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Review Article

SPERM SORTING - A COMPHRAHENSIVE REVIEW

S.M.S. Nikhil *, Sarvan Kumar G [1], Dr. Kiran Kumar Y [2], Dr. P. Venakateswar Rao [3], Dr. Ramesh Adepu [4]

** Department of Pharm.D, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana, INDIA.*

[1]Department of Pharmacology, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana, INDIA.

[2]Department of Pharmaceutics, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana, INDIA.

[3]Department of Medicinical Chemistry, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana, INDIA.

[4]Department of Pharmacy Practice, Vikas College of Pharmaceutical Sciences, Rayanigudem, Suryapet, Telangana, INDIA.

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ABSTRACT

The sex of mammalian offspring can be predetermined by flow sorting relatively pure living populations of X and Y-chromosome bearing sperm. This method is based on precise staining of DNA of sperm with the nucliecacid specific fluorospore, Hoechst 33342, to differentiate between the subpopulations of X and Y sperm. The fluoroscently stained sperm are then sex sorted using a specialised high speed sorter, MoFlo SX XDP, and collected into biologically supportive media prior to reconcentration and cryopreservation in numbers adequate for use with artificial insemination for some species or for invitro fertilization. Sperm sorting can provide subpopulations of X- or Y-bearing bovine sperm at rates in the 8000 sperms/s range while maintaining; a purity of 90% such that it has been applied to cattle on a commercial basis. The sex of offspring has predetermined in a wode variety of mammalian species including cattle, swine, horses, sheep, goats, dogs, cats, deer, elk, dolphins, water buffalo as well as in humans using flow cytometric sorting of X- and Y- sperm.

KEYWORDS: *Sperm, Sorting, Flow Cytometer, Sex linked diseases.*

INRTRODUCTION

Sperm:

Sperm is the male reproductive cell and is derived from the Greek word sperma (meaning "seed"). In the types of sexual reproduction known as anisogamy and its subtype oogamy, there is a marked difference in the size of the gamets with the smaller one being termed the "male" or sperm cell. A uniflagellar sperm cell that is motile is referred to as a spermatozoon whereas a non-motile sperm cell is referred to as a spermatium. Sperm cells cannot divide and have a limited life span, but after fusion with egg cells during fertilization, a new organism begins developing, starting as a totipotent zygote. The human sperm cell is haploid, so that its 23 chromosomes can join the 23 chromosomes of the female egg to form a diploid cell in mammals, sperm develops in the testicles and is released from the penis (Fig. 1).

Anatomy:

The mammalian sperm cell consists of a head, neck, a midpiece and a tail. The head contains the nucleus with densely coiled chromatin fibres, surrounded anteriorly by an acrosome, which contains enzymes used for penetrating the female egg. The neck contains the sperm centriole the midpiece has a central filamentous core with many mitochondria spiraled around it, used for ATP production for the journey through the female cervix, uterus and uterine tubes. The tail or "flagellum" executes the lashing movements that propel the spermatocyte [4].

During fertilization, the sperm provides three essential parts to the oocyte: A signalling or activating factor, which causes the metabolically dormant oocyte to activate [1]; The haploid paternal genome [2]; The centriole, which is responsible for forming the centrosome and microtubule system [3].

As the sperm arrive at the egg, it is the egg that chooses the sperm and pulls it towards her. The selected sperm actually tries to swim away from the egg but is tethered to the egg by female hormones. The membrane around the egg literally opens up and swallows the sperm. Semencontains hundreds of millions of sperm, the egg will admit only one. The other ones will die within three to five days and are absorbed by the body (Fig. 2).

Origin:

The spermatozoa of animals are produced through spermatogenesis inside the male gonads (testicles) via meiotic division. The initial spermatozoon process takes around 70 days to complete. The spermatid stage is where the sperm develops the familiar tail. The next stage where it becomes fully mature takes around 60 days when it is called a spermatozoon. Sperm cells are carried out of the male body in a fluid known as semen. Human sperm cells can survive within the female reproductive tract for more than 5 days post coitus. Semen is produced in the seminal vesicles, prostate gland and urethral glands [4].

Sperm quality:

Sperm quantity and quality are the main parameters in semen quality, which is a measure of the ability of semen to accomplish fertilization. Thus, in humans, it is a measure of fertility in a man. The genetic quality of sperm, as well as its volume and motility, all typically decrease with age [4].

DNA damages present in sperm cells in the period after meiosis but before fertilization may be repaired in the fertilized egg, but if not repaired, can have serious deleterious effects on fertility

***Corresponding author:**

S.M.S. Nikhil

Department of Pharm. D,

Vikas College of Pharmaceutical Sciences,

Rayanigudem, Suryapet, Telangana-508376, INDIA.

** E-Mail: sms.nikhil123@gmail.com*

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and the developing embryo. Human sperm cells are particularly vulnerable to free radical attack and the generation of oxidative DNA damage.

The postmeiotic phase of mouse spermatogenesis is very sensitive to environmental genotoxic agents, because as male germ cells form mature sperm they progressively lose the ability to repair DNA damage. Irradiation of male mice during late spermatogenesis can induce damage that persists for at least 7 days in the fertilizing sperm cells, and disruption of maternal DNA double-strand break repair pathways increases sperm cell-derived chromosomal aberrations. Treatment of male mice with melphalan, a bifunctional alkylating agent frequently employed in chemotherapy, induces DNA lesions during meiosis that may persist in an unrepaired state as germ cells progress though DNA repair-competent phases of spermatogenic development. Such unrepaired DNA damages in sperm cells, after fertilization, can lead to offspring with various abnormalities (Fig. 3).

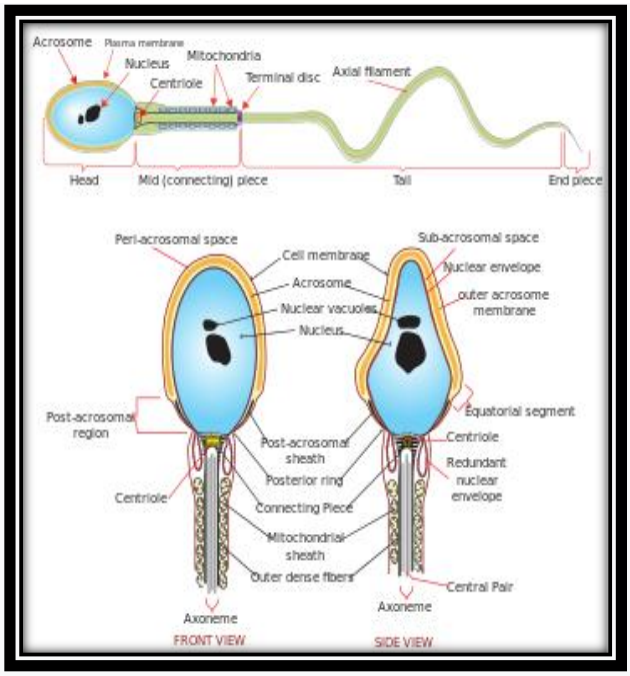

Fig. 1: Diagram of a human sperm cell

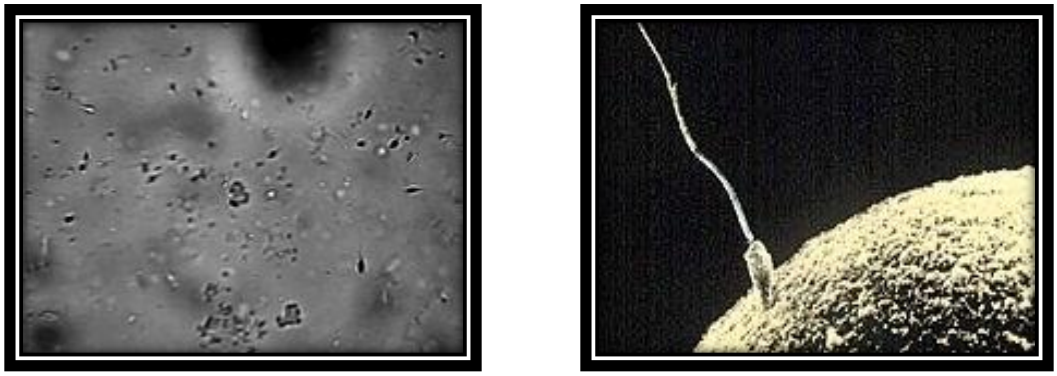

Fig. 2: Sperm and egg fusing

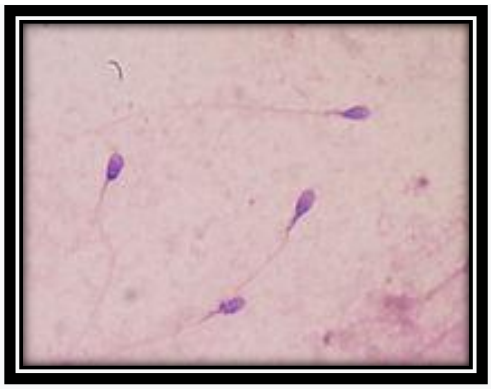

Fig. 3: Human sperm stained for semen quality testing

Sperm size:

Related to sperm quality is sperm size, at least in some animals. For instance, the sperm of some species of fruit fly (*Drosophila*) are up to 5.8 cm long about 20 times as long as the fly itself. Longer sperm cells are better than their shorter counterparts at displacing competitors from the females seminal receptacle. The benefit to females is that only healthy males carry good genes that can produce long sperm in sufficient quantities to outcompete their competitors [4].

Introduction to Sperm Sorting:

Wolfgang Godhe in 1968-1969 proposed flow cytometer. Fluoroscence could be accurately measured from the flat surface of the nucleus, nucleus specified dye 4-6-diamidino-2-phenylindole (DAPI) to reflect DNA content. Initially they got dead sperm cells, but later Johnson et al altered the staining process that living sperms could be sorted according to the DNA content. The membrane permeant bisbenzimidazole fluoroscent dye, hoescht 33342 readily differentiated into between the two populations of living sperm according to the their DNA content. This initial advancement in staining with hoescht was followed by development of the ability to sort living sperm into populations of their X and Y sperm population set 85-90% purity. The membrane impermeant DNA specific dye propidium iodide (PI) was added during the staining process to identify dead and injured sperm [3].

Flow Cytometric Sorting of X and Y Sperm:

Semen is collected and prepared for sorting using carefully cleansed equipment in sterilizwd media, then the semen sample is examined for volume and antibiotic in 20microlitre/ml of semen, add Tylosin(100 microgram/ml), Gentamycin (500microgram/ml) and linco-spectin (300/600 microgram/ml) final concentration are added to the raw semen. Next raw semen is then sorted upto 7hrs with aliquotes diluted approx hourly to a sperm concentration of 200*10^6 sperm/ml [1].

Sperm Staining:

An aliquot of the sperm which had been diluted to 200*10^6 sperm/ml in staining TACP + stained with 50.4 microlitre Hoeschst 33342 and placed in a 34 degree celcius water bath, 100 microlitre/ml of 20% eggyolk in a TALP is added and final concentration is 2%. stained sperm samples are then filtered using 50microlitre sterile nylon mesh filters to remove clumped sperm, media aggregates and seminal debris. Identification of nonviable sperm is accomplolished by adding 2 micolitres/ml of a FDMC#40 food coloring in TALP to quench Hoeschst staining of the DNA in membrane damaged sperm [1].

Sorting Equipment:

After stoichiometrically staining the sperm with Hoeschst 33342, they are pumped in a stream infront of a UV laser beam (wave length 355nm) to excite the Hoechst 33342 stained DNA to differetiate between the X and Y chromosome bearing sperm. Most stained sperms are oriented as they pass infront of the laser so that the flat surface of sperm nucleus can be used to precisely measue the DNA content. This orientation of the sperm nucleus is accompolished using the hydrodyanmicc forces of the pressured sheath fluid that surrounds the sample stream as is pumped into the nozzle of the flow sorter. The emitted fluorescence (460nm) of the oriented sperm is digitally quantified by dual ortogonal photodetector which are situated at 0 and 90 degree to the laser beam. This system can provide sorted subpopulation of X and Ybearing sperm at rate of 8000 sperms/sec at a purity of 90%. Then the sperm exit the orienting nozzle, the stream the is broken into individual droplets by a piezo-electric crystal vibrator either a negative or positive charge is appied to droplets containing sperm relative to their DNA content. The charged dropletsthen pass infront of two charged plates, one positive and one negative, such that they are deflected into one of three collection tubes, X and Y sperm and one for undetermine DNA content, membrane-damaged sperm with quenched fluoroscent or no sperm [1].

Re-Concentration of Sperm:

To acheive required concentration for IVF. The sperm that had been sorted into the medium in the catch tuber are then slowly cooled to 5 degree celcius to prevent cold shock by placing the catch tube containing the prepared sperm in a 600ml beaker containing room temperature(18-20) water prior to placing the beaker in a 5 degree celcius room for approx 1.5 hrs. Then 12% glycerol is added in two equal portions 15 minutes apart. Then cooled sperm sample is then centrifuged at 5 degree celcuis at 850* of for 20 min to concentrate sperm. The supernatant is removed carefully by pouring off, leaving a 400-500 microlitre sperm pellet containing > 90% of the sorted sperm. Then motility is checked by in injecting into the sample. If 70% progressive motility is observed then they are packed at a concentration of 10*10^6 sperm/ml which results in a dose of 2.1 * 10^6 sperm per straw in a volume of 210microlitre. The sperm are packed into the 0.25 ml French straws and then cry preserved [1].

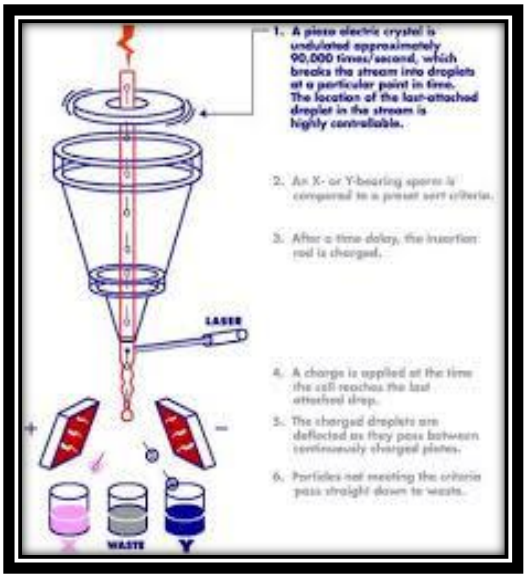

Fig. 4: Sorting Equipment

List of Pros of Gender Selection:

1. It gives the couple the option to plan a family: [2]

Perhaps one of the positive sides of applying the gender selection method is the opportunity of would-be parents to raise the number of children they want. In some countries, limiting the size of a family is encouraged. In fact, in China, there was a 1979 law which prohibited families to have more than one child although over the years, there had been some changes. Also, there are couples who want a specific gender for a first-born. With this practice now available,

mothers need not have to give birth multiple times just so they can have a baby with the gender they prefer.

2. It makes it easier for parents to take care of the kids:

Another advantage of sex selection which proponents find important is the capacity of parents to start a family. With having just enough number of children to raise, couples will be more capable to take care of their kids and give them good education as well as proper nutrition.

3. It is advisible for parents with genetic disorder problems:

There are medical conditions that are genetically related and gender specific that a mother or a father can pass on to a child.if this a concern pre selection of gender before conception is an effective alternative to prevent this conditions.

4. It gives parents enough time to prepare for the babies coming:

Supporters also find it practical to know the gender of the baby of an expectant mother, so it will be easier for them to get ready for the coming new born like preparing the nursery, buying clothes or hand down the old clothes and stuff of an old child with the same gender.

5. In instances where parents have lost the child, this can be a way to get over the loss:

For some pro-gender selections supporters the pain of a couples who have suffered death in the family, say ,a child ,will be relieved if they will be conceive a child with the same gender as the child they have lost .although it might not necessarily replace the other child ,the new born with the same gender can lessen the grief and give the family the chance to move on.

List of cons of Gender selection:

1. It is a breach of morals and ethics: [2]

One of the issues opponents are raising is the ethical and moral implication of breaking the law of the nature that is the traditional way of conception without interfering with the gender of the unborn child.

2. It is an impractical expense:

Another contention of critics is the high cost of scientifically implanting an embryo with a specific gender .the amount is around $20,000 on the treatment alone .these is one top of the other expenses the couples need to save for .they also refute the accuracy of the method since it is not 100%, except for the IV with PGD which is not allowed for every couple who dream to have a child .these only permitted on parents after they have passed the criteria like the need to prevent genetic issues.

Uses:

Sex selection might help to pevent se associated heritable diseases such as Duchene muscular dystrophy in males and Haemophilia in females.

Sex ratio balance:

The most common X-linked recessive disorders are:

- **Red-green color blindness**, a very common trait in humans and frequently used to explain X-linked disorders.[4] Between seven and ten percent of men and 0.49% to 1% of women are affected. Its commonness may be explained by its relatively benign nature. It is also known as daltonism.

- **Hemophilia A**, a blood clotting disorder caused by a mutation of the Factor VIII gene and leading to a deficiency of Factor VIII. It was once thought to be the "royal disease" found in the descendants of Queen Victoria. This is now known to have been Hemophilia B (see below).

- **Hemophilia B**, also known as **Christmas Disease**, a blood clotting disorder caused by a mutation of the Factor IX gene and leading to a deficiency of Factor IX. It is rarer than hemophilia A. As noted above, it was common among the descendants of Queen Victoria.

- **Duchenne muscular dystrophy**, which is associated with mutations in the dystrophin gene. It is characterized by rapid progression of muscle degeneration, eventually leading to loss of skeletal muscle control, respiratory failure, and death.

- **Becker's muscular dystrophy**, a milder form of Duchenne, which causes slowly progressive muscle weakness of the legs and pelvis.

- **X-linked ichthyosis**, a form of ichthyosis caused by a hereditary deficiency of the steroid sulfatase (STS) enzyme. It is fairly rare, affecting one in 2,000 to one in 6,000 males.

- **X-linked agammaglobulinemia (XLA)**, which affects the body's ability to fight infection. XLA patients do not generate mature B cells. B cells are part of the immune system and normally manufacture antibodies (also called immunoglobulins) which defends the body from infections (the humoral response). Patients with untreated XLA are prone to develop serious and even fatal infections.

- **Glucose-6-phosphate dehydrogenase deficiency**, which causes nonimmune hemolytic anemia in response to a number of causes, most commonly infection or exposure to certain medications, chemicals, or foods. Commonly known as "favism", as it can be triggered by chemicals existing naturally in broad (or fava) beans.

Less common disorders:

Theoretically, a mutation in any of the genes on chromosome X may cause disease, but below are some notable ones, with short description of symptoms:

- Adrenoleukodystrophy; leads to progressive brain damage, failure of the adrenal glands and eventually death.
- Alport syndrome; glomerulonephritis, endstage kidney disease, and hearing loss.
- Androgen insensitivity syndrome; variable degrees of under virilization and/or infertility in XY persons of either gender.
- Barth syndrome; metabolism distortion, delayed motor skills, stamina deficiency, hypotonia, chronic fatigue, delayed growth, cardiomyopathy, and compromised immune system.
- Blue cone monochromacy; low vision acuity, color blindness, photophobia, infantile nystagmus.
- Centronuclear myopathy; where cell nuclei are abnormally located in skeletal muscle cells. In CNM the nuclei are located at a position in the center of the cell, instead of their normal location at the periphery.
- CharcotMarieTooth disease (CMTX2-3) disorder of nerves (neuropathy) that is characterized by loss of muscle tissue and touch sensation, predominantly in the feet and legs but also in the hands and arms in the advanced stages of disease.
- CoffinLowry syndrome; severe mental retardation sometimes associated with abnormalities of growth, cardiac abnormalities, kyphoscoliosis as well as auditory and visual abnormalities.
- Fabry disease; A lysosomal storage disease causing anhidrosis, fatigue, angiokeratomas, burning extremity pain and ocular involvement.
- Hunter's Syndrome; potentially causing hearing loss, thickening of the heart valves leading to a decline in cardiac function, obstructive airway disease, sleep apnea, and enlargement of the liver and spleen.
- Hypohidrotic ectodermal dysplasia, presenting with hypohidrosis, hypotrichosis, hypodontia.
- Kabuki syndrome; multiple congenital anomalies and mental retardation.
- Spinal and bulbar muscular atrophy; muscle cramps and progressive weakness.
- Lesch-Nyhan syndrome; neurologic dysfunction, cognitive and behavioral disturbances including self-mutilation, and uric acid overproduction (hyperuricemia).

- Lowe Syndrome; hydrophthalmia, cataracts, intellectual disabilities, aminoaciduria, reduced renal ammonia production and vitamin D-resistant rickets.
- Menkes disease; sparse and coarse hair, growth failure, and deterioration of the nervous system
- Nasodigitoacoustic syndrome; mishaped nose, brachydactyly of the distal phalanges, sensorineural deafness.
- Nonsyndromic deafness; hearing loss.
- Norrie disease; cataracts, leukocoria along with other developmental issues in the eye.
- Occipital horn syndrome; deformations in the skeleton.
- Ocular albinism; lack of pigmentation in the eye.
- Ornithine transcarbamylase deficiency; developmental delay and mental retardation. Progressive liver damage, skin lesions, and brittle hair may also be seen.
- Siderius X-linked mental retardation syndrome; cleft lip and palate with mental retardation and facial dysmorphism, caused by mutations in the histone demethylase PHF8.
- Simpson-Golabi-Behmel syndrome; coarse faces with protruding jaw and tongue, widened nasal bridge, and upturned nasal tip.
- Spinal muscular atrophy caused by UBE1 gene mutation; weakness due to loss of the motor neurons of the spinal cord and brainstem.
- Wiskott-Aldrich syndrome; eczema, thrombocytopenia, immune deficiency, and bloody diarrhea.
- X-linked Severe Combined Immunodeficiency (SCID); infections, usually causing death in the first years of life.
- X-linked sideroblastic anemia; skin paleness, fatigue, dizziness and enlarged spleen and liver.

CONCLUSION

Although gender selection is ongoing practice, there are still moral and ethical considerations debated upon by proponents and opponents .the privilege of choosing the gender of child a couple desires to have seems to be the most logical choice for some, but it also bring up the question of morality when it comes to the embryos not selected in the process. At the end of the day, the burden and the decision lie on the hopeful parents. In the meantime, this will still be a controversial issue
There are medical conditions that are genetically related and gender specific that a mother or a father can pass on to a child. If this is concern, pre-selection of gender before conception is an effective alternative to prevent this condition

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