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Flow cytometric sorting of human sperm: MicroSort® clinical trial update

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Abstract

This report provides a summary of MicroSort® efficacy in separation of X- from Y-chromosome bearing human sperm (XSort® and YSort®, respectively), clinical outcomes, and the sex of the resultant babies when sorted sperm were used for intrauterine insemination (IUI), in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). Clinical trial participants were married couples seeking reduced X-linked genetic disorder risk or family balancing. Sperm were stained with Hoechst 33342, sorted by flow cytometry, then used or cryopreserved for subsequent use. Fluorescence in situ hybridization (FISH) analysis determined the post-sort enrichment (purity) for X- and Y-bearing sperm. Birth and pediatric records were evaluated for incidence of congenital malformations. Between June 1994 and January 2007, patients underwent 3629 IUI cycles, 1642 IVF/ICSI cycles with fresh embryo transfer (ET) and 99 frozen embryo transfer (FET) cycles after MicroSort®. Of 5871 total sorts, 74.9% were XSort® and 25.1% were YSort®. IVF/ICSI fertilization rate was 70.7% and 93.8% of 2PN embryos cleaved. The pregnancy rates for IUI, IVF/ICSI, and FET were 15.6, 32.0, and 33.3%, respectively, while miscarriage rates were 15.7, 14.3, and 33.3%, respectively. Post-sort purity averaged 87.9% (XSort®) and 73.4% (YSort®). A total of 1125 clinical pregnancies yielded 943 babies born and 167 ongoing pregnancies. For babies born, XSort® resulted in 92.0% females and YSort® yielded 81.5% males. Postnatal follow-up showed a 2.6% major congenital malformation rate, with no recurrent pattern or clustering of malformations. FISH results confirmed MicroSort® enrichment of X- and Y-bearing sperm populations that closely corresponded with the sex of the resultant child. Fertilization, cleavage, spontaneous abortion, and pregnancy rates as well as incidence of major congenital malformations were comparable to those in literature reports utilizing unsorted sperm.

Keywords: Human; Sperm; Sexing; Flow cytometry; Babies; IVF

1. Introduction

Sorting human sperm by flow cytometry is a pre-conception method of sex selection that can be employed to reduce sex-linked disease risk or to balance the sex distribution of children in a family (family balancing). Utilizing the 2.8% difference in DNA content between X- and Y-bearing human sperm [1,2], the detection of differential fluorescence emitted by stained X-chromosome bearing sperm vs. Y-chromosome bearing sperm allows the identification and subsequent recovery of highly enriched populations of X- or Y-bearing sperm.

The Genetics & IVF Institute (GIVF) was granted an exclusive license by the United States Department of Agriculture to the patented flow cytometric sperm separation technology for development and use in humans in 1992. Institutional Review Board approval was received in 1993 to initiate clinical studies for application of flow cytometric sperm sorting to couples

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at risk for having children with sex-linked disease. The clinical study was expanded in 1995 to offer family balancing to couples. The United States Food and Drug Administration (FDA) approved an Investigational Device Exemption in 2000 for GIVF to conduct a clinical trial of safety and efficacy of the MicroSort® Sperm Separation Technology.

Fresh- and frozen-thawed human sperm have been sorted to yield populations enriched in X-bearing (Xsort®) or Y-bearing (YSort®) sperm [3]. MicroSort® sperm have been successfully used to achieve pregnancies after intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). Early results from the clinical trial reported the first human pregnancies and babies resulting from the use of flow cytometrically sorted human sperm for IUI, IVF, or ICSI [4–6]. In this paper the updated results of the ongoing MicroSort® clinical trial are reported.

2. Materials and methods

2.1. Subject selection

In this Institutional Review Board- and FDA-approved clinical trial, participants were primarily fertile, married couples who met inclusion criteria and who sought reduced genetic disease risk or balanced sex distribution among their children. Couples with infertility undergoing IVF for other indications that qualified and desired participation were also included. Family balancing is defined as a desire for a child whose gender is the least frequent among all the living children of the family. Couples meeting inclusion criteria underwent a clinical consult and signed informed consent before being accepted as clinical trial participants. Current protocol requires both partners to be tested for HIV-1, hepatitis B surface antigen and hepatitis C antibody. Male partners were encouraged to provide results of a recent semen analysis. Other reproductive testing of the husband or wife was performed as clinically indicated.

2.2. IUI treatment cycles

Clinical trial participants with presumed normal fertility often initially attempted pregnancy using IUI. Cycle monitoring was performed using either ovulation predictor kits or more intensive monitoring consisting of frequent daily transvaginal sonography (e.g., GE Voluson 730 Pro, GE Healthcare, Waukesha, WI, USA) coupled with serum progesterone (P4), estradiol (E2), and luteinizing hormone (LH) testing. Most participants received empiric oral clomiphene citrate therapy and daily monitoring; participants have also been given the option of using a natural cycle (no medication for ovulation induction) and urinary LH testing for monitoring, if appropriate. Gonadotropin-stimulated cycles were occasionally utilized for participants desiring to increase the likelihood of conception. Participants were usually inseminated 28–52 h after detection of the LH surge or greater than 36 h after human chorionic gonadotropin (hCG) administration. Follicular size was at least 15 mm and usually greater than 20 mm at the time of hCG administration.

2.3. IVF/ICSI treatment cycles

Treatment cycles using IVF or ICSI were performed on participants using various gonadotropin-stimulated cycle protocols that were in standard use at GIVF as well as at multiple national and international collaborator sites. Freshly sorted sperm samples were primarily used at GIVF for IVF/ICSI whereas sorted, frozen samples were used exclusively by collaborators. Due to the low number of sperm available, frozen sorted samples were typically thawed and used for ICSI without further processing.

2.4. Sperm separation and staining

Study participants provided either fresh or frozen semen for sorting. Prior to evaluation and processing, freshly collected semen was allowed to liquefy at 35 °C for 30 min; frozen specimens were thawed at room temperature (~20 to 23 °C) in a laminar flow hood for 15 min. All semen was evaluated for volume, concentration, percentage motile, progression (grades 0–4), and viability (eosin dye exclusion) before and after processing. Semen was processed to recover motile sperm and to remove undesirable seminal components by centrifugation through either glass wool columns or, after 1998, discontinuous density gradients (ISolate, 50%, 90%, Irvine Scientific, Santa Ana, CA, USA). After processing, recovered sperm were washed and the pellets resuspended in serum albumin-supplemented medium (BWW or Ham's F-10; Irvine Scientific, Santa Ana, CA, USA) and then stained for 1 h at 37 °C with Hoechst 33342 (Calbiochem-Behring Corporation, La Jolla, CA, USA) at a final concentration of 9 μM as previously described [1].

2.5. Flow cytometric separation

Sperm were sorted by flow cytometry as previously described [1]. Briefly, stained sperm were sorted using