(MP44-14) Sorting of sperm with ChromaSelect does not skew the ratio of X to Y chromosome bearing sperm.

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Background

- ChromaSelect is a highly sensitive and specific method of identification and isolation of intact and live human sperm from ejaculate, epididymal or testis specimens with a low concentration (up to 100 sperm /ml).
- As Chr Y ch is much smaller than X, it is theoretically possible that Y or X bearing sperm will show different fluorescence intensity and thus could result in a skewed selection of one of the sexes in sorted sperm.

Objective

- To evaluate the ratio of Chr X and Y bearing sperm in intact, live sperm and dead/apoptotic sperm fraction after ChromaSelect identification and sorting.

Methods

- Ejaculated human sperm were resuspended to a concentration of 10x106/ml in MHM-C medium, stained using ChromaSelect protocol, and then sorted on SONY-FX500 fluorescent activated cell sorting (FACS).
- Three groups of samples were prepared: CONTROL – unsorted but stained sperm, LIVE- after sorting intact sperm, and DEAD: after sorting apoptotic and dead sperm based on specific gates.
- Sperm from each group were placed on microscope slides and number of sperm bearing X, Y, and Chr 8 was counted in at least 500 sperm from each group using FISH with Vysis CEP X (DXZ1) Spectrum Green, CEP Y (DYZ3) Spectrum Orange, and Vysis CEP 8 (D8Z2) Spectrum Aqua DNA.
- Sperm specific decondensation protocol for FISH was developed by us.
- The automatic FISH protocol for peripheral WBCs was not adequate for the hybridization of DNA probes in sperm. Only sperm with one signal from Chr 8 were used in the analysis.
- Four channels epifluorescent microscope was used to acquire images that were then processed using the Bioimaging station. Z-stage deconvolution was used to assure a single signal from each probe was counted. DAPI was used for counterstaining. Experiments were performed in triplicates. JIM 14 was used for statistical analysis.

Results

- The rate of aneuploidy in any of the specimens was less than 0.5% and was negligible.
- The mean ratio of X:Y sperm for CONTROL was 0.99 (49.7% of X positive sperm), for LIVE was 0.99 (49.72 % of sperm had Chr X) and for DEAD was 1.03 (50.68% sperm had Chr X).
- The total number of sperm counted was 829 in CONTROL, 720 in LIVE, and 916 in DEAD groups.
- There was no statistically significant difference in the number of X or Y chromosomes bearing sperm between any of the groups. (Chi-square)

	Sperm_ before_sorting (count)	Sperm_ before_sorting (%)	Sperm_ after_sorting (count)	Sperm_ after_sorting (%)
Sperm counted	2274		4542	
Chr 8 present	2274	100.0%	4542	100.0%
Chr X present	1143	50.3%	2280	50.2%
Chr Y present	1131	49.7%	2262	49.8%

Table 1. Summary of results of sperm FISH with probes against Chr. 8, Chr. X and Chr. YB before and after sorting. Live and dead populations were combined for after sorting group, but there was no difference in FISH between dead and live sperm after sorting. Chi-square statistics showed p=0.95 hence, there was no statistically significant difference in a number of sperm bearing X and Y chr before and after sorting.

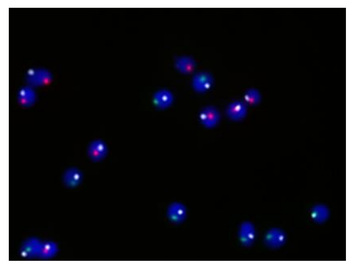

Figure 1. Results of FISH analysis of human sperm after sorting with probes against Chr. 8 (white signal), Chr. Y (red signal), and Chr. X (green color). Magnification x400

Conclusions:

- Sorting of human sperm using the ChromaSelect method does not skew the ratio of Chr X or Y carrying sperm hence it will not result in any change in the sex ratio in children born using sorted sperm.

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