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Sexing of Spermatozoa

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Introduction:

The latest breakthrough in animal reproduction is sexing of semen. Predetermination of sex in livestock offspring is great demand and importance for profitable dairy industry. With the changes that have taken place in animal husbandry over the past generation. The application of sex pre-selection to production systems has become increasingly important. Semen from many mammalian species can be sexed by flow cytometry or cell sorting machine at about 90% accuracy without damaging them (Sidel, 2003).

Use of sexed bovine frozen semen gives new opportunities to all sectors of artificial insemination industry,

- 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 established method that has been commercially used in cattle (Rath *et al*., 2013). This technology is an important tool for the dairy and beef industry and it leading to greater supply of replacement heifers and the consequent hastening genetic gain (De Vries *et al*., 2008 and Chebel *et al*., 2010). This technology has improved by increasing sorting rates and conception rates over the past decade (Schenk *et al*., 2009).

Successful use of sexed semen requires excellent management of cattle and careful handling of semen and use of a skilled inseminator (Seidel, 2007). Advances in semen sex sorting have enabled incorporation of this technology into commercial operations (Norman *et al*., 2010). Sexed semen is now

gaining popularity amongst the dairy and beef industries and is reaching a point of commercialisation (Seidel, 2012). Its commercial application is also seen in several other species (Seidel, 2012). Successful use of sexed semen requires good managemental practices, proper preservation of semen and requires skilled manpower.

Sperm Sexing:

1) To produce calves of desired sex in both dairy and beef cattle
2) Herd replacement and herd extension can be done very quickly
3) To ensure birth of heifers when progeny testing of young bulls is desired
4) Combination of super-ovulation and insemination with sexed semen further increases the desired calf crop
5) In-vitro fertilization programmes, one dose of sexed sperms can be used to produce many embryos of desired sex

Difference between X and Y Spermatozoa:

Parameter	Difference
DNA content	Less in Y sperm
Size	X sperm is larger
Motility	Y sperm is faster
Surface charge	X sperm is negative
Cell surface antigen	H-Y antigen on Y sperm

Benefits of Sex-Sorted Semen:

- Determination of sex at the earliest stage can reduce the management cost thorough selective management of superior bulls or cows.
- Quick replacement and extension of herd.
- Calves of desired sex can be produced. 90:10 female to male ratio or vice-versa can be ensured.
- Dystocia can be reduced by preventing production of male calves.
- Selective culling.
- It lowers the cost of progeny testing programs and embryo transfer and enhances the value of genetic markers.
- Fewer quantity of sexed sperm is used in genetically superior dairy females for heifer replacement

Methods of Sperm Sexing:

There are several methods of sperm sexing,

1) Identification of H-Y antigen (Eichwald and Silmer, 1955)
2) Albumin gradient (Ericsson *et al*., 1973)
3) Free-flow electrophoresis (Kaneko *et al*., 1984)
4) Detection of sex specific proteins (Blencher *et al*., 1999)
5) Centrifugal counter current distribution (Ollero *et al*., 2000)
6) Volumetric differences (Van Munster, 2002)
7) Percoll density gradient
8) **Flow- cytometry:** Gledhill *et al*. (1976) first attempted to separate X and Y sperm by analytical flow cytometry. First successful separation of sperms was made in mammals (Pinkel *et al*., 1982). Sex sorting process by flow cytometry is the most efficient method to separate X from Y spermatozoa in a large scale (Rath *et al*., 2013; Seidel, 2014).
9) Other methods are,
   - Quinacrine mustard staining.
   - Raman micro-spectroscopy
   - Albumin Gradient
   - Sperm Sorting based on Volumetric Differences
   - Swimming Patterns under Laminar Flow
   - Free flow electrophoresis
   - Counter Current Galvanic Separation
   - Immunological Sexing of Semen
   - Polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH)
   - Hormonal Manipulation for Sexing of Spermatozoa

Principle of Flow Cytometry:

This is the best technique which is based on difference in DNA content between X and Y spermatozoa. X spermatozoa contain more DNA. Difference in DNA content for most mammals are in the range of 3-4.2% (Johnson et al., 2000).

Mechanism 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[2-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5-bis-1H-benzimadazole trihydrochloride) 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 uncharged droplets pass straight through. Uncharged droplets may contain multiple sperm, damaged material, or cells that were not

aligned in proper direction.

Speed of Cell Sorting:

The speed of cell sorting machine as reported by Seidel *et al*., 1999 are as follows:

1) Standard speed system: 35000 cells/h
2) High speed cell sorters: 15 milli
3) Accuracy of sorting is 85-95%

Pregnancy Rate with Sexed Semen:

- The use of sexed semen resulted in 45% conception in heifers and 28% in cows (Vries *et al*., 2008).
- Higher pregnancy rate was obtained in heifers for sexed cryopreserved semen with 7-20 times more sperm per dose when deposited in uterine body (Seidel *et al*., 1999).
- Pregnancy rate in heifers for 2×10^6 sexed sperm per insemination was 56% and in control with 10×10^6 unsexed sperm was 61% (Garner and seidel, 2003).
- In some cases, pregnancy rate for sexed sperm was about 60-80% of those found with unsorted control sperm when sperm per dose were same for both.
- Use of sexed semen has reduced dystocia cases in heifers.

Factors Affecting the Sorting Efficiency of Flow Cytometer:

1) Orientation of sperm head
2) Angle of sperm presentation towards the excitation source, 450 is adequate
3) Orientation of fluid in the nozzle
4) Even with the correct orientation of cell and fluid 20-40% of live sperms are not measurable, passes directly to the waste tube
5) Optical techniques
6) Speed of computer processor

Problem of Sperm Sexing:

High cost of equipment, high cost of maintenance, lack of skilled manpower, about half of the sperm sample is unsexable, low sorting efficiency, low pregnancy rates and the process is very slow.

Demerits of Sexed Semen Technology:

- High cost of maintenance of sexed sorting machine like flow cytometry and lower sorting speed and efficiency
- Higher cost of sexed semen then conventional semen
- Delayed sexual maturity in heifers under Indian condition
- The conception rate with sex sorted semen is 10–15% less than conventional semen, which is more detrimental condition in our country where total coverage of AI is less than 25% of breed able population (Abdalla *et al*., 2014).

- Sexed semen contains only 2–4 million sperms/dose as compared to conventional semen which contains 20 million sperms/dose which will be challenge under Indian field condition
- Sperm from some bulls had higher tolerances for sorting, freezing and thawing than from other bulls also every bull's semen cannot be sexed due to inherent abnormalities in the sperm cells.
- Lack of skilled manpower.
- Lack of awareness about sex semen to farmers. The major problem is that it needs highly specialized, non-portable equipment which is quite costly for routine use.

Future Challenges and Possibilities:

1) To reduce the cost of sperm sexing

2) To develop two or more nozzled flow cytometer

3) Isolating a protein marker characteristic of X or Y sperms

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.

References:

Blecher, S.R., Howie, R., Li, S., Detmar, J., Blahut, L.M.(1999).A new approach to immunological sexing of sperm. Theriogenology 52. 1309–1321. https://doi.org/10.1016/ S0093-691X(99)00219-8 • Campanile BG, Gasparrini D, V.

Chebel R.C., Guagnini F.S., Santos J.E.P, Fetrow J.P., Lima J.R. (2010). Sex-sorted semen for dairy heifers: effects on reproductive and lactational performances. J. Dairy Sci. 93:2496-2507. https://doi.org/10.3168/jds.2009-2858.

De Vries A, Overton M, Fetrow J, Leslie K, Eicker S, Rogers G. (2008). Exploring the impact of sexed semen on the structure of the dairy industry. J. Dairy Sci. 91:847-856. https://doi.org/10.3168/jds.2007-0536

Eichwald, E.J, Silmer, C.R. (1955). Commun. Transplant Bull. 2: 148-149

Ericsson, R.J., Langevan, C.N., Nishino, M. (1973). Isolation of fractions rich in Y spermatozoa. Nature. 245: 421-424. https://doi.org/10.1038/246421a0

Galli, A., Balduzzi, D. (2009). Fertility of sexed bovine semen. Proceeding: 44° International Symposium of Animal Production "Impact and perspectives of biology and technology of Animal Reproduction"

(Eds G.F. Greppi, S. Mura), p. 77, Milan.

Garner DL and Seidel Jr GE (2003). Past, present and future perspectives on sexing sperm. Can J Anim Sci.; 83:375-84.

Gledhill, B.L., Lake, S., Steinmetz, L.L., Gray, J.W., Crawford, J.R, Dean, P.N. (1976). Flow micro fluoro metric analysis of sperm DNA content: effect of cell shape on the fluorescence distribution. J. Cell Physiol. 87:367- 376. https://doi. NEAcademic Publishers US Advances in Animal and Veterinary Sciences July 2017 | Volume 5 | Issue 7 | Page 297 org/10.1002/jcp.1040870312.

Johnson LA. (2000). Sexing mammalian sperm for production of offspring: the state-of-the-art. Anim. Reprod. Sci.60/61:93- 107. https://doi.org/10.1016/S0378-4320(00)00088-9.

Norman, H.D., Hutchison, J.L.,Miller, R.H. (2010). Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J. Dairy Sci. 93:3880-3890. https://doi.org/10.3168/jds.2009- 2781.

Ollero, M., Perez-Pe, R., Argallo, I., Moralanes, S., Osalda, J., Muino-Blanco, T.,Cebrain-Perez, J. (2000). Separation of ram spermatozoa bearing X and Y chromosome by centrifugal countercurrent distribution in an aqueous of two phase system. J. Androl. 21:921-928.

Pinkel, D., Gledhill, B.L, Lake, S., Stephenson, D., Van Dilla, M.A. (1982). Sex preselection in mammals? Separation of sperm bearing Y and "O" chromosomes in the vole Microtusoregoni. Science. 218:904-905. https://doi. org/10.1126/science.6753153.

Rath, D., Barcikowski, S., De Graaf, S., Garrels, W., Grossfeld, R., Klein, S., Knabe, W., Knorr, C., Kues, W., Meyer, H., Michl, J., Moench-Tegeder, G., Rehbock, C., Taylor, U. andWashausen, S. (2013). Sex selection of sperm in farm animals: status report and developmental prospects. Reproduction. 145:R15-R30. https://doi.org/10.1530/ REP-12-0151.

Schenk, J.L., Cran, D.G., Everett, R.W, Seidel, G.E. Jr. (2009). Pregnancy rates in heifers and cows with cryopreserved sexed sperm: effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. Theriogenology, 71:717-728. https://doi.org/10.1016/j. theriogenology.2005.04.026 • Schenk, J.L., Suh, T.K, Seidel.

Seidel GE (2003). Economics of selecting for sex: the most important genetic trait. Theriogenology; 59:585-598.

Seidel Jr GE. Sexing mammalian spermatozoa and embryos – state of the art. J Reprod Fertil Suppl. 1999; 54:477-487.

Seidel, G. E., Jr. (2007).Overview of sexing sperm. Theriogenology 68:443-446. https://doi.org/10.1016/j. theriogenology.2007.04.005.

Van Munster, E.B. (2002).Interferometry in flow to sort unstained X- and Y-chromosome bearing bull spermatozoa. Cytometry. 35: 125-128. https://doi.org/10.1002/ cyto.10064