

## Flow cytometric sexing of mammalian spermatozoa

### Preben Christensen

Department of Production Animals and Horses, Section for Reproduction, Royal Veterinary and Agricultural University, Dyrlaagevej 68, 1870 Frederiksberg C, Denmark.

Tel: +45 35 28 29 70

([PC@kvl.dk](mailto:PC@kvl.dk))

Being able to preselect the sex of offspring at the time of conception ranks among the most sought-after reproductive technologies of all time. Sex preselection that is based on flow cytometric measurement of sperm DNA content to enable sorting of X- from Y-chromosome-bearing sperm has proven to be reliable with many species at greater than 90% purity. Offspring of the predetermined sex in both domestic animals and human beings have been born using this technology since its introduction in 1989.

The method involves treating sperm with the fluorescent dye, Hoechst 33342, which binds to the DNA and then sorting them into X- and Y-bearing sperm populations with a flow cytometer modified specially for sperm. With the advent of high-speed cell sorting technology and improved efficiency of sorting by a new sperm orienting nozzle, the efficiency of sexed sperm production is significantly enhanced. Under typical conditions the high-speed sperm sorter with the orienting nozzle (HiSON) results in purities of 90% X- and Y-bearing sperm at 18 million sperm per h for each population. This represents a 50-fold improvement over the 1989 sorting technology using rabbit sperm.

Today, sexed sperm are commercial available for cattle. Initial experiments for this species involved deep intra uterine insemination with low dose (2 million sperm) but more recent experiments with normal intra uterine inseminations have shown similar success rates. In comparison to conventional insemination with 15 million sperm, pregnancy rates are still approximately 10 percentage units lower. At present, it is unclear if this reduction in fertility is related to the difference in number of sperm per dose or reflects damage to sperm during sorting. Up to now, several thousands of healthy calves have been born after this technology.

The method have also been applied for other species such as sheep, horses, exotic and endangered species and have in some cases been used in combination with in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI). The most important application of sexing in the human population is to minimize sex-linked genetic disease. A more controversial application is sex selection for purposes of family gender balancing.