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Sexed semen technology in cattle: A revolutionary technique in Indian dairy industry

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Abstract

Desired sex either female or male which is produce from semen having X or Y bearing sperm is known as sexed semen. Sexed semen increase the genetic progress in a herd by increases the number of superior heifers and good male germplasm from elite bulls which is used for future breeding programme. Various methods are developed based on density gradient centrifugation or swim-up, sex specific antibodies, free flow electrophoresis and flow cytometry that efficiently separate bovine semen into fractions containing higher concentrations of X or Y chromosome bearing sperm. Flow cytometry is the only proven method for semen sexing to be commercially viable more than 90% accuracy to produce calves of desirable sex. Other methods for sex sorting of sperm (Albumin Gradient/ Percoll gradient/ Gradient swim down, Centrifugal counter current distribution, Free flow electrophoresis, Identification of H-Y antigen, Genetic approaches etc.) have also emerged though these techniques further needs fine tuning for commercial viability. This paper aimed to review the sexed semen methodology, utility of sex sorted semen, benefits and limitation of sex sorted semen.

Keywords: Flow cytometry, sexed semen, sperm sorting

Introduction

The animal husbandry sector now has been become a commercial venture in many developing countries. Its play a very crucial role in national growth as well as livelihood generation of rural population. The commercial sexed semen technique is now considered as most advance technique for milk and animal by-products production. The advancement of commercial sexed semen and its role in animal husbandry was summarised by (Garner and Seidel, 2008; Schenk *et al.*, 2009 and Kumar *et al.*, 2017). The first attempt was made in 1976, to separate X and Y sperm by analytical flow cytometry [22]. Briefly, the basic technology was developed in the early 1980s at the United States Department of Energy's Lawrence Livermore Laboratory in California using procedures that required de-membrating sperm, resulting in non-viable sperm [19]. The first sex-selected calf was born in 1999 by using frozen sexed semen through AI. This process became commercially available in 2004 through 'Sexing Technologies' with labs in Texas, Ohio, Wisconsin and Brazil. In India, a government of West Bengal organisation, paschim Banga Go Sampad Bikash Sanstha (PBGSBG), high speed semen sorter or flow cytometer (Influx, Becton Dickinson, Biosciences, San Jose, CA, USA) initiated sorted of semen was installed on 15th August, 2009 under RKVY with a total outlay of Rs.2.90 crores, during 2007 and completed in Nov., 2009 at Frozen Semen Bull Station, Haringhata. They reported first male calf Shreyas was born on 1st Jan 2011 by using sexed semen. After that female calves were also successfully born to sexed semen [2]. The purity of X-sorted semen was found to be higher compared to Y-sorted semen [4]. By application of sexed semen, female calves are ensured in about 90% of the cases in contrast to the 49% average frequency obtained with conventional semen [13, 35]. Sexed semen is nowadays used for many cattle breeders.

Methods of sperm sexing

There are several approaches of sperm sexing and significant among them are listed as follow:

1. The Albumin gradient [15]
2. The Identification of H-Y antigen [14]
3. The Free-flow electrophoresis [28]
4. The Detection of sex specific proteins [5]
5. The Centrifugal counter current distribution [36]

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6. The Volumetric differences [48]

7. The Percoll density gradient

8. The Flow- cytometry

To separate X from Y spermatozoa in a large scale the flow cytometry is the most efficient method [38, 45].

Albumin gradient

The basic principle of Gradient swim down method is difference in ability of X and Y bearing spermatozoa to swim down in a gradient solution. This method depends on the natural movement of spermatozoa. First of all the discontinuous bovine serum albumin medium is prepared. This medium becomes progressively less concentrated moving from top to bottom. The semen sample is placed onto the top of the medium, and the tube is incubated at 37 °C for one hour. The most motile sperm move downward into the gradient during migration. Due to small size of Y chromosome bearing spermatozoa have high motility and exhibiting greater downward swimming velocity than X chromosome bearing spermatozoa. Hence the isolation of fraction of semen from specific part of albumin gradient shows higher proportion of X or Y spermatozoa at different gradients but the success rate is reported to be around 75% [3, 30].

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 [25, 24, 23, 5, 30].

Sperm sorting by swim-up procedure

The spermatozoa with small size Y chromosome swim faster than spermatozoa with X chromosome and this difference was used by various scientists for sperm sorting [50, 36]. In this method 81% success rate was recorded [10, 30].

Sperm sorting by free flow electrophoresis

The difference in surface charges in spermatozoa (the spermatozoa with X chromosome has a negative charge while spermatozoa with Y chromosome has a positive charge) is used in electric field separation for sperm sorting [34, 28, 30].

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 [11, 31, 30]. These differentially expression of proteins can be used as molecular markers to differentiate X- and Y-sperm and sorting as well. In upcoming, differentially expression of proteins contained in sperm membrane can be capable for the development of new methods for sperm sexing and also to identification of X- and Y-sperm.

Centrifugal counter current distribution

The first effort to fractionate X and Y sperm populations was performed by using Counter current distribution (CCD) [8].

Counter current distribution (CCD) is a chromatography process with a mobile (upper) phase and stationary (lower) phase. The cell sample is divided in a systematic way with 1 system and the 2 phases, brought into contact with fresh opposite phases. Counter current distribution (CCD) machine on the basis of the invention [1] is required for this method. The apparatus with 60 chambers set in a circle, which allows transfers of the upper (mobile) phases relative to the lower (stationary) phases. The CCD can be achieved during centrifugation by keeping the bottom with denser phases in the outer half while the lighter (upper) phases are in the inner half of each chamber. Because no elution or pumping of any phase takes place, the overall process consists of a circular multistep transfer of 60 upper- over 60 bottom-batch phases. Each transfer in this centrifugal-enhanced CCD includes shaking the phases at unit gravity to thoroughly mix them and then separating them by centrifugation (1000g). After the phases have separated and while they are still rotating at full speed (1000g), the upper (inner) phases are transferred to the next chambers. The new cycle can be performed after deceleration.

Volumetric differences

The difference in volume between unstained X- and Y-chromosome-bearing sperm heads could be detected using interference microscopy in visible light. Differential interference contrast (DIC) microscopy was used to measure these volume differences. No staining was necessary, and measurements were done using visible light of 550 nm [49, 50, 48].

Sperm sorting by percoll density gradient method:

Sedimentation density of X chromosome bearing spermatozoa is higher and settles in the bottom of column while the Y chromosome bearing spermatozoa remain in high proportion at the top of column during sperm sorting. The success rate is reported to be from 86% to 94% [32, 47].

Sperm sorting by flow cytometry

Flow cytometry is a useful technique in sperm sorting and is based on the fact that X-bearing (female) sperm contain 3.8 percent more DNA than Y-bearing (male) sperm [27]. In flow cytometry fluorescent dyes are used to stain DNA in sperm sorting [7, 39]. By measuring DNA content of individual sperm cells the sex of future progeny can be predetermined as if they contain the larger X chromosome or smaller Y chromosome. Initially this technique, sperm are stained with a non-toxic, DNA-binding dye (Hoechst 33342) and then pumped in a stream in front of UV laser beam having wavelength of 351 - 364 nm and the bright blue fluorescence emitted is detected and analysed [26]. Individual spermatozoa in stream of individual droplets is broken by crystal vibrator and illuminated spermatozoa emitted bright fluorescence which is measured rapidly by a photo-multiplier tube as the sperm flow past in single file [20]. To ensure adequate illumination, the sperm stream is oriented at the appropriate angle for accurate measurement of a 4% difference in fluorescence [46]. The relative fluorescence of X and Y chromosome bearing sperm population is analysed by high speed computer and are then sorted by DNA content by introducing opposite charges on droplets containing X chromosome bearing sperm than Y chromosome bearing sperm [41]. These droplets falls on previously charged deflect or plates thus separated into two streams and then collected separately. By using electrostatic

deflection separation of X and Y chromosome bearing droplets are done and collected separately for further processing [44]. An uncharged droplet passes through as waste and is discarded [41]. Uncharged droplets may contain multiple sperm, damaged material, or cells that were not aligned in proper direction [30, 33]. This method of sex specific spermatozoa sorting is the most popular and consistently proven to be effective among various methods.

Table 1: Difference between X and Y spermatozoa

| Parameter | Difference |
|--|-----------------|
| Size of X sperm | Larger |
| DNA content | Less in Y sperm |
| Presence of cell surface antigen H-Y antigen | Y sperm |
| Surface charge of X sperm | Negative |
| Motility of Y sperm | Faster |

Limitation of sex sorted sperm by flow cytometry

Slow speed of the process relative to the number of viable sperm required for artificial insemination is the major limitation of flow cytometry. As individual sperm is passing through a nozzle which is analysed by the detector, there are physical limits in how many sperm can be evaluated accurately [21]. Currently the speed of passage is around 80Km/hr during which about 30000 sperm can be evaluated per second under ideal conditions. Thus, it would take 1-2 hr to sort the number of sperm in a typical artificial insemination dose [45].

Strategies employed in flow cytometry procedure to improve fertility

Due to dye, physical trauma and exposure to laser light sperm damaged during sorting process [18] and due to this acrosomal membranes also damaged which results in decreased motility [9]. Various strategies like that reduced sorting pressure and use of pulse lasers decreases damage to sperm and increases fertility [40]. Advances in semen preservation protocols during and after sorting may result in increased pregnancy rates [37].

Advantage of using sexed semen in dairy cattle

- This also enables rapid herd expansion without the risk of introducing diseases that occur with purchased animals [42].
- Female to male ratio with 90:10 or vice-versa is ensured [35].
- Reduced dystocia by preventing production of male calves [16].
- The Production of superior bulls.
- The cost of progeny testing programs is lowers and enhances the value of genetic markers of embryo transfer [12].
- Selective culling.
- By using sexed semen, selection intensity can be increased by choosing genetically superior dams of replacements which accelerate the rate of genetic gain in dairy herds [51, 29].
- The principal benefit of using sexed semen is production of calves of desired sex [43, 12].
- It is possible to reduce the incidence of difficulty in first calvers (heifer calves are lighter than male calves) and additional replacement heifers for herd expansion may offer benefits in terms of improved biosecurity by increasing herd size while maintaining a closed herd [51].

Limitation of sex-sorted semen

1. Cost factor and Low conception rate

Due to high cost of machine, doses of sexed semen are costly and as compared to conventional semen, conception rate is 10-20% lower in sorted sexed-semen [35, 30]. Low dose rate and physical or chemical stress on sperm during sorting process lower the conception rate in sexed semen [17]. The sorting stresses include high dilution rate, staining with the dye, mechanical forces, UV laser light beam, and higher fluidic pressure during projection into the collection tube and centrifugation [18]. In addition to these factors, site of semen deposition in genital organ also affects the conception rate in sexed semen. Conception rate is more when sexed semen deposited in body of uterus (45%) rather than horn of uterus (32%) in buffaloes heifers [6].

2. Non-commercial availability of the sorting technology

It is a major limitation that orienting nozzle which is a patent product is not available commercially.

3. Efficiency and speed of machine

The sorting speed of the machine is low (6000 sperms per second) and if we run it for 24 hours maximum doses (2X 10⁶ sperms per dose) which can be produced is 259.2 [27]. If frozen semen is produced then it can be produced 129 doses (4 X 10⁶ sperms per dose). This is due to non-precision detection of DNA content an about 30% sperms/ejaculate during the sexing process will be rejected and out of the detected Y bearing sperms are 50%; The semen of bull having good genetic merit by 70% doses harvesting will be reduced through this method.

4. Need for standardization in Indian conditions

Currently under Indian conditions, there is a need of standardize the lower dosage of spermatozoa and site of deposition for artificial insemination with good conception rate. Standardization of the technique with respect to different breeds of indigenous cattle and buffaloes are also required because the effectiveness of utilizing DNA content differences

between the X- and Y-chromosome bearing sperm not only depends on relative DNA differences, but also on the capability to precisely orient these gametes at the time of measurement in the flow cytometer/cell sorter [18].

5. Low number of indigenous elite bulls

The low number of indigenous elite bulls and lack of good quality ejaculates from indigenous cattle and buffalo will limit the options for semen sexing.

6. Need of highly skilled manpower

For excellent result, experienced and proven AI technicians are required for inseminations. Furthermore, there is optimum record performance and managerial condition is important which will support the success of AI with sexed semen.

Strategies for optimization the conception rate by using sexed semen

1. Implementation of sexed semen in healthy cycling females bearing good body condition score and highly fertile females animals (having AI pregnancy rates with conventional semen \geq 60%) will be beneficial [33].
2. It will be more beneficial when sexed semen used by experienced AI technicians. Be extremely careful with

storing, handling and thawing of the straws.

3. While using fixed time AI, make sure that a high percentage of animals were in heat before doing AI.
4. Above all, optimal use of sexing technology requires excellent and careful animal management (nutrition, disease control, estrus detection, semen handling, and insemination technique) ^[33].

Conclusions

The flow cytometry is the most common and good technique for sperm sexing. Currently government mainly focused on this technology and a large number of researches will be out to develop this technique in collaboration with other laboratories to make it feasible in India. Furthermore, high cost and low fertility related constraints limit its wider use all over the country. The sexed semen must be utilized in highly fertile herd and in healthy cycling females with good body condition score. When the cost of sex sorted semen is decrease with enough fertility rates than this technology will be accepted as most popular techniques in many regions of India.

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