSL, VCL and ALH were observed in fresh as compared to frozen-thawed sperm in all age groups of buck. Thus, age of bucks should be considered as one of the factors that contribute to better sperm velocity which might affect fertilizability of the sperm. Optimizing the freezing technique is also essential in order to obtain satisfactory results after artificial insemination.

Key words: Age, Buck, Computer automated semen analyzer (CASA), Goat, Semen freezing.

INTRODUCTION

Anatomically, bucks would continue to produce good quality semen from 9 months to 4 years of age (Nelson and Mukherjee, 1973). Dowsett and Knott (1996) indicated that most of the semen and sperm characteristics were influenced by age. It was noted that stallions either under 3 years or older than 14 years of age produced poor quality semen.

The advantages of computer evaluations of sperm movement include the availability of objective data for each individual sperm, high precision and accuracy in the recording samples containing a higher percentage of motile sperm, detection of small differences in sperm movement, less stressful and within a reasonable cost. It also allows a large number of sperm to be analyzed individually within short intervals (Rijsselaere et al., 2003). In spite of these advantages, CASA is not used commonly and routinely in veterinary practice (Klimowicz et al., 2008).

In several species, semen cryopreservation has been shown to modify sperm motility parameters (Nunez-Martínez et al., 2006; Flores et al., 2008). Cryopreservation might cause damage to sperm due to altered membrane structure and function which would reduce fertilizability of frozen semen (Watson, 2000). Cryopreservation leads to a decrease in sperm motility measured objectively by computer-aided methods in the goat (Dorado et al., 2009) and other animal species (Thurston et al., 2001; Martinez-Pastor et al., 2005).

There is limited research on motility analysis of goat sperm by CASA system. Therefore, the current study was carried out to evaluate the effects of freezing on sperm velocity in four different age groups of Jermasia buck.

*Corresponding author’s e-mail: nhhpasum@um.edu.my and address: Centre for Foundation Studies in Science, University of Malaya, 50603 Kuala Lumpur.
1Faculty of Medicine, Universiti Teknologi MARA, 40000 Shah Alam, Selangor.
MATERIALS AND METHODS

This study was conducted at the farm of Institute of Biological Sciences, University of Malaya, Kuala Lumpur. Semen collection and evaluation started from January 1999 to December 2000. Four different age groups of male Jerumasia goat (young: <1 year; mature: e<1.0-2.5 years; old: e<2.5-4.5 years and very old: e<4.5 years) were used as semen donors. Forty bucks were used with ten bucks for each age group with ten replicates for each group. Semen was collected from these bucks once a week. Upon arrival at the laboratory, Tris Citric Acid Egg Yolk Extender (TCAYE) was added to semen samples and the fresh extended semen was evaluated for sperm velocity using The Hamilton-Thorn Integrated Visual Optic System (HTM-IVOS Version 10.7). The extended semen was then subjected to slow cooling using a programmable freezer (Sheldon Manufacturing, Inc) prior to plunging into liquid nitrogen (-196°C), thawed and evaluated for sperm velocity using the same semen analyzer.

The settings used for the sperm image analyses were as follows: frames acquired, 30 at 60 Hz; temperature set to 37°C with 2292 illumination intensity. The parameters measured were curvilinear velocity (VCL in μm/s), average path velocity (VAP in μm/s), straight line velocity (VSL in μm/s), amplitude of lateral head displacement (ALH in μm), beat cross frequency (BCF in Hz), straightness (STR in %) and linearity (LIN in %). Finally, stored velocity track for each individually of all sperm were displayed and printed for more detailed analysis. The percentages of sperm velocity were determined according to World Health Organization (WHO, 1999).

Statistical analysis of data obtained was performed on a microcomputer using Statistical Package for Social Sciences (SPSS) programme. Data were analyzed through analysis of variance (ANOVA) and Duncan’s Multiple Range Test with significant levels of P<0.05.

RESULTS AND DISCUSSION

Various sperm velocity characteristics of both fresh and frozen-thawed sperm for four different age groups: young (<1 year), mature (e<1.0-2.5 years), old (e<2.5-4.5 years) and very old (e<4.5 years) of Jerumasia bucks have been presented in Table 1. In general, mature age group showed significantly (P<0.05) higher sperm velocity characteristics as compared to that observed in the other age groups. Fresh sperm of young and mature bucks had significantly (P<0.05) higher VAP, VSL, VCL and ALH values than that of the older age groups. Fresh and frozen-thawed sperm velocity characteristics varied significantly among individual in all age groups. Sperm velocity characteristics were also observed to be significantly (P<0.05) higher in fresh than frozen-thawed sperm in all age groups.

Significant variation in the effects of freezing in different age groups of buck was probably due to the individual variation. Batista et al. (2009) indicated individual differences in freezability based on evaluation of post-thawed goat semen. The ability of sperm to withstand cryopreservation depends on some consistent property of the sperm or seminal plasma from the individual buck. Muino et al. (2009) confirmed that there was a specific pattern of movement in each sperm subpopulation of bull’s ejaculate.

Pregnancy outcome studies have revealed that paternal aging caused an increase in preimplantation loss, percentage of low weight fetuses and postnatal mortality (Serre and Robaire, 1998). These observations strongly suggested the occurrence of structural or functional or both changes in the sperms with increasing age (Serre and Robaire, 1998). The structural elements or movement characteristics of the sperm or both were altered during aging as a consequence of altered testicular and epididymal age dependent changes (Syntin and Robaire, 2001).

In the present study, generally all age groups of buck showed higher fresh sperm values for VAP, VSL, VCL and ALH than the frozen-thawed sperm. These velocity characteristics were more affected by the freezing process and indicated that these sperm characteristics measured different aspects of cell physiology. In particular, the physiological basis for these velocity characteristics was more sensitive to cryobiological damage. Similar observations were also observed in frozen-thawed sperm of lion and tiger (Patil et al., 1998), and leopard (Jayaprakash et al., 2001). According to Woolley and Richardson (1978), alteration in sperm movement was associated with reduction in mitochondrial function.
During freezing and thawing, sperm mitochondria underwent damages. The sperms were unable to generate ATP through mitochondrial respiration which resulted in the decrease of sperm movement (Viswanath and Shannon, 1997).

Present study indicated that STR and LIN values in frozen-thawed sperm were significantly higher than the fresh sperm in all age groups of buck. In ram, Moses et al. (1995) observed significant reduction of motile sperm, ALH, STR and LIN after freezing and thawing. According to Anel et al. (2003), STR and LIN were the commonest primary predictive variables for predicting the post-thawed percentage of motile sperm. The present result for the STR and LIN were in contradiction to previous reports probably due to the use of egg yolk in the extender (TCAYE). Iritani et al. (1964) reported that the deleterious interaction between egg yolk and the bulbourethral gland secretions exists in goat semen but did not exist in other species, such as the bull, boar or ram. Previous study reported that without seminal plasma, sperm maintained their motility in egg yolk diluents. However, if neat semen was added to egg yolk media, sperm would die due to coagulation of egg yolk (Roy, 1957). Yolk-coagulating enzyme from the bulbourethral gland would act as a catalyst that hydrolyzed egg yolk lecithin into fatty acids and lysolecithin (Iritani and Nishikawa, 1963). The hydrolysis caused fusogenic of sperm membrane which induced the acrosome reaction (Upreti et al., 1999) and decondensation of chromatin (Sawyer and Brown, 1995) which was toxic to the sperm (Aamdal et al., 1965).

CONCLUSION

This study has provided essential fundamental descriptions of goat sperm velocity characteristics which may be applied for breeders to increase productivity and profitability of goat production and other livestock animals.

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REFERENCES


