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Totipotent Stem Cells Scope and Limitation: A Review

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Last century has witnessed a tremendous progress in emergence of the different embryo biotechnologies for the faster reproduction and genetic improvement of livestock. Latest among them is use of stem cells as a nuclei donor for cloning. Stem cells would be of great value for large scale cloning. Stem cells are unspecialized cell with remarkable ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. The era of stem cells starts with findings by Canadian scientists Ernest A. McCulloch and James E. Till in the 1960s. Based on the potential to differentiate into different cell types, stem cell are of different types like totipotent, pluripotent, multipotent and unipotent. Totipotent (also known as omnipotent) stem cells can differentiate into embryonic and extraembryonic cell types i.e. such cells can construct a complete, viable, organism. Zygote as well as Cells produced by the first few divisions of the fertilized egg is considered as totipotent. Recently use of embryonic stem cells proves its worth in genetic improvement of livestock and as advanced breeding technology.

Artificial insemination (AI) is the most effective technique currently being used for the genetic improvement of animals. This technology enables the widespread use of outstanding sires with valuable genetic make up for breeding up gradation programme in livestock. But in this technology only the genetic quality of superior male is being used and takes a long time for genetic up gradation. Embryo transfer technology (ETT) offers the use of both male and female genetic quality to be propagated effectively to produce suitable offspring with important economic traits .In this technology the females of superior genetic merit are super ovulated, their eggs fertilized in vivo using semen from genetically outstanding male, then the resultant embryos are transferred to synchronized recipient mothers. The main objective of ET is the improvement of animal population through maximum utilization of superior females in contrast to AI where maximum utilization of male was the prime concern. The main limiting factor in application of embryo transfer technology is the unpredictable ovarian response and embryo recovery. Difficulty in synchronization of follicular wave emergence and superovulatory treatment limits the ovarian response and embryo yield. Thus the economical feasibility of the application for the technology in field condition becomes less viable due to fewer yields of transferable embryos.

In vitro embryo production (IVP) can be made either from the ovaries of

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slaughtered donors on large number or from living animals using ovum pick up (OPU). OPU is a method of collection of oocyte from live animal with help of ultrasound scanner with a transvaginal probe and needle guidance system and a suction pump. OPU offers most flexible and repeatable technique to produce embryos form any given live donor. Drawback of OPU is higher cost compared to MOET, Lower number of oocyte availability and drastic fall of follicle number and the requirement of specialized laboratory equipments to perform all the steps of embryo production. From a particular breed, oocytes may be collected from ovaries and matured, fertilized and cultured in vitro to propagate a particular breed following embryo transfer.

Cloning can be used to multiply animals of a high genetic merit. Embryo splitting, and cloning through nucleus transplantation to egg cells or embryo cells whose genetic material has been removed (nucleus transfer) are the two artificial cloning methods. Embryo splitting changes neither the age nor the totipotency of the cells used. The (two) embryos from the splitting are in the same stage of development, exactly the same age as the undivided embryo would have been and genetically complete identical. The nuclear transfer technique of cloning takes a different approach by transferring the genetic program (the cell nucleus with the desired genetic material) from a totipotent blastomer or no longer totipotent cell (embryonic, foetal or even a differentiated body cell) to an unfertilised egg cell whose nucleus has previously been removed. This technique basically offers the possibility of replicating an adult individual and their genetic program. The result is a new individual whose existence does not derive from the fertilisation of an egg cell by a sperm cell. Various cell types such as embryonic cells, fibroblasts, mammary glands, cumulus cells, oviductal cells, leucocytes, granulose cells, germ cells and liver cells have been used as donor for production of cloned animals (Brem and Kuhholzer, 2002).

The major breakthrough in mammalian nuclear transfer occurred by a modified technique of Willadsen (1986) who produced full term lambs from the transplanted nuclei of blastomeres from 8-16 cell staged embryos. By the time electrofusion technique was developed (Prather *et al.,* 1987) reported the production of two calves from the implantation of early cleaved bovine blastomere into enucleated oocyte. For cloning in cattle, initially embryonic blastomers were used as donor nuclei (Bondioli 1993) as these were thought to be relatively undifferentiated, readily programmed and likely to support full term development of the fetus. Several reports of production of offspring from blastomere cloning are available (Bondioli *et al.,* 1990; Johnson *et al.,* 1995; Willadsen and Polgen, 1981).

Development of nuclear transfer technology to produce clone animals after the birth of Dolly (Wilmut *et al.,* 1997) opened a new era in the field of reproductive biology. Successful somatic cell cloning has now been achieved in several mammalian species as reported by birth of offspring in sheep, cattle, goats, pigs and mice (Wilmut *et al* 1997, Kato *et al.,* 1998.) respectively. Cloning based on nuclear transfer has not always succeeded or been successful in the long term. Many of the embryos created in this way die, not infrequently shortly before or after birth. However, the surviving animals also frequently have "deficits" which hamper their development and have a deleterious effect on their health.

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Further expanding the potential of cloning was the development and use of embryonic stem cell as a source of donor nuclei. Embryonic stem cells are derived from the inner cell mass of an yo at blastocyst stage and are thought to be relatively undifferentiated. Ramirez-soils et al (1993) reported that mouse embryonic stem cell divided indefinitely in culture without differentiation could be modified genetically.

The establishment of embryonic stem cell (ES) lines was achieved in mice from the late stage inert cell mass (ICM) of mouse blastocyst (Evans and Kaufman, 1981) or morula (Eistetter, 1989) and it would be of great value for large scale cloning. Chereny *et al* (1993) developed strategies for isolation and characterization of bovine embryonic stem cells and concluded that primordial germ cells may provide an alternative avenue for the generations of pluripotent cell lines from domestic animals. Bovine embryonic cell cultures have been established from ICM cells, morulae and precompaction 16-20 cell stage embryo (First *et al* .,1994) .Sims and First (1994) reported the birth of four calves from the use of 16-27 day loose suspension cultured bovine ICM cells in nuclear transfer.

Embryonic stem cells have been developed in bovine (Saito *et al.*, 1992) and have been used as a source of donor nuclei. There are evidence of stem cells contributing to somatic tissues of chimeras in pigs (Wheeler *et al.*, 1994; Shim *et al*., 1997) and cattle (Cibelli *et al*., 1997). A number of cloned animals have been produced from the blastomere cells ,inner cell mass and stem cells after culture (Heyman and Renard,1996; Campbell *et al*., 1996). It has been observed that stem cell as donor nuclei produce larger number of embryos as well as clones compared to somatic cells.

There is assumption that there is a role of stem cell of particular tissue in the somatic cell cloning by the use of that particular type of cell. The somatic cell of fetal origin provides better result in nuclear transfer than adult somatic cell. Serum starved fibroblasts cells are more efficient as donor than serum unstarved fibroblasts cell. There is more chance of stem cell fusion in the serum starved group as stem cell number increase in this group (Majumdar and Bag 2006)

Stice *et al* (1996) working with blastomeres from morula, or the ICM of blastocyst further cultured disaggregated blastomere in plate containing mitomycin – C blocked fibroblast cells, isolated embryonic cell lines and through nuclear transfer procedures produced embryos that develop through early organogenesis.

The possibility of generating genetically identical offspring in livestock increased considerably from the first embryo splitting experiment (Willadsen, 1979) up to the results obtained after the birth of dolly (Wilmut *et al.*, 1997).Using nuclear transfer to multiply genetically superior livestock; however today in the field of biomedicine appears to be the first major commercial opportunity for cloning technology. Nuclear transfer technology can produce transgenic livestock faster and more efficiently than conventional microinjection technology.

For faster multiplication of superior germplasm, cloning is the best method .Successful somatic cell cloning has been achieved in several mammalian species .The overall efficiency of the technique ,however, remains low because only a very limited percentage (0.5-5%) of the reconstructed embryo results on full term development. This is due to high frequency of post implantation development arrest , which can occur after the transfer of blastocyst that appear to be morphologically normal in mice (Wakayama and Yanagimachi, 1999) and in cattle (Kato *et*

al., 2000). These long lasting effects of cloning are associated with fibroid fetal membrane ,excessive accumulation of allantoic fluid and increased fetal birth weight (Hill *et al*., 1999). This syndrome is similar to large offspring syndrome (LOS), that causes dystocia which has been reported in sheep and cattle (Kurip and Dass, 1997). Frequency and occurrence of late gestation losses from cloned embryos of cattle were comparatively studied for adult somatic clone, fetal somatic clones ,embryo cloning and in vitro fertilization (IVF) by Heyman *et al* (2002). They reported incidence of loss between days 90 of gestation and calving was 43.7% for adult somatic clones and 33.3% for fetal somatic clones compared with 4.3% after embryo cloning and 0% in the control IVF group. Prior to these Heyman *et al* (1997) observed that the incidence of late abortion was relatively low in (10%) after embryonic cloning and that LOS was limited to 3% of the calves born.

Somatic cell cloning has been proved to be less efficient till now. It is expected that early embryonic cells derived from early stage embryo are more efficient nuclear donor for production of cloned animals. Blastomeres isolated from embryo derived from best combination of sire and dam are the best source to be used as donor in cloning .Further there will be less loss due to abortion and having hardly any large offspring syndrome. But the blastomere cells are limited in number to be used as donor. Embryonic stem cell derived by the culture of blastomere cells from precompact morulae are of having similar characteristics as blastomere cells. Therefore there is a need to develop large number of blastomere derived from stem cell from 16-32 cell morulae that further to be used as nuclear donor.

India is having large population of low yielder cattle and buffalo that not only consume large quantity of feed and fodder but also release more methane that is contributing a lot in global warming. Interestingly, very few rare cattle and buffalo have developed in different corner of the country that produce very high quantity of milk per lactation. These milch animals have not only of high genetic merit but also have adapted very well in the specific region so they are in less stress. We need to multiply these animals very fast with the latest reproductive techniques viz. Embryo cloning by nuclear transfer technique using the totipotent stem cells as nuclear donor.

The decisive factors in the economic efficiency and expediency of cloning for animal breeding in agriculture are the effectiveness of the cloning technique and the (breeding) value of the genetic material available for cloning. If techniques for cloning adult animals can be developed into a routine procedure, this would also have implications for animal production, whose extent would largely be decided by the cost of cloning. As long as the procedure is still very expensive, only isolated and extremely valuable, top-performance animals will be cloned, e.g. in the event of the loss (from age or disease) of the services of a very valuable breeding animal, it could be replaced by a clone of itself.

Totipotent stem cells provide an opportunity to reduce the cost of cloning and chance to get a high valued genetic material via In vitro fertilization and following stem cell culture of the morulae stage. Totipotent stem cell lines can be produced using techniques of hormonal stimulation of folliculogenesis, ultrasound guided oocyte collection, in vitro fertilization of these oocytes with adopted high milk mother bulls spermatozoa to form precompact morulae (8-16 cells) and then these blastomere would be cultured blocking genetic progression to stem cell lines from all the embryos produced from those few high milk producing cows and buffaloes. They could be sexed, cryopreserved while few embryos could be

made out of these stem cells as nuclear donor by electro fusion or blastomere packing techniques and transferred to recipient to get few calves born in different region of the country. Their production potential can be checked using micro array techniques or on 1st calving and those showed high production potential can be multiplied in large scale to replace the low producing. The rest of the stem cell could be cryopreserved for further use in embryo cloning. A group of high yielding animals should be used for making stem cell lines and embryo cloning. Embryos from all the animals should be propagated by transferring them in a restricted area stopping AI to get animals of different genetic make up both in male and female to keep the genetic variability. This technique of totipotent stem cell development and cryopreservation may help in quick up-gradation of different indigenous breeds of animals of the country.

Recently, embryo like structures could be developed from pluripotent stem cell culture in our laboratory. These embryos developed to blastocyst stage but having very few trophoblast cells and they are very smaller in size. A new media has been developed in this laboratory that can increase the cell size around ten times bigger than the original one. We feel embryo size can be increased by culturing the cells in this media followed by embryo production. Still the problems of fewer number of trophotblast cells may not be overcome using pluripotent stem cells. Replacement of pluripotent stem cells by totipotent stem cells may help in overcoming this problem. We therefore are now engaged in producing totipotent stem cells, their culture in new media developed to increase the cell size and further develop embryos in culture as above technique for transfer in live animals. This will open up a way where cumbersome procedure of micromanipulation could be bypassed, without the threat of gene pollution.

But cloned embryos developed by fusion of totipotent stem cells to enucleated oocytes or culture may have another problem. The production potential of mother may not reflect in the cloned animal as during fertilization reorganization of gene occur that may mask some important gene related to production. We therefore looking into the opportunity of using parthenogenetic cells as this may be done faster by making totipotent stem cell from diploid heterozygous parthenogenetic cell and further culturing of these totipotent cells to develop high producing animal. The normal parthenogenesis lead the formation of haploid cell but this could be manipulated to form diploid homozygous or heterozygous cells. But both homozygous as well haploid cells contain many lethal genes that could be activated at any point of their development which will culminate in the death of the cell. That is why parthenogentic animals normally conceive but do not give birth to offspring. Recently birth of few vertebrate animals has been reported parthenogenitically. It seems heterozygous parthenoids, if could be produced the problems of higher number of lethal genes activation could be decreased, leading to development of parthenogenetic animals as well as stem cells that will be similar in nature of normal blastomere developed stem cells, capable of giving birth of animals that will have similar production potential. In order to increase the production potential of the animals many times, to meet the requirement of the rapidly increasing human population, and same time to decrease the number of animals, the above described strategy could be a promising technology in the 21st century. The technique envisages the increase in the production potential of individual animal many times with the decrease in animal population, thus decreasing methane production with manifold increase of production solving the

feed and fodder problem facing by the country and world as a whole.

REFERENCES

Becker AJ, McCulloch EA and Till JE. 1963. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* **197**: 452–4.

Bondioli KR, Westhusin ME and Looney CR. 1990. Production of identical bovine offspring by nuclear transfer. *Theriogenology* **33**: 165-74.

Bondioli KR. 1993.Nuclear transfer in cattle. *Molecular Reprduction and Development* **36**: 274

Brem G and Kuhholzer B. 2002. The recent history of somatic cell cloning in mammals. *Cloning stem cells* **4**: 57-63

Campbell KHS, McWhir J, Ritche WA and Wilmut I. 1996. Sheep cloned by nuclear transfer from a cultured cell line. *Nature* **380**: 64-66

Cherney RA, Stokes TM, Brandon MR and Willams RL. 1993. Strategies for isolation and characterization of bovine embryonic stem cell. *Reproduction and Fertilization Development* **6**: 569-575.

Cibelli JB, Stice SL, Kane JJ, Golueke PJ, Jerry J, Dickinson ES, Gao XY, Ponce deLe, Dickinson H and Elves PM. 1997. Production of germ line chimeric bovine fetuses from transgenic embryonic stem cells. *Theriogenology* **47**: 241

Eistetter HR. 1989. Pluripotent embryonic stem cell lines can be established from mouse morulae. *Development Growth and Doffer* **31**: 275-282

Evans MJ and Kaufman MH. 1981). Establishment of culture of Pluripotent cells from mouse embryos. *Nature* **292:** 154-6.

First NL, Sims MM, Park SP and Kent-First MJ. 1994. System for Production of Calves from Cultured Bovine Embryonic cells. *Reproduction and Fertilization Development* **6**: 553-562.

Heyman Y, Chavatte-Palme P, LeBourhis D, Camos S, Vignon X and Renard JP. 2002. Frequency and occurrence of late gestation losses from cattle cloned embryos. *Biological Reproduction* **66**: 6-13

Heyman Y, Marchal J, Chesne P, LeBouhis D, Deniau F and Renard JP. 1997. Outcome of pregnancy after single or twin transfer of cloned embryos in cattle .In: proceedings of the 13th AETE meetings,Yon,France,156.

Heyman Y and Renard JP. 1996. Cloning of domestic species. *Animal Reproductive Science* **42**: 427-436

Hill JR, Roussel AJ, Cibelli JB, Edwards JF, Hooper NL, Miller MW, Thompson JA, Loomey CR, Westhusian ME, Robl JM and Stice SL. 1999. Clinical and pathological features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology* **51 :**1450-1465

Johnson WH, Laskutoff NM, Plante Y and Betteridge KJ. 1995. Production of four identical calves by the separation of blastomere from an in vitro derived four cell embryos. *Veterinary Research* **137:** 15-16

Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, Yasue H and Sunoda Y. 1998. Eight calves cloned from somatic cells of a single adult. *Science* **282**: 2095-2098

Kato Y, Tani T and Tsunoda Y. 2000. Cloning of calves from various somatic cell types of a male and female adult new born and fetal cows. *Journal of Reprodiction and Fertilization* **120**: 231-237

Kurip TAM and Deu Dass JHG. 1997. In vitro produced and cloned embryos:

effects on pregnancy parturition and offsprings. *Theriogenology* **47:** 43-52

Majumdar AC and Bag S. 2006. Application of Stem cells in health and production .In: Natl.Sym.on Technological interventions for livestock improvement and production, Feb.17-19, 2006, New Delhi.

Prather RS, Barnes FL, Sims ML, Robl JM, Eyestone WH and First NL. 1987. Nuclear transplantation in bovine embryo: assessment of donor nuclei and recipient oocyte. *Biological Reproduction* **43**: 292

Ramirez-soils R, Davis AC and Bradley A. 1993). *Method Enzymology* **225**: 855.

Saito S, Strelchenko N and Niemann H. 1992. Roux's Arch. *Developmental Biology* **201**: 134

Shim H, Gutierrez AA, Chen LR, BonDurant RH, Behboodi E and Anderson GB. 1997. Isolation of pluripotent stem cells from cultured porcine primodial germ cells. *Biological Reproduction* **57:** 1089-1095

Siminovitch L, McCulloch EA, Till JE. 1963. The distribution of colony-forming cells among spleen colonies. *Journal of Cellular and Comparative Physiology* **62**: 327–36

Sims MM and First NL. 1994. Production of fetuses form totipotent cultured bovine inner cell mass cells. *Proc Natural Academic Science USA* **90:** 6143-6147

Stice SL, Strelchenko NS, Keefer CL and Mathews L. 1996. Pluripotent bovine embryonic cell lines direct embryonic development following nuclear transfer. *Biological Reproduction* **54**: 100-110

Wakayama T and Yanagimachi R. 1999. Cloning of male mice adult tail tip cells. *Nature Genetics* **22:**127-128

Wheeler MB. 1994. Development and validation of Swine Embryonic Stem cells- A Review. *Reproduction and Fertilization Development* **6**: 563-568.

Willadsen SM. 1979. A method for culture of micro-manipulated sheep embryos and its use to produce monozygotic twins. *Nature* **227**: 298-300

Willadsen SM and Polge C. 1981. Attempts to produce monozygotic quadruplets in cattle by blastomere separation. *Veterinary Research* **108**: 211-213

Willadsen SM. 1986. Nuclear transplantations in sheep embryos. *Nature* **320**: 63-65

Wilmut I, Schnienke AE, Mc Whir J, Kind AJ and Campbell KHS. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature* **385:** 810-813