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Livestock Reproductive Techniques

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Genetic improvements of farm animals through assisted reproductive technologies like artificial insemination, superovulation, *in vitro* fertilisation, 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 as well as to reduce the generation intervals in farm animal. Reproductive ability and efficiency have improved significantly since the introduction of artificial insemination. These alternative reproductive methods are available not only for manipulation of reproductive processes but also powerful tools in overcoming the spread of vertically transmitted diseases. These technologies have also been used to conserve the indigenous breeds. The successful reproductive technologies namely AI and embryo transfer required for application 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 a significant 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.

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1. INTRODUCTION

Failure of reproduction can leads to a great economic loss in the 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 significant problem. The synchronisation of Oestrus, Multiple ovulation (Superovulation), Embryo transfer, In-vitro fertilisation and Cloning which are important potential tools for reproductive improvement in livestock [1]. Third and fourth generation technologies (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 [2]. Keeping all these points in view, the present paper summarises the potential achievements of these assisted reproductive techniques(ART) in cattle breeding, which will be helpful for improving the current status of livestock reproduction.

2. ARTIFICIAL INSEMINATION (AI)

First successful insemination was performed by Spallanzani, [3] in a bitch nearly 200 years ago. Pioneering efforts to AI were begun in Russia in 1899 byIvanoff [4]. He practised 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 [5] revolutionised the AI program through worldwide transport of semen. As a modern technology, AI with fresh or frozen semen is 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 [6]. Dominantly, AI technology uses outstanding males, dissemination of superior genetic germplasm, improve the rate and efficiency of genetic selection, the introduction of new germplasm by import of semen in compared to live animals and thus, reducing the international transport costs [7]. The frozen semen even after the donor is dead has been used and reduces the risk of spreading sexually transmitted diseases. This will also help in improving 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 [7]. 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 [8]. In India, semen production for AIhas increased from 22 million (1999-2000) to 67 million straws (2011-2012) and the number of inseminations has increased from 20 to 54 million. As per the impact analysis report submitted by NABARD, the overall conception rate has increased from 20% to 35% [9]. The conception rate in AI programme in developing countries is minimal due to scarcity of proper management and technical skill of AI provider and therefore 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 [10]. The AI technique is a powerful tool for augmenting the reproductive and productive performance of livestock.

3. CRYOPRESERVATION OF EMBRYOS AND GAMETES

It was the attainment of successful protocols for semen preservationthat 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 [2]. Short fertile lifespan of mammalian oocytes hence, storage of unfertilized oocytes would generate a readily available source, which allows the experiments to be carried out at a convenient time and could, therefore, be of practical importance. Live offspring's (at least 25 species) resulted from the transfer of cryopreserved embryos or oocytes [11]. Preservation of oocytes reduce the risk factors and expense that involved in the transport of live animals, hazards of disease transmission and also provides insurance against natural disasters. Preservation of oocytes of endangered species safeguards from the danger of extinction. Vitrification is a simple, faster, less expensive technology than slow freezing. Vitrification of germplasm was introduced by Rall and Fahy [12]. According to Vajta et al. [13] its more more effective than slow freezing for material more sensitive to chilling. Cryopreservation of oocytes by vitrification was attempted with variable

success in bovine [14], equine [15], swine [16] and buffalo [17]. 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 [18]. Recently, high-security verification device [19], fibre plug [20], pipette tip [21], Cryo-E [22], vitrification spatula [23], sealed pulled straw [24], Rapid-i [25], Cryopepette [26] and plastic blade [27] has been introduced for more convenient and better results.

4. 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 [1]. 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 [28,29] introduced the concept of MOET and demonstrated how well designed MOET programmes could be 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 [30]. The progress achieved during the past 25 years has positioned commercial bovine embryo transfer as a large international business [31,32]. 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 [33]. Multiple ovulation and embryo transfer (MOET) leads to the production of multiple progenies from genetically superior females. However, ET and AI can be very useful, provided that good production practices (husbandry, nutrition, and management) are in place [34]. 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 [31]. 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 last two decades [33,35]. 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 [31]. 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 [2]. 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 1000 oocytes could be collected from a heifer/cow/year) and following *in vitro* embryo production up to 300 *in vitro* produced, embryos can be obtained per year [36].

5. *In-vitro* FERTILISATION (IVF)

The first IVF followed by birth of offspring was achieved in the rabbit [37]. Now, unfertilized eggs are fertilised in the laboratory and cultured for a few days until they have developed into early embryos. These are then transplanted into the recipient cow that has normal oestrous cycles [1]. Early stages of bovine embryo development show many similarities with human embryos. Therefore, bovine embryos are used as a model organism [38]. *In vitro* embryo production technologies not only help in the 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 the developmental potential of embryo, including the pattern of cytogenetic disorders, epigenetic modifications, and gene expression during the development [39]. In 2009, more than 292 thousand IVP embryos were transferred world-wide [18], 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 [40].*In vitro* produced embryos were used to facilitate breeding of transgenic bulls. Frequency of transgene transmission varied from 3% to 54% between bulls [41]. However, the practical use of IVEP is limited by high production costs and the low overall efficiency under field conditions.

6. 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 ofEmbryo 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 [42] 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 was performed worldwide, mainly in dairy cattle, with 62% being transferred in North America and Europe, 16% in South America and 11% in Asia [43]. 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 [44]. According to International Embryo Transfer Society Data Retrieval Committee, 8 lakh embryos in cattle [45], 25 thousand in sheep, 7 thousands in goat, 30 thousand in pig and 12 thousand in horses [33] were transferred worldwide (two thirds as in vivo derived embryos and one third as *in vitro* produced) with 55-70% conception rate [45]. Approximately 61% of embryo transfer work in the USA continues to involve beef cattle [46]. Recently, Breeding farm in Himachal Pradesh the birth male calves named “Gaurav” (2010) and “Saurabh” (2011) [47,48] and two female calves “Ganga” and “Jamuna” in 2012 at livestock farm [49], Kotlabarog, H.P. World’s first ever Mithun calf through embryo transfer technology was born at the National Research Centre [50] on Mithun, Jharnapani, Nagaland.

7. CLONING

The cloned animal is an exact photocopy in every way of its parent; it has the same exact DNA [1]. 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 [51]. First animal obtained by somatic cloning was a sheep, “Dolly” [52], using a cultured adult somatic cell with an enucleated oocyte. Since then, SCNT was used successfully for cloning cattle [53], goat [54], pig [55], and horse [56]. Microinjection of DNA into the pronuclei of recently fertilised 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 the conservation of the available genetic diversity. list of a 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 [57] hasnamed 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.

8. 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 [58] pig [59], goat [60], cattle [53] and sheep (Simon et al*.,* 1998) were produced. The use of recombinant DNA techniques is to introduce new characters (ie. 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 antitrypsin in milk (Kues and Niemann 2004). An important achievement was the 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 the production of environment-friendly transgenic individuals which are used to understand various

physiological processes in farm animals and humans (Niemann et al., 2005).

9. STEM CELL TECHNOLOGY

Stem cells are having various applications like a model for developmental biology, gene therapy, organ transplantation, drug development, chimera production and in the field of regenerative medicines [61]. Its application in large animal models in which the embryo stem cell technology can be tested for tissue-specific differentiation [62] and cell therapy of various tissues and organs. Attempts have been made to establish embryo stem cell lines from mammals like rat [63], pig [64], mink [65], bovine [66], equine [67], sheep [68], rabbit [69], and rhesus monkey [70]. Therefore, identification of reliablemarkers 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 was 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 [71,72]. The successful transplant of testicular tissue containing spermatogonial stem cells (SSCs) used in goat and pig is readily adapted in cattle [73,74]. By transplanting SSCs from elite bulls into lesser bulls followed by natural service, elite genetics could be disseminated more widely [75]. 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 [76]. Herrid et al., [75] demonstrated that male germ cell transplantation between the unrelated bull calves and between cattle breeds could also be successful.

10. SEXING OF SEMEN

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 by using Loop-Mediated Isothermal Amplification (LAMP) reaction [77]. 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 [78]. The bovine Y-chromosome specific sequences are conserved amongst buffalo, Indian zebu and Taurus cattle [79]. 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 [80]. 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 [81] using fresh and frozen-thawed spermatozoa in several mammalian species: cattle [82], goat [83], pigs [84] sheep [85]. The sex sorting process by flow cytometry is the most efficient method to separate X from Yspermatozoa in a large scale [81,86,87]. Advances in semen sex sorting have enabled incorporation of this technology into commercial operations [88,89]. 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 [90] Schenk 2009; Larson et al., [91]; Sales et al., [92]; Soares et al., [93]; Sa Filho et al., [94] Seidel,[87]. Semen and embryo sexing have not been reported in the field in any of the developing countries, except China.Sa Filho et al*.,* [94] showed that overall P/AI rates were reduced with sex-sorted sperm compared with non sex-sorted sperm. Seidel and Schenk [95] 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 with sex-sorted sperm by literature, most part of the researches with heifers indicates that conception rate after AI upon oestrous 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.

11. NANOTECHNOLOGY

Nanotechnology is a recent advancement in cellular and molecular biotechnology. Nanotechnology applies the nanoscale principles and techniques to understand and transform biosystems (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 [96]. 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 [97]. It is a useful technique in farm animal breeding and reproduction. Microfluidic and nanofluidic [98,99] are recent tools to simplify traditional procedures of *in vitro* fertilization (IVF) and *in vitro* embryo production [100]. Oocyte manipulation under *in vitro* condition can also become feasible with the advent of this technique [101]. Glasgow et al., [102] first established the manipulation and movement of an embryo in a microfluidic environment. Also, be used in the sorting of sperm and eggs. In farm animal breeding heat detection can be done by implanting a nanotube [103] under the skin to detect the changes in the level of estradiol in the blood. Braydich-Stolle et al., [104] 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 [105]. Some other reports show the support of nanoparticles in disease diagnosis [106,107,108,109]. Nucleic acid engineering-based probes and methods offer powerful new ways to deliver therapeutic or preventative treatment for particular diseases [110]. Illumination of the body with infrared light raises the cell temperature to about 55°C, which 'burns' and kills a tumour [111]. Nanotechnology is employed in the treatment of African animal trypanosomosis [112]. 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 [97]. The immunological properties of a novel nano-bead adjuvant in a sheep (large-animal) model were investigated [113].

12. CONCLUSION

There is a gradual revolution in assisted reproductive techniques after the introduction of artificial insemination 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 some extent for use of superior females.Now a day's cloning, transgenesis and sexed semen technology give a new hope through the production of animal, which has 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 standardise these techniques for wider application for the welfare of mankind.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Balaji N. Sri, Chakravarthi P. Vikrama. Use of assisted reproductive technologies for Livestock Development Veterinary World. 2010;3(5):238-240.
2. Bertolini M, Bertolini LR. Advances in reproductive technologies in cattle: From artificial insemination to cloning. Rev. Med. Vet. Zoot. 2009;56:184-194.
3. Spallanzani L. Dissertations relative to the natural history of animals and vegetables. Trans. by T. Beddoes in Dissertations Relative to the Natural History of Animals and Vegetables. J. Murray, London. 1784; 2:195-199.
4. Ivanoff EI. On the use of artificial insemination for zootechnical purposes in Russia. Agric. Sci. 1922;12:244-256.
5. Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature. 1949;164:666.
6. Lohuis MM. Potential benefits of bovine embryo-manipulation technologies to genetic improvement programs. Theriogenology. 1995;43:51-60.
7. Verma OP, Kumar R, Kumar A, Chand S. Assisted reproductive techniques in farm animal – from artificial insemination to nanobiotechnology. Vet. World. 2012;5(5): 301-310.

8. Boa-Amponsem K, Minozzi G. The state of development of biotechnologies as they relate to the management of animal genetic resources and their potential application in developing countries. Background Study Paper. 2006;33.
9. Annual report, (2012-2013). Department of Animal Husbandry, Dairying & Fisheries Ministry of Agriculture, Government of India, New Delhi.
10. Baruselli PS, Sales JN, Sala RV, Vieira LM, Sa Filho MF. History, evolution and perspectives of timedartificial insemination programs in Brazil. Anim Reprod. 2012; 9:139-152.
11. Gajda B, Smor¹g Z. Oocytes and embryos cryopreservation-state of art and recent development in domestic animals. Journal of Animal and Feed Sciences. 2009;18: 371-387.
12. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at -196 0C by vitrification. Nature. 1985;313: 573-575.
13. Vajta G, Holm P, Greve T, Callesen H. Overall efficiency of *in vitro* embryo production and vitrification in cattle. Theriogenology. 1996;45:683-689.
14. Hochi S, Ito K, Hirabayashi M, Ueda M, Kimura K, Hanada A. Effect of nuclear stages during IVM on the survival of vitrified- warmed bovine oocytes. Theriogenology. 2000;49:787-796.
15. Hurt AE, Landim-Alvarenga GE, Siedel JR, Squires EL. Vitrification of immature and mature equine and bovine oocytes in ethylene glycol, ficoll and sucrose solution using open-pulled straws. Theriogenology. 2000;54:119-128.
16. Huang WT, Holtz W. Effect of meiotic stages, cryoprotetants, cooling and vitrification on cryopreservation of porcine oocytes. Asian Aust J. Anim. Sci. 2002;15: 485-493.
17. Sharma G. Taru, Loganathasamy K. Effect of meiotic stages during *in vitro* maturation on the survival of vitrified-thawed buffalo oocytes. Veterinary Research Communication. 2006;1-13.
18. Stroud B. The year 2009 worldwide statistics of embryo transfer in domestic farm animals. IETS Newsletter. 2009; 48:11-21.
19. Camus A, Clairaz P, Ersham A, Van Kappel AL, Savic G, Staub C. Principe de la vitrification: Cine´tiques comparatives. The comparison of the process of five different vitrification devices. Gynecologie, Obstetrique & Fertilite. 2006;34:737-745.
20. Muthukumar K, Mangalaraj AM, Kamath MS, George K. Blastocyst cryopreservation: Vitrification or slow freeze. Fertility and Sterility. 2008;90:426-427.
21. Sun X, Li Z, Yi Y, Chen J, Leno GH, Engelhardt JF. Efficient term development of vitrified ferret embryos using a novel pipette chamber technique. Biology of Reproduction. 2008;79:832-840.
22. Petyim S, Makemahar O, Kunathikom S, Choavaratana R, Laokirkkiat P, Penparkkul K. The successful pregnancy and birth of a healthy baby after human blastocyst vitrification using Cryo-E, first case in Siriraj Hospital. Journal of the Medical Association of Thailand. 2009;92:1116-1121.
23. Tsang WH, Chow KL. Mouse embryo cryopreservation utilizing a novel high-capacity vitrification spatula. Bio. Techniques. 2009;46:550-552.
24. Yavin S, Aroyo A, Roth Z, Arav A. Embryo cryopreservation in the presence of low concentration of vitrification solution with sealed pulled straws in liquid nitrogen slush. Human Reproduction. 2009;24:797-804.
25. Larman MG, Gardner DK. Vitrifying mouse oocytes and embryos with super-cooled air. Human Reproduction. 2010;25: 265.
26. Portmann M, Nagy ZP, Behr B. Evaluation of blastocyst survival following vitrification/warming using two different closed carrier systems. Human Reproduction. 2010;25:i261.
27. Sugiyama R, Nakagawa K, Shirai A, Sugiyama R, Nishi Y, Kuribayashi Y, Inoue M. Clinical outcomes resulting from the transfer of vitrified human embryos using a new device for cryopreservation (plastic blade). Journal of Assisted Reproduction and Genetics. 2010;27:161-167.
28. Smith C. Theriogenology. 1988a;29:203-212.
29. Smith C. World Anim. Rev. 1988b;65:2-10.
30. Bondoc OL. *Anim. Breed. Abst*. 1989;57: 819-82.
31. Mapletoft RJ, Hasler JF. Assisted reproductive technologies in cattle: A review. Rev Sci Tech. 2005;24:393-403.
32. Lonergan P. State-of-the-art embryo technologies in cattle. Soc Reprod Fertil Suppl. 2007;64:315-325.

33. Thibier M. Data retrieval committee annual report: Transfer of both in vivo derived and *in vitro* produced embryos in cattle still on the rise and contrasted trends in other species in 2005. Embryo Transfer Newsletter. 2006;24:12-18.
34. Chakravarthi PV, Sri Balaji N. Use of assisted reproductive technologies for livestock development. Veterinary World. 2010;3(5).
35. Galli C, Duchi R, Crotti G, Turini P, Ponderato N, Colleoni S. Bovine embryo technologies. Theriogenology. 2003;59: 599-616.
36. Presicce GA, Xu J, Gong GC, Moreno JF, Chaubal S, Xue F, Bella A, Senatore EM, Yang XZ, Tian XC, Du FL. Oocyte source and hormonal stimulation for *in vitro* fertilization using sexed spermatozoa in cattle. Vet Med Int. Published online 2010 September 5; 2011.
37. Thibault C. Comptes Rendus de la Societe de biologie. 1954;9-10:789-790.
38. Niemann H, Wrenzycki C. Alterations of expression of developmentally important genes in preimplantation bovine embryos by *in vitro* culture conditions: Implications for subsequent development. Theriogenology. 2000;53:21-34.
39. Galli C, Lazzari G. The manipulation of gametes and embryos in farm animals. Reprod. In Domestic Animals. 2008;43:1-7.
40. Sirard MA, Richard F, Blondin P, Robert C. Contribution of the oocyte to embryo quality. Theriogenology. 2006;65:126-136.
41. Eyestone WC. Production and breeding of transgenic cattle using *in vitro* embryo production technology. Theriogenology. 1999;51:509–517.
42. Seidel GE. (Jr.). Superovulation and embryo transfer in cattle. Science. 1981; 211:351-358.
43. Madan ML. Animal biotechnology: applications and economic implications in developing countries. Rev. Sci. Tech. Off. Int. Epiz. 2005;24(1):127-139.
44. Konig S, Bosselmann F, Borstel UUvo., Simianer H. Genetic analysis of traits affecting the success of embryo transfer in dairy cattle. Journal of Dairy Science. 2007;90:3945-3954.
45. Thibier M. The worldwide statistics of embryo transfer in farm animals. Embryo Transfer Newsletter. 2009;27(4): 13-19.
46. Stroud B. The year 2011 worldwide statistics of embryo transfer in domestic farm animals. IETS Newslet. 2012;50:16-25.
47. Cattle Breeding Farm, Bagthan, Himachal Pradesh. 2010 and 2011.
48. Director of Animal Husbandry, Cattle Breeding Farm, Bagthan, Himachal Pradesh; 2012
49. Livestock farm, Kotlabarog, Himanchal Pradesh; 2012.
50. National Research Centre on Mithun, Jharnapani, Nagaland; 2012.
51. Das SK, Majumdar AC, Sharma GT. *In vitro* development of reconstructed goat oocyte after somatic cell nuclear transfer with foetal fibroblast cells. Small Rumin. Res. 2003;48:217-225.
52. Wilmut I, Schnieke AE, McWhir J. Viable offspring derived from foetal and adult mammalian cells. Nature. 1997;385:3. DOI: 10.1038/385810a0
53. Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, Ponce De Leon FA, Robl JM; 1998.
54. Baguisi A, Behboodi E, Melican DT, Pollock JS, Destrempes MM, Cammuso C. Production of goats by somatic cell nuclear transfer. Nature Biotechnology. 1999;17: 456-61.
55. Polejaeva IA, Chen SH, Vaught TD, Page RL, Mullins J, Ball S, Dai Y, Boone J, Walker S, Ayares DL, Colman A, Campbell KH. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature. 2000;407:86-90.
56. Galli C, Lagutina I, Crotti G, Colleoni S, Turini P, Ponderato N, Duchi R, Lazzari G. Pregnancy: A cloned horse born to its dam twin. Nature. 2003;424:635-636.
57. National Dairy Research Institute, Karnal, India (NDRI). June 2009. Retrieved 2010-05-18.
58. Gurdon JW, Ruddle FH. Integration and stable germline transmission genes injected into mouse pronuclei. Science. 1981;214:1244-1246.
59. Hammer RE, Pursel VG, Rexroad C, Wall RJ, Bolt DJ, Ebert KM, Palmiter RD, Brinster RL. Production of transgenic rabbits, sheep and pigs by microinjection. Nature. 1985;315:680-683.
60. Ebert KM, Selgrath JP, Di Tullio P, Smith TE, Memon MA, Vitole JA, Gordan K. Transgenic production of variant human tissue type plasminogen activator in goat milk: Generation of transgenic goat and analysis of expression. Biotechnology. 1991;9:835-838.

61. Bajada S, Mazakova I, Richardson BJ, Ashammakhi N. Updates on stem cells and their applications in regenerative medicine. Tissue Eng. Regen. Med. 2008;2:169-183.
62. Brown BD, Gentner B, Cantore A, Colleoni S, Amendola M, Zingale A, et al. Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. Nat. Biotechnol. 2007; 25:1457-1467.
63. Iannaccone PM, Tabom GU, Garton RL, Caplice MD, Brenin DR. Pluropotent embryonic stem cell from the rat are capable of producing chimeras. Dev Bid. 1994;163:288-292.
64. Wheelar MB. Development and validation of swine embryonic stem cell: A review. ReprodFertili Dev. 1994;6:563-568.
65. Sukoyan MA, Votalin SY, Golubitsa AN, Zhelezova AI, Semenova LA, et al. Embryonic stem cell derived from morula, inner cell mass and blastocyst of mink, Comparison of their pluripotencies, Mol Repro Dev. 1993;36:148-158.
66. Strelchenko N. Bovine pluripotent stem cells. Theriogenology. 1996;45:131-140.
67. Saito S, Ugai H Sawai K, Yamamoto Y, Minamihashi A, et al. Isolation of embryonic stem- like cells from equine blastocycts and their differentiation *in vitro*. FEBS Lett. 2002;531:389-396.
68. Notarianni E, Galli C, Lauric S, Moor RM, Evans MJ. Derivation of pluripotent, embryonic cell lines from pig and sheep. J Reprod Feriil. 1991;43:255-260.
69. Graves KH, Moreadith RW. Derivation and characterisation of putative pluripotential embryonic stem cells from preimplantation rabbit embryo. Mol, Reprod Dev. 1993;36: 424-433.
70. Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, et al, Isolation of aprimate embryonic stem cell line Proc Natl Acadsci USA. 1995;92:7844-7848.
71. Hwang WS, Ryu YJ, Park JH, Park ES, Lee EG, Koo JM, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. Science. 2004;303:1669-1674.
72. Klimanskaya I, Chung Y, Beckers S, Lu SJ, Lanza R. Human embryonic stem cell lines derived from single blastomeres. Nature. 2006;444:481-485.
73. Honaramooz A, Behboodi E, Blash S, Megee SO, Dobrinski I. Germ cell transplantation in goat. Mol. Reprod. Devel. 2003;64:422-428.
74. Joerg H, Janett F, Muller S, Graphodatskaya D, Suwattana D, Asai M, Sranzinger G. Germ cell transplantation in a klienefelter bull. Biol. of Reprod. 2003; 69:1940-1944.
75. Herrid M, Vignarajan S, Davey R, Dobrinski I, Jonathan RH. Successful transplantation of cells into hetrologous recipient. Reproduction. 2006;132:617-624.
76. Hill JR, Dobrinski I. Male germ cells transplantation in livestock. Reprod. Fertility and Dev. 2006;18:13-18.
77. Zoheir KMA, Allam AA. A rapid method for sexing the bovine embryo. Anim. Repro. Sci. 2010;119:92-96.
78. Garner DL. Flow cytometric sexing of mammalian sperm. Theriogenology. 2006; 65:943-957.
79. Apparao KBC, Pawshe CH, Totey SM. Sex determination of *in vitro* developedbuffalo (*Bubalus bubalis*) embryos by DNA amplification. Molecular Reproduction and Slowdevelopment. 1993;36:291-296.
80. Lopatarova M, Cech S, Krontorad P, Holy L, Hlavicova J, Dolezel R. Sex determination in bisected bovine embryos and conception rate after the transfer of female demi-embryos. Vet. Med. 2008;53: 295-603.
81. Garner D, Seidel GJ. History of commercializing sexed semen for cattle. Therio-Genology. 2008;69:886-95.
82. Seidel GJ, Schenk J, Herickhoff L, Doyle S, Brink Z. Insemination of heifers with sexed sperm. Theriogenology. 1999;52: 1407-20.
83. Parrilla I, Vazquez J, Roca J, Martinez E. Flow cytometry identification of X- and Y-chromosome-bearing goat spermatozoa. Reprod. Domest. Anim. 2004;39:58-60.
84. Grossfeld R, Klinc P, Sieg B, Rath D. Production of piglets with sexed semen employing a non-surgical insemination technique. Theriogenology. 2005;63:2269-77.
85. De Graaf S, Evans G, Maxwell W, Cran D, O'Brien J. Birth of offspring of pre-determined sex after artificial insemination of frozen thawed, sex-sorted and re-frozen-thawed ram spermatozoa. Theriogenology. 2007;67:391-398.
86. Rath D, Barcikowski S, De Graaf S, Garrels W, Grossfeld R, Klein S, Knabe W, Knorr C, Kues W, Meyer H, Michl J,

Moench-Tegeder G, Rehbock C, Taylor U, Washausen S. Sex selection of spermin farm animals: Status report and developmentalprospects. Reproduction. 2013;145:R15-R30.
87. Seidel GE Jr. Update on sexed semen technology in cattle. Animal. 2014;8(suppl 1):160-164.
88. De Vries A, Overton M, Fetrow J, Leslie K, Eicker S, Rogers G. Exploring the impact of sexed semenon the structure of the dairy industry. J Dairy Sci. 2008;91:847-856.
89. Norman HD, Hutchison JL, Miller RH. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J Dairy Sci. 2010;93:3880-3890.
90. Schenk JL, Suh TK, Seidel GE Jr. Embryo production from superovulated cattle following insemination of sexed sperm. Theriogenology. 2006;65:299-307.
91. Larson JE, Lamb GC, Funnell BJ, Bird S, Martins A, Rodgers JC. Embryo production insuperovulated Angus cows inseminated four times withsexed-sorted or conventional, frozen-thawed semen. Theriogenology. 2010;73:698-703.
92. Sales JNS, Neves KaL, Souza AH, Crepaldi GA, Sala RV, Fosado M, Campos Filho EP, Faria M, Sa Filho MF, Baruselli PS. Timing of insemination andfertility in dairy and beef cattle receiving timed artificialinsemination using sex-sorted sperm. Theriogenology. 2011;76:427-435.
93. Soares JG, Martins CM, Carvalho NaT, Nicacio AC, Abreu-Silva AL, Campos Filho EP, Torres Júnior JRS, Sá Filho MF, Baruselli PS. Timing ofinsemination using sex-sorted sperm in embryoproduction with *Bos indicus* and *Bos taurus* superovulated donors. Anim Reprod Sci. 2011;127:148-153.
94. Sa Filho MF, Girotto R, Abe EK, Penteado L, Campos Filho EP, Moreno JF, Sala RV, Nichi M, Baruselli PS. Optimizing the use of sex-sortedsperm in timed artificial insemination programs forsuckled beef cows. J Anim Sci. 2012;90:1816-1823.
95. Seidel GE, Schenk JL. Pregnancy rates in cattle with cryopreserved sexed sperm: Effects of sperm numbers per inseminate and site of sperm deposition. Animal Reproduction Science. 2008;105(1-2): 129-38.
96. Num SM, Useh NM. Nanotechnology applications in veterinary diagnostics and therapeutics. Sokoto Journal of Veterinary Sciences. 2013;11(Number 2).
97. Chauhan RS, Sharma G, Rana JMS. Nanotechnology in health and disease. Bytes and Bytes, Bareilly, UP, India. 2010; 1-11.
98. Schuster T, Cho B, Keller L, Takayama S, Smith G. Isolation of motile sperm from semen samples using microfluidics. Reprod. Biomed. 2003;7:73-79.
99. Eijkel TCJ, Berg DVA. Nanofluidics: what is it and what can we expect from it? Microfluid Nanofluid. 2005;1:249-267.
100. Suh R, Phadke N, Ohl D, Takayama S, Smith G. *In vitro* fertilization within microchannels requires lower total numbers and lower concentrations of spermatozoa. Hum. Reprod. 2006;21:477-483.
101. Beebe D, Wheeler M, Zeringue H, Walters E, Raty S. Microfluidic technology for assisted reproduction. Theriogenology. 2002;57:125-135.
102. Glasgow I, Zeringue H, Beebe D, Choi S, Lyman J, Chan N. Handling individual mammalian embryos using microfluidics. IEEE Trans. Biomed. Eng. 2001;48:570-578.
103. O'Connell MJ, Bachilo MS, Huffman BC, Moore CV, Strano SM, Smalley RE. Band gap fluorescence from individual single-walled carbon nanotubes. Science. 2002; 297:593-596.
104. Braydich-Stolle L, Hussein S, Schlager JJ, Hofmann MC. *In vitro* cytotoxicity of nanoparticles in mammalian germ-line stem cells. Toxicological Science. 2005; 88:412-419.
105. Tripp RA, Alvarez R, Anderson B, Jones L, Weeks C, Chen W. Bioconjugated nanoparticle detection of respiratory syncytial virus infection. International Journal of Nanomedicine. 2007;2(1):117-124.
106. Na HB, Song IC, Hyeon T. Inorganic nanoparticles for magnetic resonance imaging (MRI) contrast agents. Advanced Materials. 2009;21(21):2133-2148.
107. Jackson P, Periasamy S, Bansal V, Geso M. Evaluation of the effects of gold nanoparticle shape and size on contrast enhancement in radiological imaging. Australas Physics, Engineering, Science and Medicine. 2011;34(2):243-249.
108. Schlachter EK, Widmer HR, Bregy A, Lonnfors-Weitzel T, Vajtai I, Corazza N, Bernau VJ, Weitzel T, Mordasini P,

Slotboom J, Herrmann G, Bogni S, Hofmann H, Frenz M, Reinert M. Metabolic pathway and distribution of superparamagnetic iron oxide nanoparticles: *In vivo* study. International Journal of Nanomedicine. 2011;6:1793-1800.

109. Huang J, Zhong X, Wang L, Yang L, Mao H. Improving the magnetic resonance imaging contrast and detection methods with engineered magnetic nanoparticles. Theranostics. 2012;2(1):86-102.
110. Luo D. Yearbook of science and technology. McGraw-Hill, New York. 2003; 93-95.
111. Hirsch LR, Stafford RJ, Bankson JA. Proc. Natl Acad. Sci. USA. 2003;100(23):13549-13554.
112. Kroubi M, Daulouede S, Karembe H, Jallouli Y, Howsam M, Mossalayi D, Vincendeau P, Betbeder D. Development of a nanoparticulate formulation of diminazene to treat African trypanosomiasis. Nanotechnology. 2010; 21(50):1-8.
113. Scheerlinck JPY, Gloster S, Gamvrellis A, Mottram PL, Plebanski M. Systemic immune responses in sheep, induced by a novel nano-bead adjuvant. Vaccine. 2006; 24(8):1124-1131.