Application of Embryo Technology for Faster Multiplication of Genetically Superior Indigenous Cattle and Buffaloes

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Introduction

When conservationists have been paying attention to propagate certain species of wild animals, the wealth of genetic resources in domesticated animals was largely ignored. Native cattle breeds are being lost due to haphazard crossbreeding with exotic (*Bos taurus*) cattle and unplanned breeding of native non-descript stocks. Comparatively higher milk producing exotic breeds of cattle like Jersey and Holstein Friesian were used for crossbreeding over many decades to meet the rising demand of the nation for milk and milk products. This practice, over a period, has largely replaced our native breeds which are well-adapted in our agro-climatic conditions and adjust productivity in adverse conditions. These breeds can thrive in resource poor areas where availability of feed and fodder is scarce and can withstand various diseases due to their high disease resistance. Cross breeds, however, are productive only in ideal, disease-free conditions in resource rich areas.

It has been observed that our native cattle and some of buffalo breeds are deteriorating in terms of both quality and quantity and that the propagation of such breeds has not been done for want of application of adequate technologies and breeding services. So conservation and propagation of indigenous breeds of cattle and buffalo have become the subject of prime importance and application of Assisted Reproductive Technologies (ART) is the only way to produce high performing progenies using the "Elite" parents of these breeds.

Majority of dairy farmers prefer to rear high yielding milch cows and buffaloes. The need of the hour is to identify the best milk yielders among indigenous cattle and buffaloes available in the country and subject them to the technologies by which they could be multiplied at a faster rate. ART such as artificial insemination (AI), multiple ovulation and embryo transfer (MOET), Ovum Pick Up (OPU), in-vitro embryo production (IVEP), embryo freezing and cryo-preservation of embryos are seen to be potential solutions for faster multiplication, dissemination and preservation of elite bovine germplasm. These technologies have been applied successfully to enhance the reproductive ability of farm animals out of which MOET and IVEP have been found to be very promising. With the application of these technologies, we can increase the number of offspring being produced by high genetic merit cattle or buffalo females in her life-time.

Embryo Technology

Embryo technologies are a combination of assisted reproduction, cellular and molecular biology and genomic techniques. The classical use of these technologies in livestock breeding is to increase the number of superior genotypes. So these are important tools to improve the genetic makeup of livestock at a faster rate and simultaneously enhance their reproductive efficiency. These technologies provide an opportunity to make use of the best genetic contribution from dam as well as sire sides.

MOET refers to the technology by which a genetically superior donor (cow or heifer) is subjected to multiple ovulation and released ova (eggs) are fertilized by artificial insemination or natural service, fertilized ova (embryos) are collected non-surgically from the reproductive tract of the donor and transferred into the uterus of another female (recipient), which is mostly genetically inferior. Through multiple ovulation, a donor can be made to produce more number of ova in an estrus cycle by treating it with hormones. Thus, the harvest of embryos can be increased manifold through MOET. A cow or buffalo with high milk production and other desirable traits is considered as donor. A good donor should have normal reproductive organs, should be healthy, younger in age, regularly cycling, free from uterine pathology and major diseases. Recipient can be of any breed and may be of poor genetic material. An ideal recipient is highly fertile female with sound reproductive organs, which can carry the calf for full term. Like donors, recipients should also

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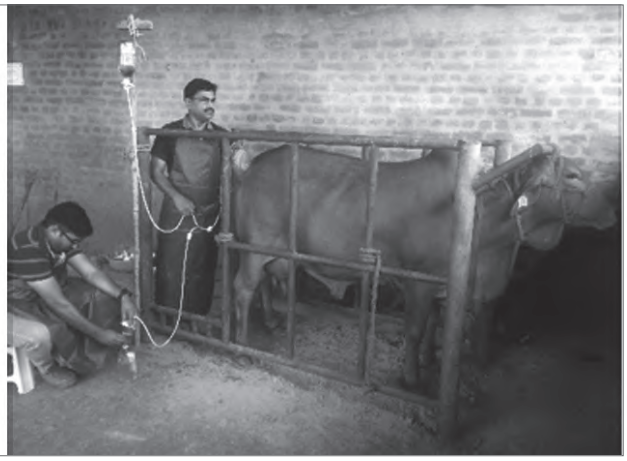
Sahiwal Donors



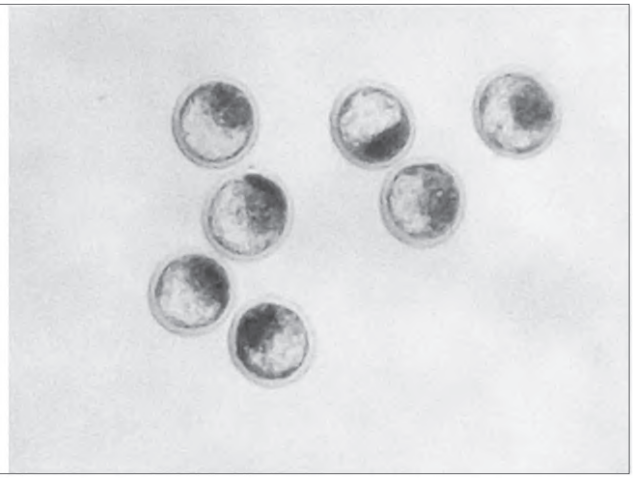
Embryo Recovery from the Donor

be free from diseases.

In-vitro embryo production (IVEP) involves recovery of immature oocytes (OPU) from a donor's ovaries, their maturation (IVM), fertilization (IVF) and development (IVC) to embryo under the controlled laboratory conditions outside the body. Immature oocytes are retrieved from the donor animal through trans-vaginal ovum pick-up (OPU) using ultrasound machine. It can be performed twice per week and can be repeated for 6-8 weeks with very little risk of health or fertility of the donor. However, frequency can be decided depending upon the requirement of embryos and production of oocytes by the donor. This provides an opportunity to produce multiple embryos from a cow/buffalo in a short period. The recovered immature oocytes are placed in an incubator where temperature, humidity and environmental gases are controlled for 22-24 hours to stimulate maturation. On second day, the sorted sperms are placed with matured oocytes and are co-incubated for 18-20 hours. After completion of fertilization, the fertilized ova (zygotes) are cultured for 7 days to allow development of embryos prior to their transfer in to suitable recipients/freezing for future use.

Ovum Pick-up from a Donor

IVF Lab



Embryos

Indian Experience

In nineteen eighties, this technology attracted the attention of Indian scientists and veterinary practitioners engaged in the field of animal reproduction. Early work in India is reported by Zawar (1988). Around this time, Government of India launched a massive project on "Cattle Herd Improvement for Increased Productivity using ETT" with active participation of country's leading institutes like National Dairy Development Board, National Dairy Research Institute, Indian Veterinary Research Institute, National Institute of Immunology and Frozen Semen Production & Training Institute, Hessarghatta. Under this project, enough work was done on multiple ovulation and embryo transfer (MOET), open nucleus breeding system (ONBS), in-vitro maturation (IVM), in-vitro fertilization (IVF), in-vitro culture (IVC), embryo splitting, embryo sexing etc.

During the project period, Regional and State level ET labs were established and various technical protocols were standardized for superovulation, embryo production, grading, freezing and transfer in cattle and buffaloes. Substantial embryo transfer work was carried out in organized dairy farms and at the door step of progressive dairy farmers. Many goals were achieved in terms of creating modern infrastructure for production of bovine embryos and allied technologies, training facilities and a pool of trained manpower. This project formed a concrete foundation for further ET work in India.

A pioneer work on embryo transfer in buffaloes was also done. The successful embryo transfer in buffalo was reported at Sabarmati Ashram Gaushala (SAG), Gujarat (Misra *et al*, 1988). The first buffalo calf from frozen thawed embryo was also born at SAG (Kasiraj *et al*, 1993). Taneja *et al* (1993) attempted production of monozygotic buffalo twins through transfer of bisected embryo halves into six recipients, out of which three got pregnant. The birth of the first buffalo calves using *in-vitro* maturation, *in-vitro* fertilization and *in-vitro* culture of embryo (Madan *et al*, 1994) was also reported in India.

In the beginning, there were many limitations in practicing this technology in India, *viz.* low super-ovulatory response and viable embryo recovery in buffaloes, availability of biologicals and other consumables, lack of trained practitioners and high cost of ET born calves.

Current Scenario

To increase the milk production and productivity of our dairy animals, breed improvement using modern reproductive technologies is essential. Top milk producing animals are to be identified and multiplied at a faster pace to achieve maximum genetic gain over a short period. This is possible when we extensively use MOET and IVEP in cattle and buffaloes. Keeping this in mind, Department of Animal Husbandry & Dairying (DAHD), Government of India (GoI) is funding a project "Breed Improvement by Modern Reproductive Technologies" as one of the components of Rashtriya Gokul Mission (RGM) scheme which is meant for development and conservation of indigenous bovine breeds. As a part of the project, establishment and strengthening of 30 ET/ IVF labs across the country have been sanctioned for propagation of elite animals and to meet the demand of bulls of indigenous breeds. Out of 30 labs sanctioned, 17 labs have been made functional and work is going on at remaining 13 labs. Out of 17 labs, 9 have started producing embryos through IVF technology.

Moreover, DAHD has recently sanctioned an 'Accelerated Breed Improvement Programme' as a part of RGM. This project will be implemented through

ET born Kankrej calves

ET born Sahiwal calves

identified Milk Unions/Milk Producer Companies, State AH Departments, State Livestock Development Boards etc. across the country. NDDB will act as Principal Implementing Agency and would be responsible for its implementation and monitoring. The project envisages establishment of two lakh pregnancies over a period of three years using bovine IVF embryos produced from very high genetic merit females and sexed semen of high genetic merit bulls, in the recipients of interested farmers. The project will benefit about two lakh farmers getting an incentive of ₹5000 per pregnancy from GoI.

In addition, several private bovine breeding companies such as Godrej Maxximilk, ABS India, Tropical Animal Genetics, Palamur Biosciences etc. are also using embryo technology to produce and supply bovine embryos and related services to dairy farmers.

Now there is a demand for bovine embryos in the country. To meet this demand, sufficient infra-structure, logistics and trained manpower are available. Farmers are also demanding the sex known embryos to produce only female progeny. This is necessary to avoid the birth of male progeny which is of no use in the current scenario when most of the agricultural operations have become mechanized.

Applications of Embryo Technology

- Rapid multiplication of important indigenous breeds of cattle and buffaloes. A good donor can easily produce 8 to 10 calves in a year through MOET and 20 to 25 calves through IVEP.
- Genetic improvement of organized dairy herds (cows/buffaloes) by breeding only top performers using ET.
- As an alternative to conventional field Progeny Testing (PT)) programmes. Sire evaluation can be made accurate on the basis of full sibs, half sibs produced by ET and pedigree details. Moreover, this is less time consuming than conventional method because of reduction in the generation interval.
- For preservation of germplasm (in the form of frozen embryos) of endangered breeds of cattle and buffaloes which are facing threat of extinction.
- Production of IVF embryos for maximum utilization of best germplasm of cattle and buffaloes available in the country.
- Production of calves of desired sexes. This is needed to have more number of milk producing animals in the population as well as to economize the Breeding Bull Production programme in the country.
- For import and export of bovine germplasm in the form of frozen embryos. As embryos are almost disease free, they reduce the risk of disease transmission. So import of embryos is much safer than import of live bovines.
- Embryo technology provides a platform for conducting embryo cloning, embryo sexing, cryopreservation, injection of genes, production of transgenic animals, etc. which can further help in enhancing the productivity of indigenous cows and buffaloes.

Benefit of Embryo Technology to Indian Dairy Farmers

There is a vast scope of genetic improvement in cattle and buffaloes reared by the farmers in respect of milk production and other traits. Embryos produced from elite dam and sire using *in vivo* or *in vitro* technologies

can be very effective in boosting the productivity of dairy animals, when used to produce next generation calves in organized dairy farms and at farmers' place. These embryos can be produced at standard labs as well as in the field using genetically elite cows/buffaloes of farmers and transferring these embryos in the recipients owned by them. This practice has received overwhelming response from the farmers. The main focus is to propagate high milk producing cows of Gir, Sahiwal, Tharparkar, Red Sindhi, Rathi, Khillar and other indigenous cattle breeds and buffaloes. The female calf so produced is retained by the farmer whereas male calf is used for breeding purpose locally or supplied to semen stations in country. Now farmers can also opt for sex known embryos for producing female calves to increase the number of milk producing animals in their herd. Sex known bovine embryos and sexed semen are available across the country.

Future Scope

Many dairy advanced nations use embryo technology on a mass scale and produce huge number of *in vivo* and *in vitro* embryos for production of better dairy animals. Mass scale production greatly brings down the cost of embryo transfer, which is paid by the farmers. Since there is no alternative to embryo technology, it has a promising future in mass production of genetically superior dairy animals of cattle and buffalo breeds in India. A sound infra-structure has been created across the country for production of conventional and IVF embryos. Exclusive manpower is being identified and trained to apply this technology on a mass scale in future. Trained manpower will be capable of producing and transferring embryos at the institutional farms as well as at farmers' door step. The success of this technology depends upon mass scale embryo production, availability of ET services in an organized way and the paying capacity of dairy farmers. The cost of ET will also come down with recovery of more viable embryos per flush or more oocytes per OPU session as well as increase in pregnancy rate after embryo transfer. The government funded project like 'Accelerated Breed Improvement Program (ABIP)' and private breeding companies will make this technology available to farmers on mass scale and in an organized way. Moreover, DAHD under RGM scheme of GoI has approved a project to facilitate establishment of 'Breed Multiplication Farms' through entrepreneurship model for making available high genetic merit heifers of cattle and buffalo breeds to farmers. The technology will be helpful to provide ET born sex known progeny to farmers. However, farmers can only pay for ET services, if available, at an affordable price. So mass embryo production, preservation of endangered native breeds of cattle and buffaloes and production of animals of desired sex by using sexed semen have enormous potential and good scope in India.

Conservation of Toda Buffalo using ET

Limitations

- Expensive technology. Levy of 18% GST on providing ET services has made it more expensive for the dairy farmers.
- Most of laboratory equipment/instruments and biologicals/consumables are to be imported and attract substantial custom duty.
- Lack of trained manpower for embryo production and transfer.
- Availability of suitable recipients
- Inadequate recovery of viable embryos/flush in case of buffaloes
- Less pregnancy rate in field condition particularly with frozen embryo transfers.
- Not suitable during monsoon (hot and humid climate) when reproductive efficiency of donors/recipients goes down.

Conclusion

The application of embryo technology is numerous in bovine reproduction to augment reproductive efficiency of valuable females, genetic improvement, planned mating, sex selection of progeny and income of dairy farmers. However, it is still not widely used despite its potential benefit. The main reasons for this are lack of adequate awareness of the technology among dairy farmers, technological skills associated with the entire process and high cost. However, the technology has good scope for

commercial use in the country especially when GoI and private companies are putting sustained efforts to make this technology available at an affordable cost. In fact, this technology is capable of providing an opportunity for producing best dairy animals quickly and thereby increasing the income and living standard of Indian dairy farmers.

References

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