Sperm Sexing Technology and Prospects of Sexed Semen for Indian Dairy Industry

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The FAO (Food and Agriculture Organization) has recognized that production of pre-sexed livestock by sperm or embryo sexing, when combined with other biotechnologies, genomics, proteomics or phenomics, for example, sperm-mediated gene transfer, offers a promising breeding strategy to help meet the increased demand for food production. Sex pre-selection also decouples the number of dairy replacement heifers from those required for milk production.

Sex of the calf is an important aspect of animal husbandry production. For example, dairy cattle and buffaloes farmers prefer female cattle for milk production, while the bulls are selectively bred due to their greater genetic influence and economic importance than females. Sex selection in an animal can be achieved before insemination by sorting for X and Y chromosome bearing spermatozoa based on their unique characteristics. Scientists have demonstrated significant differences in structure, morphology, motility and energy metabolism between X and Y sperm.

Several ways have been attempted to shift the gender ratio for a calf crop in an animal, but the idea of sex selection to favour male or female offspring has been researched since the advent of artificial insemination. In India, the need to preselect the gender of the young one is gaining much importance day by day, especially in dairy cattle and buffaloes. With the decreasing land productivity, crop yield and other feed resources one hand and steady increase in demand and consumption of the high-quality dairy product, on the other hand, there is an urgent need to expand the genetic merit of our cattle and buffaloes.

In 2012 India's population reached more than 120 million and was increasing day by day. To meet food demand associated with this population growth; it will be necessary to produce pre-sexed livestock by sperm or embryo sexing; which offer a promising breeding strategy to meet the increased demand for food production and nutritional security. So far, it has been demonstrated that the application of sexed bovine sperm using AI is effective in altering the sex ratio and rapidly expanding dairy herds carrying high genetic value animals. Thus, the practical application of sexed sperm in indigenous cattle and buffalo breeding would be of great interest both in biological and economic terms. Besides the long-term

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benefit, farmers can profit directly from the use of sexed spermatozoa by producing optimal proportions of males and females in their animal production systems. The use of sexed spermatozoa increases the rate of genetic progress, especially in combination with the genomic selection of sires and easier culling decisions. It also has the potential to improve herd management and reduce the incidence of dystocia by avoiding male calves. But, the superior male can be produced by sex-sorted spermatozoa from the 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. Increased bio-security can be attributed to keeping a herd closed by not purchasing as many animals from outside for a replacement.

Several attempts have been made to develop a method that efficiently separates bovine semen into fractions containing higher concentrations of X- or Y-bearing sperm. These technologies include sex-specific antibodies, centrifugation, and flow cytometry. Of these attempts, the only method proven to be commercially viable is flow cytometry till date. In the recent years, there have been several advances in computer science, biophysics, cell biology, and applied reproductive physiology, which have led to the development of systems that could accurately measure fluorescent signals emitted by the spermatozoa treated with specific fluorescent dyes. This development showed the way to measure the small difference in DNA content between X and Y chromosome-bearing sperm. The technique flowcytometry sorting method is not 100% efficient, but it does shift the ratio to about 85 to 95 per cent of the desired sex. As with any other market-driven technology, sex sorting may evolve to become more efficient and less costly, and research is being continuously taken up to fine-tune the technology to reach maximum efficiency and accuracy in developed countries.

Most of the studies on fertility of sex-sorted bull spermatozoa have been carried out in highly organised farms abroad. In cattle, the conception rate of AI using sexed sperm, with one-tenth the sperm number of non-sexed sperm, is around 70-80% of those achieved by non-sexed sperm in heifers. The most important variables in the extent of use of sexed semen are pregnancy rate and the cost per straw, whereas the economic benefit will greatly depend on the baseline fertility of the herd and other key management factors. However, such studies on crossbred and buffalo bulls are very limited.

Since, cattle slaughter is banned nearly all over India, half of the calves (i.e., 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. Majority of the farmers are such that their conscience does not allow them for rearing for meat & selling them for slaughter. Farmers rear these calves till the dams are in milk for the letdown process of the milk and become restless, as they do not contribute anything on the agricultural farm after that. We certainly need technology to significantly reduce the ratio of birth of male-calves on the farms for the sustainability of dairy farming.

Another aspect of the use of sexed semen is the production of superior genetically desirable females for a replacement which will significantly influence the profitability of a dairy enterprise. Sex-sorted semen allows farmers to skew the sex ratio of their animals' offspring significantly, so herd reproductive management is no longer limited by balancing the need for an adequate supply of replacement heifers against the necessity of achieving pregnancies to start new lactations. Sex pre-selection will accelerate genetic gain, improve herd bio-security, and reduce the incidence of dystocia and the number of superfluous bull calves a major industry welfare issue. Fewer cows would be required to produce daughters for progeny testing, and greater opportunity would exist for cross-breeding of older, genetically inferior cows because a steady supply of genetically superior replacement heifers is ensured by this method.

Sex predetermination of offspring in farm animals is of commercial importance. For example, the female is the choice animal for dairy industry whereas the male is the first choice in the meat industry. A direct method for sex predetermination in animals is based on the sorting of X- and Y-sperm cells before insemination. Spermatozoa are separated into X and Y-bearing populations based on the presence of X or Y-chromosomes, respectively. If the egg, containing only X-chromosome, is fertilised by an X-spermatozoa the offspring will be a female; if is fertilised by a Y-spermatozoa, the offspring will be a male. Therefore, "sex-sorted" spermatozoa can be used in conjunction with artificial insemination or in vitro fertilisation to produce offspring of the desired sex. Dairy farms which are producing and marketing milk can exploit sexed semen for production of replacement heifers from elite

cows of high genetic merit and beef calves from remaining population of cows. It can also reduce calving difficulty in first calvers.

Conclusions

Slaughter of the cow is banned in most of the states in India. Extra males are very difficult to dispose of. So sex selection towards female will help in producing near about 92 to 95% female and strengthen the food production and nutritional security. Production of the superior male through sex selection will further contribute to improving the production potentiality of dairy animals. Practically, not much work has been done in this direction in our country. Even in the developed world, research efforts on the subject came about after flow cytometry, and cell sorting became available as a handy tool. A lot of research needs to be carried out to develop this technique in our country in collaboration with other laboratories to make it feasible in our country. Because of the expense involved with sorting sperm, research has focused on developing improved sorting technology in term of more sperm/sec and on methods to inseminate females with reduced sperm dosage per unit (1.8×10^6) compared to conventional AI (8 to 10×10^6 viable progressive motile sperm at the time of AI). So with 50 to 60% viability after post-thaw in a standard dose of straw around 20×10^6 viable motile sperm packed initially before cryopreservation. Use of sex-sorted spermatozoa with lower doses in a combination of multiple ovulation embryo transfers, In-vitro fertilisation, Gamete intra-fallopian transfer and Sperm intra-fallopian transfer may help to overcome the lower fertility problem. The sex sorting through flow cytometry is refining day by day by increasing the speed of sorting, but the fertility results are still compromised – the lack of availability of technology and negative impacts on fertility limiting the use of sex-sorted spermatozoa throughout the world. Introduction of inert gold nanoparticle and harmless deflection technique will improve quality and fertilising capacity of sex-sorted spermatozoa. The success of sperm sexing technologies depends on simple, efficient and highly accurate technology having practical and economic feasibility with a less deleterious effect on germplasm.