Current status, scope and constraints of sexed semen - An Indian perspective

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ABSTRACT
The aim of sexed semen is to produce a calf of a specific sex. The use of sexed semen increases the rate of genetic gain not only from the daughter-dam path but also through production of superior male from elite cows for future breeding. Gender selection using sexed semen from genetically elite bulls is imperative to meet the projected demand of 191.3 million tones of milk by 2020 in the country. The demand of sex semen in dairy cattle is also increasing in order to dispose the large number of unproductive males, to ensure required number of progenies per bull under progeny testing programme and to reduce the replacement cost on heifers. In India, this technique is gradually been adopted by many states like Punjab, Haryana, West Bengal, Kerala etc. However, high cost and lower fertility limits its wider use across the country. Furthermore, there is need to standardize the lower dosage of spermatozoa, site of deposition for AI with good conception rate in Indian conditions. Optimal use of sexing technology also requires excellent and careful animal management (nutrition, disease control, estrus detection, semen handling, and insemination technique) for obtaining higher efficiency. More research needs to be carried out to make it feasible in order to extend this technique in our country.

Key words: Application, Constraints, Scope, Sexed semen, Status.

The aim of sexed semen is to produce a calf of a specific sex. The predetermined the sex has valuable impact on livestock industry because of its economic gains. Females are essential for milk production and the production of calves, while males are usually required for meat production because of the better feed conversion efficiency and lean-to-fat ratio (Espinosa-Cervantes and Córdova-Izquierdo, 2012). In addition, males of high genetic merit are still required as sires in artificial insemination programs. The sexed sperm can be further used to increase the reproductive rates in animals and subsequently increase rates of genetic gain not only from daughter-dam path through possible higher selection intensity and accuracy of selection but also through production of superior male from elite cows for future breeding.

History: Preselecting the gender of offspring in both human and animals has been of keen interest since the beginning of recorded history. Theories for controlling the sex of offspring have been established since the era of the Greek philosophers when Democritus, 470-402 BC, suggested that the right testis produced males, whereas the left testis produced females. Some believed that females developed on the left side of the uterus and males on the right. All these attempts were derived from folklore and lacked a scientific basis and hence didn’t succeed. Scientific efforts in the right direction started early in the 20th century when first documented microscopic identification of sex chromosomes was reported by Guyer (1910). One of the first sensible scientific studies to be conducted to control prenatal sex was reported by J. L. Lush in 1925. The basis for Dr. Lush’s research was the possible differential density of X- and Y-bearing sperm in the rabbit. Later, many physical methods of separation of X- and Y-chromosome based on differences in the mass and motility, swimming patterns, surface charges, volumetric differences, centrifugal countercurrent distribution, and immunologically relevant properties reported (Table 1) but failed to convincingly demonstrate the result of distortion in the sex ratios of animal offspring in a practical sense.

The only method proven to be commercially viable with promising results till date is flow cytometry/cell sorting. The first attempt to separate X and Y sperm by analytical flow cytometry was initiated in 1976 by Gledhill (1976). Pinkel et al. (1982) first time successfully separated mammalian sperm. But the methods were found to be destructive because the tails were removed by sonication leaving sperm biologically unusable. In 1980’s a breakthrough in semen sexing technology was made by USDA researchers in the Lawrence Livermore Laboratory in California. This method works adequately since the X chromosome is larger than the Y and therefore takes up more of the DNA-specific stain.
The differences in DNA content between the X- and Y-chromosome-bearing sperm in different species have been reviewed elsewhere (Garner and Seidel, 2003). In *Bos indicus*, the average X-Y sperm difference is 3.73%. Whereas, differences in DNA content for Murrah and Nili-Ravi buffalo were 3.59% and 3.55% respectively (Lu et al., 2007). This means an optimum sorting accuracy and sorting rate would be more difficult for buffalo sperm compared to that of bovine sperm, whose difference in DNA content between X- and Y-sperm is slightly higher. Breed-wise differences in DNA content between X- and Y-chromosome of cattle and buffalo are summarized in Table 2.

**Technique:** The sperm sorting procedure involves staining the sperm with a dye (Hoechst 33342) that binds specifically to the DNA. The diluted mixture passes through a flow cytometer in a fine stream; and a vibrating crystal breaks the stream into droplets. The stained sperm are illuminated by 351 and 364 nm lines of an argon laser beam for fluorescence. The X-sperm glow brighter than the Y. A computer, quantifies the fluorescence of the sperm and assigns the sperm droplet as either X or Y, or uncertain. 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 collected into separate test tubes (Sharpe and Evans 2009). The current sorting accuracy is about 90% for each sex (Johnson and Welch, 1999).

**Status of semen sexing in India:** In India, Paschim Banga Go-Sampad Bikash Sanstha (PBGSBS), a Government of West Bengal organisation, initiated sorting of semen using high speed semen sorter or flow cytometer (Influx, Becton Dickinson, Biosciences, San Jose, CA, USA) installed on 15th August, 2009 under RKVY with a total outlay of Rs. 2.90 crores, during 2007 – 08 and 2008 – 09 and completed in November, 2009 at Frozen Semen Bull Station, Haringhata. They reported first male calf named Shreyas, born on 1st Jan 2011 using sexed semen. Later female calves were also successfully born to sexed semen. They are currently in a position to produce 40-50 sexed semen straws per day. The conception rates observed were 20.7% in cows and 35.3% in heifers using sexed semen. The purity of X-sorted semen was found to be higher compared to Y-sorted semen (Biswas et al., 2013). Kerala also reported birth of two sexed semen calves to Jersey crossbred heifers and Holstein Friesian crossbred cows respectively at Vakkavu in Nenmara, Palakkad under a pilot project jointly taken by Kerala Livestock Development Board (KLDB) and Department of Animal Husbandry. The sexed semen of HF and Jersey were imported by KLDB from Canada @ Rs.1250 per dose. The Kerala Livestock Development Board has imported 650 doses of sexed semen doses.

The ABS India is providing Holstein and Jersey sexed semen. Prime Bovine Genetics in collaboration with Sexing Technologies provides sexed semen of Holstein Friesian, Jersey, Brown Swiss and Gir crossbreds. But, the Planning commission, Govt. of India has recently assigned the responsibility for sorting of the sex of semen in cattle to independent companies such as Sexing Technologies to bull studs (Select sires, Genex, Accelerated Genetics, CRV, ABS Global WWS and Prime Genetics etc.)

### Table 1: Potential differences between X and Y Spermatozoa (Source: Prasad et al., 2010)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>X spermatozoa</th>
<th>Y spermatozoa</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Larger</td>
<td>Relatively smaller</td>
<td>Percoll method</td>
</tr>
<tr>
<td>Motility</td>
<td>Swim slower</td>
<td>Swim faster</td>
<td>Swim up</td>
</tr>
<tr>
<td>Surface charge</td>
<td>Migrate to cathode fast</td>
<td>Migrate to cathode slow</td>
<td>Free flow electrophoresis</td>
</tr>
<tr>
<td>Sperm surface</td>
<td>Absence of HY antigen</td>
<td>Presence of HY antigen</td>
<td>Immunological sexing</td>
</tr>
<tr>
<td>DNA</td>
<td>More DNA</td>
<td>Less DNA</td>
<td>Flow cytometry</td>
</tr>
</tbody>
</table>

### Table 2: Differences in the DNA content between X- and Y- bearing spermatozoa among different breeds of cattle and buffaloes (Source: Prakash et al., 2014)

<table>
<thead>
<tr>
<th>Breed</th>
<th>Difference in DNA content</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holstein Friesian</td>
<td>3.98%</td>
<td>Garner et al., 1983; Garner, 2001; 2006</td>
</tr>
<tr>
<td>Jersey</td>
<td>4.24%</td>
<td>Garner et al., 1983; Garner, 2001; 2006</td>
</tr>
<tr>
<td>Angus</td>
<td>4.05%</td>
<td>Garner et al., 1983; Garner, 2001; 2006</td>
</tr>
<tr>
<td>Hereford</td>
<td>4.03%</td>
<td>Garner et al., 1983; Garner, 2001; 2006</td>
</tr>
<tr>
<td>Brahman</td>
<td>3.73%</td>
<td>Garner et al., 1983; Garner, 2001; 2006</td>
</tr>
<tr>
<td>Murrah</td>
<td>3.59%</td>
<td>Lu et al., 2007</td>
</tr>
<tr>
<td>Nili Ravi</td>
<td>3.55%</td>
<td>Lu et al., 2007</td>
</tr>
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</table>
NDRI, Karnal. This ambitious project of 55 crore budget for semen sexing of cattle will aim to multiply the indigenous and crossbred cows in the country by providing sexed semen at farmers’ doorsteps.

Imported sexed semen is now available to farmers in some states with Punjab in forefront. The cost of one dose of sexed semen varies between Rs. 1000/- to 1500/-. In Punjab the farmers are being charged Rs 600/- per straw with the state subsidizing the remaining 50% of the total cost of Rs 1200 per straw. Moreover, Artificial Insemination co-operatives from USA are in collaboration with Progressive dairy farmers in Punjab for providing breeding solution that promises 90% female calves.

**Farmer’s view point:** Information about the opinion of farmers regarding sexed semen is scanty. A survey conducted in 2012 among 871 progressive farmers in Ahmednagar and Pune districts of Maharashtra showed that 88% of them favored the use sexed semen if it could be made available to them. The semen dose cost was expressed as important matter and two third of the fraction indicated the cost should be up to Rs. 500 per insemination (Jeyakumar et al., 2015).

In order to implement the sorted semen program on a generalized scale, it is needed to initially build up a field level experience for getting prepared for any operational problems likely to crop up during implementation. Realizing the need of large scale experience with an objective of understanding rural level factors in success of this technology, a large scale two year field experiment partly sponsored by Bill Gate Foundation USA and cooperation of different state governments is being planned. Initially the frozen sexed semen will be imported and used at village level and the data gathered will be thoroughly analyzed for understanding the role of different factors affecting the success rate and so, arriving at a strategy to improve and standardize the implementation approach. This information could be used as a guideline to plan for taking up the sorted semen activity within country for indigenous and crossbred cattle and buffalo (Jeyakumar et al., 2015).

**Sexed buffalo semen:** Encouraging results from the use of sexed semen through AI have been reported in Murrah and Nili Ravi buffaloes by the scientists of Guangxi University. Lu et al. (2007) obtained 94% and 89.5% accuracy of sorted X and Y sperm of Murrah and Nili-Ravi buffalo respectively by a modified BD FACS Vantage SE flow cytometer (Becton, Dickinson and Company). Sexing accuracy was found to be 82.8% (Lu et al., 2010). In terms of conception rate significant difference was observed between AI with sexed semen from different bulls, but not between sexed and non sexed semen. Also no difference was found between conceptions rates in heifers and parous buffaloes (Companile et al., 2011).

Haryana Livestock Development Board in collaboration with Navasota (USA) is planning to introduce sexed semen technology in Murrah buffalo.

**Scope of semen sexing technology in India**

In Indian context, the sexed semen has following potential applications

1. **To realize the increasing demand of milk production:**

The projected demand of milk by 2020 is estimated to be 191.3 MT. To meet the increasing demands, it is necessary to substantially increase the number of elite female which can achieved by shifting the sex ratio towards females. The sexed semen bearing X chromosome could be used in elite cows to produce superior high yielding cows at a faster rate than the conventional unsexed semen.

2. **Production of superior breeding bulls:**

In our country there is shortage of superior breeding bulls. The projected additional frozen semen doses required per year (millions) is 48, 9.6 and 52.8 for indigenous, crossbred and buffalo respectively (Mohanty et al., 2013). By introducing sexed male sperm, superior bulls could be produced from the limited number of elite cows available. But, superior male can be produced by sex sorted spermatozoa from superior dam, which will be a great boost for semen station which is the need of the hour for increasing the frozen semen productivity in the country.

3. **Progeny testing:** Sexed semen technology ensure required number of progenies per bull under progeny testing programme, thereby increases the accuracy of bull testing. The sexed female sperms could be used in test mating so as to ensure the production of required number of daughters in shortest time, thus increasing the genetic gain.

4. **Reducing the number of unproductive young bulls:**

The percentage of total males used for breeding and allied activities are 36.6, 64.8 and 29.56 in case of crossbred, indigenous and buffalo respectively (Mohanty et al., 2013). Thus there is large number of unproductive young bulls. Since, cattle slaughter is banned nearly all over India, male calves produced through artificial insemination are of little use in terms of either future breeding bulls or bullocks in the agricultural fields as a source of farm power. The use of sexed semen can solve the problem of production of unwanted male progenies.

5. **Replacement and expansion of herds:**

Sexed semen is an excellent way to expand the dairy herd without spending large amount on replacement and virtually a breeder need not to buy new heifers.

The benefits of sexed semen can be further amplified by coupling it with assisted reproductive techniques like multiple ovulation embryo transfer technology, In-vitro fertilization, Gamete intra-fallopian transfer and Sperm intra-fallopian transfer. These technologies may help to overcome the lower fertility problem.
Constraints

1. High cost: The machines costs around 4 to 5 crore and along with it royalty should be paid for each dose of semen produced.

2. Commercial availability of the sorting technology: The technology is not fully commercially available. The firm holding patent of orienting nozzle does not sell it and it is a major hindrance.

3. Lower sorting speed and efficiency: The sorting speed of the machine is 6000 sperms per second (Johnson, 2000) and if we run it for 24 hours maximum doses (2 X 10^8 sperms per dose) which can be produced is 259.2. If frozen semen is produced then it will be 129 doses (4 X 10^8 sperms per dose).

   From an ejaculate 30% sperms will be rejected during the sexing process due to non-detecting precisely for difference in DNA content and out of the detected sperm 50% will be Y bearing; so semen doses harvesting from a bull of good genetic merit will be reduced by 70% through this technology.

4. Low conception rate: Conception rate is 10-20% lower in sorted sexed semen compared to conventional semen (Norman et al., 2010). The lower conception rate in sexed semen may be affected by several factors but the primary factors are low dose rate and physical or chemical stress on sperm during sorting process (Frijters et al., 2009). The sorting stresses include high dilution rate, staining with the dye, mechanical forces, UV laser light beam, and higher fluidic pressure during projection into the collection tube and centrifugation (Garner, 2006). In addition, site of deposition of semen in uterus also affects the CR in sexed semen. Conception rate is more when sexed semen deposited in body of uterus (45%) rather than horn of uterus (32%) in buffaloes heifers (Campanile et al., 2011).

5. Need for standardization in Indian conditions: The technology of sexed semen being patented and used in the western countries, the standardization and production caters mainly to the Bos taurus dairy and beef breeds. Although the technology is claimed to have been tried and used in Bos indicus breeds like Gir in Brazil and some states have adopted the use of sex semen, its use under Indian tropical condition is still to be verified as impact of adopting the technology for genetic improvement of cattle has not been reported. There is a need to standardize the lower dosage of spermatozoa and site of deposition for AI with good conception rate under Indian conditions. Moreover, attribute that differs among the sperm of mammals is the shape of the head. The effectiveness of utilizing DNA content differences between the X- and Y-chromosome carrying sperm depends not only on relative DNA differences, but also on the ability to precisely orient these gametes at the time of measurement in the flow cytometer/cell sorter (Garner, 2006). This makes sex-sorting of sperm not only different for each mammalian species and breed. Hence, standardization of the technique with respect to different breeds of indigenous cattle and buffaloes is also required.

6. Low number of elite bulls: The low number of elite bulls will limit the options for semen sexing in genetically superior bulls.

7. Lack of skilled manpower: Experienced and proven AI technicians are required for inseminations. Furthermore, developing such a scheme for tropical developing countries is constrained by small flock size, indiscriminate mating and absence of pedigree and performance recording.

8. Lack of good quality ejaculates from indigenous cattle and buffalo

Practices for optimization the conception rate on using sexed semen

i. Use sexed semen only in herds where the AI pregnancy rates with conventional semen are consistently 60% or more.

ii. Use only in healthy cycling females (heifer and cows).

While using fixed time AI, make sure that a high percentage of animals were in heat before doing AI.

iii. Use only experienced AI technicians

Be extremely careful with storing, handling and thawing of the straws.

   Above all, optimal use of sexing technology requires excellent and careful animal management (nutrition, disease control, oestrus detection, semen handling, and insemination technique).

CONCLUSIONS

In Indian condition there is need to standardize the lower dosage of spermatozoa, site of deposition for AI with good conception rate in our conventional system. A large number of research need to be carried out to develop this technique in collaboration with other laboratories to make it feasible in India. Furthermore, high cost and fertility related constraints limit its wider use all over the country. So, the main target should be focused to use of sex sorted spermatozoa in good quality heifers and the cows with excellent reproductive and productive performance to achieve good results. There is also immense requirement to develop instruments to transfer sex sorted spermatozoa non-surgically and to train the skilled manpower in above area to achieve good results. As this technology has much more advantages, so, it is being enthusiastically accepted in many regions of India and it is expected to bloom further in other regions too with decline in cost.
REFERENCES


