Tikrit University
College of Veterinary Medicine

Sperm sexing

Subject name: Reproductive techniques

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Lecturer name: Dr.Ali Aziz Abd

Academic Email: aliaziz2235@tu.edu.iq

Lecturers link

Sperm sexing or Sperm sorting: is a gender-selection technique that improves of choosing embryo's sex by mean of selection what type of sperm cell is to fertilize the ovum.

Advantages of sperm sexing

1. Sperm sorting have been routinely used in assisted reproductive technologies; artificial insemination (AI), in vitro fertilization and embryo transfer.

2. Dairy farmers will be able to avoid the birth of dairy bull calves because the females are essential for dairy products, while male calve are usually required for beef production (better feed conversion efficiency and lean-to-fat ratio of males).

3. Avoiding freemartins in multiple births.

4. In human; use of separation technology could help in reducing selective abortion of female fetuses or infanticide, which is currently reported from some societies.

General Aspects

- Semen contains roughly 50% each of male and female sperm.
- As in many other mammals, including humans, the Y sex chromosome in cattle is smaller than the X-chromosome; X-chromosome contains ~4% more DNA than Y-chromosome.
- A normal commercial dose of sexed bull semen contains only 10^7 spermatozoa per ml (2.1×10^6 spermatozoa per dose in 0.25 c.)

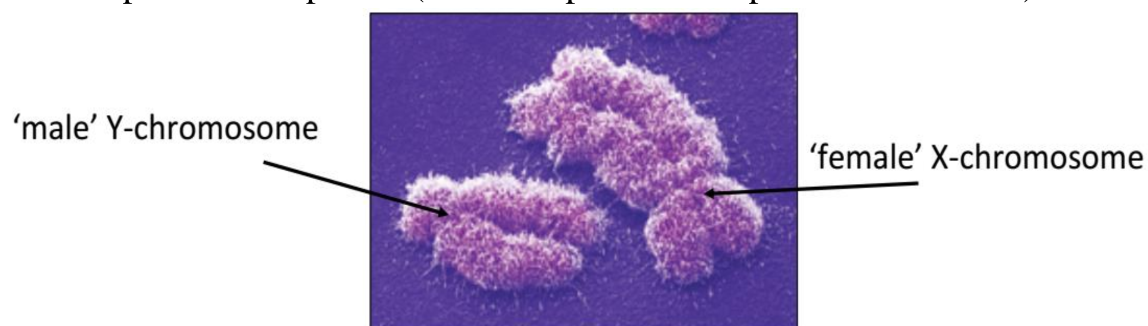


Fig. 1: X and Y Chromosomal shape

- Different collection-frequency regimes made a significant contribution to the variation in Y-chromosome content in successive ejaculates from individual bulls. There appeared to be some association between sexual rest and a large amount of variability in Y-chromosome content. The authors suggest that it may be **possible to devise bull management routines that would maximize the observed variation in Y-**

chromosome content and eventually lead to a method of manipulating the sex ratio of calves on the farm.

Methods for Separation of x and y- bearing Spermatozoa

1. Centrifugal Counter Current distribution based on density characteristics

The difference in density between X-bearing bovine spermatozoa and Y-bearing bovine spermatozoa to be only 0.0007 g/cm^3, hence this feature was also not suitable to be exploited as a characteristic to sex sperm. Some scientists attempt to Separation the ram spermatozoa by centrifugal counter current distribution using an aqueous two-phase system.

2. Albumin gradient

mean separation of X and Y-bearing spermatozoa by using an albumin gradient, this method was effective in increasing the proportion of **spermatozoa with motility** and elimination of abnormal forms, there was no much difference in the ratio of X- Y- bearing spermatozoa.

3. Percoll density gradient

Semen is layered on top of a percoll column and spermatozoa are allowed to penetrate the column. This technique was not effective in separation of X or Y-bearing spermatozoa.

4. Free flow electrophoresis

It is based on the possibility that the electric charge on the surface of X-bearing spermatozoa differs from that of Y-bearing spermatozoa **uses an electric feld to separate spermatozoa into the two major classes**. Inseminations with semen separated by this technique found a birth rate of 50.4% female calves in inseminations by semen enriched in X-bearing spermatozoa. Another drawback of this technique was an associated reduction in motility of the sperm after being subjected to electrophoresis.

5. Counter Current galvanic Separation

Each sperm will have an individual sedimentation velocity that will be influenced by physical forces such as size, shape, mass, specific gravity and difference in density between cell and suspending medium. The selection can be further enhanced by the **application of a suitable micro-ampere current that will attract Y-bearing spermatozoa to the anode and X-bearing spermatozoa to the cathode** emulates the same technique but could not succeed in producing any significant alteration of sex ratio.

6. Immunological Sexing of Semen (Presence of H-Y antigen)

Immunization of male and female rabbits by injecting sperm subcutaneously was done to raise antibodies to **sperm membrane (H-Y antigen) proteins**. The anti-sperm antisera obtained from the female rabbit were putative "anti-Y" and those obtained from male rabbit were "anti-X" antisera. Sperm doses after suitable treatment were mixed with either of these antisera and incubated for 60 min at 38.5C and 5% CO^2. It was found that only the "anti-X" antisera resulted in agglutination of spermatozoa whereas the "anti-Y" antisera failed to show any agglutination in the spermatozoa. The agglutinated sperm population was separated from the free-swimming sperm by glass wool filtration and the free-swimming sperm population (potentially Y-bearing spermatozoa) was isolated. Bovine embryos were produced *in vitro* using the isolated sperm population and blastocysts were sexed cytogenetically. The results indicated that 92% of the sexed embryos were male thus marking the technique as one of the potential methods of sperm sorting.

7. Flow cytometry

Separation of x and y- bearing Spermatozoa is possible on the basis of their relative DNA content. Flow cytometry permits separation after measuring DNA content based on the fluorescence emission intensity after staining with a DNA-specific fluorochrome. The basis for the selection of the procedure is the expected differences in nuclear consistency of X- and Y chromosome-bearing spermatozoa (on average larger head, neck and tail, a higher dry mass, and 3–4 % more DNA in X chromosome-bearing sperm). The differences in DNA content between the X- and Y chromosome-bearing sperm of the human is approximately 2.8 %; bulls, 3.8 %; boars, 3.8 %; rams, 4.2 %; bucks, 4.4 %; dogs, 3.9 % and stallions, 3.7 %. The sperm are prepared with a DNA-specific fluorochrome stain (**Hoechst** 33342 can bind to the adenine–thymine region of nucleic acids). Fluorescence signals are emitted when Hoechst 33342-stained

sperm are illuminated by an argon laser. **Fluorescence detectors at a 90° angle measure the fluorescence signals of the sperm to allow separation based on the difference in DNA content between the X and Y chromosomes** (**the signal at 90° is used to determine which sperm are properly oriented for accurate evaluation, whereas the signal at 0° is used to determine DNA content).**

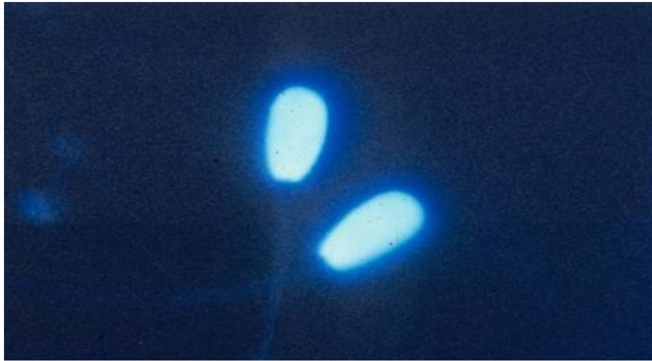
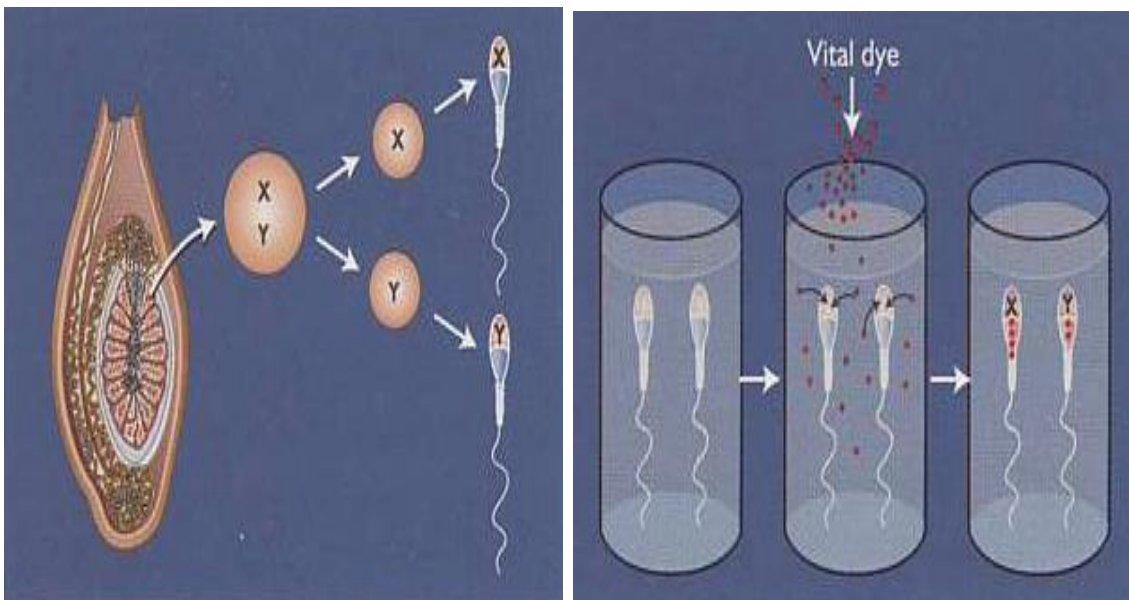
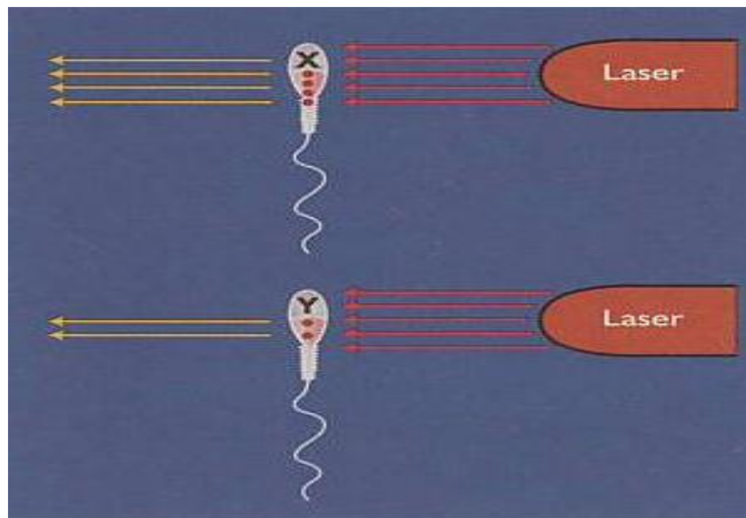


Fig. 2: Sperm stained with a harmless, DNA-binding dye (Hoecsht 33342).

The dyed sperms are then placed in the flow cytometer and the sperm enter the flow chamber one at a time and then each sperm is evaluated individually. The dyed sperm are subjected to a laser beam; the X chromosome-bearing sperm emit more intense light due to the high adsorption of fluorescing dye. The computer recognizes this light intensity and can assign the sperm as either X or Y, or uncertain. The sperm then drop sequentially through the droplet charging collar of the apparatus, where the droplets are assigned with their charge (positive or negative). The sperm sequentially pass through an electromagnetic field where they are drawn to either the positive or negative or no charge side based on their assigned charge.





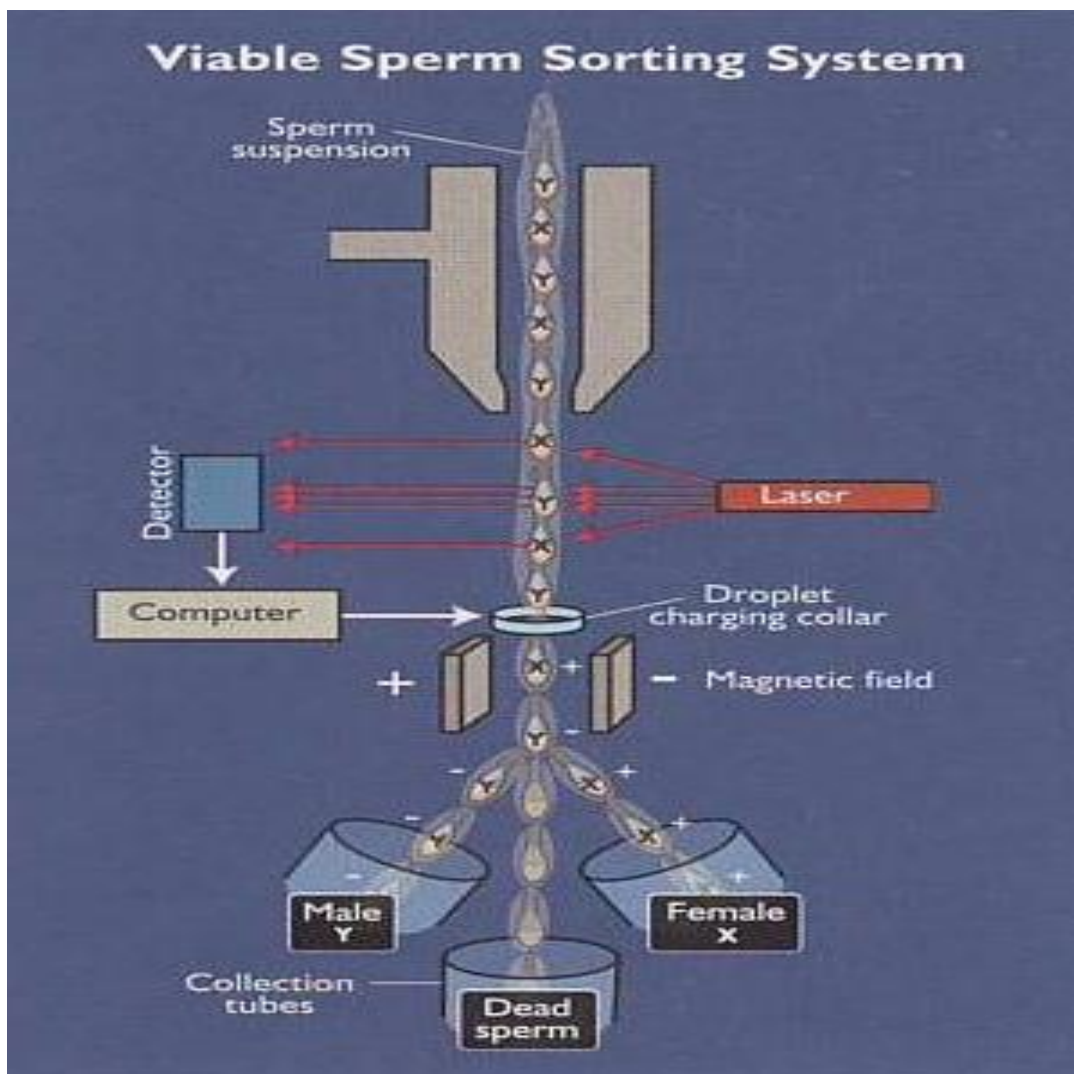


Fig. 3: Schematic diagram of main components of a typical flow cytometer

Limitations of Sperm Sexing

1. Percentages of motile sperm post- thaw are diminished (<10%) by the flow cytometric sorting process.

2. Higher laser intensities are found to damage sperm more than the lower intensities.

3. The UV-excitable DNA specific stain is cytotoxic and or mutagenic effects on sperm.

4. The fluorochrome dyes reduced embryonic viability and mid-gestation pregnancy rate because the limitation associated with their viable lifespan in the female genital tract.

5. Spermatozoa are exposed to high pressure (40– 50 psi). Negative effects of high pressure on post-thaw motility. Centrifugation after sorting also presents stress to spermatozoa and may cause an increase in lipid peroxidation. The natural defense against oxidation provided by seminal plasma is lost by high dilution during sorting. A major reason for reduced sperm survival seems to be the presence of reactive oxygen species (ROS). Oxygen radicals are known to cause a decrease in motility and induce pre-capacitation, as well as, damage to the membrane system by lipid oxidation, especially when the seminal plasma content is reduced after extensive dilution and washing of spermatozoa..

6. Procedure is too slow to produce adequate numbers for use in artificial insemination (a sperm-sexing system could only sort 12–20 million sperm/h of cattle, sheep, swine, and horses).

7. The procedure is potentially invasive and requires specialized, expensive and immobile equipment and highly skilled operators.

8. Less than half of heifers became pregnant as compared to AI with unsorted semen from the same bulls (decrease the conception rates of lactating cows and embryonic loss between 1 and 2 months of gestation).