

# Application of Sex-selected Semen in Heifer Development and Breeding Programs

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## KEYWORDS

• Sexed semen • Heifers • Economics • Fertility

## KEY POINTS

- Measuring DNA content of individual sperm with a flow cytometer/cell sorter is the only feasible method for sexing mammalian sperm.
- With excellent management, skilled inseminators, and appropriate handling of semen, fertility of sexed semen is 70% to 90% of fertility of unsexed control semen in the same herds.
- The accuracy of sexing sperm routinely is set at about 90% of the desired sex, sorting at higher accuracy is more costly.
- It is unlikely that a different method for practical application for sexing sperm will be found in the next 3 to 5 years, but incremental improvements of sexing procedures are constantly being developed.

## BRIEF HISTORY OF SEXED SEMEN

Being able to choose the sex of offspring of people has been a goal for millennia; for example, Democritus in Greece mentioned this issue and made recommendations nearly 2500 years ago. This also has been a goal of cattle producers for centuries. However, only recently have scientific principles been applied to the problem; dozens of methods of sexing sperm have been attempted over the past 60 years. These include centrifugation, electrophoresis, manipulating pH, separation by the swimming speed of sperm, using antibodies, etc. Unfortunately, all of these methods have failed to date, either because they are not effective, severely damage sperm, or otherwise are problematic, despite hundreds of studies and hundreds of mostly useless patents.

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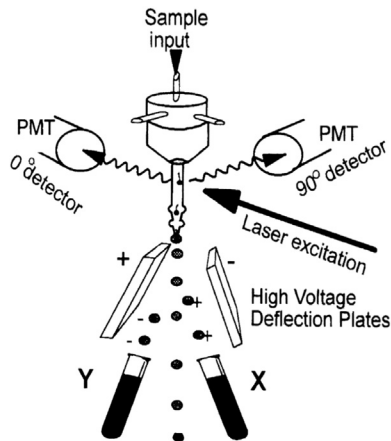
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However, there is one efficacious method, which despite limitations and imperfections, is about 90% accurate and is available commercially for cattle.<sup>1</sup> This method, measuring DNA content of individual sperm by a process termed flow cytometry and then sorting the sperm based on DNA content, was pioneered by about a dozen scientists and first convincingly demonstrated to alter the sex ratio of offspring by a team headed by Lawrence Johnson at a US Department of Agriculture laboratory in Beltsville, Maryland. Currently, this is the only method available that is efficacious in any practical sense, and it is unlikely to be replaced commercially by any other method in the next several years, and maybe not for decades.

### HOW ARE SPERM SEXED?

Bovine X-chromosome-bearing sperm (which result in females) contain about 4% more genetic material (DNA) than Y-chromosome-bearing sperm (which result in males). Sperm are incubated for about half an hour with a DNA-binding dye that emits fluorescent light when stimulated by a particular wavelength of ultraviolet light, which usually is provided by a laser. The sperm then flow past the laser and fluorescence detectors in a device called flow cytometer/cell sorter. The fluorescence of each individual sperm is evaluated by computer into 3 categories: probably X-sperm, probably Y-sperm, or unable to distinguish if X or Y.<sup>2</sup> As illustrated in **Fig. 1**, droplets are formed at the tip of the nozzle of the cell sorter, and those droplets containing putative X-sperm are given a positive charge, those droplets containing putative Y-sperm are given a negative charge, and those droplets containing no sperm or sperm with indistinguishable sex chromosomes are not charged at all. The droplets exit the nozzle at about 80 km per hour and pass by strong positive and negative electric fields (see **Fig. 1**). Because opposite electrical charges attract, the positively charged X-sperm droplets veer toward the negative electric field, the negatively charged droplets veer toward the positive electric field, and the uncharged droplets fall straight. In this way, droplets can be sorted into 3 test tubes. In the process, the sperm become highly diluted, so they must be concentrated by centrifugation and removal of the excess fluid, after which they are packaged in straws and frozen.<sup>2</sup> This complicated process damages the sperm slightly, so they are slightly less fertile than unsexed sperm.



**Fig. 1.** Sorting by charge. (From Johnson LA, Welch GR, Rens W. Beltsville sperm sexing technology: High speed sperm sorting gives improved sperm output for in vitro fertilization and AI. *J Anim Sci* 1999;77(Suppl 2):213–20.)

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