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Sex-Sorting of Bovine Spermatozoa – Concepts and Methods

R Selvam

Livestock Farm Complex, Veterinary College and Research Institute,

Tirunelveli – 627 358, Tamil Nadu, India.

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Abstract

The production of desired sex will be the one of the crucial factors to increase the genetic improvement and farmer's income in dairy farming. The various methods are developed to separate X-chromosome bearing sperm and Y-chromosome-bearing sperm based on different principles and methods such as based on the fluorescence of the Y chromosome, differences in mass and motility, differences in swimming pattern, differences in surface charge, differing cell surface antigenic determinants and differing DNA content that efficiently separate bovine semen into X or Y chromosome bearing sperm. Among all the techniques discussed flow cytometry is the best method which effectively separates X and Y bearing spermatozoa, for accurate prediction of the sex and to produce calves of desirable sex.

Introduction

Sex-Sorting technology in livestock is highly significant for predetermining the sex of the animals. The production of desired sex will be the one of the crucial factors to increase the genetic improvement and farmer's income in dairy farming. Recent technologies in sex sorting of sperm are very much important in commercial dairy cattle reproductive management. In dairy farming production of female calf is very much economic than the male calf. The sex determination is genetic in nature. The mammals have two sex chromosomes X and Y and the males develop due to the male-dominant effect of the sex chromosome Y. The Y-chromosome carries in particular specific genes having effect on the development of the male organs in XY embryos – one of these genes is SRY-gene (sex-determining factor Y). The X-chromosome contains genes responsible for the development of female reproductive organs and reproduction. In fertilization a sperm either contributes an X or a Y chromosome. The egg always contributes an X chromosome. Hence, if the sperm contributes X chromosome, the resulting offspring is a female and if it contributes Y chromosomes, the resulting offspring is a male. The separation of the Y sperm from the X sperm

is possible due to the differences on the DNA content of these spermatic cells, X sperm has about 4% more genetic material than Y. Y-chromosome is smaller than X-chromosome. Various techniques have been applied to separate X-chromosome bearing sperm and Y-chromosome-bearing sperm based on different principles and methods as follows.

Separation of X and Y sperm

1. based on the fluorescence of the Y chromosome:
2. on the basis of differences in mass and motility:
3. on the basis of differences in swimming pattern
4. on the basis of differences in surface charge
5. based on differing cell surface antigenic determinants H-Y antigen
6. on the basis of differing DNA content

1. Separation of X and Y sperm based on the fluorescence of the Y chromosome

A. F-body Fluorescence

In this phenomenon, when spermatozoa are stained with the fluorochrome dye quinacrine, results in fluorescence of sperm with Y chromosome alone. But is not applicable to the semen of food producing animals, whose Y-chromosomes do not fluoresce more brightly than their other chromosomes

B. B-body Fluorescence

In the bull and other mammals, the 'Y' chromosome bearing spermatozoa, stained" with quinacrine mustard, can be identified by brightly fluorescing, Spots termed B'-bodies.

C. DNA-Probing

A number of DNA probes specific for sequences on the Y-chromosome now exist. This technique is much more rapid and accurate than karyotyping. DNA Probes will probably become the preferred method used to confirm the success or failure of any semen sexing technique.

2. Separation op X-and Y-bearing spermatozoa on the basis of differences in mass and motility

In this the basic principle is the Y-bearing spermatozoa being smaller, swim faster and that x-bearing spermatozoa being larger and heavier, sediment faster.

A. Albumin gradient

If semen is layered on top of a discontinuous albumin gradient derived from the human serum or bovine serum, the smaller Y-bearing spermatozoa should have a greater ability to penetrate an interface between fluids and to swim faster than X bearing spermatozoa in fluids of high density and viscosity.

B. PercolI Density Gradients

Percoll consists of colloidal silica particles coated with polyvinyl pyrollidone. The percoll is set up in a discontinuous density gradient, similar to an albumin gradient, spermatozoa layered on top of the column may be allowed to penetrate the column naturally. The extent of this penetration is depended upon their mass and motility. Mammalian x-bearing spermatozoa are heavier than are Y-bearing spermatozoa. Therefore, they should have a greater sedimentation velocity when centrifuged. Consequently, a higher of X-bearing spermatozoa should be found in the heavier Percoll fractions at the bottom of the column with most of the Y-bearing spermatozoa being found in the lighter fractions nearer the top of the column.

3. Separation of spermatozoa on the basis of differences in swimming pattern

This is based on the fact that Y-bearing spermatozoa swim differently and "more quickly than X-bearing sperm.

A. Laminar flow fractionation

Spermatozoa separated into X and Y sperm using a specially developed cylindrical flow column with laminar flow velocity gradients

4. Separation of spermatozoa on the basis of differences in surface charge

A. Free-flow Electrophoresis

There are differing electrical charges on the cell membranes of X and Y bearing spermatozoa or differing amounts of net charge. If semen is subjected to an electrical field, some sperm do move towards the anode and some towards the cathode. The spermatozoa which migrated towards the anode were X-bearing and must have had a higher net negative charge than the Y-bearing spermatozoa. The difference in net negative charge seems to be due to more surface glycoproteins rich in neuraminic acid on the X-sperm cell membrane. But major disadvantage is the motility of the sperm following electrophoresis is lost

B. Counter current galvanic separation

It involves the use of a specially designed forced convection streaming galvanic cell which enhance the separation of X and Y bearing sperm. sperm with X or Y chromosome, differing slightly in density and volume are introduced into a steady counter current influenced by the velocities of the streams. So, this hypothesis would move the X and Y- bearing sperm over a period of time, at different velocities, with the lighter Y sperm lagging and accumulating at distance below the X sperm.

5. Separation of spermatozoa based on differing cell surface antigenic determinants: (h-y) antigen

Histocompatibility - Y antigen (H-Y) is found in male tissues of many mammalian species, except erythrocytes and premeiotic germ cells. H-Y is secreted by the cells of the sertoli lineage. It is also present in the plasma membrane of spermatozoa in many mammalian species. The expression of H-Y antigen on the surface of these haploid cells is due to expression of the Y-chromosome. This could be used to separate Y chromosome sperm.

6. Separation of spermatozoa on the basis of differing DNA content

A. Flow Cytometry Method

Flow cytometry is the most effective and scientifically valid of all the techniques used to separate semen. The technique of flow cytometry relies on the fact that the chromatin in the head of the spermatozoa can be stained with a DNA-specific fluorescent dye. These stained cells can then be passed rapidly in single file through a flow cytometer consisting of one or more illuminating light beams. This causes stained spermatozoa to fluoresce. They can be separated into different fractions since the degree of fluorescence is directly proportional to the amount of DNA within each cell. Because the X-chromosome is larger than the 'Y'-chromosome and Contains more DNA X-bearing spermatozoa will fluoresce more than Y-bearing spermatozoa.

Methodology

The spermatozoa are treated with a DNA dye (fluorochrome). X bearing sperm absorb more dye than Y bearing sperm. They therefore emit more intense light when excited by a laser. Sperm also are treated with a dye that greatly suppresses the signal from dead sperm. Dead sperm are therefore identified and rejected. Once spermatozoa enter the flow cytometer chamber, they pass single-file through a small nozzle. At a region just outside the nozzle, an excitation laser beam activates the fluorescent dye in each sperm and each live sperm produces an emission with an intensity that is directly related to the quantity of DNA within the sperm head. X-bearing live

sperm produce more intensity. A light sensing device is coupled to a computer that determines the intensity of light emission by each sperm and the order of passage of each sperm through a column below the nozzle. When the sperm pass by charged plates, they are assigned either a positive or negative charge depending on their DNA content (X or Y chromosome). When the microdroplet containing a single sperm passes through an electromagnetic field the computer applies an appropriate charge and directs the droplet (and sperm) to one side or the other Dead sperm are discarded into the centre tube. Thus, at the conclusion of the separation process there are three vessels that contain sperm. One contains a high proportion of X, one contains a high proportion of Y chromosome bearing sperm and one contains dead sperm.

Disadvantages

i. Flow cytometry are very expensive to buy and maintain
ii. Mutagenic effect: DNA binding dyes such as bind loosely to the DNA in the genome but may still cause chromosomal aberrations
iii. Flow cytometry causes low sperm viability and fertility.
iv. To facilitate access of the DNA stain to the genome, the integrity of the cell membrane has to be compromised by digestion with papain or by light sonication. Digestion with papain results in a high % of cell loss. Sonication increases cell survival rates but at the expense of sperm motility. Light sonication causes loss of sperm flagellum and these spermatozoa are not suitable for conventional of insemination techniques.

Conclusions

The various methods are developed to separate X-chromosome bearing sperm and Y-chromosome-bearing sperm based on the fluorescence of the Y chromosome, differences in mass and motility, differences in swimming pattern, differences in surface charge, differing cell surface antigenic determinants and differing DNA content that efficiently separate bovine semen into X or Y chromosome bearing sperm. Among all the techniques discussed flow cytometry is the best method which effectively separates X and Y bearing spermatozoa, for accurate prediction of the sex and to produce calves of desirable sex.

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