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Molecular Techniques for Potential Application of Sex- Sorted Semen in Dairy Cattle

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Abstract

The genetic progress and farmer profitability in livestock sector depends on the selection of off spring of desired sex. This now a days can be achieved by artificial insemination after diagnosing estrous cycle simultaneously using sex-sorted semen. This type of semen is not genetically manipulated it is entirely natural. The difference in spermatozoa carrying X and Y chromos ome, is the content of DNA, X chromosome contains more DNA as compared to Y. The sex – sorted semen can be attained by either flow cytometry, albumin gradient, immunological sexing of sperm, precoll density gradient, to name a few. The most successful being flow -cytometry. But, this technology is hindered by many factors like high costs, complexity of operation and lower pregnancy rates than with traditional semen. Despite the odds, sexed semen will contribute to increased profitability of dairy and beef cattle production in terms of milk, meat and other essential products.

Keywords: Artificial Insemination, Sex -Sorted Semen, Flow Cytometry, DNA.

Introduction

Availability of sex sorted sperm is the major break through in reproductive technology. The profitability of dairy industry depends on large extent on the predetermination of sex in live stock off spring. The most successful method of sorting male producing sperm from female producing sperm is flow cytometry. This can be done with 90% accuracy and without damaging gamete (Sidel 2003). Proper preservation of sperm, skilled manpower, and good management is required for successful implementation of this technique. The commercial application of sex-sorted sperm is gaining prominence.

The difference in various parameters in X and Y sperm are-

DNA content is more in X sperm. Difference in DNA content for most mammals are in the range of 3-4.2% (Johnson et al., 2000). motility of Y sperm is faster, X sperm is larger in size, the surface charge of Y sperm is negative and There is H-Y antigen on Y sperm

Aim of the Study

Demonstrate that flow cytometry is one of the most reliable methods for sperm sexing.

Utility of Sperm Sexing

1. To produce calves of desired sex in both dairy and beef cattle
2. 90:10 females to male ratio or vice-versa is certain to be achieved.
3. Fast replacement of herd and extension of herd.
4. To ensure birth of heifers when progeny testing of young bulls is desired
5. Sexed semen when used in combination with super-ovulation and AI increase the needed calf population.
6. In in-vitro fertilization programs, one dose of sexed sperms can be used to produce many embryos of desired sex .

Quinacrine Mustard Staining

Very intense fluorescence to certain regions of chromosome are produced by Quinacrine mustard staining (Caspersson, 1968). Quinacrine staining was earlier used to verify X- or Y-sperm enrichment, in which the presumed Y-chromosome bearing sperm exhibit a fluorescent spot or F body, and the presumed X-chromosome bearing sperm remain unstained (Barlow and Voss, 1970). Quinacrine fluorescence is not universal property of all mammalian Y-chromosome. Therefore, it is considered an inappropriate approach for selection of sperm for most mammalian species

Raman Micro-Spectroscopy

As reported by De Luca et al., (2014) distinguishing of X- and Y-bovine sperm can be done based on Raman spectroscopy. The differences between X- and Y-sperm can be identified using the spectral component in the sperm (DNA, protein, lipids, etc.). The main biochemical differences between X- and Y-sperm lies in the nucleus. The three nuclear regions, viz., acrosomal, middle, and neck Regionshave raman peak position and consistent relative intensities in Y-sperm. The main variations of Raman peaks were observed due to DNA content together with the sex membrane (De Luca et al., 2014). According to him ramanspectrsoscopy is a reliable tool for the progress of a highly efficient and noninvasive technique for sperm sexing.

Centrifugal Counter Current Distribution based on Density Characteristic

Ollero et al., (2000) have attempted to sex ram spermatozoa by centrifugal counter current distribution using an aqueous two-phase system. 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 sperm sexing.

Albumin Gradient

Using albumin gradientericsson et al., (1973) first successfully separated X and Y bearing spermatozoa. Moruzzi (1979) reported that Y chromosome is smaller than X chromosome. This method assisted in selecting the spermatozoa with high motility and eliminated unusual forms, but there was not much discrimination in the ratio of X and Y spermatozoa. (Maxwell et al.,) (1984)

Sperm Sorting based on Volumetric Differences

The difference in sperm head volume and in DNA content between X and Y-bearing bovine spermatozoa was used by Van Munster et al., (1999). He demonstrated this by using interference microscopy and subsequent image analysis.

Swimming Patterns under Laminar Flow

The Y-bearing spermatozoa swim differently and more quickly than X-bearing spermatozoa in a column of flowing media. The demerit with this techniqueis, only 10 % of the total number of spermatozoa placed in the system survived. (Sarkar et al., 1984).

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).

Free flow electrophoresis

The electric charge on the surface of X-bearing spermatozoa differs from that of Y-bearing spermatozoa, this fact was used, to separate spermatozoa into the two major classes by applying an electric field. (Kaneko et al., 1984). Successful inseminations with semen separated by this technique were not attained.

Counter Current Galvanic Separation

Application of a suitable micro-ampere current attracts Y-bearing spermatozoa to the anode and X-bearing spermatozoa to the cathode (Bhattacharya, 1977). However, it could not succeed in producing any significant alteration of sex ratio.

Immunological Sexing of Semen

Immunization of male and female rabbits by injecting sperm preparations with Freund‟s incomplete adjuvant subcutaneously was done to raise antibodies to sperm membrane proteins. The anti-sperm antisera obtained from the female rabbit were putative “antiY” and those obtained from male rabbit were “anti-X” antisera. Sperm doses after suitable treatment were mixed with either of these antisera and incubated for 60 min at 38.5C and 5% CO_2. It was found that only the “anti-X” antisera resulted in agglutination of spermatozoa whereas the “anti-Y” antisera failed to show any agglutination in the spermatozoa. The agglutinated sperm population was separated from the free-swimming sperm by glass wool filtration and the free-swimming sperm population (potentially Y-bearing spermatozoa) was isolated. This method has to be validated by further experiments and another constraint is that the method was successful in isolating Y-bearing spermatozoa only and attempts to isolate X bearing spermatozoa by agglutinating Ybearing spermatozoa was not successful. (Blecher et al., 1999).

Polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH)

The accurate identification of X- and Y-sperm has now become possible with the introduction of PCR and FISH and this has opened the way for assessment of sorting purity of different sperm sexing methods (Flaherty and Matthews, 1996). The sex of individual sperm and sex ratios of sperm in semen sample has been identified by using specific - DNA sequences on X and Y sperm (Wang et al., 2011). Accurate determination of the sex ratio using single sperm PCR necessitates analysis of a large number of individual sperm. However, sex ratio of semen can be determined more simply and accurately by quantitative real-time PCR (qPCR) (Parati et al., 2006). However, its application to populations of cells is of limited use in the assessment of sex selection methods. Moreover, it is labor intensive to be used for screening large number of individual sperm (Flaherty and Matthews, 1996). Single and double label FISH can be used for the direct visualization of sex chromosomes in individual sperm. FISH precisely identifies the sex chromosome of individual sperm using specific probes conjugated with fluorescence molecule for the X- and Ysperm (Flaherty and Matthews, 1996). The application of double FISH using X- and Ychromosome-specific probes has allowed a more accurate assessment than single label FISH (Han et al.,1993) The main advantage of FISH compared to flow cytometry reanalysis and single cell PCR evaluation is that it is highly qualitative and quantitative (Parrilla et al.,2003).

Flow- cytometry for Sperm Sorting

Separation of X and Y sperm by flow cytometry was first attempted by Gledhill et al (1976). Flow cytometers are the advanced cell sorters that use laser to excite fluorescent dye that binds to the DNA in spermatozoa. The DNA percent and DNA

specific dye are the major principle for sperm sexing through flow cytometry. Flow cytometry is the most effective method for attaining sex-sorted semen. (Rath et al, Seidel 2014)

The chances of getting male and female offspring are 50/50, because the sperm carrying either X or Y chromosome has same size, shape, weight and speed. Therefore, sorting semen is challenging task. In cattle X sperm has about 4% more DNA as compared to Y chromosome. There is a difference in fluorescence after using DNA – binding dye, laser light and accurate computer analysis.

A DNA specific fluorescent dye Hoechst-33342(2-(4-ethoxyphenyl)-5-(4-methyl-1- piperazinyl)-2, 5-bis-1H-benzimadazole-trihydrochloride is used. This dye binds to the A-T area of nucleic acid, after penetrating the sperm.it is then passed through the flow cytometer as droplets containing single sperm cell. The amount of dye penetrating the X sperm is more as compared to the Y -sperm. On exposing to Low wave-length laser beam the dye flourescence. A detector measures the fluorescence and analyzed by computer. The droplets are charged positively or negatively. (+/-) The uncharged drops pass through directly as they contain multiple sperms, damaged sperms., whereas charged droplets are deflected in opposite directions.

In domestic animals the differences in DNA content between X and Y bearing spermatozoa ranges from 3 – 4.5% (Johnson et al., 1987; Johnson, 2000). Success rate in this method has been reported to be 85 – 95% (Pinker et al., 1982; Johnson, 2000).

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 million cells/h
3. Accuracy of sorting is 85-95%

Conclusion

Flow cytometry is the most reliable method for sperm sexing. There is lower fertility, conception and pregnancy rates with sexed semen. Jersey heifers have lower fertility than Holstein heifers with sexed semen. Conception rate with sexed semen decreases with progress of service number. Use of sexed semen reduced the losses from dystokia in heifers and stillbirth frequency in cows and greatly increases the potential sexed offspring from a sire. However, it results in reduction in the number of transferable embryos. Use of pulse lasers and reduced sorting pressure increasesfertility in sexed semen. Sexed semen should be used in highly fertile herd and in healthy cycling females bearing good body condition score.

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