Sex Preselection in Domestic Animals - Current status and Future prospects

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Abstract

Sex preselection is a subject that has held man's attention for generations. The most effective way to achieve sex predetermination is to resolve X and Y chromosome bearing sperm populations. One of the most reliable methods of sorting spermatozoa is flow cyto-fluorimetric analysis, which is based on the difference in the DNA content of the X and Y sperm populations. The difference in the DNA content between X and Y spermatozoa of mammals range from 3.5 to 4.2 per cent. Sorting of variable spermatozoa by flow cytometer have been improved over time and the sorting rate with the recent high speed cell sorter is about 11 million sperms of each sex per hour with purity of 90 per cent. Sort re-analysis enables the laboratory validations of the purity of the sex-sorted spermatozoa. Cryo-preservation of the sorted spermatozoa has also been successful without affecting the viability and fertility. Sexed semen has a wide range of applications in animal breeding by increasing the selection pressure for replacement females and in providing more number of female offspring for progeny testing of breedable males consequently increasing the accuracy of selection. Moreover use of sex-sorted semen will also help in the conservation of endangered species. Development of the instrument for increasing the sorting rate and also purity of sorting without affecting the viability and fertility is still an active area of research.

Key words: Sex preselection, Spermatozoa, Fertilization, Domestic animal, Chromosome.

Introduction

The aim of preselecting the sex of offspring and there by to alter the sex ratio is attractive for centuries. In animal industry, predetermining the sex has been a goal of livestock producers for generations. Predetermination of sex of an offspring is of commercial importance. Female is the choice animal for dairy industry where as the male is the first choice in meat industry and also in A.I. programs. A range of methods has been advocated, often based on mythology or superstition. Recently, however, it has become possible to predetermine gender through an understanding of the differences between X- and Y-chromosome bearing spermatozoa and by sexing concepts before embryo transfer. The ability to resolve X and Y chromosome bearing sperm populations have important applications for controlling the sex of offspring of agriculturally important animals.

Sexing of Spermatozoa

Sexing of Spermatozoa can be used as a wonderful tool to alter the sex ratio and there by to enhance the milk production and to meet the growing demand for milk. Out of the various procedures available for pre and post-fertilization sex determination, the most elegant method of achieving an alteration in sex ratio is to separate spermatozoa into X and Y bearing spermatozoa as the sex of the offspring is determined by the sex chromosome with in the spermatozoa. The methods/ procedures to separate spermatozoa in to X and Y bearing fractions have been based on differences between each subpopulation on the basis of mass, motility, DNA content, surface charge and sperm cell surface antigenic determinants.

I. Physical Methods (on the basis of Mass and Motility):

a) Separation based on size and shape

The X-chromosome bearing sperm has been postulated to be necessarily larger than the Y-chromosome bearing sperm. It has been reported that the Y-chromosome is smaller on average than the X-chromosome by 3.9 per cent and that a 3-4 per cent difference in sperm DNA content is theoretically equivalent to a 1 per cent difference in sperm head radius (McEvoy 1992).However no repeatable experimental evidence exists to substantiate these hypotheses and their reliability is doubtful.

Table-1. Potential Differences between X and Y Spermatozoa

Parameter	X Spermatozoa	Y Spermatozoa	Method
DNA	More DNA	Less NDA	Flow Cytometry
Size	Larger	Relatively smaller	Percoll method
Motility	Swim slower	Swim faster	Swim Up
Surface Charge	Migrate to cathode fastly	Migrate to cathode relatively slowly	Free flow electrophoresis
Sperm Surface	Absence of HY Antigen	Presence of HY antigen	Immunological sexing

b) Separation on the basis of Swim up

Laminar flow fractionation:

Y-bearing spermatozoa swim differently and more quickly than X-bearing spermatozoa. Fluid flow rates can be used to accentuate this difference, and therefore enhance the separation of the two subpopulations. Success has been reported using human spermatozoa in a specially developed cylindrical flow column with laminar flow velocity gradients. But the ability of the technique to separate X- and Y-spermatozoa is highly dependant on the initial individual semen casting doubt on the feasibility of this technique to separate semen effectively.The semen sample is overlaid with an appropriate volume of a suitable medium and left in an incubator for 30-60 minutes during which time the progressively motile sperm swim up into the media. During swim up it has been postulated that Y-bearing spermatozoa reach the top of the medium before X-bearing ones. But Han et al. (1993) and De Jonge et al. (1999) found no significant change in the ratio of X-Y spermatozoa in the swim up procedure.

Albumin gradients

Theoretically, the Y-bearing spermatozoa being smaller swim faster. If semen is layered on top of a discontinuous albumin gradient derived from bovine or human serum, the smaller Y-bearing sperm have a greater ability to penetrate the interface between fluids and to swim faster than the X-bearing sperm in fluids of high density and viscosity. The fractions of semen isolated from specific parts of the column are expected to be either X or Y enriched.

Percoll gradients

Percoll consists of colloidal silica particles coated with polyvinyl pyrollidine. When percoll is set up in a discontinuous gradient, spermatozoa layered on top of the column are allowed to penetrate the column naturally; the extent of this penetration is a function of both their mass and motility. Alternatively, centrifugation can be done to minimize the effect of motility and maximize the difference in mass.

II. Predicting differences in surface charge:

(a) Free-flow electrophoresis:

It has been suggested that there are differing electrical charges on the cell membrane of X- and Y-bearing spermatozoa, or differing amounts of net charge. It has been postulated that when semen is subjected to electrical field on an electrophoresis plate, subpopulations of X- and Y-bearing spermatozoa should separate perpendicular to the electrical field according to their net electrical charges and difference in motility. The spermatozoa which migrate towards anode are X-bearing, having higher negative charge than Y-bearing spermatozoa, due to a relatively greater concentration of neuraminic acid containing glycoproteins on the cell membrane surface of X spermatozoa.

(b) Counter current galvanic separation:

It involves the use of specially designed forced convection streaming galvanic cell which is claimed to enhance the separation of X-and Y-bearing spermatozoa.

However, these techniques are ineffective in separating as high variations have been observed in different research experiments. Validity of the concept that X- and Y-bearing spermatozoa have differing genetically influenced surface net electrical charges is questionable, since both populations are in close contact with each other and bathed in the same seminal fluid.

III. Predicting differences in cell surface antigenic determinants:

Histocompatibility-Y antigen (H-Y) is found in male tissues of many mammalian species with the exception of erythrocytes and premeiotic germ cells. H-Y antigen present in the plasma membrane of many mammalian species. If the expression of H-Y antigen on the surface of these haploid cells is due to expression of the Y-chromosome, then this could be used to separate HY+ spermatozoa, presumably carrying the Y-chromosome, from HY- spermatozoa carrying the X-chromosome. There is circumstantial evidence that one or more structural genes responsible for H-Y expression may be autosomally located. It has been postulated that H-Y antigen is absorbed on to the cell membranes of X- and Y-bearing spermatids during association with sertoli and their products. It was concluded that H-Y was present on the cell membranes of both X- and Y- chromosome bearing spermatozoa under normal circumstances hence the prospects of sexing mammalian semen using H-Y antibody are poor.

IV. Predicting the difference in DNA content:

The DNA difference between X and Y sperm of domestic livestock ranges from 3.5 to 4.2 per cent. Therefore, this difference in DNA content can be used

to distinguish and select X from Y bearing sperm (Garner et al., 1983).

Flow cytometry – Fluorescence activated cell sorting:

The technique of flow cytometry relies on the fact that the chromatin in the head of the spermatozoon can be stained with a DNA-specific fluorescent dye. These stained cells can then be passed rapidly in single file through a flow cytometer containing light beam. This causes stained spermatozoa to fluoresce, and they can be separated into different fractions since the degree of fluorescence is directly proportional to the amount of DNA within each cell. As X chromosome contains more DNA, X-bearing spermatozoa fluoresce more than Y-bearing spermatozoa. Thus, flow cytometry has been used to sort spermatozoa into a bimodal distribution, with differences in DNA content between the peaks ranging from 3.5-4.5% in domestic animals. Purities of separated bull, ram and boar semen can be as high as 90%. Flow cytometry is the most effective and scientifically valid of all the techniques used to separate semen. However, a number of problems currently preclude its use on a commercial basis. They are:

1) Low sorting rate: Full day's sort of intact spermatozoa will only yield 20 X 106 spermatozoa.

2) Freeze storage of sorted semen: Effect of freeze storage of sorted semen has not been investigated.

3) Expense: Flow cytometer is very expensive to purchase and maintain.

4) Mutagenic effects: DNA binding dyes such as Hoechst 33342 bind loosely to the DNA in the genome but may still cause chromosomal aberrations. Fluorochrome dyes are potential cause of low embryo viability and hence low pregnancy rates.

5) Low sperm viability/ fertility: To facilitate access of the DNA stain to the genome, the integrity of the cell membrane has to be compromised either by digestion with papain or by light sonication. Digestion with papain results in a high percentage of cell loss, where as light sonication increases cell survival rate but at the expense of sperm motility as sonication causes loss of sperm flagellum, necessitating sperm microinjection.

Of all the techniques discussed above, Fluorescence activated cell sorting by flow cytometry is the only available reliable and accurate method which effectively separates X- and Y-bearing spermatozoa, enabling accurate prediction of the sex ratio at birth.

Sexed semen will contribute to increased profitability of dairy and beef cattle production in a variety of ways. It could be used to produce offspring of the desired sex from a particular mating to take advantage of differences in value of males and females for specific marketing purposes. Commercial dairy farmers, those who produce and market milk, could use sexed semen to produce replacement daughters from genetically superior cows. The impact on annual genetic progress in milk yield of using sexed semen technology both in matings to produce commercial replacement heifers and in matings of elite parents to produce bulls for progeny testing has been predicted. Compared to mating system using artificial insemination with regular semen, annual genetic progress is increased by 15 per cent.

The most ideal route for preselection of sex would be sexing of spermatozoa. Of all the semen sexing techniques available till date, flow cytometry is the only method which effectively separates X- and Y-bearing spermatozoa in spite of its limitations such as low sorting rate, mutagenic effect etc. Even though attempts were made to separate spermatozoa based on differing cell surface antigenic determinants such as H-Y antigen, they were not successful, as H-Y antigen is not synthesized by cellular machinery of sperm and is absorbed on to the cell membranes of X- and Y-bearing spermatids during association with sertoli and their products. Instead of H-Y antigen research workers should search for a sperm plasma membrane protein/ antigen whose synthesis is dependant on Y-chromosome.

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