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APPLICATION OF SPERM SEXING TECHNIQUE IN FARM ANIMALS: A REVIEW

Veterinary Science

Abhay Kumar Meena* M.V.Sc. Scholar Department of Veterinary Gynaecology and Obstetrics, Post Graduate Institute of Veterinary Education and Research (PGIVER), Rajasthan University of Veterinary and Animal Sciences (RAJUVAS),Bikaner, Rajasthan, India. *Corresponding Author

Chetan Prakash Bamnia B.V.Sc College of Veterinary and Animal Science, Bikaner Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), Bikaner, Rajasthan, India.

ABSTRACT

In animal husbandry sector, farmers have a constant urge to produce young animals of preferred sex in order to meet the rising demand for meat and milk, which eventually benefits farmers financially. In the animal production industry, sexual pre-selection has a significant economic impact. Sexed sperm improves a herd's genetic advancement by increasing the number of excellent heifers and good male germplasm from exceptional bulls used in future breeding programmes. Sex-sorted sperm is not genetically changed or engineered. It's a natural product based on the premise of DNA content differences between X and Y spermatozoa, with X spermatozoa having more DNA. Flow cytometry is the most accurate method for sperm sexing. The purpose of this review is to provide insight into the value of sex-sorted sperm.

KEYWORDS

Sperm sexing, Flow cytometry, Sex-sorted sperm, Sorting efficiency.

INTRODUCTION

Desired sex either female or male which is produce from semen having X or Y bearing sperm is known as sexed semen. Sexed semen helps a herd improve genetically by increasing the quantity of outstanding heifers and good male germplasm from elite bulls that may be used in future breeding programmes. The commercial sexed semen technology is currently widely regarded as the most advanced method for producing milk and animal by-products. Traditionally, the sex is determined by random coupling of gametes during fertilisation, and the expected sex ratio in the progeny is likely to be about 1:1. Female calves are ensured in roughly 90% of cases when sexed semen is used, compared to a 49 percent average frequency when conventional semen is used (DeJarnette *et al.,* 2009; Norman *et al.,* 2010). All aspects of the artificial insemination industry benefit from the use of sexed bovine frozen semen, including cross-breeding, inbreeding, progeny testing, embryo transfer, genetic markers, and genome selection. (Galli and Balduzzi, 2009). Sex sorting of sperm cells by flow cytometry is an established method that has been commercially used in cattle. (Rath *et al.,* 2013). This technology has improved by increasing sorting rates and conception rates over the past decade. (Schenk *et al.,* 2009).

BENEFITS OF SEX – SORTED SEMEN

- Production of calves of desired sex. (Seidel, 2007; De Vries *et al*., 2008)
- Quick replacement and extension of herd.
- Selective culling.
- Production of superior breeding bulls as the country has limited elite cattle and buffalo bulls (<0.1% of total).
- 90:10 females to male ratio or vice-versa is ensured. (Norman *et al*., 2010)
- Lowers the cost of progeny testing programs and embryo transfer and enhances the value of genetic markers. (De Vries et al., 2008)
- Reduced dystocia by preventing production of male calves. (Fetrow *et al.,* 2007)
- Sex determination at an early stage can lower management costs by allowing superior bulls or cows to be managed selectively.

METHOD OF SPERM SEXING

There are several methods of sperm sexing and important amongst them are listed as:

I. Identification of H-Y antigen.
II. Albumin gradient.
III. Free-flow electrophoresis.
IV. Detection of sex specific proteins.
V. Centrifugal counter current distribution.
VI. Volumetric differences.
VII. Percoll density gradient.
VIII. Flow- cytometry.

Table 1: X-and Y- Sperm: How they Differ? (Sharpe et al.,2009)

Parameter	Y- Bearing Spermatozoa	X- Bearing Spermatozoa
DNA Content	Comparatively Lower	Comparatively higher
Bovine spermatozoa	3.7-4.2 % lesser than X	3.7-4.2 % greater than Y
Other mammals	2.3-7.5 % lesser than X	2.3-7.5 % greater than Y
Size	Comparatively smaller	Comparatively Larger
Motility	Comparatively faster	Comparatively slower
Surface Charge	Comparatively positive	Comparatively negative
Cell surface antigen	HY-Antigen	..

Traditional refrigerated storage method

X bearing spermatozoa are heavier than Y bearing spermatozoa. When the extended semen is stored for 1-2 days, the bottom fraction is expected to have more X bearing spermatozoa. Use of the bottom fraction for Al was thus believed to result in more female offspring.

I. Identification of H-Y antigen

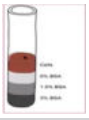
- The identification of H-Y antigen is also one of method for Sperm sorting.
- In this method the specific antibody (against surface protein) of Y chromosome bearing spermatozoa (against H-Y antigen) is also used an option for sperm sorting through affinity chromatography or magnetic bead.
- This sperm sorting technique is applied at large scale with efficacy observed by many workers.

(Kumar N *et al*., 2017)

II. Albumin Gradient

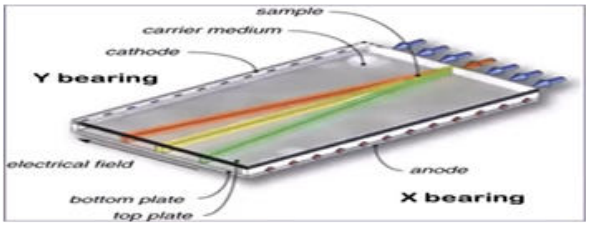
- Successful separation of X and Y-bearing spermatozoa using an albumin gradient was first reported by Ericsson *et al*., (1973).
- Moruzzi (1979) reported that Y chromosome is smaller than X chromosome.
- Maxwell *et al*., (1984) recorded that though the method was effective in increasing the proportion of spermatozoa with motility and elimination of abnormal forms, there was no much difference in the ratio of X- Y bearing spermatozoa.

Swim Down Procedure –



- Differences in the ability to swim down in a gradient solution.
- Since Y bearing spermatozoa are smaller in size and have high motility, they exhibit a greater downward swimming velocity than x chromosome bearing spermatozoa.

III. Free flow electrophoresis



(Kaneko *et al.*,1983, Kwon and Bowser, 2016)

- Based on the presence of electric charges (Zeta potential) the surfaces on of spermatozoa.
- Surface of X spermatozoa are charged negative, while the surface of Y spermatozoa is charged positive.
- Based on electric field of separation, X and Y spermatozoa are separated using the differences in the surface charges.

IV. Detection of sex specific proteins

- The detection of sex-specific proteins (SSP) in X- or Y-sperm can be used to develop an immunological method for sperm separation.
- The protein profiles of X- and Y-sperm are differential expression of proteins.
- These proteins with differential expression may be affect phenotype, the sperm functions, interaction between oocyte and sperm, and to development of zygotic embryo.

V. Centrifugal Counter Current Distribution based on Density Characteristic

- Meistrich (1982) found the difference in density between X-bearing bovine spermatozoa and Y-bearing bovine spermatozoa to be only 0.0007 g/cm3, hence this feature was not suitable to be exploited as a characteristic to sex sperm.
- Ollero *et al.,* (2000) have attempted to sex ram spermatozoa by centrifugal counter current distribution using an aqueous two-phase system.

VI. Sperm Sorting based on Volumetric Differences

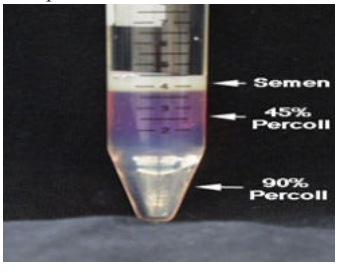
- Van Munster *et al.,* (1999) used interference microscopy and subsequent image analysis to demonstrate a difference in sperm head volume that matched differences in DNA content between X and Y-bearing bovine spermatozoa.

VII. Percoll Density Gradient

- Semen is layered on top of a percoll column and spermatozoa are allowed to penetrate the column.
- This technique was not effective in separation of X or Y-bearing spermatozoa.

(Iwasaki, 1988)

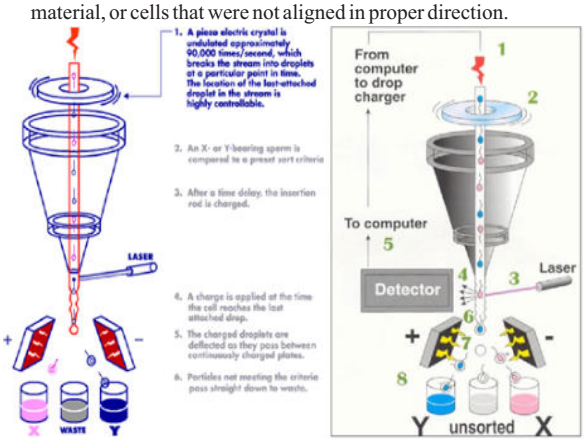
- Differences in the sedimentation density.
- Due to high sedimentation density of X bearing spermatozoa, it settles in the bottom of column while Y bearing spermatozoa remain at the top of column.



(Source: http:// www. ansci. wisc. edu/j jp1/ ansci repro/ lab/ lab10/ lab11 2003/ percoll.html)

VIII. MACHANISM OF FLOW CYTOMETRY

- Principle of this method is based on the fact that X-bearing (female) sperm contain 3.8 percent more DNA than Y-bearing (male) sperm (Johnson, 2000).
- Before sorting, the sperm cells are stained with a fluorescent dye (Hoechst-33342 (a DNA binding fluorochrome) and then passed through the flow cytometer as droplets containing the single sperm cell.
- X-bearing sperm shine brighter than the Y-bearing sperm when illuminated with laser for fluorescence.
- A positive or negative charge is then applied to the droplets. Charged (+/-) droplets are deflected in opposite directions and un-charged droplets pass straight through.
- Uncharged drop- lets may contain multiple sperm, damaged material, or cells that were not aligned in proper direction.



(Source: google image)

Sorting efficiency

Using flow cytometry based sorter, 15 straws each of X and Y sex per hour per instrument. 360 straws (2million sperm each) of X sex can be produced from a machine if it runs continuously for 24h. If input rate is 40,000 sperm / sec then sorting rate 8000 "x" sperm/sec and 8000 "Y" sperm /sec and waste is 24,000sperm /sec. 40 % of total input sperm are sorted into either X and Y bearing while 60 % is wasted (could not be sorted).

- Fertility rate with sexed semen was lower than conventional semen
  (Norman *et al*., 2010; Healy *et al*., 2013; Karakaya *et a*l, 2014).
- Sexed sperms of bull had 75-80% of the fertility of conventional non-sexed, frozen-thawed semen.
  (Schenk *et al.,*2009).
- Conception and pregnancy rates with sexed sperms had reduced.
- Healy et al. (2013) reported 52% pregnancy rates to sexed semen while 58% to conventional semen.
- There occurred reduction of 4% to 38% in pregnancy rates in heifers, and 33% in postpartum cows on using sexed semen
  (Rhinehart *et al*., 2011).
- In contrast, conception rate of 69.7% (30/43) for sexed semen and 66.5% (1545/2325) for unsexed semen following AI was reported in China.
  (Lu *et al*., 2010).

TYPE OF BREED AND SERVICE NUMBER

- Jersey heifers had lower fertility (52.2%) than Holstein heifers (56.3%) with sexed semen.
- Conception risk by service number decreases from 53% for first services to 33% for seventh services
  (Kuhn *et al*., 2006).

STILL BIRTH AND DYSTOCIA

- Stillbirth frequency was reduced by sexed semen use for cows.
  (Norman *et al*., 2010).
- Use of sexed semen reduced the losses from dystocia in heifers
  (Fetrow *et al*., 2007).
- Difficult births declined by 28% for heifers and 64% for cows with sexed semen use.
- Stillbirths were more prevalent for twin births except for sexed semen bred heifer.
  (Norman *et al*., 2010).

IMPECT ON REPRODUCTION TECHNOLOGIES

- Using sexed semen in super ovulated cows resulted in 20% to 35% reduction in the number of transferable embryos when compared with unsexed semen (Hayakawa *et al.,* 2009; Larson *et al*., 2010).
- Percentage of transferable embryos on using sexed and conventional semen were18.6 and 43.5 (Schenk *et al*., 2006).
- Most of this reduction is due to an increased number of unfertilized ova.

LIMITATION OF SEX SORTED SEMEN

- Sexed semen is costly and has lower conception rates and sexing accuracy.
- Processing time is three to four times longer than conventionally processed semen.
- Pregnancy rates are about 70 to 90 percent of that with conventional semen.
- Half the sperm cannot be sorted because they are damaged or the machine could not determine X or Y. Therefore, 50% of the ejaculate is discarded.
- Lower number (20 percent to 50%) of transferable embryos produced than that from conventionally packaged semen.
- Mass insemination or timed breeding is not an acceptable breeding method.

Table 2: Sexed semen availability in India

Organization	Location	Semen from breeds
ABS India	Pune, Maharashtra	Holstein , Jersey , Sahiwal , Gir , Red Sindhi , HF Crossbreeds and Murrah and Mehsana buffaloes
Prime Bovine Genetics collaboration with Sexing Technologies (ST , USA)	Malviya Nagar , Delhi	Holstein Friesian , Jersey , Brown Swiss and Gir crossbreds
PSK (Pashu Samvardhan Kendra) –Dudh Sagar Dairy	Jagudan , Gujarat	HF , HF crossbreds , Gir , Kankrej , Sahiwal , Mehsana , Murrah
BAIF (Bharatiya Agro Industries Foundation)	Urali Kanchan , Pune , Maharashtra	HF , HF crossbreds , Jersey , Gir , Khillar , Rathi , Red Sindhi , Sahiwal , Tharparker , Jaffarabadi , Murrah
Deep Frozen Semen Production Center collaboration with Sexing technologies (ST , USA)	Shyampur , Rishikesh	HF ,Jersey, Sahiwal, Red Sindhi ,Hariana, Gir and Murrah

CONCLUSIONS

- Flow cytometry is the most accurate method for sperm sexing.
- Sexed Semen has a reduced rate of fertility, conception, and pregnancy.
- Jersey heifers with sexed semen had lower fertility than Holstein heifers.
- With increasing service numbers, the rate of conception with sexed semen drops.
- However, the quantity of transferrable embryos is reduced.
- In sexed semen, the use of pulse lasers and lower sorting pressure promotes fertility.
- In highly fertile herds and healthy cycling females with good body condition scores, sexed semen should be employed.
- Use of sexed semen reduced the losses from dystocia in heifers and stillbirth frequency in cows and greatly increases the potential sexed offspring from a sire.

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Conflict of Interests

There is no conflict of interest.

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