Cryopreservation of Goat Sperms Collected From Different Regions of the Epididymis

[1]K. U. E. Perera, [2]H. B. S. Ariyaratne

[1]Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka

[2]Professor of Veterinary Physiology, Dean of the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka

Abstract: ***Cryopreservation of epididymal sperms in domestic and non-domestic animals has a number of important practical applications such as preservation of valuable genetic material from non-breedable animals. In the present study, the motility, morphology and viability of sperms obtained from head, body and tail of the epididymis were studied before and after freezing using eleven samples of goat epididymis collected from the Kandy abattoir soon after slaughter. To assess the changes of morphology and viability, smears stained with Eosin and Nigrosin were used. Statistically significant differences were observed in the motility and viability of sperms in the head, body and tail of the epididymis (P< 0.05) before and after freezing. Before freezing, the highest motility and viability were detected in sperms collected from the tail of the epididymis. The values observed were 75.45± 5.22% and 78.18± 5.65% for motility and viability respectively which reduced up to 39.09± 8.31% and 37.72± 4.96% after freezing. The lowest motility and viability were observed in sperm of the head of the epididymis, which were 5.27± 2.72%, and 47.27± 10.52% respectively and reduced up to 1.00± 0.89% and 24.18± 6.90% after freezing. The motility and the viability of the sperms in the body of the epididymis were found to be 26.36± 16.29% and 49.54± 8.57% and reduced up to 4.36± 2.38% and 32.54± 6.79% respectively due to freezings. Freezing resulted in increased head and tail abnormalities of sperms such as small heads, acrosomal defects, coiled tails and bent tails (P<0.05) in sperms collected from all three parts of the epididymis. A significant difference was detected in the proportion of sperms having proximal cytoplasmic droplet among the head, body and tails of the epididymis (P< 0.05), whereas no such difference was observed for the distal cytoplasmic droplet in sperms collected from different regions (P> 0.05) before freezing. Similar trend was also observed in sperms collected from these regions after freezing. Analysis of variance revealed that a significant interaction existed between the treatment and the region of the epididymis for the parameters of motility, viability and acrosomal defects (P< 0.05). From these observations, it was concluded that the cryopreservation was most successful when the sperms were obtained from the tail of the epididymis.***

Keywords: Acrosomal defects, Cryopreservation, Cytoplasmic droplet, Epididymis, Sperms

1. Introduction

Cryopreservation of the semen is the most widely used bio-technique for dissemination of genetic material over the population through artificial insemination and long term preservation of valuable genetic material. Semen collection using artificial vagina and preservation of semen in usual manner is not possible in some animals. Fractures due to traumatic injuries, certain disease conditions like urolithiasis and aggressive behavior of certain bucks are some of the common problems, which result in the loss of their breeding ability. Most of the farmers tend to rear bucks for the breeding purpose. Once they lose their breeding ability, these animals are directed for slaughter even though they bear good genetic background. Cryopreservation of epididymal sperms can be used in order to preserve the genetic merits of these animals.

Objectives of this study are to evaluate the viability and morphological changes that occur in sperms obtained from the epididymis before and after freezing, to determine whether there is any significant change in viability and morphology in different regions (Head, Body and Tail) of the epididymal sperms and to determine whether there is a statistically significant interaction between the freezing and the region of the epididymis with regard to morphology and viability.

2. Materials and Methods

Eleven samples of epididymis were obtained from eleven goats soon after slaughter from Kandy Abattoir for this study. The samples were immersed in a flask containing water at the temperature of 37°C and quickly transported to the Central Artificial Insemination Station at Kundasale for the processing.

Sperm collection and dilution were carried out at 37°C. Multiple incisions were made along the three regions of the epididymis using a sharp blade in order to collect sperms. Tri buffer extender was used as the diluent with the dilution rate 1:5v/v. Sperms obtained from the three regions were loaded in 0.25ml straws. These straws were then kept at 4°C for 4 hours and quickly transferred in to the programmable freezer where the temperature drops from 4ºC to -10ºC over 3 minutes and from -10ºC to -100ºC over 2 minutes. Temperature further drops from -100ºC to -140ºC over 2 minutes. Then these straws were plunged in to liquid N -196°C for cryopreservation.

Microscopic evaluation was carried out under 10X of the phase- contrast microscope. Sperm motility before and soon after freezing was determined visually and expressed as a percentage (0- 100%).

Morphological examination and live dead count of sperm cells were done by Eosin-Nigrosin stain to find out the effect on the viability during freezing. Two hundred sperms were

counted in each sample before and soon after freezing while examining the morphological abnormalities of the head, mid piece and the tail under 100X of the light microscope. Sperms with abnormal heads (swollen heads, small heads), proximal cytoplasmic droplet, distal cytoplasmic droplet, free heads, singly bent tails and coiled tails were counted and expressed as a percentage in each region separately.

3. Results and Discussion

All the data is expressed as means and standard error of the mean (mean±SE). Statistical analysis was performed by using 2-Way Analysis of Variance (ANOVA) in order to find out whether any significant difference in the above parameters before and after freezing, whether any morphological changes occur in the three regions of the epididymis and to find out whether there is any interaction between freezing and the sperms obtained from the three regions of the epididymis. If the P value is less than 0.05 ($P<0.05$), the observation is considered to be statistically significant.

The results revealed that there is a significant difference in number of live sperms and the motility of sperms in the samples before and after freezing ($P< 0.05$). It also revealed that there is a statistically significant difference in number of live sperms and motility of sperms among head, body and the tail of the epididymis ($P<0.05$). The results also revealed that there is an interaction between the treatment and the region of the epididymis ($P<0.05$). Our observations show that the number of live sperm and sperm motility is markedly influenced by the temperature which agrees with the study reported by (Datta *et al.*, 2009). Similar studies performed by (Peterson *et al.*, 2007; Blash *et al.*,2000) further prove the reduction in sperm motility and number of live sperms in low temperature. Furthermore, the two parameters vary with the region of the epididymis.

No significant difference was observed in the number of sperms with proximal cytoplasmic droplet in the samples before and after freezing ($P> 0.05$), however, a statistically significant effect was observed in the number of sperms with distal cytoplasmic droplet in the samples before and after freezing ($P<0.05$). Number of sperms containing proximal cytoplasmic droplet varies with the region of the epididymis ($P<0.05$). Results of this study showed a reduction in the number of sperms having proximal cytoplasmic droplet as it moves towards the tail. In the present study, no variation was observed in distal cytoplasmic droplet among different regions of the epididymis ($P> 0.05$) which does not agree with the observation of Tijik, Arman and Taktaz, (2007). No interaction was observed in our study between the region and the treatment with the proportion of sperms having proximal cytoplasmic droplet while slight interaction was observed between the region and the treatment with the sperms having distal cytoplasmic droplet ($P< 0.05$).

There is a statistically significant difference existing in number of sperms with small heads and the acrosomal defects in the samples before and after freezing ($P< 0.05$). Similarly, the number of sperms those with small heads and acrosomal defects was depended on the region of the epididymis ($P<0.05$). Furthermore, statistically significant interaction was observed between the region of the epididymis and the treatment with the acrosomal defects ($P< 0.05$) whereas no significant interaction was observed with the number of sperms having small heads ($P>0.05$). Small changes in the overall size, acrosomal area and width at the base of the head markedly reduce fertilizing ability of sperms (Arthur, 1964). Morphology and the stability of the acrosome also play an important role in fertilization. Changes in normal morphology of sperm heads such as small heads and acrosomal defects markedly increased during freezing in liquid N_2 as seen in this study may potentially lead to poor fertilizing ability.

Analysis of variance revealed that there was a significant effect in the number of sperms with coiled and bent tail in the samples before and after freezing ($P<0.05$) No significant difference was observed in the number of bent tail sperms with the region ($P> 0.05$) whereas a slight variation was detected in the number of coiled sperms with the region ($P< 0.05$). Analysis of variance further revealed that there is no interaction between the treatment and the region with the number of coiled and bent sperms ($P> 0.05$). These results implied that there is a significant increase in coiled and bent tail after freezing which may potentially lead to reduce the sperm motility after freezing. Coiling, bending, and looping are some of the common responses of sperms to noxious stimuli. These defects may arise during spermatogenesis or epididymal transit as well as in certain disease conditions such as testicular degeneration (Arthur, 1964). In our study, the tail defects may have mainly occurred due to cold shock when reducing the temperature up to -196ºC. If the extender is unable to maintain a constant environment, hypotonic stresses and pH changes cause tail abnormalities during cryopreservation of sperms. Tail abnormalities have a great influence on sperm motility, which plays an important role in fertilization.

A significant difference was observed in the number of free heads with the region ($P< 0.05$). Slight variation was detected in the number of free heads in the samples before and after freezing ($P< 0.05$). Analysis of variance of our results revealed that there is no significant relationship between the treatment and the region with the number of free heads ($P> 0.05$). Free heads can be seen in the instances where the sperms remain in the epididymis for a long period of time and it is commonly seen in old animals as well as the animals with congenital defects (Arthur, 1964). In the present study, more free heads were observed in the head of the epididymis while less were detected in tail of the epididymis that may due to presence of more immature sperms in the head which are more susceptible to freezing. Beyond the conventional methods of sperm analysis, most of the developed countries widely use Computer Assisted Sperm Analysis method (CASA) for semen evaluation. The most important characteristics such as rate of forward progress, lateral movement of the sperm head and the characteristics of the flagellar beat that are important in fertilization can be measured using this technique. This is largely practiced in evaluation of thoroughbred stallions as well as AI stud bulls though not used as much in other species because of the high cost involved. Fluorescent markers are used to stain live sperms and also to demonstrate the correlations with the fertility. In vitro fertilization is the latest technique used in many developed countries in order to evaluate the sperm function.

Though there are many studies done in order to evaluate the bull semen using above techniques, only a few studies have been carried out for goats. Further studies should be performed with the aid of more accurate techniques in order to improve the goat farming in Sri Lanka.

4. Conclusion

From the results of this study, it can be concluded that the viability of the sperms was the highest after freezing in the sperms collected from the tail of the epididymis under the conditions used in this study.

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Author Profile

Dr. K.U.E Perera received her BVSc degree from the Faculty of Veterinary Medicine and Animal science, University of Peradeniya, Sri lanka in 2012. She worked as the Research Assistant in the Department of Pathobiology in the same institute in 2012. Currently she is working as the Assistant Lecturer, Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka.

Professor H.B.S. Ariyaratne received his BVSc and M Phil degrees from the Faculty of Veterinary Medicine and Animal Science of the University of Peradeniya, Sri Lanka in 1983 and 1989 respectively. He obtained his PhD degree from the University of Tennessee, U.S.A in 2000 and Post Doctoral training from the same institute in 2003 and 2007. Currently he is the Professor of Veterinary physiology and the Dean of the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka.