Gender Preselection in Farm Animals

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Every living being has a set of paired chromosomes, which carry all the genetic material necessary to maintain life and also to propagate new life.

All but one pair of chromosomes are called autosomes and carry genes for all the characteristics of the body, such as skin, hair and eye color, mature size, and body characteristics. The remaining pair are called sex chromosomes. They carry the genetic material that specifies gender. One sex chromosome is called X, the other Y.

A sperm from the male or an egg from the female contains one of each pair of autosomes; in addition, in mammals the egg always contains an X chromosome, while the sperm always carries either an X or Y chromosome.

When a sperm and egg unite and the sperm carries the Y chromosome, the offspring is male (XY); however, if the sperm carries an X chromosome when it unites with the egg, the resulting offspring is female (XX).

In most animals, including humans, the ratio of males to females is 50:50. Because the determination of sex, or gender, takes place when a sperm fertilizes an egg, preselection of gender by selecting the sperm that fertilize eggs must be done before the sperm are used for insemination.

In all bird species, including turkeys and chickens, the female determines the sex of offspring. In birds, the sex chromosomes are exactly the same in all sperm, so poultry sperm cannot be manipulated to preselect the sex of offspring.

Purpose of Gender Preselection

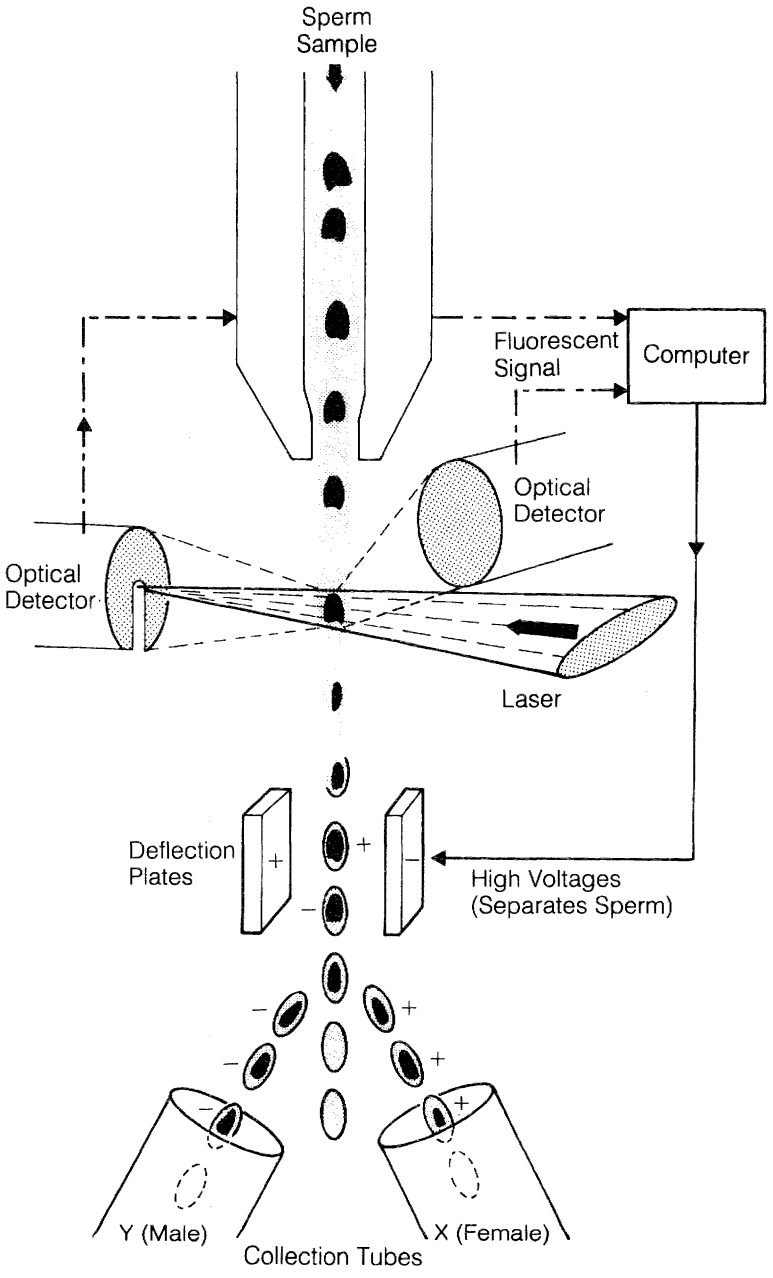
Gender of animal offspring is important to livestock producers. Selection of gender by separating X-bearing sperm from Y-bearing sperm before semen is used in artificial insemination could give farmers the choice of sex of offspring.

Because the dairy farmer has little use for most bull calves, the use of sexed semen to produce only females would make milk production more efficient. Swine farmers would produce pork more efficiently if they were able to market only female swine because females grow faster than males.

In beef cattle and sheep breeds, the male grows at a faster rate than the female and hence is preferred for meat production.

In addition, the ability to specify male or female offspring should shorten the time required for genetic improvements, since desirable traits are often associated with one or the other parent. Planning the sex of cattle offspring is already practiced on a limited basis. This procedure consists of removing embryos from the cow, identifying their potential gender, and re-implanting only those of the desired gender. However, an ability to separate sperm into male-producing and female-producing groups before they are used for artificial insemination could enhance the overall value of offspring produced by embryo transfer.

Cell Separation Process



History of Gender Preselection

Interest in controlling the sex of offspring dates back at least to Hippocrates (460–377 B.C.). In the 20th century, particularly since 1950, many attempts have been made to determine differences between Y-bearing sperm and X-bearing sperm, particularly with regard to cattle. Most techniques that have been tested have been aimed at distinguishing subtle physical differences, such as swimming ability, size, shape, density, and weight of the sperm. Any such physical differences, however, are small, and the methods used to separate X- from Y-bearing sperm are not sufficiently precise to detect the differences.

Nevertheless, some entrepreneurs have tried to capitalize on the interest in controlling sex by selling so-called "sexed" semen. As one might expect, they fail to follow up to determine the outcome of live births from that supposedly "sexed" semen.

In short, no valid practical method exists today for separating a sample of livestock semen into X-bearing or Y-bearing sperm, and regardless of the claims, no practical method exists for even enriching a sample of livestock semen for either X- or Y-bearing sperm.

Sexing Semen by DNA Content

The only established and measurable difference between X and Y sperm that is known and has been proved to be scientifically valid is their difference in deoxyribonucleic acid (DNA) content. The X chromosome is larger and contains slightly more DNA than does the Y chromosome. The difference in total DNA between X-bearing sperm and Y-bearing sperm is 3.4 percent in boar, 3.8 percent in bull, and 4.2 percent in ram sperm.

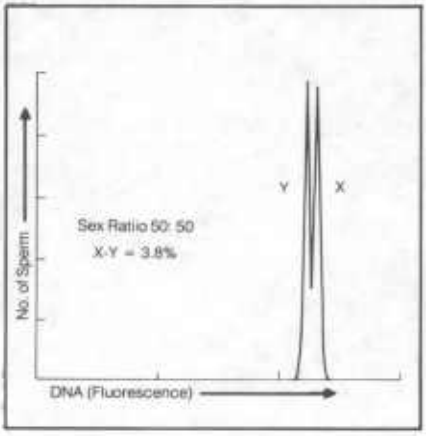
Steve Brown, ARS

Flow cytometers are advanced cell sorters that use lasers to excite fluorescent dye to separate X and Y chromosome-bearing sperm.

The amount of DNA in a sperm cell, as in most body cells, is stable. Therefore, the DNA content of individual sperm can be monitored and used to differentiate X- and Y-bearing sperm.

Instruments to Measure Sperm DNA

Instruments to measure DNA in microscopic particles and cells, such as sperm and blood cells, have been de-



veloped during the past 10 years. These instruments, called flow cytometers, measure the amount of fluorescent light given off when the sperm, previously treated with a fluorescent dye, pass through a laser beam. The dye stains the DNA. The fluorescent light is collected and analyzed by computer. Because the X chromosome contains more DNA than the Y chromosome, the female sperm (X) takes up more dye and gives off more light than the male sperm (Y).

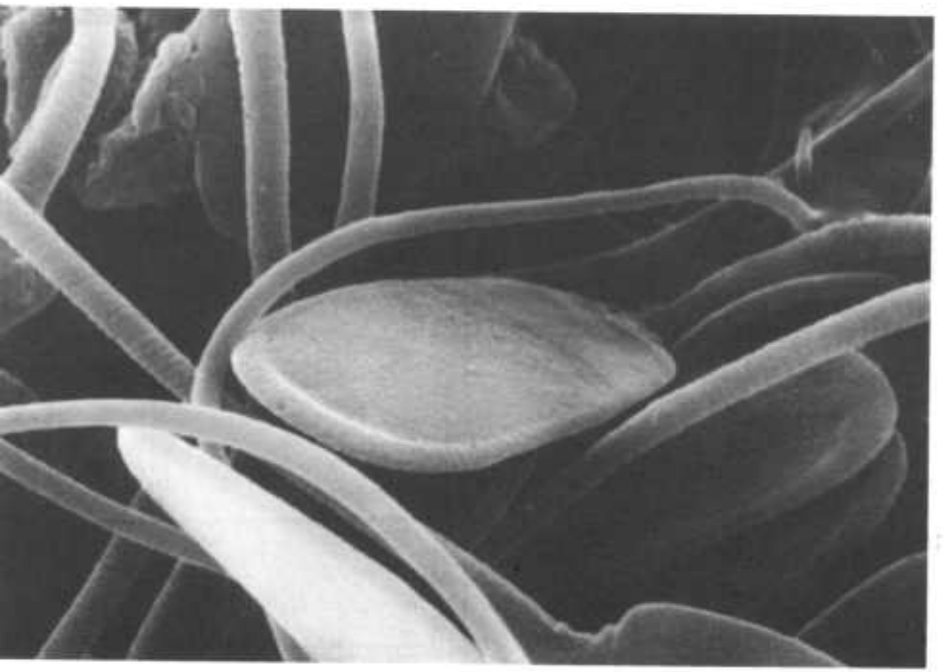
For small differences in DNA to be detected between X and Y, the sperm must pass single file through the laser beam, which measures the DNA content of individual sperm. Hundreds of sperm pass through the beam each second, and 10,000 sperm per sample are routinely analyzed. From this analysis, the ratio of X to Y sperm in a sample of semen can be determined.

Flow cytometric methods can verify any change in the X to Y sperm ratio in a sample of semen. Flow cytometry also is useful in determining whether a particular procedure used in an attempt to change the X–Y ratio is actually changing the ratio. Before the advent of this flow cytometric procedure, the experimental semen had to be used to artificially inseminate many females, and the offspring counted to determine the ratio of males to females. This is an expensive and time-consuming procedure.

Now the potential sex ratio can be determined before the semen is used for artificial insemination.

Flow Sorting of X- and Y-Bearing Sperm

Flow cytometry systems also can be used to separate cells. After sperm pass in front of the laser beam to de-



Spermatozoa from swine, magnified 10,000 times.

termine their DNA content, which takes only a fraction of a second, each sperm can be encased in a droplet of liquid. The droplet containing an individual sperm can then be given either a positive or negative electrical charge, depending on the amount of DNA that was measured (X- or Y-bearing sperm). Next, the droplets pass between two steel deflection plates with high voltage, one positive and one negative. Each plate will attract the oppositely charged (+ or −) droplet, pulling it out of the center stream of droplets and into a tube below.

Using this type of cell sorter, the sperm can be separated into groups containing only X- or Y-bearing sperm. The sorted sperm, however, are not intact; during preparation for DNA measurement, the sperm tails are removed. Only the head of the sperm (nucleus) containing the DNA is passed through the flow cytometry system. So the sorted sperm are not capable of fertilizing an egg. Research is currently being conducted to separate intact sperm capable of fertilizing an egg into X- and Y-bearing groups.

Future Prospects

Once the problems of separating two intact groups of sperm (X and Y) are solved, efforts will be made to identify some other factor on the surface of sperm that can be used to differentiate female from male sperm. Although flow cytometry and cell sorting using DNA as a marker are useful for the numbers of sperm required for research, these procedures are not applicable to separating the billions of sperm required for artificial insemination programs. A surface marker, however, might be used to separate the large numbers of sperm needed for artificial insemination.

Membrane Research: New Approach to Treatment of Gastrointestinal Illnesses

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Diarrhea and other gastrointestinal illnesses cause high livestock mortality rates and result in significant economic losses to the livestock industry. These losses were passed on to consumers in the form of higher prices.

During the past few years, however, research on the function of the epithelial membrane has begun to provide information in both basic and applied physiology. (This membrane of cellular tissue covers a surface or lining of a tube or cavity of an animal, serving to enclose and protect other parts of the body, to produce secretions and excretions, and to function in assimilation. Gut linings are an example.) Studies are leading to new approaches for the treatment of diarrhea and acute and chronic bowel injuries. Significant therapeutic advances based on this new knowledge are now anticipated. Some of these studies will now be discussed.

Membrane Function

The study of epithelial function is especially difficult because of the many different types of cell in the mucous membrane of an animal's gastrointestinal tract as well as the complexity of