New Accuracy in Sex Selection

For more than a decade, it has been our goal to provide livestock producers the opportunity to predetermine the sex of offspring to increase reproductive efficiency, says ARS animal physiologist Lawrence A. Johnson.

Now, what may have once appeared impossible to accomplish is possible.

In 1989, Johnson, head of the Germplasm and Gamete Physiology Laboratory in Beltsville, Maryland, first developed a method to separate living female-producing (X-chromosome) and male-producing (Y-chromosome) sperm based on their DNA content. USDA has patented this method, called the Beltsville Sperm Sexing Technology, and licensed it to several firms for commercial development for livestock reproduction and in human medicine.

Initially, Johnson proved the method worked with rabbits, pigs, and cattle, but he could only sort 1.5 million to 2 million sperm per day with 75- to 90-percent accuracy. [See "Good Breeding: From Simple Beginnings to Genetic Engineering," Agri*cultural Research,* November 1991, pp. 4-9.] The technology has come a long way since that initial breakthrough.

The principles on which the method is based are still the same, but Johnson and his colleagues have transformed the technology in a major way. They have proved its effectiveness in sheep, laboratory animals, and other species. Recently, scientists with Colorado State University and XY, Inc., of Fort Collins reported the birth of a filly from sexed horse semen.

Basic Sorting—Plus

So how does one separate something that the naked eye can't see?

Johnson uses a fluorescent dye that sticks to the DNA. The dye binds to the sperm based on how much DNA the X and Y chromosomes in the sperm are carrying. Female-producing X sperm contain 2.8 to 7.5 percent more DNA than male-producing Y sperm, depending on the species.

Sperm cells are analyzed and sorted using a flow cytometer/cell sorter. When a laser beam illuminates the dye, each sperm gives off light proportional to its DNA content and is separated into different tubes, depending on the amount of light emitted. The X sperm always glows brighter because of the greater amount of DNA.

"Cell sorters are built and sold primarily for the medical community and are used in cancer and other types of medical research," Johnson says. "Since blood cells are spherical, we had to adapt the cell sorter for domestic animal sperm, which generally have paddle-shaped heads and long tails. Then we did what had never been done before on a consistent basis: separated living sperm for use in producing offspring."

Johnson and colleagues Glenn R. Welch and Wilem Rens have made three significant enhancements to the Beltsville technology that dramatically increase the practicality and chances that the animal industry will adopt the technology.

"First," Johnson says, "we improved our sperm-processing procedures, reducing the sperm's exposure to excess dye. Second, we developed a new nozzle [patent pending] for the flow cytometer cell sorter that allows 70 percent of the sperm—compared to only 30 percent with the older version—to be properly aligned to the laser beam and sorted into the correct tube. Third, we modified a newly developed commercial high-speed cell sorter and adapted it not only for sorting sperm, but to fit our new nozzle."

The combination of these improvements has led to a 15- to 20-fold increase in production rates of sorted sperm populations. Now, 35 to 40 million of both X and Y sperm can be sorted in an average 8-hour day, compared to 1 to 2 million previously.

Johnson notes that accuracy depends on the DNA difference between the X and Y sperm, which varies with the species being sorted. This difference allows sorting to take place and determines the accuracy of a positive selection,

LAWRENCE JOHNSON

Heifer calf born to a cow inseminated with sorted X-chromosome-bearing sperm.

Animal physiologist Lawrence Johnson displays a pig born as a result of studies using sorted sperm and in vitro fertilization.

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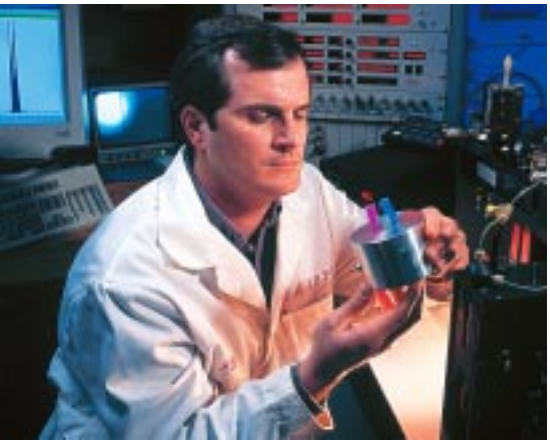

Biologist Glenn Welch operates the Beltsville high-speed sorter that separates X and Y sperm. Livestock inseminated with the sexed sperm will produce offspring of the selected sex with 90-percent accuracy.

Johnson says.

For example, boars carry about 3.6 percent more DNA in their X sperm than in their Y sperm; bulls, 3.8 percent more; and humans, 2.8 percent. The wider the difference, the easier it is to sort with greater accuracy.

Sperm-sorting technology can now be used with both conventional and deep-uterine artificial insemination in cattle.

With conventional artificial insemination, sperm are placed just inside the cow's uterus—a procedure that requires about 5 million sexed sperm and an hour of sorting time. With deep-uterine insemination, only about 300,000 sperm need to be placed to get pregnancy.

Because pigs require much larger doses of semen for artificial insemination, the scientists must use in vitro fertilization with sexed sperm. The embryo is implanted in a surrogate sow.

In a recent experiment showing the effectiveness of the sperm-sexing technology, eight litters of pigs were born at Beltsville using sorted X-chromosome sperm. Ninety-eight percent of the pigs—or 43 of 44—in the 8 litters were female. Three control litters produced at the same time with unsexed sperm resulted in equal numbers of male and female offspring.

To date, more than 500 animals have been born using sexed semen, and all have been healthy and normal. Johnson says the technology could potentially save the cattle industry millions of dollars annually.

"Sexing sperm could help farmers get the required numbers of the desired sex without producing the other sex," says Johnson. "This speeds the rate at which they can achieve genetic improvement while reducing production costs."

Johnson says the clearest example of this is with dairy cows. "Normally a farmer would use the top 40 percent of his herd to reproduce enough female replacement calves. With sexed semen, a farmer would only need to use the top 20 percent."

Another benefit of sex-sorting sperm is efficiency in conserving genetic resources. Johnson says that when storing rare germplasm for use by later generations, conservators can maintain only the sperm needed to produce sexed offspring desired for particular situations, like selecting a cow versus a bull, for example.

"Sex preselection provides an opportunity to improve management flexibility using this technology," he says.

Johnson is collaborating with several scientists around the world to help establish the technology for use in livestock and to perfect it for commercial use.—By **Tara Weaver,** ARS.

This research is part of Animal Germplasm, Resources, Conservation, and Development, an ARS National Program described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

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