SexedULTRA™, a new method of processing sex sorted bovine sperm improves post-thaw sperm quality and *in vitro* fertility

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Introduction

Since the first publications 30 years ago showing that flow cytometry was a reliable method to separate X and Y chromosome bearing sperm, the process has been subject to continual refinement. The objective has been to develop an improved sex sorted product that performs at a level comparable to the non-sorted conventional semen. This study focusses on a new sex sorting process branded "SexedULTRA™" that retains sperm integrity, improves post thaw sperm quality and in vitro embryo production compared with the previous XY method.

Material and Methods

LABORATORY TRIAL - Ejaculates from 12 bulls (Sexing Technologies, Navasota, Texas) were divided in two aliquots and processed in one of two methods: XY or SexedULTRA™. Post thaw sperm motilities were classified into percent total and progressively motile after thawing (0 h) and after a 3 h incubation at 37°C using a computer assisted sperm motility analyzer (Hamilton Thorne IVOS II system). Percent intact acrosomes (PIA) was also estimated after a 3 h incubation. Results (Figure1) were analyzed by a mixed model analysis of variance with the fixed effect of treatment and random effect of bull.

IN VITRO FERTILIZATION – Performed as a measure of sperm competence using 8 ejaculates from 2 bulls (Sexing Technologies, Laceyville, Pennsylvania). 5-10 oocytes and 5,000 motile sperm/oocyte were placed per IVF drop for the analysis. A total of three straws and a minimum of 800 oocytes per treatment group (ejaculate x treatment) were included in the comparison for development to 8 cell stage (cleavage rate) and today 7 blastocyst stage measured as total (grades 1 to 4) and freezable (grades 1 and 2) embryos. Results (Table 1) were analyzed using a mixed model analysis of variance with treatment as a fixed effect and bull, ejaculate within bull, and IVF cycle as random effects.

Results

Figure 1. LABORATORY TRIAL - Percent total motile SexedULTRA™ sperm (closed bars) was greater ($P < 0.001$) than sperm processed following the XY method (open bars) at 0 h (78.8% vs 67.2%) and 3 h (51.0% vs 39.0%) post thaw. Likewise, there was a higher percent of progressively motile sperm at 0 h (50.7% vs 44.9%) and 3 h (31.5% vs 4.4%) post thaw in the SexedULTRA™ sperm. PIA was also greater in SexedULTRA™ sperm compared to the sperm processed following the XY method (78.0% vs 64.0%).

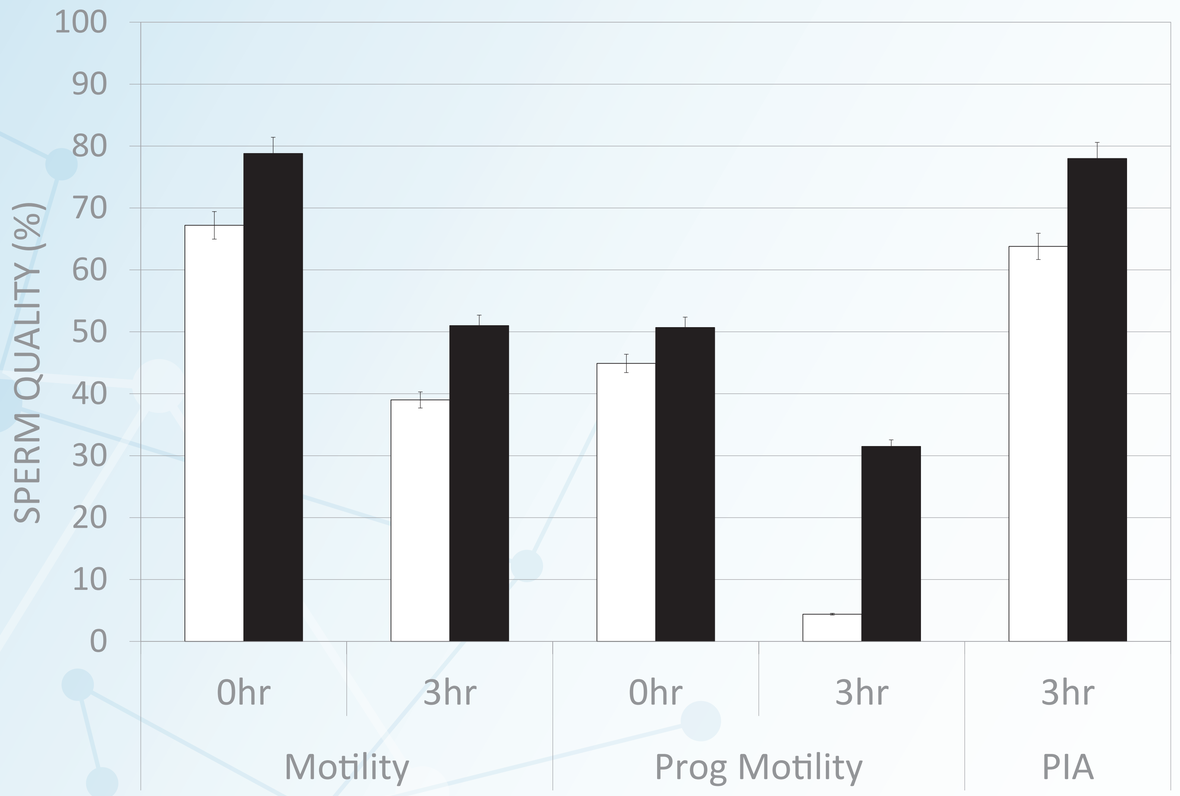

Table 1. *IN VITRO* FERTILIZATION - Total and freezable embryo numbers were significantly higher ($P<0.05$) when using SexedULTRA™ compared with XY frozen-thawed, sex-sorted sperm..

Treatment	Oocytes for IVF (n)	% 8-cell rate (n)	% Total embryos (n)	% Freezable embryos (n)
XY	5082	32.7% (1664)	18.4% (937)	9.2% (472)
SexedULTRA™	5081	34.8% (1770)	22.3% (1134)*	13.2% (669)*

*Treatments within IVF endpoint differ ($P<0.05$)

Conclusion

Maintaining a suitable environment for sperm to progress through the various steps of the sex sorting process results in better sperm quality post thaw as well as improved *in vitro* fertility. The SexedULTRA™ method confers a significant benefit in maintaining sperm integrity that, if translated into field fertility, could reduce the conception rate gap between conventional and sex sorted bovine sperm.

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