High Resolution Purity Analysis of Sex Sorted Semen and the Correlation to Field Results

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Introduction

The gender of the offspring can be predetermined by flow cytometric sorting of highly purified X- or Y-chromosome-bearing sperm subpopulations. The technology has undergone several improvements in the past ten years and it is now widely used by Bovine, Small Ruminants, Cervine, Equine and Porcine industries. To ensure the quality of the sorted product, Sexing Technologies analyzes various sperm parameters at multiple checkpoints during the sex sorting process. One of these quality parameters is sperm sex purity. The implementation of these technological advancements provide high confidence in the quality of Sexing Technologies sex sorted products, which reflects in the most recent field results.

Objectives

- ✓ Provide information about how Sexing Technologies manages the assessment of sex sorted semen purity in the laboratory.
- ✓ Review the effective outcome comparing laboratory sex purity results with offspring gender ratio.

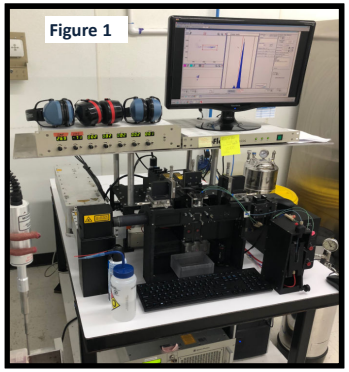
Materials and Methods

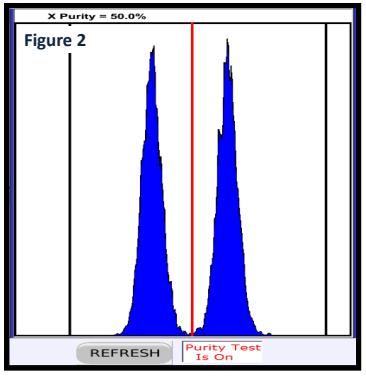
Sperm sex purity is analyzed using a Genesis-1 high resolution Analyzer (**Figure 1**), a modified MoFlo SX sperm sorter, developed for sperm analysis using Sexing Technologies proprietary technologies.

Calibration of the Analyzer is performed using certified sperm nuclei as a standard to confirm the instrument is properly aligned and validated prior to purity analysis (**Figure 2**). Certified nuclei are qualified to meet specifications of number of oriented cells for the Side Fluorescence detector path and quality of resolution on DNA content on the Forward Fluorescence detector path before running any purity analysis.

High performance sperm sorting systems known as Genesis-III are verified multiple times a day to confirm that the setup is accurate for the desired sex ratio, while optimizing and maintaining productivity. Purity Analysis of the percent of X- and Y- chromosome-bearing sperm is also performed on every batch of sex sorted sperm produced. For Purity analysis, 3,000-5,000 cells are analyzed per sample depending on the target purity.

Laboratory and field data was collected in the years of 2016-2018 from 7 bulls and 9,447 calvings.

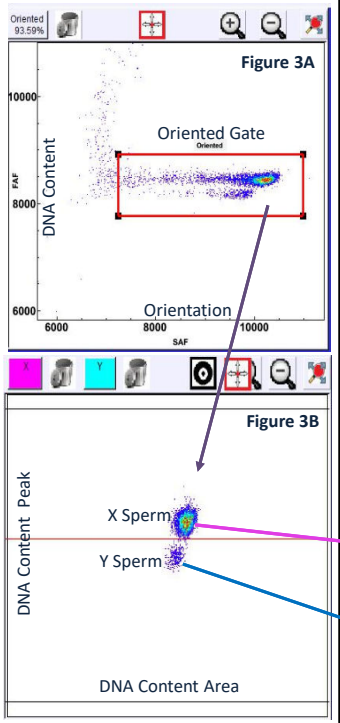


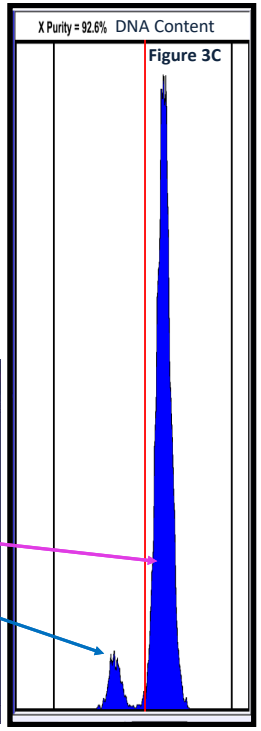


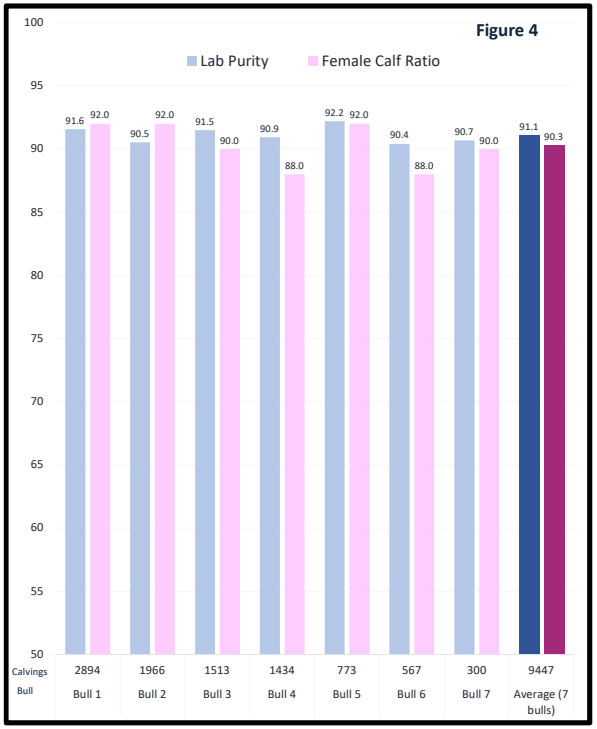
Results

With the improvements to the technology and the use of a Genesis-1 high resolution Analyzer, sperm cells properly orient within the Oriented gate (**Figure 3A**). Using gated oriented cells, more than 95% of the sperm that run through the Analyzer are then gated for X- or Y-chromosome-bearing sperm subpopulation analysis (**Figure 3B**). Lastly, sperm populations are quantified in a DNA content histogram that represents the percentage of purified X sperm (**Figure 3C**).

Field data confirmed that a sperm purity of 91.1±0.2% analyzed using the aforementioned methods, resulted in an *in vivo* female calf percent of 90.3±0.2% (**Figure 4**).







Conclusion

With the technological advancements made by Sexing Technologies and the implementation of these improvements into sex purity analysis methods and instrumentation, there is very high confidence in the assessment of sex sorted semen products meeting the desired purity as it highly correlates to field results.

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