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Semen Sexing: A Promising Technology for Improvement of Livestock Productivity

Ankita D. Verma[1]*, Anshu R. Ahlawat[2], Pravin N. Chaudhary[3]
[1]Assistant Professor, College of veterinary Science & A. H., Kamdhenu University, Navsari
[2]Associate Professor, College of veterinary Science & A. H., Kamdhenu University, Anand
[3]Assistant Research Scientist, Bull Mother Farm, Kamdhenu University, Amreli

Introduction

India is the highest milk producing country in the world with around 23% share in global milk production. This milk production had increased over the period of time and the major reason behind it is sustainable collective efforts by government and the stakeholders all together. At the same time, some constraints are certainly present and they continuously change every year as well. From last few years, we have been facing the problem of non-producing animals, crunch in feed and fodder supply, difficulty in maintaining the optimum production. Sustainable livestock production systems, whether for milk, meat, draft, or replacement animals, rely on efficient reproductive performance and vigilant monitoring. One of the major aspects of growing milk production is performance by female animals and that to with superior genetic background which hints us towards crucial management of male animals. Hence, sexed semen technology is a promising technology helpful in propagation of animals with desired sex. It will not only give us the direction to work with but also would be beneficial to channelize the resources in better way.

Technology

The objective of utilizing sexed semen is to selectively produce calves of a specific sexual category. Its application not only enhances the genetic progress along the dam-daughter axis but also allows for the generation of superior males from elite cows for future breeding purposes. The adoption of sexed semen, particularly from genetically elite bulls, is of paramount importance in meeting the anticipated demand of our country for upcoming years.

Benefits of Sex sorted semen:

- Generation of Offspring of the Preferred Gender
- Efficient herd replacement and expansion
- Maintaining a ratio of 90:10 (female to male or vice versa)
- Selective culling
- Production of bulls with high genetic merit
- Reduced dystocia by less production of male calves
- Marked reduction in progeny testing programs and embryo transfer technology

Methods of semen sexing

Semen sexing involves the separation of X and Y chromosome-bearing sperm cells to determine the desired sperm type for fertilizing the egg cell. Methods for sexing of semen have basic principle of Y bearing spermatozoa being different in many ways compared to X bearing spermatozoa. The following are some methods described in brief along with their principles.

- Physical methods
  - Separation based on size and shape: The X-chromosome bearing sperm has been postulated to be necessarily larger than the Y chromosome bearing sperm.
  - Separation on the basis of Swim up: Y-bearing spermatozoa swim differently and more quickly than X-bearing spermatozoa. Fluid flow rates can be used to accentuate this difference, and therefore enhance the separation of the two subpopulations.

- Predicting differences in surface charge
  - Free-flow electrophoresis: It has been proposed that there are differing electrical charges on the cell membrane of X- and Y containing spermatozoa, or variable amounts of net charge.
  - Counter current galvanic separation: It involves the use of specially designed forced convection streaming galvanic cell which is claimed to enhance the separation of X-and Y-bearing spermatozoa.
- **Predicting differences in cell surface antigenic determinants:** Histocompatibility-Y antigen (H-Y) is found in male tissues of many mammalian species with the exception of erythrocytes and premeiotic germ cells.
- **Predicting the difference in DNA content:** The DNA difference between X and Y sperm of domestic livestock ranges from 3.5 to 4.2 per cent. Thus, this variation in DNA content can be used to differentiate X carrying sperm cells.

Flow cytometry

Flow cytometry has been found to be widely used for sexing of semen. The principle of flow cytometry for sex sorting relies on the differences in DNA content between X and Y chromosomes in the target cells, typically sperm cells. In most species, including humans, X and Y chromosomes have different DNA content, with the X chromosome containing more DNA than the Y chromosome. By measuring the DNA content of individual cells as they pass through a flow cytometer, it is possible to distinguish between X-bearing (female) and Y-bearing (male) cells based on their fluorescence profiles (Figure 1). The technique of this involves the following steps:

1. **Sample Preparation:**
   - Sperm samples are collected and processed to remove debris and impurities.
   - The sperm cells are then stained with a DNA-specific fluorescent dye, such as Hoechst 33342 or DAPI, which binds to the DNA in the cell's nucleus.
2. **Flow Cytometer Setup:**
   - The flow cytometer is calibrated and set up for the specific fluorescent dyes used in the staining process.
   - A laser is used to excite the fluorescent dye within the stained sperm cells.
3. **Sorting Procedure:**
   - The stained sperm sample is introduced into the flow cytometer.
   - Individual sperm cells are carried in a stream of fluid, and they pass through the laser beam one by one.
   - When a sperm cell passes through the laser, the fluorescent dye in its nucleus emits fluorescence, and the detector measures the intensity of this fluorescence.
4. **Data Analysis:**
   - The flow cytometer records the fluorescence intensity for each sperm cell.
   - The differences in DNA content between X and Y chromosomes result in distinct fluorescence profiles for X-bearing and Y-bearing sperm.
   - A computer system analyzes the fluorescence data for each sperm cell and classifies them as X or Y based on their fluorescence intensity.
5. **Sorting:**
   - After classification, a sorting mechanism, such as electrostatic deflection or a high-speed air jet, is used to physically separate the X and Y sperm cells into different collection chambers.
6. **Collection:**
   - The collected sperm cells, now sorted by sex, can be used for various applications, such as artificial insemination or in vitro fertilization.

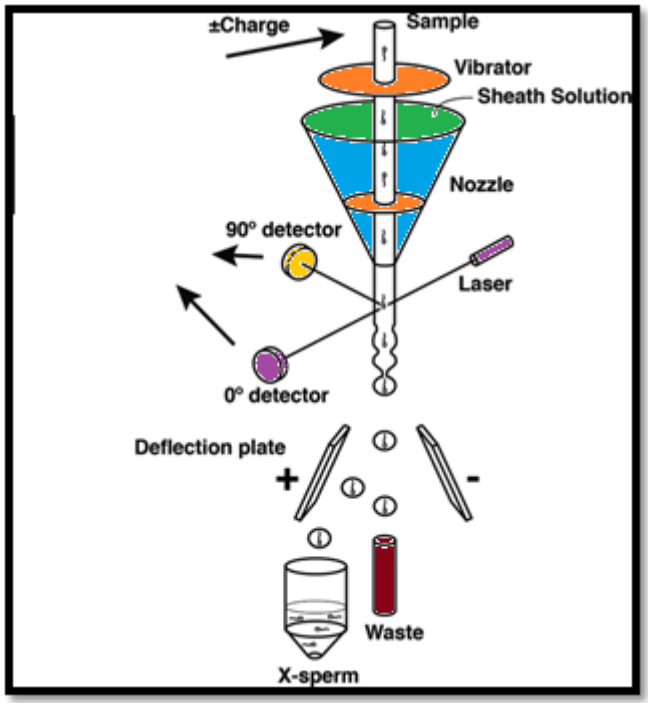

Figure 1: Schematic diagram showing principle of Flow cytometry for sexing of semen

Limitations in practical field:

Improving the utilization of sexing technology in India's livestock and animal husbandry sector is crucial. Sexed semen technology offers numerous advantages, but it's important to adapt it to the specific challenges and conditions in India to achieve satisfactory conception rates. Here are some key considerations for implementing sexed semen technology effectively in the Indian context:

- **Standardization of spermatozoa dosages:** Determining the appropriate dosage of sexed semen for artificial insemination is critical. This dosage should be standardized based on the specific requirements of Indian breeds and environmental conditions. Factors like the quality of semen, the age and health of the recipient animals, and the genetic characteristics of the animals should all be considered when establishing dosing guidelines.
- **Quality control:** Ensuring the high quality of sexed semen is essential. Proper collection, processing, and storage of semen are vital to maximize its effectiveness. Quality control measures should be put in place to minimize any negative impacts on conception rates.
- **Optimizing site of deposition:** The choice of the site for artificial insemination can significantly influence conception rates. Different breeds and species may require different approaches. Considering the reproductive anatomy and physiology of Indian livestock is essential. This might involve research and trials to determine the most effective deposition methods for different species and breeds.
- **Environmental factors:** Indian conditions can be quite diverse, and climate, temperature, and humidity can affect the success of artificial insemination. It's essential to adapt the technology to accommodate these factors, which may require the development of specialized equipment or protocols.
- **Training and education:** Farmers and technicians need training to effectively use sexing technology. Proper training and education programs should be developed to ensure that individuals involved in artificial insemination are well-versed in the technology and its application in Indian conditions.
- **Research and collaboration**: Continuous research and collaboration with academic institutions, government agencies, and private sector stakeholders are essential. This can help identify best practices and innovations that can be applied to improve sexing technology in India.
- **Economic viability:** It's important to ensure that the adoption of sexing technology remains economically viable for Indian farmers. Cost-effective solutions and government support or subsidies may be required to make the technology accessible to a wider range of livestock keepers.
- **Regulation and quality assurance**: Developing and enforcing standards and regulations for sexed semen production and distribution is crucial. This ensures that the technology is used responsibly and that the quality of semen is maintained.

- Additionally, achieving higher efficiency through this technology necessitates meticulous animal management, including proper nutrition, disease control, estrus detection, semen handling, and insemination techniques.

Conclusion:

Being different than other livestock husbandry, dairy industry is mainly reliable on productivity of female animals because of production of milk. To target this fact, production of only female animals can be achieved through biased reproduction and is possible due to semen sexing technology. There are various methods through which sperm cells can be sorted for X & Y chromosome. Among many technology available, flow cytometry is useful for this sorting and is based on the principle of X chromosome bearing sperm cells being heavier due to more DNA content. There are certain limitations of the technique especially in Indian context and are needed to be addressed for better results and impact of this technology over Indian dairy industry.

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