

Sperm Sexing - An Assisted Reproductive Technology, Its Role in Improving Rural Economy

Background and Objectives

West Bengal, although having a large cattle population (199.5 lakh as per the 18th Livestock Census 2007-08), is traditionally a low milk producing state due to a negligible buffalo population and the absence of any recognized breed of cattle. 80% of cattle population in West Bengal is of indigenous non-descript type, which is a very poor milk yielder – averaging 400 litres in 300-lactation days.

In the year 2002, when Paschim Banga Go-Sampad Bikash Sanstha came up, a total of 35.15 lakh MT of milk was produced, with a per capita availability of 119 gm. By the year 2009 – 10, the scenario changed considerably with the production of milk rising to 43 lakh MT, with a per capita availability of 131.40 gm. The primary reason for the change is increase in Artificial Insemination coverage in rural areas by “*Prani Bandhus*” – trained, self-employed AI Workers, along with other Government Employees. However, as the actual requirement of milk is about 57 lakh MT, there is need to increase per cattle milk production.

Increasing the overall milk production in the State required targeted quantitative, qualitative and genetic improvement of cattle population along with strengthening of infrastructure for collection and processing of milk from the rural producers and marketing to the urban consumers by co-operatives, private sectors as well as government.

To increase overall numbers and improve the breeds of cattle, Paschim Banga Go-Sampad Bikash Sanstha undertook production of sexed sperm by introducing ‘**BD Influx high speed cell sorter**’ **in the Frozen Semen Laboratory, at the Haringhata Farm**. This project was taken up under RKVY with a total outlay of Rs. 2.90 crores, during 2007 – 08 and 2008 – 09 and completed in November, 2009.

Intervention

In order to bring about rapid increase in numbers of better-breed milk producing bovines, a system was introduced whereby insemination can be planned to produce a specific sex of cattle. The process involves pre-determination of sex by sorting 'X' and 'Y' chromosome-bearing live sperm cells using the DNA content of sperm as the discriminatory parameter. A flow-cytometer/cell sorter is used to detect the difference in the total DNA content between 'X' and 'Y' chromosome bearing sperm, separate them and create frozen semen straws accordingly.

This intervention would help achieve the following objectives:

- Insemination can be planned to produce animals of predetermined sex.
- Destruction of undesired bull calves would be avoided.
- Superior quality male calves, as per parameters laid out by Govt. of India, can be planned for Frozen Semen Bull Stations to enable production of elite cows in Bull Mother Farms using semen of superior elite bulls.
- Dairy herd can maintain elite quality cows along with production of female replacement heifers by using 'X' semen of Superior Dairy Bulls.
- Intensity of selection can be high so that genetic gain per generation is enhanced.
- Large number of sexed embryos can be produced using Multiple Ovulation & Embryo Transfer and In-Vitro Fertilization, resulting in production of large number of female calves, which would ultimately boost milk production.
- Large number of female calves can be maintained by progressive farmers in rural areas, with little limitations.
- "Breeding Policy" of the State can be implemented by upgrading non-descriptive cows zone-wise using 'X' Frozen Semen Straws of Sahiwal and Gir Breeds.

- Potential for an immense effect on “Field Progeny Testing”, where data from small number of cows will be required within a short period.

The Scientists of the Sanstha have dedicated substantial effort to modify the BD Influx high speed cell sorter to separate the ‘X’ and ‘Y’ - chromosome bearing sperm from billions of bovine sperm cells and cryo-preservation of sexed sperm cells. After tremendous efforts the procedure of sorting sperm was standardized (staining procedure, sorting procedure and cryo - preservation of sexed sperm) in the month of March, 2010. Two lasers operating at 488 nm (blue laser) and 355 nm (UV laser) are used for coarse calibration and fine calibration of cell sorter before actual sorting. Subsequently the UV laser is used to excite the Hoechst 33342 stain of sperm cells. The stained ‘X’ sperm shows more fluorescence than ‘Y’ sperm because of the higher DNA content in it. The emitted fluorescence is captured by different photomultiplier tube (PMT) and the analog signal is converted to a digital signal. The difference in fluorescence of ‘X’-chromosome bearing sperm and ‘Y’ - chromosome bearing sperm can be accurately analyzed by observing the two peaks in the appropriate software.

Purity of sorted ‘X’ and ‘Y’ chromosome bearing sperm is determined by polymerase chain reaction (PCR) by amplifying both bovine ‘X’ chromosome specific (PLP) and ‘Y’ chromosome specific (SRY) gene. Two pairs of primer and probes have also been developed for quantification of sorted ‘X’ and ‘Y’ chromosome bearing sperm thereby improving the sorting efficiency and sorting rate.

Outcome

The first pre-determined sexed male calf in the country, named Shreyas was born on 01.01.11 by using sorted ‘Y’ chromosome bearing sperm. Till date, five female calves and three male calves have been born by using sorted ‘X’ and ‘Y’ chromosome bearing sperms at Bull Mother Farm, Paschim Banga Go-Sampad Bikash Sanstha, located at Haringhata Farm.

Till now, 598 'X' sorted and 622 'Y' sorted Frozen Semen Straws have been produced and Eighty-one (81) animals have been inseminated using Thirty Six (36) 'Y'- bearing and Forty-five (45) 'X'-bearing Frozen Semen Straws. Out of these eleven animals inseminated by 'Y'-bearing sperm and fifteen animals inseminated by 'X'-bearing sperm were found pregnant.

Currently the production capacity of this fluorescence activated cell sorter is 10 to 12 million sperm of each sex per hour. After standardization and cryo-preservation of sexed sperm, the current levels of production are @ 40-50 nos. of Frozen Semen Straws in a day, with a target of production of at least 100 Frozen Semen Straws in a day.

This state-of-art assisted reproductive technology for pre-determination of bovine sex is expected to enhance creation of assets for enhancement of the rural economy by producing desired female dairy calves in rural areas, which will ultimately augment milk production, create employment generation in rural areas, and improve livelihood of the rural population of West Bengal.



“High Speed Cell Sorter Machine”



“Shreyas”, the 1st predetermined male calf born on 01.01.11 at 2.36 A.M. by sorted “Y” chromosome sperm



Predetermined female calf born on 28.01.11 at 3.50 P.M. by sorted “X” chromosome sperm



Predetermined female calf born on 31.01.11 at 9.30 P.M. by sorted "X" chromosome sperm



Predetermined female calf born on 05.02.11 at 4.17 A.M. by sorted "X" chromosome sperm