Short communication

SOCIO-ECONOMIC IMPACT OF SEXING OF SEMEN

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ABSTRACT

Nowadays sexing of semen is slowly developing among dairy producers and farmers and benefit both the farmers and industry. Sexing of semen is used to sort out the healthy as well as more specific traits in which spermatozoa are separated into X-bearing or Y-bearing chromosome populations through either selection or sorting. The sex-sorted semen is collected from genetically superior animals so the result of milk production and quality of meat will get up to the expected level. The use of sex-sorted semen mainly produced the female heifer in dairy farms or herds which reduced the purchasing number and price of cattle from outside. Collectively, the rate of genetic change has been expected to increase by not more than 15% as a result of the increased selection of dams through sexed semen.

Key words: Sexed semen, India, Breeding technique, X-chromosome, Female calf

INTRODUCTION

Sexing of semen is semen in which sperm is selected based on their progeny to produce either male or female with 80-90%accuracy. Technically it is the breeding technique in which the sperms X and Y bearing chromosome separated for desired progeny (Neculai-Valeanu and Ariton, 2021). The desired sex of progeny is (X- bearing chromosome or Y- bearing chromosome) produce by selection or killing of unwanted chromosomes of sperm by newly developed technology like flow cytometry or fluorescent dye technique. The breeding technique of sexing semen is immensely economical for dairy producers for milk production as well as meat production. Although some demerits using the sexing of semen that semen straw more expensive, the concentration of spermatozoa is low, not easily available in everywhere and the fertility rate is low compared with conventional straw of semen.

SPERM SEXING METHODS

There are several methods is for sexing of semen these are 1) physical method (mass and motility) which is classified based on their size and shape and separation based on their swimming up. 2) predicting differences in surface charge, methods are Free-flow electrophoresis Counter current galvanic separation 3) Predicting differences in cell surface antigenic determinants, these are Histocompatibility -Y antigen (H-Y), Sex specific proteins (SSPs).4) Predicting the difference in DNA content these are Flow cytometry and Lumisort.

METHODS OF SPERM SEXING

Several methods are available for the process of sexing the spermatozoa these are quinacrine mustard staining in which stain is produced by fluorescence in some parts of chromosomes. Centrifugal counter current distribution is a technique based on the differences in density between X and Y chromosomes. The albumin gradient method is based on the size of the chromosome of X and Y spermatozoa that are separated. Another method is to separate the X and Y spermatozoa based on the volumetric difference. The swimming pattern under laminar flow in which Y motility is higher than X spermatozoa separated. Other methods are electrophoresis, PCR flow cytometry, immunological sexing of semen., Among all the methods mentioned above the flow cytometry is one of the best methods and is commonly used

Table: 1Difference between X and Y chromosome

Objectives	X chromosome	Y chromosome	method
DNA content	More in X –sperms (3.8%)	Less in Y sperms	Flow cytometry
size	X sperm is larger	Y sperm is smaller	Percoll method
Motility	X sperm is slower	Y sperm is faster	Swim-up
Surface charge	Negative for x sperm	Positive for Y sperm	Free-flow electrophoresis
Presence of cell surface HY antigen	Absent	Present	Immunological method

SWIM-UP TECHNIQUE

In this technique, Y-bearing spermatozoa are swimming differently and more quickly than X bearing chromosomes in flowing media. The disadvantage of this technique is only 10% of total spermatozoa are placed systematically and recovered and the success rate was recorded up to 81% (Jameel, T. (2008).

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ALBUMIN GRADIENT METHOD

The y chromosome is smaller than the X chromosome, it is a separation that occurs based on the motility of sperm. Most 85% of spermatozoa have the Y chromosome at the lowermost portion of the tube. It is a method very effective in increasing the proportion of spermatozoa with motility and elimination of abnormal sperm (Beernink *et al*., 1993). No many different ratios of X and Y bearing spermatozoa.

PERCOL DENSITY GRADIENT

According to this technique, X chromosome bearing spermatozoa has a higher density than Y chromosome bearing spermatozoa based on that density X spermatozoa is located at the lower most portion and Y spermatozoa are uppermost layer portion during sperm sorting. The success rate of this technique is 86-92% (Henkel and Schill, 2003). The added 2-fold diluted semen with HEPES-buffered isotonic salt solution Layering over personal gradients (1.11gm/ml & 1.06gm/ml) and Centrifuge at 250 g for 20 min. Separation of X – spermatozoa is occurred up to 75% by this method.

CELL SURFACE ANTIGEN DETERMINANTS

In this method sperm is sorted by identification of H-Y antigen. Through the chromatography and magnetic bead using the specific antibody against Y chromosome bearing spermatozoa for reduction of sperm. It is used for large scale separation of sperm sorting, and efficacy is reported more than 90%.

FREE-FLOW ELECTROPHORESIS

The X chromosome bearing sperm has a negative charge and the Y chromosome bearing sperm has a positive charge. Hence, the significant difference in surface charges in spermatozoa are used for sorting the sperm by electric field separation (Ishijima *et al*.,1992). The difference in surface charge of X and Y sperms are due to their fatty acid composition present on the membrane and which is not well established.

IMMUNOLOGICAL SEXING OF SEMEN

The male and female rabbits are immunized by the injection of sperm preparation with Freund's incomplete adjuvant subcutaneously for raising their antibody to sperm protein. Then antisera were collected from a male called anti X while from female called as anti Y antisera, after suitable treatment and mixed with either antiserum and incubated for 60 min at 38.5 c and 5% co2. The result is obtained from that technique agglutination occur antisera X while antisera Y failed to occur to agglutination due to vigorous and quick motility of Y chromosome bearing spermatozoa. However low viability, low enrichment high cost has been commercially prohibited (Quelhas, *et al*., 2021).

COUNTERCURRENT GALVANIC SEPARATION

In this method, suitable microampere current is applied which attracts Y –bearing spermatozoa to the anode and X-bearing spermatozoa to the cathode. But the method of this technique did not provide a significant result of sex ratio.

QUINACRINE MUSTARD STAINING

In this method the stain of quinacrine stains certain part of the chromosome of spermatozoa. In ancient days quinacrine stains were used for finding the enrichment of X and Y spermatozoa. The result of this method for Y chromosome bearing spermatozoa exhibited the fluorescent part of F body while X chromosome bearing spermatozoa are unstained. However, that method is revealed the false positive and false negative in interphase cells and that techniques produce misleading. But it has no universal property of all mammalian Y chromosomes.

RAMAN MICRO SPECTROSCOPY

This is the technique highly efficient and most non-invasive technique for semen sexing. In this method, spectral components (protein, lipids) are used to find the differences between X bearing and Y bearing chromosome spermatozoa. In this technique, the nucleus reveals the main difference between X and Y spermatozoa and Raman peaks were observed due to DNA content together with the sex membrane (Huang *et al*.,2014). Raman peak position is acrosome, neck, middle regions in Y sperm.

POLYMERASE CHAIN REACTION

In this technique, specific sequences of DNA of X and Y sperms have been used to find the individual sperm and it is very accurate method for finding or identification of X or Y sperm. Sex ration can also be identified from the single sperm in a semen sample. However, sex ratio can be determined accurately using quantitative real-time PCR.

CURRENT STATUS, SCOPE AND CONSTRAINTS OF SEXED SEMEN – INDIAN PERSPECTIVE

In India, Paschim Banga Go Sampad Bikash Sanstha (PBGSBS) is a government of West Bengal Organization first developed sex sorting semen through flow cytometer or high-speed semen sorter. They reported by using sex-sorted frozen semen born male calf name was Shreyas on Jan 1th 2011. The organization have been produced the number of sex-sorted semen straw 40-50 per day and that organization reported the conception rate of sex-sorted semen for cows 20.7% and heifer 35.3%. The purity of X sorted semen is found to be higher than Y sorted semen. In India, that technique is gradually developed day by day and established in

many states like Punjab, Haryana, west Bengal, Kerala. Etc.

FLOW CYTOMETRY

In India only method used for sorting the desired sex is by flow cytometry, which is commercially available and proven to get a success rate compared to another method of sex sorting. In this method, the X chromosome contains more DNA than the Y chromosome so therefore takes more DNA specific stain. The principle of flow cytometry technique is the based-on size of the DNA content difference between X chromosome bearing spermatozoa and Y chromosome bearing spermatozoa and X chromosome contain more contain DNA. In this technique sperm cells are stained with a fluorescent dye (Hoechst 33342) DNA binding fluorochrome and then passed to allow through-flow cytometer as a droplet before that vibrating crystal breaks the stream into droplets. The X bearing sperms are more shine than Y bearing sperms because the content of DNA and size is larger when illuminated with laser for fluorescence. Then positive or negative charges are applied to the droplets, uncharged droplets may pass through straight and charged droplets is deflected in opposite direction and contents of uncharged droplets is may multiple sperm, damaged materials, or cells and accuracy of semen sexing 85-95%.

Input rate	40,000 sperm/second (X and Y sperm)
Sorter speed	55-60mph
Pressure	40-60 psi
Fluid stream travelling	60 miles /hr
Droplet formation frequency	60-70 kHz (16-14) microsecond
Droplet	70,000-80,000/s
Nozzle speed	80km/hr
Sorting achiever rate	8000 sperms/second
Sexed semen of AI	2×10^6 spermatozoa
Production capacity	10-14 straws /hr/instrument
Cost of the process of sexed semen	Rs 1908-3543
Accuracy of semen sexing	85-95%

LIMITATIONS OF FLOW CYTOMETRY TECHNIQUE

- Hoechst stain (33342) is a stain that is mutagenic and perhaps maybe cytotoxic at a higher dosage of stain.
- The dye. stain, pressure, laser light, electrical charging and changes in the medium collectively lead to the effect or damage of spermatozoa during the sorting process.
- During sex sorting, the procedure may induce the damage of plasma membrane and premature capacitation and occur change the glycosylation of membrane proteins because plasma membrane is very crucial for in-vivo fertilization
- Motility of sexing of spermatozoa is affected ~~by when sorting procedure~~ sperm exposed to stain, laser light and electric charges.
- The mitochondrial activity is decreased by exposure to laser and causes a decrease in the production of ATP.
- The addition of specific dye causes chromatin de-condensation to occur.
- High sorting pressure of 40-60psi and high speed(55-60mph) causes damage to the sperm DNA during the sperm sorting procedure.
- The power of UV rays 200MW or more causes destruction of chromatin integrity than sperm exposed 125 MW.
- By electrical charging and deviation depolarization occurs in sperm and further reduce the mitochondrial activity of sperm and reduce embryonic viability.
- If changes in pH and osmolarity cause a decrease in the sperm fertilizing ability ~~during the sorting process~~.

Different between sex-sorted semen and conventional semen

Objectives	Sexing of semen	Conventional semen
Definition	Produce the desired progeny by sorting or killing unwanted sperm with 80-90% accuracy	Produce the progeny either male or female with no further process
Concentration of sperm	2- 4 million spermatozoa	15- 20 million spermatozoa
Insemination technique	Intra uterine or intra tubal	Intra uterine
Cost of straw	Rs 1500- 4500	Rs 15-20
Availability	Not more than place	Available in all over India
Conception rate	10 – 15 %	40-50%
Cost of technique	The highly expensive technique-used	The usual technique used Not expensive
Sperm abnormalities	More than 50% of dead sperm and proximal droplets are present	Less than 50% of sperm abnormalities are present after freezing
Freezing	Freezing ability is low for longer time preservation	Freezing ability is higher than sexing semen straw
Insemination	Good conception rate with heifer than pleuriparous animal	Equal conception rate with heifer and pleuriparous animal

(Mallory *et al*.,2013)

BENEFIT FOR FARMERS AND LIVESTOCK OWNERS

- India is one of the largest populations of the country in the world and the largest milk producers for the past few years, yet not satisfied the people's requirement of milk consumption, so it is immensely for milk production.
- Technique able to increase the number of animals in dairy farm or herd through the production of female progeny by using of sex-sorted semen.
- The use of sex-sorted semen is not only producing the desired sex of progeny but also produce the superior quality of trait of the bull through the basis of selection of index.

- Technique is able to produce desired sex either male or female, so that technique enhances the requirements of milk and milk products through the female calf and by male calf meet requirements of meat production as well as leather production.
- In a dairy farm, uneconomic due to born male calf with feeding and management, so use of sex-sorted semen able to gain more income.
- Using this breeding technique that able to increase the cattle population and increase our national economic growth.
- Able to produce superior quality progeny by using sex-sorted semen.
- The use of sex-sorted semen prevents the culling of an unwanted male calf by the production of the female calf through sexing.
- The mostly dystocia is common in animal carrying a pregnancy with a male calf so using sex-sorted semen able to reduce the dystocia through female progeny.
- Highly useful where male calf sending to a slaughterhouse or not possible to culling
- The cost of the heifer calf is more expensive than bull calf which is one of the businesses in the dairy industry.
- The sexing of semen is used where transport is impossible to carry the bull of superior quality trait character.

SHORT TERM EFFECT ON SOCIO-ECONOMIC STATUS UNECONOMIC TO THE FEED AND MANAGEMENT

Every farmer wants to female calf so by sex-sorted semen can be able to predetermine desired sex. So that this breeding technique able to prevent the birth of male calf led to saving the feed cost and management cost during pregnancy.

PROGENY TESTING

Sexed semen technology ensures the required number of progenies per bull under the progeny testing program~~me~~, thereby increasing the accuracy of bull testing. The sexed female sperms could be used in test mating to ensure the production of the required number of daughters in the shortest time, thus increasing the genetic gain.

SALE OF *IN VITRO* PRODUCTION OF AN EMBRYO

Production and sale of *in vitro* production embryo are one of the businesses in dairy industries it may be either male or female embryo. But in the case of using sex-sorted semen can be able to produce the desired sex which causes lead to further increase cost of an embryo.

THE INCREASED COST OF SEX-SORTED SEMEN STRAW

That sex-sorted semen straw is too expensive than conventional straw so which is highly profitable for production companies if selling is too much for in the dairy industry.

TYPE OF BREEDER AND SERVICE NUMBER

Among the breed of cattle jersey breed (52.2%) had a lower fertility rate than Holstein heifers (56.3%). Conception risk by service number decreases from first service (53%) to seventh service (33%).

IMPACT ON REPRODUCTIVE TECHNOLOGIES

In super ovulated cows using that sex-sorted semen resulted in a 20-30% of reduction in the number of embryos compared to conventional semen (percentage of the transferable embryo for sex-sorted semen 18.6% and for unsexed semen 43.5 %) reason for that increased number of unfertilized ova due to reduced number of spermatozoa.

IVF (in-vitro fertilization) that process require only 600-1500 sorted sperm to fertilize an oocyte. Progeny rate from IVF cultured embryo fertilized with sexed semen range from 30%-50%.

LONG TERM EFFECT ON SOCIO-ECONOMIC STATUS TO CHALLENGE THE DEMAND OF MILK PRODUCTION

The project is planned by 2020 to increase the milk production up to 191.3 million tons, so want to meet the requirements of milk production need an elite female with an increased number of female animals so used the X chromosome bearing spermatozoa semen for the production of superior quality of females.

Production of superior breeding bulls

In our country, there is a reduced number of superior bulls. By introducing sex-sorted semen of Y chromosome bearing spermatozoa will lead to increase production of elite bulls for a particular time.

SALE OF FEMALE HEIFER

Cost of female calf is too costly than male calf by this increases the economy of the dairy industry by selling of female calf.

TO DECREASE THE NUMBER OF UNPRODUCTIVE YOUNG BULLS

In India cattle slaughter is banned and agricultural work is replaced by mechanization and so bull and male

calf are roaming and unnecessary killed, so using sex-sorted semen can reduce the number of born of unproductive males or unwanted male pregnancy.

REPLACEMENT AND EXPANSION OF HERDS

Sex sorted semen can be able to facilitate the expand the herd or replacement the herd with reduced days and without spending maximum money on farming.

STILLBIRTH AND DYSTOCIA

Stillbirth and dystocia are more common in heifer respectively with male calf than pupiparous animal. So, by using sex-sorted semen can reduce the calving difficulties and stillbirth by born female calves (6% dystocia for conventional semen 4.3% for sex-sorted semen).

PERFORMANCE OF CALVES

Calves produced by gender selected semen are vigour, birth weight, calf health, weaning weights, or mortality rate before weaning good compared to products is derived from conventional semen.

ECONOMIC ASPECT

Sex sorted semen straw is too expensive and not easily available than conventional straw. So, it is high profit for the straw production companies.

CONSTRAINTS

- High cost of equipment's which is around 4-5 crore.
- The technology for sex-sorted semen is currently fully not available in any places of India.
- Low conception rate with sex-sorted semen is comparing to unsexed semen
- Lack of skilled manpower and experienced man
- Lack of good quality ejaculate from indigenous cattle and buffalo.

LIMITATION OF SEX-SORTED SEMEN

- The cost of the sex sorting semen straw is mostly expensive than conventional semen straw (cost of sexing of semen straw is 1200-1500 and conventional straw is 15-20)
- The conception rate with sex-sorted semen is very less compared with conventional straw reduced to 10 -15%
- The concentration of sperm is very less compared with conventional straw (sexed semen straw is 2-3 million and conventional straw is contained 20 million straw)
- By using sex-sorted semen able to get a good conception rate in heifer compared with multi-time calved animals so sexed semen is not widely used.
- There is no special operation for insemination of sex-sorted semen so following insemination like this usual intrauterine deposition.
- Processing time is very longer than conventionally processed semen
- Mass insemination or timed breeding is not an acceptable breeding method

DON T USE SEXED SEMEN

- Don't use the sexed semen into the cattle of repeat breeder
- Animal suffering from any reproductive diseases (pathological and congenital)
- Animal with a poor genetic trait like poor body condition
- If a cow has more than 3 lactation young heifers which has been still not reached the mature weight
- Animal with stray mating
- Animal which has silent heat or sub estrous cycle or estrus induction or estrus synchronization
- Freshly calved cow which is less than 100 days postpartum

CONCLUSION

- Reduce the expensive rate of sex-sorted semen straw which is easily chance to get a very poor farmer.
- The establishment of sex-sorted semen bank laboratory should be located in a certain place all over India.
- Enhancement of scheme about sex-sorted semen, and it should reach the level of the poor farmer
- Should be start research again about increase the conception rate of sex-sorted semen than the previous conception.
- The insemination technique is the same as using conventional straw, so try to implement some new techniques for sexed semen straw breeding technique to further increase the conception rate.
- The arrangement of the training program~~me~~ for farmers and dairy producers about teaching, description, advantages, knowledge, growth of the individual economy due to sex-sorted semen all over India.
- Should be recommended only for highly fertile herds.

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