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Review Article

Reproductive Augmentation in Livestock

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A B S T R A C T

Genetic improvement of farm animals is a prime concern over the years for breeders, researchers, farmers. Several reproductive technologies have been employed to achieve this goal. Assisted reproductive technologies like artificial insemination, super ovulation, in vitro fertilization, embryo transfer, embryo cryopreservation, cloning, transgenesis, sexing of semen and embryos, stem cell technology, embryo genomics, micro and nanotechnology have been introduced to overcome reproductive problems. These technologies are used to increases the offspring from selected females and to reduce the generation intervals in farm animal. Reproductive ability and efficiency has improved significantly since the introduction of artificial insemination. These technologies have also been used to conserve the indigenous breeds. These alternative reproductive techniques are available not only for manipulation of reproductive processes but also proved to be powerful tools in overcoming the spread of vertically transmitted diseases. The successful reproductive technologies such as AI and embryo transfer need to be applied on a large scale, emerging biotechnologies such as MOET, IVF and cloning provide powerful tools for rapidly changing the animal populations. These advanced reproduction technologies will definitely play an important role in the future perspective and visions for efficient reproductive performance of livestock.

Keywords

Assisted reproductive technology (ART), Artificial Insemination, Embryo Transfer, Cryopreservation, Cloning, Multiple Ovulation and Embryo Transfer (MOET), IVF, Transgenesis, Stem cell technology, Semen sexing, Nanotechnology

Introduction

Productivity is the key to growth and reproduction is backbone of animal production (Verma *et al.*, 2012). Livestock sector is one of the growing industries which contribute major income to the dairy farmers across the country (Balaji and Chakravarthi, 2010). Failure of reproduction can leads to great economic loss in livestock industry. The majority of this loss occurs because cows do not become pregnant during a defined breeding season, infertility due to low conception rate and high embryonic mortality rate remains a major problem.

In order to make the animals as better productive population, the science has given so many novel reproductive techniques viz. Synchronization of Oestrus, Multiple ovulation (Super ovulation), Embryo transfer, In-vitro fertilization and Cloning which are important potential tools for reproductive improvement in livestock (Balaji and Chakravarthi, 2010). Third and fourth generation technologies such as sexed semen or embryos, cloning, transgenesis, stem cell biology and molecular diagnosis have the potential to enhance the influence

of superior animals on production, but their commercial applications have been limited (Bertolini and Bertolini, 2009). Keeping all these points in view, the present paper summarizes the potential achievements of these assisted reproductive techniques (ART) in cattle breeding, which will be helpful for improving the current status of livestock reproduction.

Artificial insemination (AI)

Artificial insemination is the first generation ART, which has been in use for more than 200 years. This is a techniques where semen is deposit in female's vagina by artificial means. On historical point of view first successful insemination was performed by Spallanzani, (1784) in a bitch. Pioneering efforts to AI were begun in Russia in 1899 by Ivanoff (1922). He practiced AI in domestic farm animals, dogs, foxes, rabbits, and poultry. Later on this technique was performed by various researchers worldwide in different species. Use of frozen semen (Polge *et al.*, 1949) revolutionized the AI program through worldwide transport of semen. As a modern technology, AI with fresh or frozen semen has been the most successful and efficient reproductive technology in animal production for the last six decades. The use of AI had a major impact on genetic improvement programs in developed countries, associated with 1.0 to 1.5% annual rates of genetic gains in dairy cattle (Lohuis, 1995). AI technology maximizes the use of outstanding males, dissemination of superior genetic germplasm, improve the rate and efficiency of genetic selection, introduction of new germplasm by import of semen rather than live animals and thus, reducing the international transport costs (Verma *et al.*, 2012). Use of frozen semen even after the donor is dead and it reduces the risk of spreading sexually transmitted diseases. This will also help in improving bio security and limit the risk for transmission of diseases from farm to farm if semen is processed according to set health standards. Earlier AI was performed by using the semen from exotic breeds for increasing the production of local livestock populations through cross breeding (Verma *et al.*, 2012). But in present scenario semen of indigenous and local breeds is also used for the purpose of conserving the indigenous breeds. At present more than 100 million cattle, 40 million pigs, 3.3 million sheep and 0.5 million goats are artificially inseminated worldwide every year (Boa Amponsem and Minozzi, 2006). In India, semen production for AI has increased from 22 million (1999-2000) to 67 million straws (2011-2012) and the number of inseminations have increased from 20 million to 54 million. As per the impact analysis report submitted by NABARD, overall conception rate has increased from 20 % to 35 % (Annual report 2012-2013). The conception rate in AI programme in developing countries is very low because lack of proper management and technical skill of AI provider and therefore the desired effect in terms of animal improvement has not been achieved so far. In Brazil, it has been recently reported that almost 60% of the AI performed are made at fixed time (Baruselli *et al.*, 2012). The AI technique is a powerful tool for augmenting the reproductive and productive performance of livestock.

Cryopreservation of Embryos and Gametes

It was the attainment of successful protocols for semen preservation that made AI thrive as an accessible reproductive technology that allowed the widespread use of genetically superior sires (Gordon, 1994). Frozen semen boosted the dairy industry, for making AI simpler, economical, and

successful, with more than 60 percent of dairy cows in the USA bred by AI (Bertolini and Bertolini, 2009). Short fertile life span of mammalian oocytes hence, storage of unfertilized oocytes would generate a readily available source, which allow the experiments to be carried out at convenient time and could therefore be of practical importance. Live offsprings of at least 25 species resulted from transfer of cryopreserved embryos or oocytes (Gajda and Smorg, 2009). Preservation of oocytes reduce the risk and expense involved in transport of live animals, hazards of disease transmission and also provides insurance against natural disasters. Preservation of oocytes of endangered species safeguards from danger of extinction. Vitrification is a simple, faster, less expensive technology than slow freezing. Vitrification of germplasm was introduced by Rall and Fahy (1985). According to Vajta *et al.,* (1996) its more effective than slow freezing for material more sensitive to chilling. Cryopreservation of oocytes by vitrification was attempted with variable success in bovine (Hochi *et al., 2000),* equine (Hurt *et al.,* 2000), swine (Huang and Holtz, 2002) and buffalo (Sharma and Loganathasamy, 2006).

During the year 2009, more than half the embryos collected in North America were frozen prior to transfer and more than 95% were frozen in ethylene glycol for direct transfer (Stroud, 2009). Recently, high-security verification device (Camus *et al.*, 2006), fiber plug (Muthukumar *et al.*, 2008), pipette tip (Sun *et al.*, 2008), Cryo-E (Petyim *et al.*, 2009), vitrification spatula (Tsang & Chow, 2009), sealed pulled straw (Yavin *et al.*, 2009), Rapid-i (Larman & Gardner 2010), Cryopepette (Portmann *et al.*, 2010) and plastic blade (Sugiyama *et al.*, 2010) has been introduced for more convenient and better results.

Multiple Ovulation and Embryo Transfer (MOET)

A cow normally produces only one egg per oestrus cycle and the gestation period is 40 weeks. On an average, a cow produces only 2-3 calves in her lifetime (Balaji and Chakravarthi, 2010). Thus, without intervention, the rate at which a particular desirable cow can be used to improve the genetic status of a herd is slow. Smith (1988 a, b) introduced the concept of MOET and demonstrated how well designed MOET programmes could led to increased selection intensity and reduced generation intervals, resulting in improving genetic gains. Embryo transfer is now commonly used to produce artificial insemination sires from highly proven cows and bulls (Bondec, 1989). The progress achieved during the past 25 years has positioned commercial bovine embryo transfer as a large international business (Mapletoft, 2005 and Lonergan, 2007). In 2005, approximately 1.3 lakh bovine females were flushed, for more than 6 lakh bovine embryos being transferred, representing a 10% worldwide increase over the previous year, with North and South America and Asia accounting for 45%, 21%, and 19% of the total worldwide activity, respectively (Thibier *et al.,* 2006). Multiple ovulation and embryo transfer (MOET) leads to the production of multiple progeny from genetically superior females. However, ET and AI can be very useful, provided that good production practices (husbandry, nutrition, and management) are in place. One of the limiting factors associated with MOET technology is the variability and lack of predictability in follicular development response and embryo production following a superovulatory treatment (Mapletoft, 2005). In reality little progress was attained, as the average number of transferable embryos per donor and the side effects on the reproductive

performance of the donors remain unchanged in the past two decades (Thibier *et al.*, 2006, and Galli *et al.*, 2003). As for AI, the use of MOET schemes forced the development of oestrus or ovulation synchronization protocols that have facilitated and shortened considerably the whole process. Fixed-time ET and direct ET of frozen embryos are satellite procedures currently in broad use world-wide. However, MOET programs are expensive, mostly due to the cost of labour and hormone treatments (Bertolini and Bertolini, 2009). For these reasons, MOET will probably continue to be more intensively used by elite cattle producers. Use of transvaginal, ultrasound-guided follicular puncture for oocyte retrieval (commonly named ovum-pick-up, OPU) may make MOET more effective since it waives super ovulation and AI treatments, by the collection of oocytes (up to 1 thousand oocytes can be collected from a heifer/cow per year) and following in vitro embryo production up to 300 in vitro produced, embryos can be obtained per year. (Presicce *et al.*, 2011).

In-vitro Fertilisation (IVF)

The first IVF followed by birth of offspring was achieved in the rabbit (Thibault, 1954). Now, unfertilized eggs are fertilized in the laboratory and cultured for a few days until they have developed into early embryos. These are then transplanted in to the recipient cow that has normal oestrous cycles (Balaji and Chakravarthi, 2010). Early stages of bovine embryo development show many similarities with human embryos. Therefore, bovine embryos are used as a model organism (Niemann and Wrenzycki, 2000). In vitro embryo production technologies not only help in production of high genetic merit animals but also provide an excellent source of embryos for embryo sexing, cloning, nuclear transfer and transgenesis. Through IVF we can analyse developmental potential of embryo, including the pattern of cytogenetic disorders, epigenetic modifications, and gene expression during the development (Galli and Lazzari, 2008). In 2009, more than 292 thousand IVP embryos were transferred world-wide (Stroud, 2009), but this is accounted for almost entirely by the increase in activity in Brazil where IVP of embryos is done primarily in Bos indicus cattle. In spite of continuous efforts to improve bovine *in vitro* embryo production (IVP), its efficiency is still low, since only 30% to 40% blastocyst development has been obtained from oocytes after *in vitro* maturation, fertilization and embryo culture (Sirad *et al.*, 2006). In vitro produced embryos were used to facilitate breeding of transgenic bulls. Frequency of transgene transmission varied from 3% to 54% between bulls. (Eyestone, 1999). However, the practical use of IVEP is limited by high production costs and the low overall efficiency under field conditions.

Embryo transfer

Embryo transfer techniques allow superior female livestock to have a greater influence on the genetic advancement of a herd or flock as well as gives an opportunity to utilize the genetic contribution of both male and female at the same time, With the help of Embryo transfer or Multiple ovulation and embryo transfer techniques (Nicholas and Smith, 1983) faster improve merit of livestock, rapid expansion of elite animals, genetic gain, accelerated herd development and conservation of rare genetic stocks could be achieved. Seidel (1981) suggested that through the use of embryo transfer the genetic gain could be increased three to four times if dairy replacements were selected from the top 10% of the herd. In 2002, more

than 5 lakh Embryo transfer were performed worldwide, mainly in dairy cattle, with 62% being transferred in North America and Europe, 16% in South America and 11% in Asia (Madan, 2005). Based on high genetic correlation and due to the higher heritability for flushed ova, indirect selection on flushed ova will increase selection response in transferable embryos by about 22% compared with direct selection on transferable embryos (Konig *et al.,* 2007). According to International Embryo Transfer Society Data Retrieval Committee, 8 lakh embryos in cattle (Thibier, 2009), 25 thousand in sheep, 7 thousands in goat, 30 thousand in pig and 12 thousand in horses (Thibier, 2006) were transferred worldwide (two thirds as in vivo derived embryos and one third as in vitro produced) with 55-70% conception rate (Thibier, 2009). Approximately 61% of embryo transfer work in the USA continues to involve beef cattle (Stroud, 2012). Recently, Breeding farm in Himachal Pradesh the birth male calves named "Gaurav" (2010) and "Saurabh" (2011) and two female calves "Ganga" and "Jamuna" in 2012 at livestock farm, Kotlabarog, H.P. World's first ever Mithun calf through embryo transfer technology was born at the National Research Centre (2012) on Mithun, Jharnapani, Nagaland.

Cloning

Animal Cloning is the development by which an entire organism is reproduced from a single cell taken from the parent organism and in a genetically alike. This means the cloned animal is an exact photocopy in every way of its parent; it has the same exact DNA (Balaji and Chakravarthi, 2010). It can be used for the conservation as well as propagation of endangered species. Cloning using somatic cells offers opportunities to select and multiply animals of specific merits (Das *et al.,* 2003). First animal obtained by somatic cloning was a sheep, "Dolly" (Willmut *et al.,* 1997), using a cultured adult somatic cell with an enucleated oocyte. Since then, SCNT was used successfully for cloning cattle (Cibelli *et al.,* 1998), goat (Baguisi *et al.,* 1999), pig (Polejaeva *et al*., 2000), and horse (Galli *et al.,* 2003). Microinjection of DNA into the pronuclei of recently fertilized ova is the most common technique used to produce genetically engineered livestock. In remote areas, where sampling and storage of adequate samples of semen and embryos is not practical, one could use clone samples from diverse animals for conservation of the available genetic diversity. list of cloned animal, first cloned camel, "Injaz", a female, (2009) and second cloned camel, Bin Soughan, a male, (2010) were born at the Camel Reproduction Centre in Dubai, United Arab Emirates. Introducing a new technique "Hand guided Cloning Technique" world's first buffalo female calf GARIMA(2009) and a male buffalo calf, "SHRESTH" (2010), female calf was born from cloned buffalo GARIMA, NDRI has named the newborn female calf "MAHIMA", male buffalo "SWARN" born from the somatic cell of semen (2013), female buffalo "PURNIMA" (2013), "LALIMA" (2014), Male cloned calf named "RAJAT" (2014) have been born at NDRI, Karnal India.

Transgenesis

The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome, in contrast to spontaneous mutation. Initial demonstration was "super mice" in 1980s. These mice were able to produce the human protein tPA to treat blood clots. First transgenic animals like mouse (Gurdon and Ruddle, 1981) pig (Hammer *et al.,* 1985), goat (Ebert *et al.,*

1991), cattle (Cibelli *et al.,* 1998) and sheep (Simon *et al.,* 1998) were produced. The use of recombinant DNA techniques is to introduce new characters (i.e. genes) into organisms (including humans) that was not present previously. Transgenic farm animals can be used both in breeding and biomedicine (Robl *et al.,* 2007; Wells, 2010). Transgenic animals show individuals are improved in quantitative, qualitative traits and they are resistance to disease. Some examples live sheep with integrated keratin-IGF-I gene and higher production of wool (Kues and Niemann 2004), sheep and goat with antitrombin III and antitripsin in milk (Kues and Niemann 2004). An important achievement was production of transgenic cows resistant to *mastitis* (Wall *et al.,* 2005). Transgenic domestic pigs are used in studies on xenotransplants, (Niemann *et al.,* 2005). Scientist are going on for production of environment-friendly transgenic individuals which are used to understand various physiological processes in farm animals and humans (Niemann *et al.,* 2005).

Stem cell technology

Stem cells are characterized by their self-renewal capacity through mitotic cell division for indefinite proliferation *in vitro* in an undifferentiated, pluripotent state. Embryonic stem cells possess the *in vitro* and *in vivo* capacity to differentiate into any specialized cell type, from *in vitro* formation of embryoid bodies to *in vivo* differentiation into somatic and germ cell lineage (Choi and Anderson, 1998). Stem cells are having various applications like, model for developmental biology, gene therapy, organ transplantation, drug development, chimera production and in the field of regenerative medicines (Bajada *et al.,* 2008). Its application in large animal models in which the embryo stem cell technology can be tested for tissue-specific differentiation (Brown *et al.,* 2007) and cell therapy of various tissues and organs. Attempts have been made to establish embryo stem cell lines from mammals like rat (Innanecone *et al.,* 1994), pig (Wheelar, 1994), mink (Sukoyan *et al.,* 1993), bovine (Strelchenko, 1996), equine (Saito *et al.,* 2002), sheep (Notarianni *et al.,* 1991), rabbit (Graves and moreadith, 1993), and rhesus monkey (Thomson *et al.,* 1995). Therefore, identification of reliable markers for characterisation of embryo stem cell is of great importance in order to exploit their potential. Embryo stem cell mediated gene transfer has some distinct advantage over other transgenic methods. Production of chimera, several lines of ES cells were obtained from (1) inner cell mass of blastocysts, (2) single blastomeres isolated from embryos at earlier stages of development, or even from (3) one-cell stage embryos (Hwang *et al.,* 2004, Klimanskaya *et al.,* 2006). The successful transplant of testicular tissue containing spermatogonial stem cells (SSCs) used in goat and pig is readily adapted in cattle (Honaramooz *et al.,* 2003, Joerg *et al.,* 2003). By transplanting SSCs from elite bulls into lesser bulls followed by natural service, elite genetics could be disseminated more widely (Herrid *et al.,* 2006). This system could create an alternative to artificial insemination for the use in elite sires in the cattle industry in areas where AI is not practicable (Hill and Dobrinski, 2006). Herrid *et al.,* (2006) demonstrated that male germ cell transplantation between the unrelated bull calves and between cattle breeds could also be successful.

Sexing of semen

Predetermination of the sex of offspring would provide a greater number of males or females, which will help in selection of

individuals with top genetic makeup for improvement in next generation (Plummer and Beckett, 2006). The sexual differentiation of embryo is determined by the presence or absence of elements normally located on the Y chromosome. Some of the techniques for sexing are i) chromosomal analysis of embryos ii) immunological detection of embryonic H- Y antigen iii) use of Y-specific probes iv) Fluorescence in situ hybridization (iv) rapid sexing method for pre-implantation embryos of bovine using Loop-Mediated Isothermal Amplification (LAMP) reaction (Zoheir and Allam, 2010). Another way is the sorting of semen, one sperm at a time, into males and females, using staining procedure and detecting by laser beam with the help of standard flow cytometry equipment (Garner, 2006). The bovine Y- chromosome specific sequences are conserved amongst buffalo, Indian zebu and Taurus cattle (Apparao *et al.,* 1993). Thus, the use of bovine Y-chromosome specific primers, demonstrate the sex of buffalo or Indian zebu cattle embryos. Efficient embryo biopsy method has also been developed (Lopatarova *et al.,* 2008). Embryos can be sexed with the help of a DNA probe in early embryonic stage. Rate of survival and conception rates are high from biopsied embryo and reflects the minimal damage of the embryos during the process. Recently advances in semen sexing, using fluorescence activated cell sorter (FACS) offspring of pre-determined sex have been successfully produced (Garner *et, al.,* 2008) using fresh and frozen-thawed spermatozoa in several mammalian species: cattle (Seidel *et al.,* 1999), goat (Parrilla *et, al.,* 2004), pigs (Grossfeld *et al.,* 2005) sheep (de Graaf *et al.,* 2007). The sex sorting process by flow cytometry is the most efficient method to separate X from Y spermatozoa in a large scale (Garner and Seidel, 2008; Rath *et al.,* 2013; Seidel, 2014). Advances in semen sex sorting have enabled incorporation of this technology into commercial operations (De Vries *et al.,* 2008; Norman *et al.,* 2010). Despite the significant advances in sex-sorting sperm using flow cytometry in cattle, lower pregnancy per AI (P/AI) and reduced *in vivo* embryo production is achieved when compared to the rates obtained with non sex-sorted sperm (Schenk *et al.,* 2006, 2009; Larson *et al.,* 2010; Sales *et al.,* 2011; Soares *et al.,* 2011; Sa Filho *et al.,* 2012; Seidel, 2014).Semen and embryo sexing have not been reported in the field in any of the developing countries, except China. Sa Filho *et al.,* (2012) showed that overall P/AI rates were reduced with sex-sorted sperm compared with non sex-sorted sperm. Seidel and Schenk (2008) observed a lower pregnancy rate when using sex-sorted sperm (31% to 42%) than non sex-sorted sperm (43% to 62%). Although the greater variability on the pregnancy outcomes of cattle inseminated of with sex-sorted sperm by literature, most part of the researches with heifers indicates that conception rate after AI upon estrous detection with sex-sorted sperm is about 70% to 90% (according to the farms handling) from the conception obtained following the use of conventional semen.

Nanotechnology

Nanotechnology is recent advancement in cellular and molecular biotechnology. Nanotechnology applies the nanoscale principles and techniques to understand and transform bio systems (living or non-living) which use biological principles and materials to create new devices and systems integrated from the nanoscale. It is engineering at the molecular (groups of atoms) level (Num and Useh, 2013). This technology allows researchers to handle biological materials and media in minute quantities usually nanoliters or picoliters. It

is classified by the size of the materials being developed and used, not by the processes being used or products being produced (Chauhan *et al.,* 2010). It is useful technique in farm animal breeding and reproduction. Microfluidic and nanofluidic (Schuster *et al.,* 2003; Eijkel *et al.,* 2005) are recent tools to simplify traditional procedures of in vitro fertilization (IVF) and in vitro embryo production (Suh *et al.,* 2006). Oocyte manipulation under in vitro condition can also become feasible with advent of this technique (Beebe *et al.,* 2002). Glasgow *et al.,* (2001) first established the manipulation and movement of an embryo in a microfluidic environment. Also be used in sorting of sperm and eggs. In farm animal breeding heat detection can be done by implanting a nanotube (O'Connell *et al.,* 2002) under the skin to detect the changes in the level of estradiol in the blood. Braydich-Stolle *et al.,* (2005) found that nanoparticles of silver negatively affect gametogenesis in mice, and therefore this element should be avoided when using animals destined for reproduction. Functionalized nanoparticles can provide direct, rapid, and sensitive detection of viruses and thereby bridge the gap between current cumbersome virus detection assays and the need for more rapid and sensitive detection of viral agents (Tripp *et al.,* 2007). Some other reports show the support of nanoparticles in disease diagnosis (Na *et al.,* 2009; Jackson *et al.,* 2011; Schlacter *et al.,* 2011; Huang *et al.,* 2012). Nucleic acid engineering-based probes and methods offer powerful new ways to deliver therapeutic or preventative treatment for particular diseases (Luo, 2003). Illumination of the body with infrared light raises the cell temperature to about 55°C, which 'burns' and kills the tumour (Hirsch *et al.,* 2003). Nanotechnology is employed in the treatment of African animal trypanosomosis (Kroubi *et al.,* 2010). Nanobiotix technology used in cancer therapy, the nanoparticles are injected into the patient intravenously or intratumoral, once the particles have been internalized by the cancer cells, an external energy field is applied to activate the nanoparticles and a local physical or chemical effect then destroys the tumour cell (Chauhan *et al.,* 2010). The immunological properties of a novel nano-bead adjuvant in a sheep (large-animal) model were investigated (Scheerlinck *et al.,* 2006)

After introduction of artificial insemination there has been a revolution of new assisted reproduction techniques like super ovulation, embryo transfer, cloning, sexing of semen, stem cell technology, and nanotechnology, etc. These assisted reproduction techniques leads to greater genetic improvement in production and reproduction traits in farm animals. Likewise in A.I. has contributing lot in exploiting the superiority of males and embryo transfer technology has contributed to same extent for use of superior females. Now a day's cloning, transgenesis and sexed semen technology gives a new hope through production of animal, which have all the desired characters for improving.

It is concluded that these assisted reproductive technologies have great potential for the improvement of livestock species. There is need to standardize these techniques for wider application for the welfare of mankind.

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