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Research Paper

Study on Seminal Attributes of X- sperm Enriched Sahiwal Bull Semen

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ABSTRACT

The present study was conducted to observe the effect of percoll density gradient centrifugation on quality of semen. Ejaculates were collected by AV method from Sahiwal bulls. X-sperm enrichment was done by percoll density gradient method i.e. 7 layers (70-10%). Centrifugation was done at 750 g (22-24°C) for 15 min. The pellets obtained were diluted in EYC medium. Semen quality was evaluated in fresh semen (Control), in pellet of normal centrifugation (Group I), supernatant of centrifugation in percoll density gradient (Group II) and pellet of centrifugation in percoll density gradient (Group III). To assess the quality of enriched semen pH, mass motility, progressive motility, live spermatozoa %, abnormal spermatozoa %, HOST % and intact acrosome % were evaluated. Number of progressively motile sperms in pellet of X- enriched semen were non-significantly increased and significantly ($P<0.05$) decreased in supernatant. The abnormal spermatozoa (%) were decreased in G III as compared to G II Live spermatozoa (%) were increased in enriched semen (pellet). Number of Intact sperms decreased significantly ($P<0.05$) in supernatant of percoll density gradient centrifuged Sahiwal semen. HOST responsive sperms number was not affected after percoll density gradient centrifugation. Thus, the semen quality of X-sperm enriched semen by percoll density gradient method (7 layer 70%) was not affected hence it can be used to increase female calves' birth after A.I.

Keywords: Percoll density gradient, semen, X-enrichment, qualitative assessment, Sahiwal

Sexing of semen is separating spermatozoa into two parts i.e., X-chromosome and Y-chromosome bearing spermatozoa to produce offspring of the desired sex. This modification makes it possible to predetermine the sex of offspring prior to conception (Sharma *et al.* 2018) and it could minimize the managemental cost of rearing the male calves (Sharma and Sharma, 2016).

Either liquid or frozen sexed semen may be used for elite cattle production, genetic progress, and conservation of endangered species in wildlife through AI, embryo transfer technology, and *in-vitro* fertilization (Sa Filho *et al.* 2014). It also reduces the calving difficulty in first calvers (Seidel, 2007). Temperature and humidity negatively influenced the quality of semen (Sharma *et al.* 2017) Sexed spermatozoa result in faster genetic improvement. Isolation of X-bearing sperm is important for the availability and affordability of dam selection and heifer replacement (Weigel, 2004; De Vries, 2008). It also increases the effectiveness of AI progeny testing programs, multiple ovulations, embryo transfer (MOET), and *in-vitro* embryo production (IVP) (Weigel, 2004).

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The technique which provides maximum accuracy is the flow cytometry that separates two populations of sperm (X and Y-bearing) on the basis of DNA content with an accuracy of 90% (Garner, 2006). However, this technique has disadvantages such as equipment costs, damage to sperm during sexing (Seidel, 2003) and altered mRNA expression of embryos (Morton *et al.* 2007). Moreover, sperm survival and sperm characteristics of sexed semen with flow cytometry are poorer compared to unsexed sperms and has compromised sperm quality (Cerventes and lzquierdo, 2012). In flow cytometry, the staining of spermatozoa imposes the chemical and mechanical stress and increases the percentage of dead and defective spermatozoa by 18.6 % (Garner and Johnson, 1995). Therefore, there is an urgent need of new semen sexing technique which would overcome the limitations of flow cytometry semen sexing and does not affect the viability and longevity of spermatozoa after sexing (Sharma *et al.* 2022).

To overcome the limitations of semen sexing by flow Cytometry method, Percoll density gradient method could be an alternative to sperm sexing because more DNA content of X bearing spermatozoa can be related to more 0.06% density in those cells (Windsor *et al.* 1993). Percoll density gradient method was capable to separate X and Y-bearing sperm with lower cost and without damages to sperm viability. However, this technique had 70% accuracy when used in humans and cattle to separate sperm cells carrying an X or Y chromosome (Hossepian de Lima, 2007). Selection by Percoll™ discontinuous gradient enhances sperm motility (Parrish *et al.* 1995; Lucio *et al.* 2008), percentage of cells with normal morphology (Prakash *et al.* 1998), intact membrane and intact acrosome (Oliveira *et al.* 2011).

Thus, the present study was conducted with the aim to the enrichment of X-sperm in the semen of Sahiwal bull using the percoll density gradient method followed by qualitative assessment of X-enriched Crossbred and Sahiwal semen.

MATERIALS AND METHODS

The present study was conducted at Semen Production Center, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand-263145. A Sahiwal bull aged 7 years weighing 450-500 kg reared at the Semen Production Center was selected. Sterilization of articles was done in hot air oven at 180°C for 1 hour and rubber articles an artificial vagina etc. were autoclaved at 121°C temperature, 15 psi for 15 minutes. Total 15 ejaculates were collected. Semen samples were collected twice a week by AV method. Ejaculatory volume >3ml and sperm mass motility >+3 were selected for the study.

A 90% percoll stock solution having a density of 1.123 g/ml was prepared by adding 9 parts (v/v) of 100% percoll (Sigma-Aldrich, India) with 1 part (v/v) of Dulbecco's modified eagle's medium (DMEM) (Sigma-Aldrich, India), 0.01 g/L Gentamicin Sulfate and 6mM HEPES (Hydroxy Ethyl Piperazine Ethane Sulfonic acid) buffer (Sigma-Aldrich, India). The pH of 90% stock solution of percoll was 7.4 and osmolarity was 280-320 mOsm/kg H2O (Hossapian de Lima *et al.* 2015). Also preparation of seven gradients of percoll were prepared i.e. 70, 60, 50, 40, 30, 20 and 10% by mixing above prepared 90% stock solution with DMEM (1X). Enrichment of semen was done by discontinuous percoll density gradient method. In which for 70% 7 layer percoll preparation, 1 ml of each gradient i.e. 70, 60, 50, 40, 30, 20 and 10% percoll solution was taken in a 15 ml conical centrifugation tube with densest at the bottom (70%) and lightest at the top (10%) (Hossapian de Lima *et al.* 2015). Then 1 ml semen was layered on the top of the conical centrifugation tube containing 1ml of each percoll gradient. Conical centrifugation tube containing semen and percoll gradient was centrifuged for 20 minutes at 750 RCF at 24°C.

Semen was collected from bull twice a week through AV method and semen quality was evaluated in fresh (Control), pellet after centrifugation (Group I) and supernatant after centrifugation in percoll density gradient (Group II) and pellet after centrifugation in percoll density gradient (Group III). To assess the quality of enriched semen pH, mass motility, progressive motility, live spermatozoa %, abnormal spermatozoa %, HOST % and intact acrosome % were evaluated. pH of semen was observed using pH meter. The mass motility of semen was assessed by placing a drop of fresh semen on a clean, grease-free slide without cover slip and it was examined under low power microscope (10X) equipped with

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a biotherm stage. For the estimation of individual spermatozoa motility, the semen samples were diluted in Tris dilutor. One drop of the diluted sample (1:100) was put on a clean, dry and grease free slide. It was covered with a cover slip. The slide was examined under microscope at 40X magnification equipped with a biotherm stage. The total 200 progressively motile spermatozoa under microscope were counted randomly in different fields. The concentration of the spermatozoa was determined by the haemocytometer method. Eosin-Nigrosin staining technique was used to count the live and dead spermatozoa (Tomar, 1997).

The protocol followed for membrane integrity test was described by Jeyendran *et al.* (1984). The hypo-osmotic solution was warmed by placing it in a water bath maintained at 37°C for 5 minutes. The osmolarity of hypo-osmotic solution used was kept at 150 mOsmol/L. To know the status of acrosomal integrity of spermatozoa giemsa staining method was used as described by Watson (1975). The statistical analysis (ANOVA and Chi Square test) of data was done and results were interpreted as per statistical procedure (Snedecor and Cochran, 1994) by using SPSS computer package**.**

RESULTS AND DISCUSSION

The pH, Progressive motility %, abnormal spermatozoa (%) and HOST (%) of semen in control, I, II and III groups is presented in table 1. and acrosome integrity assessment is presented in table 2 and Fig. 1.

The pH X- enriched semen was not affected after centrifugation in percoll density gradient compared to fresh semen of Sahiwal bull. The progressive motile, live, abnormal and HOST responsive sperms % were non-significantly increased in X- enriched semen (pellet). The intact acrosome spermatozoa (%) increased and decreased significantly (P<0.05) in pellet and supernatant obtained after centrifugation in percoll density gradient.

In our study, sperm enrichment by percoll density gradient method didn't affect pH of semen but changes in pH during sorting process by flow cytometry was observed that subsequently decreased the semen quality (Gadella and Harrison, 2000; Harrison and Gadella, 2005).

Total sperm with an increase of 20% in total motility was recorded (Lucio *et al.* 2012). Further, increased motility after centrifugation (63 ± 4.36%) was compared to control (50 ± 4.47%) (Samardžija *et al.* 2006). Also, increase in motility (86%) was observed compared to control (56.55%) in buffalo bull (Abdel-Razek *et al.* 2017). Whereas, no change in progressive motility before (92.5%) and after centrifugation (90.86%) in percoll medium was observed (Promthep *et al.*, 2016). Similarly, unaffected sperm motility before (70%) and after centrifugation (73%) was reported (Resende *et al.* 2016). Contrary to our results, lower motility (45.0 ± 2.1%) in sexed semen was observed after centrifugation in percoll density gradient method as compared to unsexed fresh semen (68.3 ± 1.1%) (Buranaamnuay *et al.* 2015). Furthermore, significant

Table 1: Qualitative Assessment of Sahiwal bull semen in all the groups

Seminal Parameters	Control	Group I	Group II	Group III
pH	6.73 ± 2.04	6.68 ± 2.09	6.70 ± .33	6.78 ± 2.07
Progressive motility %	89.17 ± 2.27^{ab}	82.50 ± 2.12^{bc}	80.83 ± 3.0^{c}	92.67 ± 2.04^{a}
Live spermatozoa (%)	92.42 ± 1.99^{a}	87.83 ± 2.19^{ab}	84.51 ± 2.57^{b}	95.50 ± 1.80
Abnormal spermatozoa (%)	7.2783 ± 1.21	8.2617 ± 0.99	9.1033 ± 0.89	5.6233 ± 1.10
HOST %	52.5 ± 9.67	54.8333 ± 0.87	53.8333 ± 0.76	64.5 ± 1.60

Means bearing different superscripts in rows differ significantly (P<0.05).

Table 2: Acrosomal integrity assessment of Sahiwal bull Semen in all the groups

Type of Sperms	Control	Group I	Group II	Group III
Fully intact	92.80 ± 0.75^{a}	90.68 ± 1.12^{ab}	88.89 ± 0.92^{b}	95.58 ± 0.69^{c}
Partially damaged	2.88 ± 0.59^{a}	4.78 ± 0.71^{b}	5.39 ± 0.73^{b}	1.93 ± 0.45^{a}
Fully damaged	4.32 ± 0.50^{ab}	4.53 ± 0.72^{ab}	5.72 ± 1.02^{a}	2.48 ± 0.67^{b}

Means bearing different superscripts in rows differ significantly (P<0.05).

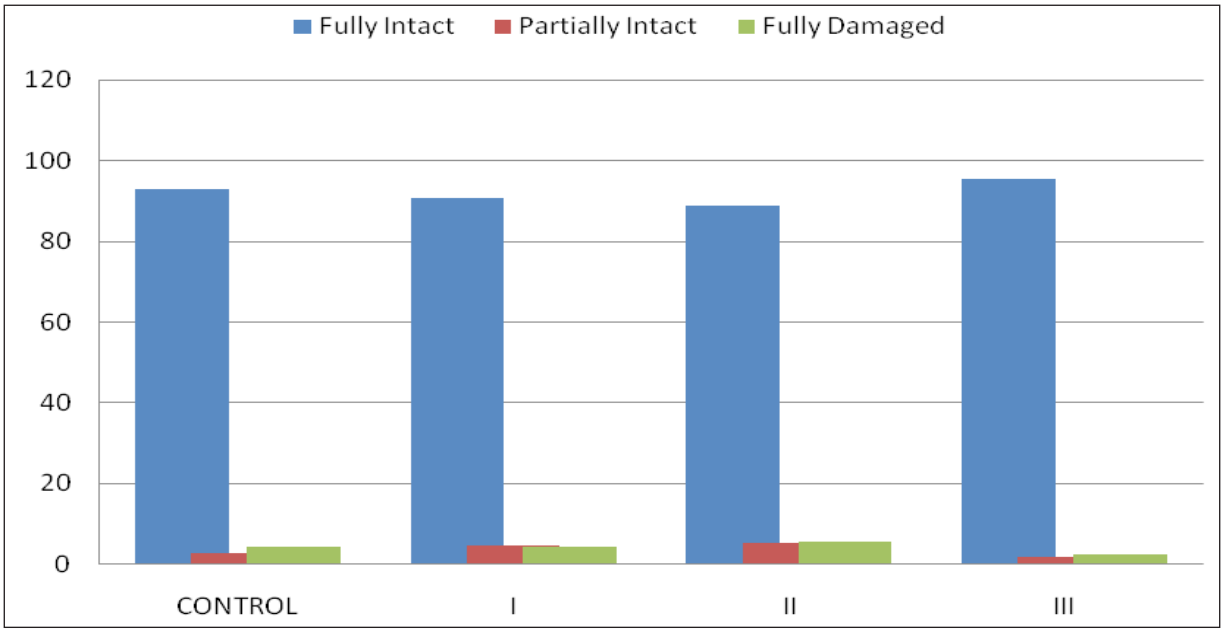

Fig. 1: Effect of X-sperm enrichment by percoll density gradient on acrosomal integrity of spermatozoa (%) of Sahiwal semen

decrease in percent motile spermatozoa was observed after separation (73.63 ± 1.95%) by percoll gradient method compared to before separation (84.78 ± 0.73%) (Malik *et al.* 2011). Whereas, only 66.7% of sexed semen samples in percoll medium showed more than 50% sperm motility (Hossepian *et al.* 2015). In flow cytometry, motility of sperms gets highly decreased compared to non-sexed semen (Hollinshead *et al.* 2004; Blondin *et al.* 2009). There was a lower motility of sexed semen (29.6%) by flow cytometry compared to non-sexed semen (58%) (Carvalho *et al.* 2009). Progressive motility varies with season and individual bull and was greater than 70% in fresh semen of Jersey bull (Lodhi *et al.* 2008). Sperm motility is reduced by 50% after freezing and thawing of sexed semen in flow cytometry and may be a probable factor behind the reduction in the sperm quality and reduced conception rate in flow cytometry (Watson, 2000).

In our study, live spermatozoa (%) were increased in percoll density gradient centrifuged semen (pellet). Similarly, decreased dead spermatozoa (21.65%) compared to control (38.76%) was recorded (Lucio *et al.* 2012). Also decrease in dead sperm and increase in live sperm (13%) and (87%) was observed compared to control (30.7%) and (69.3%) respectively in buffalo bull (Abdel-Razek *et al.* 2017). Also, beneficial effect of percoll density gradient was reported on the viability of spermatozoa after centrifugation at either 500 or 750 or 1750 ×g for 15 minutes (Zeidan *et al.* 2008). However, unaffected viability of semen (69.34%) after centrifugation in percoll gradient was observed (Kaneko *et al.* 1983). Further, viability was almost same before and after centrifugation in percoll gradient and it was 92.7% and 93.57%, respectively (Promthep *et al.* 2016). Buranaamnuay *et al.* (2015) observed reduction in viability of spermatozoa after centrifugation in percoll (66 ± 3.7%) compared to before centrifugation (87 ± 2.0%). Additionally, decreased sperm viability was observed in sexed semen when centrifuged in percoll gradient medium (Hossepian *et al.* 2015). Live sperm percentage was 74.7-86.6% in fresh semen after collection (Nasrin, 2008) and also 83.5% in fresh semen of HF bull (Hahn *et al.* 1969). Decrease in sperm abnormalities was observed (7.8%) compared to control (18.8%) in buffalo bull after centrifugation in percoll density gradient (Abdel-Razek *et al.* 2017).

Moreover, percoll method significantly improved the proportion of live spermatozoa with an intact acrosome (72.00±2.53 %) compared to control in buffalo bull (Abdel-Razek *et al.* 2017). Resende & coworkers (2010) reported no damage in acrosomal integrity when semen is centrifuged through percoll medium for enrichment of X-spermatozoa. Centrifugation in percoll medium had no effect on normal sperm morphology before and after centrifugation (Promthep *et al.* 2016). Compared to other methods of sexed semen, flow cytometry

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highly compromises the acrosomal integrity of spermatozoa (Moce *et al.* 2006). Flow cytometry reduced the percent spermatozoa with intact acrosome (37.1%) compared to non-sexed semen (60.9%) (Carvalho *et al.* 2010) which is very less than enriched semen produced in our experiment. In all mammals, capacitation and following acrosome reaction of spermatozoa are essential steps for successful fertilization and formation of a zygote (Chioham and Hunter, 2004). Possibly the acrosomal integrity status can be used as a suitable marker for fertilization ability of spermatozoa. Therefore, for in-vitro fertilization ability assessment, a reliable assay for the acrosome reaction rate evaluation of spermatozoa is needed (Boccia *et al.* 2007). Assessment of acrosomal integrity of spermatozoa gives a significant and important indication about the fertilizing capacity of spermatozoa (Boccia *et al.* 2007). Like our results, Oliveira *et al.* (2011) also reported enhancement of sperms with intact membrane after centrifugation in percoll gradient medium and it was 57.60 ± 2.04% compared to control (39.25 ± 3.63%). Increase in membrane integrity was observed (41.9 ± 9.1%) compared to control (31.2 ± 3.0%) in goat after centrifugation in percoll density gradient (Batista *et al.* 2011). Likewise, increase in HOST% was observed (39%) compared to control (23.3%) in buffalo bull (Abdel-Razek *et al.* 2017).

Malik & coworkers (2011) reported significant ($P<0.05$) decrease in percent spermatozoa with intact membrane (HOST reactive) after separation in percoll (52.79 ± 1.38%) compared to before separation (71.22 ± 0.92%). Similarly, there was lower number of HOST reactive sperms in semen after centrifugation in percoll (67.7 ± 1 1.7%) compared to non-sexed semen (86.2 ± 1.4%) (Buranaamnuay *et al.* 2015). Only 36% sperm cells with intact membrane (HOST reactive) were present in sexed semen with flow cytometry compared to non-sexed semen (58.2%) (Carvalho *et al.* 2010) which is quite less than observed in our study. Flow cytometry method of sexing highly increased the percentage of spermatozoa with damaged plasma membrane compared to non-sexed semen (Blondin *et al.* 2009; Villamil *et al.* 2012). HOST has a high positive correlation (0.80 in cattle) with fertility (Goswami, 2006). The percentage of sperms with positive HOS reaction varies with individual bull and breed (Prasad *et al.* 1999), season (Kale *et al.* 2000), individual fertility level (Jeyendran *et al.* 1984) and has strong correlation with concentration, percent live sperms, progressive motility and total acrosome (Prasad *et al.* 1999).

CONCLUSION

In conclusion, X-sperm enrichment by percoll density gradient method did not affect the quality of semen. Overall, this study strengthens the idea that enriched semen could be used to increase the birth rate of female calves following artificial insemination. For future research, further work could be undertaken to explore the potential use of enriched semen for artificial insemination to assess the sex of the foetus and its fertility in terms of conception rates in different species of livestock.

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REFERENCES

Bhat, Y. and Sharma, M. 2020. X-sperm Enrichment of Bovine Semen by Percoll Density Gradient Method and Its Effect on Semen Quality, Sex Ratio and Conception Rate. *Ind. J. Anim. Res.*, **54**(10): 1181-1187.

Garner, D.L., Gledhill, B.L., Pinkel, D., Lake, S., Stephenson, D. and VanDilla, M.A. 1983. Quantification of the X and Y chromosome bearing-sperm of domestic animals by flow cytometry. *Bio. Reprod.*, **28**: 312-321.

Hossepian de lima, V.F.M., Moreira-Filho, C.A., Ramalho, M.F.P.D.T. 2003. Processo de seleção do sexo de espermatozoides mamíferos e métodos de controle de qualidade de doses de sêmen sexado congelado. Fapesp/ UNESP/USP (Brasil). BR PI 0300604-2, 17.

Jeyendran, R.S., Van Der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fertil.*, **70**: 219-228.

Lucio, A.C., Oliveira, L.Z. and Celeghini, E.C.C. 2008. Influence of bovine subspecies in the recovered rate after the separation of X-bearing sperm by centrifugation in discontinuous Percoll™ density gradient. *Anim. Reprod. Sci.*, **6**: 336.

Oliveira, L.Z., Arruda, R.P. and Celeghini, E.C.C. 2011. Effects of discontinuous Percoll gradient centrifugation on the quality of bovine spermatozoa evaluated with computer-assisted semen analysis and fluorescent probes association. *Andrologia*., **44**: 9-15.

Parrish, J.J., Krogenaes, A. and Susko-Parrish, J.L. 1995. Effect of bovine sperm separation by either swim-up or Percoll method on success of in vitro fertilization and early embryonic development. *Theriogenology,* **44**: 859-869.

Prakash, P., Leykin, L. and Chen, Z. 1998. Preparation by differential gradient centrifugation is better than swim-up in selecting sperm with normal morphology (strict criteria). *Fertil. Steril*., **69**: 722-726.

Salisbury, G.W. and Dongen, C.G. 1964. A comparison of several methods of estimating nuclear size of spermatozoa. *J. Anim. Sci.,* **23**: 1098-1101.

Seidel, Jr. G.E. 2003. Economics of selecting for sex: the most important genetic trait. *Theriogenology,* **59**: 585 – 598.

Sharma, N., Chand, D.K., Rawat, S., Sharma, M. and Verma, H. 2018. Effect of sexed semen on conception rate and sex ratio under field conditions. *J. Entomo. and Zool. Stud.,* **6**(1): 702-705.

Sharma, M., Bhat, Y., Sharma, N. and Singh, A. 2018. Comparative study of seasonal variation in semen characteristics of buffalo bull. *J. Entomo. and Zool. Stud.,* **6**(1): 947-951.

Sharma, V., Verma, A.K., Sharma, P., Pandey, D. and Sharma, M. 2022. Differential proteomic profile of X- and Y- sorted Sahiwal bull semen. *Res. in Vet. Sci.,* **144**: 181-189.

Sharma, M. and Sharma, N. 2016. Sperm sexing in animals. *Adv. Anim. Vet. Sci*., **4**(10): 543-549.

Tomar, N.S. 1997. Artificial Insemination and Reproduction of cattle and buffaloes. 4th Edn. Saroj Prakashan, Allahabad.

Weigel, K.A. 2004. Exploring the role of sexed semen in dairy production systems. *J. Dairy Sci*., **87**: E120-E130.

Windsor, D.P., Evans, G. and White, I.G. 1993. Sex predetermination by separation of X and Y chromosome-bearing sperm: a review. *Mol. Reprod. Dev.,* **5**: 155-171.

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