

MicroSort

MicroSort, developed in 1990 by the Genetics and IVF Institute, is a form of pre-conception sex selection technology for humans. Laboratories located around the world use MicroSort technology to help couples increase their chances of conceiving a child of their desired sex. MicroSort separates male sperm cells based on which sex chromosome they contain, which results in separated semen samples that contain a higher percentage of sperm cells that carry the same sex chromosome. The technology ultimately enables couples to choose the sex of their future child by choosing semen samples that predominately contain sperm with the X chromosome for a female or Y chromosome for a male. MicroSort technology is a sperm sorting technique that provides couples worldwide a means of pre-conception sex selection.

Sex chromosomes contain the genetic material that determines a human's biological sex. Generally, humans have two sex chromosomes, one passed down from each parent. People with two X chromosomes are biologically female, while people with one X and one Y chromosome are biologically male. Because females have two X chromosomes, each of which can be individually contained in a female's egg, females can only pass down an X chromosome to their offspring. Males have both X and Y chromosomes, each of which can be individually contained in sperm cells. Males can therefore pass down either an X or a Y chromosome to their offspring. Therefore, male sperm cells are responsible for determining the sex of an offspring. If the male sperm cell that fertilizes a female egg contains a Y chromosome, the child is male. If the sperm cell that fertilizes the egg contains an X chromosome, the child is female. Sperm separation is a technique that separates sperm cells within a semen sample according to which sex chromosome the sperm cells contain. The separated sperm samples can then be used for artificial insemination, or the process of injecting sperm directly into a female's uterus for fertilization. By choosing which sperm sample fertilizes the female's egg, couples have the opportunity to choose the sex of their child prior to conception.

MicroSort is a sperm separation technology developed by the Genetics and IVF Institute, or GIVF, where IVF stands for in vitro fertilization, located in Fairfax, Virginia. Joseph D. Schulman, a physician and researcher specializing in human reproduction and genetics, founded GIVF in 1984. In 1990, Schulman adapted a technique used for animal sex selection named flow cytometry for human use. Flow cytometry is a technology that sorts cells based on what chromosomes they contain. The method involves passing cells through an electronic detection apparatus. In combination with flow cytometry, Schulman used a means of genetic testing called fluorescence in situ hybridization to develop a method for separating human male sperm cells in particular. Fluorescence in situ hybridization enables laboratory technicians to identify specific features within chromosomes using a fluorescent stain. Schulman named the technology that performed the sperm separation technique MicroSort.

MicroSort's sperm separation process utilizes the fact that human male sperm cells contain different amounts of genetic material. When Schulman developed his technique in the 1990s, prior research had shown that human X chromosomes were larger than human Y chromosomes. That means that a sperm cell containing an X chromosome contains 2.8 percent more genetic material than a sperm cell containing a Y chromosome. MicroSort uses that difference in size to differentiate sperm cells. The process begins after a laboratory technician washes the semen samples to remove seminal fluid and immotile sperm cells. The laboratory technician then recovers the motile sperm cells and re-suspends them in a medium that contains albumin, a viscous protein that surrounds human eggs that sperm cells must travel through to fertilize the egg. The sperm cells are then stained with a fluorescent material, called Hoechst 33342, and incubated for one hour at 37 degrees Celsius to allow the stain to attach to the sex chromosomes.

Once the cells are properly stained, they are ready to be separated according to the sex chromosome they carry. A flow cytometer machine analyzes the stained cells at a rate of 3000 to 3500 cells per second. The cells are suspended in a liquid within the flow cytometer, which is also equipped with argon ion lasers. The lasers excite the cells individually, which causes them to emit light due to the fluorescent stain. A filter within the flow cytometer then detects the cell's fluorescence and sorts the cells based on how much light they give off. Because sperm cells that carry an X chromosome contain 2.8 percent more genetic material than sperm cells that carry a Y chromosome, X-bearing cells produce more light when excited by the argon ion laser.

The flow cytometer then deposits the sorted sperm cells onto a cell growth medium that contains either yolk or albumin. A lab technician evaluates the separated cell samples under a microscope for post-separation motility to determine which cells have survived the separation process. Post-separation purity, or the degree to which X and Y chromosome-carrying sperm cells have been separated, is measured using fluorescence in situ hybridization. Testing post-separation purity is a crucial step in the process because it demonstrates how successfully the process has separated the sperm into X and Y chromosome-carrying samples. That sample is then stained and observed under a fluorescent microscope that shows the red X-bearing sperm cells and green Y-bearing sperm cells. The stained cells indicate the percentage of cells carrying the chromosome that will produce the desired gender in the sorted samples. That process encompasses MicroSort's method of separating sperm cells and ensuring the process's effectiveness.

In the beginning of its development, MicroSort was not utilized for pre-conception sex selection. In 1992, the United States Food and Drug Administration or FDA granted GIVF an exclusive license to use of their patented flow cytometry technology to sort human sperm cells. After a year of developing MicroSort laboratories worldwide to perform the sperm separation technique, GIVF received Institutional Review Board approval in 1993 to use MicroSort technology on couples who were at risk for passing on sex-linked diseases to their children. Sex-linked diseases are passed down from either parental sex chromosome, X or Y. For example, if couples have a known history of a sex-linked disease that affects the Y chromosome, they can use MicroSort to conceive a child using the X chromosome sample that does not have the sex-linked disease. Laboratory technicians did not initially use MicroSort as a means of sex selection, but as a means of preventing couples from passing on sex-linked diseases.

In 1995, however, the study expanded to include couples that wished to choose the sex of their child for family planning purposes. In 2000, the FDA approved an Investigational Device Exemption for the technology, which allowed GIVF to begin independent clinical trials to demonstrate the safety and efficacy of MicroSort sperm separation technology for pre-conception sex selection. The clinical trial, led by GIVF's Andrology Laboratory Director David Karabinus, included 5,871 sorted sperm samples over a thirteen-year period. Of those samples, the average post-separation purity was 87.9 percent for X chromosome-carrying sperm and 73.4 percent for Y chromosome-carrying sperm. As for sex outcome, the X chromosome-carrying sperm samples yielded 90.2 percent females and the Y chromosome carrying sperm samples yielded 81.5 percent males.

The sperm separated by MicroSort can be utilized in different ways. Physicians around the world who perform assisted reproductive procedures have partnered with laboratories that use MicroSort to help couples conceive a child using the separated sperm sample of their choice. Intrauterine insemination is the least invasive of those methods and involves a physician placing the sperm directly into the female's uterus on the day of ovulation. Sorted sperm samples can also be used for in vitro fertilization, the technique in which a female egg is fertilized by sperm outside of the human body to form an embryo. That embryo is then implanted inside the female's uterus. If couples want to store the sperm and use it in the future, the samples can be preserved using cryopreservation. Cryopreserved samples are stored in liquid nitrogen and can be used at a later time after being thawed at room temperature.

Through research on the effectiveness and safety of MicroSort, GIVF has refined specific requirements and recommendations to optimize couple's success in using the technology. The requirements and recommendations are listed on MicroSort's website to assist couples in deciding whether or not they are able to utilize the technology. To use the technology for sex selection, couples must have at least one child already and test negative for HIV, or human immunodeficiency virus, Hepati-

tis B and C, and syphilis at least six months prior to using MicroSort to decrease the spread of those diseases to offspring. GVIF also recommends that males provide sperm samples as close as possible to the use of the technology, because sperm samples tend to become unviable over time and assisted reproductive techniques require a high amount of viable cells. To ensure good semen quality, GVIF suggests that males abstain from ejaculation for two to three days, not consume alcohol during the period of abstinence, and avoid exposure to high temperatures such as hot water.

By taking the proper precautions, couples enhance their chances of achieving the technology's success rate. To date, clinical trials performed by GVIF have determined that MicroSort is 93 percent effective for producing females and 82 percent effective for producing males. The technology's website provides a disclaimer that although MicroSort does not guarantee a child of the desired sex, scientific data supports that it will significantly increase couples' likelihood of conceiving a child of the desired sex.

Despite GVIF's clinical trials that demonstrate MicroSort's safety and effectiveness, researchers have questioned the potentially harmful consequences of using MicroSort for sex selection. A study published in *Human Reproduction* in 2011 analyzed how sperm separation techniques affect the quality of sperm. The authors hypothesized that, if sperm were exposed to harsh laboratory conditions for a prolonged period of time, the unnatural environment would cause damage to the genetic material. Specifically, they were concerned that the stain used to fluoresce the genetic material within the cells, Hoechst 33342, caused mutations that could be harmful. If female eggs were then fertilized with the mutated sperm, embryonic development might be disrupted, which could potentially result in birth defects. However, MicroSort's clinical trials have shown that sorted sperm produce healthy, normal children at the same rate as unsorted sperm. Data also show that sorted sperm produces children with birth defects at a rate of 2.6 percent. That rate is similar to rate at which the general human population produces children with birth defects, which is between 3 and 5 percent.

Although the safety and efficacy of MicroSort has been scientifically supported, the technology, like many other methods of sex selection, has raised ethical concerns. In a report by the Human Fertilisation and Embryology Authority, a section of the Department of Health in the UK headquartered in London, England, the authors suggested that children who were chosen for their sex might develop psychological deficits after learning the circumstances of their birth. However, there has been no indication that children whose sex was chosen by their parents are any less loved or cared for than children who were conceived naturally. Furthermore, pregnancies involving sex selection technology such as MicroSort are less likely to be terminated than natural pregnancies.

According to their website, MicroSort continues to be the leading sperm separation technology in the world. The Genetics and IVF Institute remains the only organization to have licensing to use MicroSort technology and maintains ongoing clinical trials that work to both improve the technology and demonstrate its safety and effectiveness. As of 2018, MicroSort continues to provide couples with the opportunity to increase their chances of producing a child of their desired sex with high potential for success and no more risk than conceiving a child naturally.

Sources

1. Caroppo, Ettore. "Sperm Sorting for Selection of Healthy Sperm: Is it Safe and Useful?" *Fertility and Sterility* 100 (2013): 695-6.
2. Genetics and IVF Institute. Genetics and IVF Institute Official Website. www.givf.com (Accessed June 9, 2018).
3. Human Fertilisation and Embryology Authority. "Sex Selection: Options for Regulation." *HFEA* 7 (2003): 14.
4. Johnson, Lawrence, Glenn R. Welch, Keyvan Keyvanfar, Andrew Dorfmann, Edward F. Fugger, and Joseph D. Schulman. "Gender Preselection in Humans? Flow Cytometric Separation of X and Y Spermatozoa for the Prevention of X-linked Diseases." *Human Reproduction* 8 (1993): 1733-9.

5. Karabinus, David. "Flow Cytometric Sorting of Human Sperm: MicroSort Clinical Trial Update." *Theriogenology* 71 (2009): 74-9.
6. Said, Tamer, and Land, Jolande. "Effects of Advanced Selection Methods on Sperm Quality and ART Outcome: A Systematic Review." *Human Reproduction Update* 17 (2011): 719-33. <https://pdfs.semanticscholar.org/870d/1b60e4d338dae0990aeefc7e174ee767cfb0.pdf> (Accessed June 9, 2018).
7. Schulman, Joseph D. "An Attempted Suppression of Liberty." *Reproductive Biomedicine Online* 8 (2004): 374-5.