Haryana Vet. (March, 2024) **63(SI),** 89-91 ISSN 0033-4359 (P) **Research Article**

SEX ENRICHMENT OF CROSSBRED BULL SEMEN USING CONVENTIONAL METHODS

S. RANGASAMY*, S. BALASUBRAMANIAN[1], G. DHINAKAR RAJ[2], A. PALANISAMMI[3], D. REENA[4], D. GOPIKRISHNAN[5] and R. CHITRA[6]

[1]Department of Veterinary Gynaecology and Obstetrics, Madras Veterinary College, Chennai-600 007
[2]Department of ABT, [3]VCRI, Tirunelveli-627 358, [4]Department of VGO, VCRI, Namakkal-637 002
[5]Department of Veterinary Clinical Complex, VCRI, Udumalpet-642 126, [6]TRPVB, MMC, Chennai-51
Madras Veterinary College, Chennai-600 007
Tamilnadu Veterinary and Animal Sciences University

Received: 21.08.2023, Accepted: 14.10.2023

ABSTRACT

The sex selection of spermatozoa has been compromised due to higher cost of flow cytometric sex sorted semen doses to poor dairy farmers. The sex enrichment of semen using percoll density gradient and swim up methods were analyzed by flow cytometry. Flow cytometric analysis of sperm separated by swim up method showed the percentages of X and Y sperm to be 34.24±1.16 and 65.76±1.16, respectively and sperm separated by percoll density gradient method showed the percentages of X and Y sperm to be 65.06±1.11 and 34.94±1.11, respectively. Conventional methods of sex sorting such as swim up and percoll density gradient helped to separate around 65.00% of Y and X sperm each.

Keywords: Bull semen, Conventional methods, Percoll density gradient method, Sex Enrichment, Swim up method

How to cite: Rangasamy, S., Balasubramanian, S., Dhinakar Raj, G., Palanisammi, A., Reena, D., Gopikrishnan, D. and Chitra, R. (2024). Sex enrichment of crossbred bull semen using conventional methods. *Haryana Vet.* **63(SI)**: 89-91.

Alternative sperm sexing methods could therefore be of relevance for the benefit of small dairy farmers both in biological and economic terms (Mota *et al.*, 2013). Sperm sexing techniques were based on size and weight (Bhattacharya *et al.*, 1996), electrical surface charge (Shirai *et al.*, 1974), immunological properties (Sang *et al.*, 2011) and swimming ability (Madrid-Bury *et al.*, 2003) of X and Y chromosome bearing sperm. These techniques include differential separation through percoll (Machado *et al.*, 2009), albumin gradients (Machado *et al.*, 2009) and modified swim up (Azizeddin *et al.*, 2014).

MATERIALS AND METHODS

Sex enrichment of Y sperm by swim up method

Semen samples collected from crossbred bulls were centrifuged and washed with Sp TALP medium and further subjected to swim up method as described by Parrish *et al.* (1986). Briefly, one ml of semen was diluted with 5 ml of pre-equilibrated Sp TALP by centrifugation at 1500 rpm for 5 min at room temperature (RT). The supernatant was removed and fresh Sp TALP was added and the above procedure was repeated twice. Finally, 200 µl of the sperm pellet was layered under 1 ml of Sp TALP medium in three sugar tubes and incubated for swim up at 38.5° C in 5 per cent CO^2 in air for 1 h. At the end of incubation, the superficial layer of 0.5-0.8 ml of the medium containing the motile sperm fraction was removed from each tube and pooled in a 15 ml centrifuge tube and washed with 10 ml Sp TALP by centrifugation at 1500 rpm for 5 min at RT. The final sperm pellet was subjected to flow cytometry for determining the percentage of X and Y sperm by DNA staining method.

*Corresponding author: drrangs1976@gmail.com

Sex enrichment of X sperm by percoll density gradient method

Motile spermatozoa were obtained by the Percoll® density gradient method as described by Rosenkrans et al. (1993). Briefly, 100 per cent percoll solution was mixed with 10X salt solution (NaCl, 2.889 g; KCl, 0.238 g; KH_2PO_4, 0.116 g; $CaCl_2$, 0.112 g; HEPES, 0.163 g; 50 ml of milli Q water) to form 90 per cent (v/v) percoll solution. A 45 per cent (v/v) percoll solution was prepared from this by adding an equal volume of HEPES-TALP. The gradient was formed by pipetting 1.5 ml of 90 per cent percoll solution into a 15 ml conical tube overlaid with 1.5 ml of 45 per cent percoll solution. On to the top of the 45 per cent gradient, 400 µl of semen was layered and centrifuged at 2000 rpm at RT. After removing the supernatant, the pellet was washed with 10 ml of HEPES-TALP by centrifugation at 1500 rpm for 10 min and re-suspended in Sp TALP and subjected to flow cytometry for determining the percentage of X sperm by DNA staining method.

RESULTS AND DISCUSSION

The mean (± SE) of flow cytometric analysis of X and Y sperm population of fresh crossbred bull semen by swim up and percoll density gradient methods is presented in Table 1 and Figs. 1 and 2.

Flow cytometric analysis of 2,27,869 sperm separated by swim up method showed the percentages of X and Y sperms to be 34.24±1.16 (81,755) and 65.76±1.16 (1,46,114), respectively. Flow cytometric analysis of 1,34,173 sperm separated by percoll density gradient method showed the percentages of X and Y sperms to be 65.06 ± 1.11 (87,575) and 34.94±1.11 (46,598), respectively. The

Table 1. Flow cytometric analysis of X and Y sperm populations (Mean ± SE) of fresh crossbred bull semen by swim up and percoll density gradient methods

Spermseparation method	Number of replicates	Number of sperm cells analyzed	Sperm population (%) (Mean ± SE)	
			X sperm	Y sperm
Swim up	6	2,27,869	34.24±1.16[a] (81,755)	65.76±1.16[a] (1,46,114)
Percoll density gradient	6	1,34,173	65.06±1.11[b] (87,575)	34.94±1.11[b] (46,598)[2]

Values within parenthesis indicate sperm number
Values bearing different superscripts within columns differ significantly (P < 0.01)

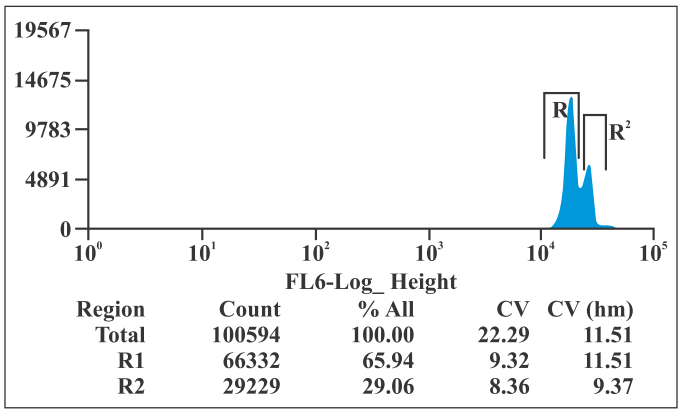

Fig. 1. Histogram showing sex sorting of swim up separated sperm (R^1 gate representing Y sperms and R^2 representing X sperms)

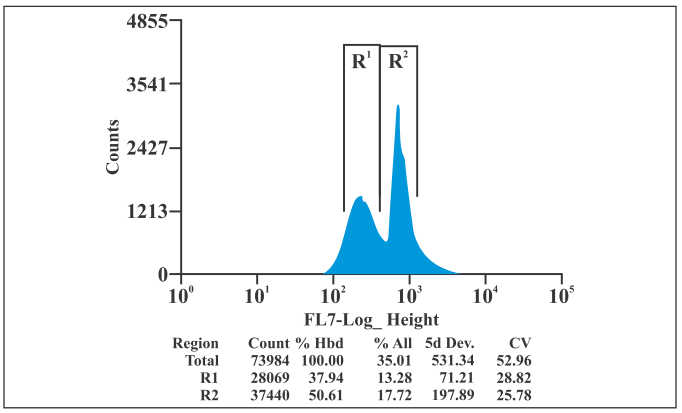

Fig. 2. Histogram showing sex sorting of percoll density gradient separated sperm (R^1 gate representing Y sperms and R^2 representing X sperms)

increased percentage of Y sperm with swim up method and X sperm with percoll density gradient method showed the sex enrichment effect of the sperm separation methods.

Azizeddin *et al.* (2014) reported that the modified swim up method was capable of separating X and Y chromosome bearing sperm, as X bearing sperm had more DNA content than Y bearing sperm which resulted in faster migration velocity of Y than the X bearing sperm (Yan *et al.*, 2006). Swim up selected the viable sperm (Lucio *et al.*, 2012) with higher motility (Henkel and Schill, 2003); normal morphology (Younglai *et al.*, 2001) and was effective in increased percentage of recovery of motile sperm (Ren *et al.*, 2004) which led to fertilization success (Rurangawa *et al.*, 2004). Modified swim up technique has previously been reported to yield sperm population which resulted in a high percentage of male births (Check *et al.*, 1989).

Ericsson (1994) reported that swim up recovery and quality in final fraction was affected by many factors including temperature, preparation of TALP solution, the isolated tube dimensions and the number of spermatozoa layered per tube. It might be speculated that X chromosome bearing sperm, owing to their higher density (due to size of the sperm head; Cui and Matthews, 1993) move out from semen sample in higher percentage (Hafez, 1991) and travel to upper nearby (1ml) layers compared to distant (4-5 ml) travel of Y chromosome bearing sperm in long narrow glass tube. It is also relevant to mention that Y chromosome bearing spermatozoa have higher forward velocity than X chromosome bearing spermatozoa (Ericsson *et al.*, 1973). Few contradictory studies are also available where no difference of X and Y chromosome bearing sperm proportion of supernatant was reported after swim-up in human (Madrid-Bury, 2003 and Yan *et al.*, 2006).

In the present study, the sperm separation by percoll density gradient method showed higher percentage of X over Y sperm. Resende *et al.* (2010) reported that the sexual deviation in favor of females using thawed semen centrifugation in percoll density gradient was 59.6 per cent. Blottner *et al.* (1993) demonstrated a deviation of 90 per cent in favour of females for *in vitro* produced embryos. In literature, results of fresh sperm sexing using percoll density gradient method; 55.7 per cent (Kobayashi *et al.*, 2004) to 74.3 per cent (Hossepian de Lima *et al.*, 2011) was in favour of females.

Flow cytometric analysis of 2,27,869 sperm separated by swim up method showed the percentages of X and Y sperm to be 34.24±1.16 (81,755) and 65.76±1.16 (1,46,114), respectively. Azizeddin *et al.* (2014) reported that the modified swim up method was capable of separating X and Y chromosome bearing sperm, as X bearing sperm had more DNA content than Y bearing sperm which resulted in faster migration velocity of Y than the X bearing sperm (Yan *et al.*, 2006).

Swim up selected the viable sperm (Lucio *et al.*, 2012) with higher motility (Henkel and Schill, 2003); normal morphology (Younglai *et al.*, 2001) and was effective in increased percentage of recovery of motile sperm (Ren *et al.*, 2004) which led to fertilization success (Rurangawa *et al.*, 2004). Modified swim up technique has previously been reported to yield sperm population which resulted in a high percentage of male births (Check *et al.*, 1989). Ericsson (1994) reported that swim up recovery and quality in final fraction was affected by many factors including temperature, preparation of TALP solution, the isolated tube dimensions and the number of spermatozoa layered per tube.

It might be speculated that X chromosome bearing sperm, owing to their higher density (due to size of the sperm head; Cui and Matthews, 1993) move out from semen sample in higher percentage (Hafez, 1991) and travel to upper nearby (1 ml) layers compared to distant (4-5 ml) travel of Y chromosome bearing sperm in long narrow glass tube.

It is also relevant to mention that Y chromosome bearing spermatozoa have higher forward velocity than X chromosome bearing spermatozoa (Ericsson *et al.*, 1973). Few contradictory studies are also available where no difference of X and Y chromosome bearing sperm proportion of supernatant was reported after swim-up in human (Madrid-Bury, 2003; Yan *et al.*, 2006 and Cesari *et al.*, 2006). The results of the program are encouraging the conventional methods of sex sorting such as swim up and percoll density gradient helped to separate around 65.00 per cent of Y and X sperms each.

REFERENCES

Azizeddin, A., F.A. Ashkar, W.A. King and T. Revay (2014). Enrichment of Y chromosome bearing bull spermatozoa by swim-up through a column. *Reprod. Domest. Anim.* **49**: 1-4.

Bhattacharya, B.C., A.D. Bangham, R.J. Cro, R.D. Keyhes and L.E. Rowson (1996). An attempt to predetermine the sex of calves by artificial insemination with spermatozoa separated by sedimentation. *Nature.* **211**: 863.

Blottner, S., M. Schwerin, M. Bottcher and C. Pitra (1993). Selective enrichment of bovine X and Y-spermatozoa by percoll density gradient. *Archiv Fur Tierzucht.* **36**: 153-162.

Cesari, A.G.G., N. Kaiser and A. Mucci (2006). Integrated morpho physiological assessment of two methods for sperm selection in bovine embryo production *in-vitro*. *Theriogenology.* **66**: 1185-1193.

Check, J.H., B.S. Shanis, S.O. Cooper and A. Bollendorf (1989). Male sex preselection: Swimup technique and insemination of women after ovulation induction. *Arch. Androl.* **23(2)**: 165-166.

Cui, K.H. and C.D. Matthews (1993). X larger than Y. *Nature.* **366**: 117-118.

Ericsson, R.J. (1994). Sex selection *via* albumin column: 20 years of results. *Hum. Reprod.* **9**: 1787-1788.

Hafez, E.S.E. (1991). Assisted human reproductive technology, reproductive health technology.

Henkel, R.R. and W.B. Schill (2003). Sperm preparation for ART. *Reprod. Biol. Endocrinol.* **1**: 108.

Hossepian de Lima, V.F.M., C.A. Moreirafilho, A.C. Lucio and M.V. Resende (2011). Selection of sex of bovine spermatozoa by centrifugation in a discontinuous gradient of percoll density. *Rev. Bras. Zootec.* **40**: 1680-1685.

Kobayashi, J., H. Oguro and H. Uchida (2004). Assessment of bovine X and Y bearing spermatozoa in fractions by discontinuous percoll gradients with rapid fluorescence *in-situ* hybridization. *J. Reprod. Dev.* **50**: 463-469.

Lucio, A.C., Resende, M.V., Dernowseck Meirelles, J.A., Perini, A.P., Oliveira, L.Z., Miguel, M.C.V., Carmo, A.S., Tomita, S.Y., Alves, B.C.A., Fazano, F.A.T. and Lima, V.F.M.H. (2012). Assessment of swim up and discontinuous density gradient in sperm sex preselection for bovine embryo production. *Arq. Bras. Med. Vet. Zootec.* **64(3)**: 525-532.

Machado, G.M., Carvalho, J.O., Filho, E.S., Caixeta, E.S., Franco, M.M. and Rumpf Dandode Man, R. (2009). Effect of percoll volume, duration and force of centrifugation, on *in-vitro* production and sex ratio of bovine embryos. *Theriogenology.* **71**: 1289-1297.

Madrid-Bury, N., Fernandez, R., Jimenez, A., Perez-Garnelo, S., Moreira, P.N., Pintado, B. and GutierrezAdan, A. (2003). Effect of ejaculate, bull, and a double swim up sperm processing method on sperm sex ratio. *Zygote.* **11**: 229-235.

Madrid-Bury, N., Fernandez, R., Jimenez, A., Perez-Garnelo, S., Moreira, P.N., Pintado, B. and GutierrezAdan, A. (2003). Effect of ejaculate, bull and a double swim up sperm processing method on sperm sex ratio. *Zygote.* **11**: 229-235.

Mota, R.R., Lopes, P.S., Marques, L.F.A., de Silva, L.P., de Resende, M.D.V. and De Almeida Torres, R. (2013). The influence of animals from embryo transfer on the genetic evaluation of growth in Simmental beef cattle by using multi trait models. *Genet. Mol. Biol.* **36**: 43-49.

Parrish, J.J., Susko Parrish, J.L., Leibfried Rutledge, M.L., Crister, E.S., Eyestone, W.H. and First, N.L. (1986). Bovine *in-vitro* fertilization with frozen thawed semen. *Theriogenology.* **25**: 591-600.

Ren, S.S., Sun, G.H., Ku, C.H., Chen, D.C. and Wu, G.J. (2004). Comparison of four methods for sperm preparation for IUI. *Arch. Androl.* **50**: 139-143.

Resende, M.V., Clucio, A.C., Perini, A.P., Oliveira, L.Z., Almeida, A.O., Gusmao, A.L. and Hossepian de Lima, V.F.M. (2010). Deviation of sex ratio and DNA integrity of bovine spermatozoa centrifuged in continuous density gradients. *Rev. Bras. Health Prod. Anim.* **11**: 260-269.

Rosenkrans, C.F., Zeng, G.Q., MeNamara, G.T., Schoff, P.K. and First, N.L. (1993). Development of bovine embryos *in-vitro* as affected by energy substrate. *Biol. Reprod.* **49**: 459-462.

Rurangwa, E., Kime, D.E., Ollevier, F. and Nash, J.P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquacult.* **234**: 1-28.

Sang, L., Yang, W.C., Han, L., Liang, A.X., Hua, G.H., Xiong, J.J., Huo, L.J. and Yang, L.G. (2011). An immunological method to screen sex specific proteins of bovine sperm. *J. Dairy Sci.* **94**: 2060-2070.

Shirai, M., Matsuda, S. and Mitsukawa, S. (1974). Electrophoretic separation of X and Y chromosome bearing sperm in human semen. *Tohoku J. Exp. Med.* **113**: 273-281.

Yan, J.H.L., Feng, Z. and Chen, J. (2006). Influence of swim up time on sex ratio of X and Y bearing spermatozoa. *European. J. Obst. Gynecol. Reprod. Biol.* **129**: 150-154.

Younglai, E.V., Holt, D., Brown, P., Jurisicova, A. and Casper, R.F. (2001). Sperm swim up techniques and DNA fragmentation. *Hum. Reprod.* **16**: 1950-1953.