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Reproductive Biotechnologies in Indian Cattle

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Abstract

In recent years there have been immense improvements in application of reproductive biotechnologies in cattle and include artificial insemination, embryo transfer, ovum pick up and in vitro fertilization, semen sexing, cloning, transgenics and xenotransplantation. The purpose of these technologies had been improvement of the genetics of animals or producing animals of benefit to human beings. The application of these technologies in indigenous dairy cows of India had been slow. This manuscript describes the status of some of the reproductive biotechnologies as applied to Indian breeds of cows.

Keywords: Reproductive Biotechnologies; Crossbreeding; Embryo Transfer; Transgenics; Xenotransplantation

Abbreviations: AI: Artificial insemination; FSDs: Frozen Semen Doses; NMBP: National Mission on Bovine Productivity; ETT: Embryo Transfer Technology; OPU: Ovum Pick up; SCNT: Somatic cell nuclear transfer

Artificial Insemination

Artificial insemination (AI) is the single most important reproductive biotechnology which had the highest impact in breed improvement of dairy cows and other livestock species [1]. In India AI was introduced in the early forties and Dr Sampat Kumaran is considered to have performed the first AI at Mysore palace dairy farm. The focus of AI had been crossbreeding with exotic breeds however; currently stress is being given to the conservation of indigenous breeds. As per Animal Husbandary Department Govt. of India Annual report 2016-17 there are presently 49, ISO certified (A and B Graded) Semen Stations in India producing around 95 million Frozen Semen Doses (FSDs) to cater the need of Artificial Insemination in the country. Currently frozen semen of Gir, Kankrej, Sahiwal, Khillar, Red Sindhi, Rathi, Tharparkar, Haryana, Deoni, Amritmahal, Hallikar is available through various sperm stations in India. The Animal husbandry department initiated the Rashtriya Gokul mission since 2014 for conservation and development of indigenous breeds of livestock in India. The 2 important objectives of this mission are upgradation of nondescript cattle using elite indigenous breeds like Gir, Sahiwal, Rathi, Deoni, Tharparkar, Red Sindhi and distribution of disease free high genetic merit bulls for natural service.

National Mission on Bovine Productivity (NMBP) will be implemented with following four components:

(i) Pashu Sanjivni: an Animal Wellness Programme encompassing provision of Animal Health cards (Nakul Swasthya Patra) along with UID identification and uploading data on National Data Base.

(ii) Advanced breeding Technology: including Assisted Reproductive Techniques- IVF/MOET and sex sorted semen technique to improve availability of disease free high genetic merit female bovines.
(iii) Creation of "E-Pashu Haat" an e-market portal for bovine germplasm for connecting breeders and farmers.
(iv) National Bovine Genomic Centre for Indigenous Breeds (NBGC-IB).

In spite of the benefits of AI and the steps undertaken by the Govt in promotion of AI adoption by farmers had been slow. In a recent study involving farmers in 4 North Indian states (UP, Uttarakhand, Haryana and Punjab) it was shown that full adoption of AI as a means of breeding was low 3.33%, 7.77%, 24.44% and 62.3% respectively in the four states under study [2]. The distance to veterinary institutions and poor education of the farmers were the important hurdles to the adoption of AI in this study suggesting stress on opening of more AI centers and farmers education. The maintenance of semen and proper timing of insemination are other two important determinants of success of AI. In order to promote AI using indigenous breeds semen efforts have to be made in selecting outstanding bulls from these breeds.

Multiple Ovulation and Embryo Transfer

Embryo transfer is a technique that can greatly increase the number of offspring that a genetically important cow can produce. Embryo transfer technology (ETT) has emerged as an important tool to improve livestock at faster rate, as in this technique, genetic contribution of both the male and female are utilized simultaneously. Thus, the most important application of ETT is the production of A1 bulls from the best proven bulls and cows available [3]. Other applications of ETT include genetic evaluation through sibling testing, conservation and preservation of the endangered breeds, creation of disease free herd, economic transport of livestock, salvage of reproductive function, production of progeny of desired sex, production of identical twins and clones and genetic modification of animals [3].

The in vivo embryo production involves superovulation of donors using ECG or FSH and recovery of embryos using non-surgical approaches. The embryos are then transferred to synchronized recipients to establish pregnancies. A large number of variables determine the ovulatory responses of cattle to super stimulation using FSH.

The first embryo transfer calf in India was born in 1987 at National Institute of Immunology New Delhi [4]. There has been a lot of work on super ovulation and embryo transfer in indigenous breeds of cows (Table 1) however; we still have to do a lot more. Problems associated with embryo transfer in indigenous cows involve those associated with embryo transfer in general and those specific to the breed for example the problems of cervical hypertrophy and fibrosis in Rathi cows limiting the passage of catheter for embryo recovery or transfer.

Breed	Mean CL	Mean TE	Mean VE	Reference
Sahiwal	9.16±4.41	-	5.33±4.19	Mishra, et al. (1997) [5]
Sahiwal	-	2.33±0.76	1.13±0.42	Prasad, et al. (2003) [6]
Ongole	9.09±0.40	5.57±0.36	1.79±0.28	Kasiraj, et al. (2000) [7]
Kankrej	-	6.7	2.9	SAG 1996-97 [8]
Rathi and its crossbred (eCG)	7.92±0.66	3.96±0.49	-	Purohit, et al. (2000) [9]
Rathi and crossbred (FSH)	7.24±0.52	5.97±0.56	-	Purohit, et al. (2000) [9]
Sahiwal	10.5	9	-	Singhal, et al. (2017) [10]
Panganur	5.29-8.14	1.14-2.57	-	Veerbramhaiah, et al. (2012) [11]
Red Sindhi (one cow)		16 embryos recovered	13 were transferable grade	Rangasamy, et al. (2015) [12]
Vechur	-	5.5	4.9	Venkatachalapathy, et al. (2000) [13]

Table 1: Superovulatory response and embryo recovery in different breeds of cattle

Invitro Embryo Production

In vitro embryo production involves the maturation of oocytes retrieved from live donors by OPU, their maturation and fertilization in vitro and development to blastocysts and transfer to recipients. Many reports have appeared on the in vitro maturation, fertilization and development of abattoir derived oocytes to blastocysts however, the benefit of in vitro embryo production lies in retrieval of oocytes from donors of known genetic merit. This involves the retrieval of oocytes by ultrasound guided ovum pick up (OPU) from live animals. The procedures of OPU retrieve oocytes from prospective donors without disturbing their physiology. However, the rate of blastocyst formation (30-60%) and calf production (10-15%) is low by in vitro procedures. Moreover, the viability of in vitro produced embryos is poor compared to in vivo developed embryos. Many reports on in vitro embryo production from abattoir derived embryos have appeared in India [14]. However reports on production of live calves from OPU-IVF derived embryos are scarce. The NDRI website shows that the first OPU-IVF Sahiwal calf was produced at NDRI in 2007. OPU derived Sahiwal oocytes were matured in vitro to yield blastocysts in one study [15]. A recent report at the website http://www.asianage.com/metros/mumbai/ mentions that the first IVF derived embryo produced from a Tharparkar cow oocyte and sperm derived from bull of the same species and placed in the uterus of a Jersey cow was born on 5th September 2017 in Pune by efforts of Dr Zawar. Hence the techniques of OPU/IVF produced calves in Indian breeds of cattle are still in infancy.

Sexing of Semen

The important discovery that female-determining (X chromosome bearing) sperm had 3-4% more DNA than male-determining (Y-chromosome bearing) sperm and when stained with fluorescent dye, X-chromosome bearing sperm glows more than Y-chromosome bearing sperm and facilitated separation of these sperm using flow cytometry [16], Thus, sperms with an or Y chromosome could be used to produce male or female embryos/animals [17]. The accuracy of this technique is high and sex of over 90% of the calves born correlated with the use of X or Y-chromosome bearing sperm rich fractions and the sexed semen is available commercially [18]. Sexed semen is currently available in many countries however there are still many limitations. ABS Technology at its website genusabsindia.com claims to make available sexed semen of Sahiwal, Gir and Red Sindhi bulls. A recent report mentioned the employment of cell sorters at Bengal and Kerala and production of live calves from sex sorted semen [19]. Also the use of sexed semen of exotic breeds is becoming popular in some Indian states like Punjab, Haryana, Kerala and West Bengal [19]. However the high cost and poor pregnancy rates are the limiting factors that have prevented the wider use of sexed semen.

Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) is a powerful technique and potentially it could be used for the multiplication of desired animals, minimizing the genetic variation in the experimental animals, production of animal models, genetically modified farm animals to make modification o f milk, growth, disease resistance, xenotransplantation etc. SCNT may be used for conservation and propagation of endangered species which are at the verge of extinction such as Cheetah, one horned rhinoceros, swamp deer, hispid hare, wild buffalo, Assam root turtle, pigmy hog etc. in India. Recently, the SCNT has emerged as a tool for production of stem cells for therapeutic purposes, popularly known as 'Therapeutic Cloning'. The efficiency of SCNT is, however, still extremely low (Dolly was one from 277 attempts) for unknown reasons [20].

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