Sex Pre-selection by Quantification of Y- Chromosome Bearing Spermatozoa in Goat Species

Sureka P*, Nilani K*, Eswaramohan T* and Balasubramaniam K**

* Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka
** Bio Tec, Thirunelvely, Jaffna, Sri Lanka

Abstract- Fractionation of female and male spermatozoa has been practiced for selection of preferred sex of offspring to increase the profit in livestock industries. Furthermore, female calves are desired by the farmers for enhancing milk production in dairy cows, whereas in goats farmers prefer male calves than female calves for beef production. Meanwhile sexed semen for AI purpose in SL has been importing from India with high cost. Thus the objective of the present study is to separate 'Y' chromosome bearing sperm through low cost method for sustaining the male goat requirement satisfying the demands of male sex goat. Sucrose stock solution (2g/ml) was prepared. It was diluted with sodium citrate buffer solution to obtain the following densities such as 1g/ml, 1.1g/ml, 1.12g/ml and 1.13g/ml. The density gradient was prepared by pippetting 0.5ml of each sucrose solution along the wall of the tube and layered without mixing one another and then 10µl of diluted semen was dropped on the top of the layer. Then it was centrifuged (at 500 x g for 12 minutes at 24°C) for sexing the semen by means of density gradient and then each layer was examined to get the percentage of Y-bearing chromosome after eluting each layers. Statistical analysis was performed by using Prism 5.04. One way ANOVA with Bonferroni test was performed to compare the percentage of 'Y' chromosome in each sucrose gradient layer. The percentage of 'Y' chromosomes decreased significantly ($P<0.05$) for 1g/ml (59 ± 2.466, 62.56 ± 2.657%), 1.11g/ml (52.33 ± 0.8819, 56.75 ± 0.866 %), 1.12g/ml (41.00 ± 1.155, 45.34 ± 1.115 %) and 1.13g/ml (33.67 ± 0.8819, 43.9 ± 1.617) in Sannan and Jamunapari respectively. The present study revealed that this technique can be considered to establish low cost semen sexing in developing countries.

Index Terms- Artificial insemination, Goat breed, Sexing, Y chromosome.

I. INTRODUCTION

Fractionation of female and male spermatozoa has been practiced for selection of preferred sex of offspring to increase the profit in livestock industries. Furthermore, female calves are desired by the farmers for enhancing milk production in dairy cows, whereas in goats farmers prefer male calves than female calves for beef production. Sperm sexing with density gradient has been performed using the physical characteristics of spermatozoa such as differences in size and shape, density, electrical surface charges, surface macromolecular protein, and different effect of atmospheric pressure to distinguish X- from Y-chromosome bearing sperms (Yan et al., 2006). There are several methods for fractionation of male and female spermatozoa namely selection of pre-implantation embryos using H-Y antigen, sex-specific antibody binding, swim-up method, density gradient separation, electrophoresis, sexing through Sephadex gel filtration method, albumin centrifugation, sedimentation and flow fractionation. In these techniques, flow cytometry has been used as the most reliable method for separating X- and Y-bearing sperm, but there are some disadvantages in flow sorting, such as damages to sperm and possibility of alteration in mRNA expression of embryos, high equipment cost and maintenance etc. Garner, (2006) stated that flow cytometry separates X and Y-bearing sperms by DNA content with an accuracy of 90%.

Centrifugation using sucrose gradient has often been used to fractionate the cell organelles from crude cellular extracts. Sucrose has been utilized as a commercial medium, low cost and readily available reagent for the density-gradient separation of goat spermatozoa. Typically, a sucrose density gradient has been established by gently overlaying less density sucrose solution on densest solution in a centrifuge tube. The particles travel through the gradient until they reach the point in the gradient at which their density matches that of the surrounding sucrose. This fraction can then be removed and subjected to further analysis (Raposo, 1996). Meanwhile sexed semen for AI purpose in SL has been importing from India with high cost. Therefore farmers in the developing countries are unable to use flow cytometrically sexed semen to manipulate the sex of offspring in cattle farming. Thus the specific objective of the present study is to separate 'Y' chromosome bearing sperm through low cost method for sustaining the male goat requirement satisfying the demands of male sex goat.

II. MATERIAL AND METHODS

Semen collection

Semen of Sannan and Jamunapari were collected with the help of an artificial vagina twice a week since October 2010 from AI centre at Thirunelvely. Warm water (39-40°C) and air were injected into an artificial vagina to maintain the proper temperature and pressure for the ejaculation. The rubber part of the artificial vagina was lubricated by Vaseline. Then semen was collected into the graduated tube when the buck mounted a doe in heat.

Sucrose density gradient preparation

Stock solution preparation

20g sucrose was weighed by using electronic balance and it was dried in the oven. It was dissolved in 10 ml of sodium citrate

buffer. The stock solution (2g/ml) was diluted with sodium citrate buffer solution to obtain following densities such as 1g/ml, 1.11g/ml, 1.12g/ml and 1.13g/ml.

Separation of 'Y' bearing chromosome of goat semen

Progressively less dense sucrose solutions were layered upon one another (1g/ml, 1.11g/ml, 1.12g/ml and 1.13g/ml). 0.5ml of each sucrose solution was loaded into the eppendorf tube by using the micropipette without mixing. Then 10µl of diluted semen was layered at the top of sucrose solution. They were then centrifuged at 500 x g for 12 minutes at 36°C. Then the layers were carefully aspirated by using micropipette one by one to transfer into the four eppendorf tubes. They were then centrifuged 700x g for 5 minutes at 36°C. Pellet was separated from each eppendorf tube. After centrifugation pellet was treated with Orcein red (2%) and Giemsa (0.75%) stain to determine the percentage of X and Y chromosomes. One drop of stained sample was placed on the slide over which cover slip was placed by using needles. Cover slip was pressed by using thumb. Then each slide was examined under Olympus microscope (oil immersion objective). Sperm motility using hemocytometer (improved NEUBAUER) and stopwatch, acrosome integrity using Giemsa (0.75%) were determined before and after sperm sexing.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism 5.04. One way ANOVA with Bonferroni multiple comparison test was performed to compare the percentage of 'Y' chromosome in each sucrose gradient layer.

III. RESULTS AND DISCUSSION

The results of present study indicated that average percentage of 'Y' chromosome was higher in less dense media (1g/ml, 1.11g/ml) than other layers. When compared with the theoretical sex ratio (50:50), higher numbers of female sperms were settled at the bottom fractions in our experiment. Sperm motility and acrosome integrity before separation were slightly higher than after the centrifugation. Sucrose was used to prepare density gradient, because sucrose does not affect the sperm cells. Before the preparation of stock solution, sucrose was placed in the oven at 55-60°C to remove the moisture content of sugar. Siméon, (2004) stated that Percoll gradient density has been used extensively for spermatozoa selection , due to Percoll's effectiveness and it's comparatively better selection compared with simple washing or swim-up preparation, but Percoll's reagents are highest cost than sucrose. During the preparation of stock solution, warmed sodium citrate buffer was used to increase the dissociation of sugar particles. Sodium citrate doesn't affect the sperm viability and maintain the pH. The addition of sodium citrate buffer helps to control the pH of the medium and regulation of the osmotic pressure. Before the dilution, stock solution was filtered with 0.2 µl filter to remove the microorganism and impurities.

Table 1: The percentage of 'Y' chromosomes decreased significantly (P<0.05) in Sannan and Jamunapari

Sucrose gradient	Goat species	
	Sannan	Jamunapari
1g/ml	59.25 ± 2.562	62.56 ± 2.657
1.11g/ml	52.00 ± 0.816	56.75 ± 0.866
1.12g/ml	41.00 ± 0.816	45.34 ± 1.115
1.13g/m	33.00 ± 0.912	43.9 ± 1.617

Table 2: Comparison of percentage of 'Y' chromosomes in different sucrose gradient layers in Jamunapari

Bonferroni's Test	P < 0.05
1g/ml vs 1.11g/ml	No
1g/ml vs 1.12g/ml	Yes
1g/ml vs 1.13g/ml	Yes
1.11g/ml vs 1.12g/ml	Yes
1.11g/ml vs 1.13g/ml	Yes
1.12g/ml vs 1.13g/ml	No

Table 3: Comparison of percentage of 'Y' chromosomes in different sucrose gradient layers in

Bonferroni's Test	P < 0.05
1g/ml vs 1.11g/ml	No
1g/ml vs 1.12g/ml	Yes
1g/ml vs 1.13g/ml	Yes
1.11g/ml vs 1.12g/ml	Yes
1.11g/ml vs 1.13g/ml	Yes
1.12g/ml vs 1.13g/ml	No

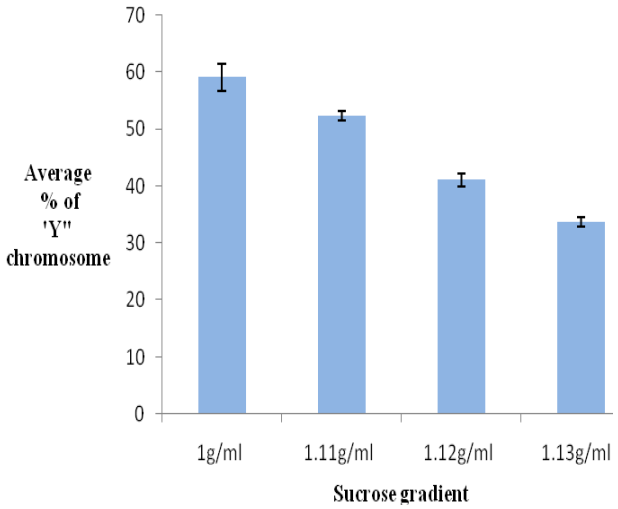

Figure 1: Average percentage of 'Y' chromosome in each density gradient layer of Sannan.

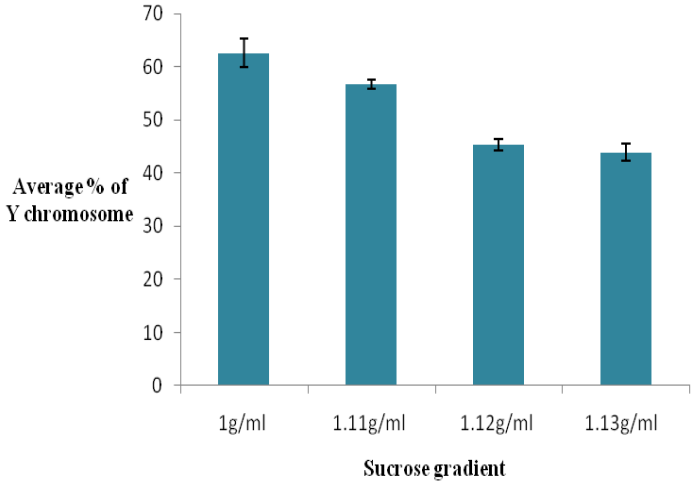

Figure 2: Average percentage of 'Y' chromosome in each density gradient layer of Jamunapari

In goat species Y-sperms are lighter than X-sperms due to the lower DNA content than female sperms. Heavier spermatozoa should settle down faster than lighter spermatozoa through sucrose gradient, therefore centrifugation time could positively influence X-bearing sperm moving down the gradient. Most of the methods for sexing were based on suggested physical differences between X and Y sperm, such as swimming velocity, density, surface charge, or presence of H-Y antigen (Amann, 1989; Johnson, 1992; Windsor *et al.*, 1993; Johnson, 1994; 1996). X chromosome was identified by its shape and thickness than Y chromosome. We considered the sperms as Y-chromosome bearing sperm in the absence of X chromosome, because identification of Y-chromosome was difficult as its shape and size was confused with the autosomes. During slide preparation some sperms head did not break, therefore it was unable to identify the X, Y chromosome in all sperms. Nail polish was used in order to prevent run off of the sample and oil intrusion into the cover slip.

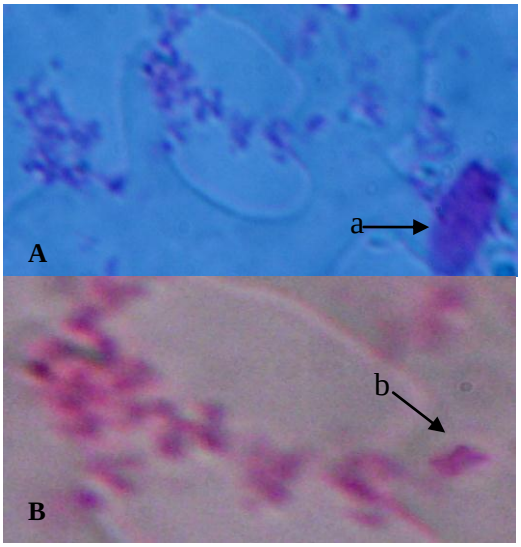

Figure 3: The assessment of 'X' chromosomes by using Giemsa (A) and Orcein red staining (B) in the goat semen, a- broken sperm head b- 'X' chromosome

Cattle have 60 chromosomes, 29 pair of autosomes and 1 pair of sex chromosomes. As in other mammals, males have an X and a Y chromosome and females have 2 X chromosomes. All of the autosomes are somewhat tear drop shaped, with the centromere at the end of the chromosome. Moruzzi, (1979) reported that sex chromosomes have the centromere in the middle of the chromsome, with the X being much larger than the Y. However Han *et al*, (1993) and Lobel *et al*, (1993) indicated that development of DNA technology is more precise methods to estimate the percentages of X and Y sperm in different fractions.

IV. CONCLUSION

Our present study suggested that the percentage of 'Y' chromosome was higher in 1g/ml solution than the other layers. Further study is needed for improvement of this gradient technique to enhance the higher meat production through male goat. There fore the present studies revealed that this technique can be considered to establish low cost semen sexing in developing countries.

ACKNOWLEDGEMENTS

We thank Dr.P. Mahadhevan (Artificial insemination centre, Thirunelvely) for providing the bull semen and Mr. P.J. Jude (reading for PhD degree in Dept of Zoology) for his help in identification of the X chromosome.

REFERENCES

[1] J.F. Moruzzi, "Selecting a mammalian species for the separation of X- and Y- chromosome- bearing spermatozoa," *Journal of Reproduction and Fertility*., 1979, 57: 319-323.

[2] M.N. Simeon, "Comparison of Six Density Gradient Media for Selection of Cryopreserved Donor Spermatozoa," *Journal of Andrology*., 2004, 25: 6.

[3] G. Raposo, H.W. *Nijman, W. Stoorvogel, "B lymphocytes secrete antigen-presenting vesicles," Journal of Experimental Medicine., 1996, 183 (3): 1161.*

[4] *R.P. Amann, "Treatment of sperm to predetermine sex," Theriogenology 1989, 31:49-60.*

[5] *L.A. Johnson, "Gender preselection in domestic animals using flow cytometrically sorted sperm.,* Journal *of* Animal Science., *1992, 70 (SuppI2):8-18.*

[6] *L.A. Johnson, "Isolation of X- and V-bearing sperm for sex preselection. In HM Charlton (ed): "Oxford reviews of reproductive biology, "Vol. 16. Oxford, UK: Oxford University Press, 1994, pp 303-326.*

[7] *L.A. Johnson, Sex preselection by flow cytometric separation of X and Y chromosome-bearing sperm based on DNA difference: a review. Reprod Int Dev 7: 1996, in press.*

[8] *D.P. Windsor, G. Evans, I.G. White, "Sex predetermination by separation of X and Y chromosome bearing sperm: a review,"* Reproduction, Fertility *and* Development., *1993, 5:155-171.*

[9] *T.L. Han, S.P. Flaherty, J.H. Ford, C.D. Matthews, "Detection of X- and Y-bearing human spermatozoa after motile sperm isolation by swim up," Fertility and Sterility., 1993, 1160:1046-1051.*

[10] *S.M. Lobel, R.J. Pomponio, G.L. Mutter, "The sex ratio of normal and manipulated human sperm quantitated by the polymerase chain reaction," Fertility and Sterility., 1993, 59:387·392.*

[11] *J. Yan, L. Huai, Z.J. Feng Chen, J.Hu, , X. Gao, Y. Qin, "Influence of swim up time on the ratio of X- and Y- bearing spermatozoa, "* European Journal of Obstetrics & Gynecology and Reproductive Biology., *2006, 129: 150-155.*

[12] *D.L. Garner, "Flow cytometric sexing of mammalian sperm,"* Theriogenology, *Stoneham, 2006, vol. 65, pp. 943-957.*

AUTHORS

First Author – Ms.Sureka Paramsothy, Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka, Email: paramsothysureka@yahoo.com
Second Author – Ms. Nilani Kanesharatnam, Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka, Email: nilanik4@yahoo.com
Third Author – Dr. Thamboe Eswaramohan, Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka. Corresponding author
Fourth Author – Prof. Kandiah Balasubramaniam, Bio Tec, Thirunelvely, Jaffna, Sri Lanka.

Correspondence Author – Dr.T.Eswaramohan (Department of Zoology, Faculty of Science, University of Jaffna, Jaffna; email: eswaramohan@jfn.ac.lk , teswaramohan@gmail.com Mobile: 0713595778), Tel.:0094(0)213737375; fax: 0094 (0) 212222685