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Review Article

SOMATIC CELL CLONING TECHNIQUE FOR PRODUCTION OF CLONED ANIMALS AND ITS APPLICATION - A REVIEW

D. MALAKAR*, Y. S. AKSHEY, A. K. DE AND R. DUTTA

Animal Biotechnology Centre
National Dairy Research Institute
Karnal-132 001, India

Animal biotechnology is a very important sector in modern biotechnology. One of the important targets of animal biotechnology is to increase the quality and quantity of animals and animal products using different assisted reproductive technologies like somatic cell nuclear transfer, stem cell technology and transgenic animal production. Somatic cell nuclear transfer (SCNT) has tremendous applications in basic biological research, species conservation, medicine and agriculture. The drawback of traditional SCNT is that its efficiency is very low (1-5 %) due to some biological & technical reasons. Oocyte enucleation is very important and pain staking step in traditional nuclear transfer technique. Moreover, this technique requires very expensive equipments like micromanipulator and other accessory tools, skilled personnel and requires more time to perform. It decreases about 15-39 % cytoplasmic volume of oocyte which results in cloning syndrome or abnormalities in cloned animals. Hand-made cloning (HMC) is a new technique, simple to perform and is advantageous over traditional nuclear transfer technique. In this method, 100% cytoplasmic volume of oocyte can be maintained, which helps in proper reprogramming of donor cell nuclei and increases cloning efficiency. Hand-made cloning technique is elaborately discussed here for improving cloning efficiency in animals.

Key words: Embryos, Hand-made cloning, Oocytes, SCNT

The 21st century seems set to see a revolution in the application of biotechnological procedures to farm animals. The current technologies such as somatic cell nuclear transfer (SCNT) and culture of stem cells are used for faster multiplication of superior germplasm. SCNT technique is also used for transgenic animal production with gene of our interest transfected somatic cells as donor. Cloning of elite animals with proven genetic background is utilized for the faster multiplication within a short period of time. Cloning or asexual reproduction is a time-honored method of reproduction; by this method we can reproduce many existing and extinct species of organisms. The use of recent embryo technologies, a combination of classical reproduction, cellular and molecular biological and

**Corresponding Author*

genomic techniques for enhancing the livestock productivity or exploiting the potential of livestock is currently under investigation.

SCNT is a technique in which the nucleus of a somatic cell is transferred into an enucleated metaphase-II oocyte for the generation of a new individual, which are genetically identical to the somatic cell donor (Campbell *et al.*, 1996a and Wilmut *et al.*, 1997). It has many potential applications in research, biomedical and agriculture/animal sciences. Many factors have been shown to contribute to this low level of efficiency such as stage of donor cell cycle, donor cell activation, lab to lab variation as well as the oocyte source and quality at the beginning of maturation (Alm *et al.*, 2005). The starting material for SCNT is oocytes. The major source oocytes for SCNT experiments is slaughter house derived oocytes which are very heterogeneous in quality and developmental competence (Malakar and Majumdar 2002, 2005). Many factors have been shown to affect the developmental potential of oocytes, including follicle size, health of the follicle (Vassena *et al.*, 2003), phase of follicular wave (Machatkova *et al.*, 2004), season (Sartori *et al.*, 2002), nutrition (Fouladi-Nashta *et al.*, 2007) and age of animals (Rizos *et al.*, 2005). The stage of the donor cell cycle is another major factor in the success of nuclear transfer in mammals (Campbell *et al.*, 1996a and Wilmut *et al.*, 1997). Donor cells arrested in the G0 or G1 stage of the cell cycle have been used to produce several cloned animal species like mice (Wakayama *et al.*, 1998), pigs (Polejaeva *et al.*, 2000 and Li *et al.*, 2013), cattle (Wells *et al.*, 1999) and cloned goat embryos (Akshey *et al.*, 2010 and Rahul *et al.*, 2011)

Earlier nuclear transfer was not based on micromanipulation. The first nuclear transfer procedure in 1894 by Jacques Loeb in sea urchin was by accidental osmotic blebbing of the cytoplasm. Over the past two decades, more scientific publications dealing with somatic cell cloning referred to micromanipulation based enucleation and somatic cell nuclear transfer (Kawase *et al.*, 2001 and Kishigami *et al.*, 2016). Nuclear transfer remained the privilege of selected laboratories that could afford the considerable investment regarding both instrumentation and skills. The idea to perform enucleation by oriented or random manual bisection of oocytes for handmade cloning technique based on earlier method of bisection of embryo and blastocyst biopsy techniques (Bredbacka *et al.* 1995 and Malik *et al.*, 2013). This enucleation procedure played a major role and 28% of blastocysts per reconstructed embryo (Akshey *et al.*, 2008, 2011a) and close to 10% of cloned calves per transferred embryo rates were obtained (Tecirlioglu *et al.*, 2005)

The successful development and application of SCNT are critically dependent on oocyte maturation, fusion of donor somatic cell with enucleated oocytes and culture of cloned embryos. "Dolly'', the first successful cloned sheep obtained from a differentiated adult mammary epithelial cell has created a revolution in science (Wilmut *et al*., 1997). Then numerous modifications for production of cloned embryos have been investigated, including different donor cell types, altered cell cycle stages of the donor cells, variations in the maturation stage of the recipient oocyte/host cell, and alterations in fusion and activation protocols (Campbell *et al*., 1996b; Akshey *et al*., 2010b and Dutta *et al*., 2011). In spite of

Table 1. List of animals that have been successfully cloned (Akhshey, 2009)

Year	Species	Donor cell type	References
1986	Lambs	Blastomeres	Willadsen, 1986
1987	Cattle	Blastomeres	Prather *et al.*, 1987
1989	Pigs	Blastomeres	Prather *et al.*, 1989
1994	Calves	ICM cells	Sims and First, 1994
1996	Sheep	Embryo	Campell *et al.*, 1996
1997	Sheep	Fetal	Wilmut *et al.*, 1997
1997	Sheep (Dolly)	Adult	Wilmut *et al.*, 1997
1998	Cattle	Fetal	Cibelli *et al.*, 1998
1998	Cattle	Adult	Kato *et al.*, 1998
1998	Mouse	Adult	Wakayama *et al.*, 1998
1999	Mouse	Embryo	Wakayama *et al.*, 1999
1999	Goat	Fetal	Baguisi *et al.*, 1999
2000	Pig	Adult	Polejaeva *et al.*, 2000
2000	Gaur	Adult	Lanza *et al.*, 2000
2001	Mouflon	Adult	Loi *et al.*, 2001
2002	Rabbit	Adult	Chesne *et al.*, 2002
2002	Cat	Adult	Shin *et al.*, 2002
2002	Zebrafish	Embryo	Lee *et al.*, 2002
2003	Rat	Fetal	Zhou *et al.*, 2003
2003	Mule	Fetal	Woods *et al.*, 2003
2003	Horse	Adult	Galli *et al.*, 2003
2005	Dog	Adult	Lee *et al.*, 2005
2006	Ferret	Adult	Li *et al.*, 2006
2007	Buffalo	Fetal	Shi *et al.*, 2007

these modifications, cloned embryo and animal production with healthy in nature is still extremely low. Subsequently different cloned animals like cattle (Cibelli *et al.*, 1998), buffalo (Shi *et al*., 2007), goat (Baguisi *et al*., 1999), mouse (Wakayama *et al*., 1998), pig (Polejaeva *et al*., 2000 and Liu *et al*., 2015), cat (Shin *et al*., 2002), mule (Woods *et al*., 2003), horse (Galli *et al*., 2003), rat (Zhou *et al*., 2003) and dog (Oransky, 2005) have been produced. Now human embryos are also being produced for therapeutic purpose (Hwang *et al*., 2005) by way of somatic cell nuclear transfer. Recently scientist of NDRI, Karnal has produced more than 10 hand-made cloned buffalos (Shah *et al*., 2009 and George *et al*., 2011). The female cloned buffaloes were delivered calves and producing milk as normal buffaloes (Saha *et al*., 2013 and Singla *et al*., 2015)

Methods are being modified in each step of somatic cell cloning. In technical aspect, traditional somatic cell nuclear transfer are carried out by using different expensive equipments like micromanipulation system and accessories like pipette puller, microfuge machine, pipette grinder etc. Nowadays there is another technique named Hand-made cloning (HMC) or Zona free cloning technique in which no such above equipments are required (Vajta *et al*., 2003 and Akshey *et al*., 2011). In this enucleation technique of oocyte step is being modified by bisecting of Hoechst 33258 stain zona free oocyte after protease treatment. The optimal yield of cloned embryo production is 10 blastocysts one person per hour (Vajta *et al*., 2003) with minimum abnormalities in cloned animal. In case of goat, no such cloning abnormalities where found (Keefer, 2008 and Akshey *et al*., 2010, 2011).

Modification in technical steps of Hand-made cloning

Removal of zona pellucida from matured oocytes :

Zona pellucida is a hard glycoprotein covering mammalian oocytes. The thickness of zona pellucida varies from species to species like cattle, sheep, goat, pig, human and mouse. For the removal of zona pellucida, several methods have been tested, including mechanical opening, chemical lysis and enzymatic digestion (Wells and Powell, 2000 and Akshey *et al*., 2010, 2011). In the enzymatic digestion method, no individual oocyte treatment is required and the procedure can be performed in quantities of 100-200 oocytes (Vajta *et al*., 2004). The effect of pronase is affected by its concentration, exposure time and thickness of zona. In case of cattle, the effective concentration of pronase for complete digestion of zona was 2 mg/ml and the time required was 15-20 min (Vajta *et al*., 2001 and Akshey *et al*., 2010). In case of goat a little less time (7 min) was reported with 2 mg/ml pronase concentration to complete digestion of zona of oocyte (Akshey *et al*., 2008, 2011).

Enucleation of oocytes for nuclear transfer :

Enucleation of zona free oocytes can be performed either by micromanipulation or by Hand- made method. The micromanipulation based procedure may consist of partial aspiration of the oocyte into a glass pipette, then controlled or rapid movements to separate the aspirated and freely extruding parts (Peura, 2003). Another possibility is to use micro blades and to perform enucleation just like embryo bisection (Booth *et al*., 2001). Random bisection is usually performed by splitting the oocyte, then separating the chromatin free cytoplasts from the karyoplast with Hoechst staining and ultraviolet (UV) light. Oriented bisection requires some easily detectable orientation point that reliably shows the presence of nuclear DNA because, in cattle, pig and sheep, cytoplasmic lipid droplets hamper the visualization of the unstained chromatin. Some chemicals, which arrest mitotic spindle formation like demecholchicine, nocodazole, clyclohexamide etc or sucrose, were used for expulsion or visualization of M-II phase maternal chromosome from matured oocyte (Fulka *et al*., 2004). For zona free work, separation of polar body from the oocytes that occurs after pronase digestion can be prevented by pre incubation of metaphase II stage zona intact oocytes in biological glue, phytohemagglutinin, before pronase digestion. The polar body oriented manual enucleation of zona free oocytes results in 90% accuracy (Akshey *et al*., 2010b, 2011a).

Fusion of enucleated oocytes with donor somatic cell nucleus:

The fusion efficiency of hand-made method in cattle was found to be even higher 90% (Vajta *et al*., 2003 and Tecirlioglu *et al*., 2005) than that of traditional nuclear transfer technique (60-80%) (Wells *et al*., 1999 and Kato *et al*., 2000). The difference between zona free and zona preserved fusion is even higher in horse 97%-100% and 65%-70% respectively (Galli *et al*., 2003 and Lagutina *et al*., 2005). Zona free cloning may provide a solution to compensate for the loss and normalize blastocyst cell number by using two cytoplasts instead of one for embryo reconstruction and this is important for further embryo development. However, carefully calculated design of fusion parameters, as well as appropriate positioning of somatic cells between the two cytoplasts is carried out one step fusion method with 94% fusion rates (Tecirlioglu *et al*., 2005; Shah *et al*., 2009 and Akshey *et al*., 2010b, 2011a).

Activation of fused oocytes:

Activation is another important parameter affecting blastocyst production of hand-made cloned embryos. It can be carried out by various agents like Ca ionophore, ethanol, 5-aza-2'-deoxycytidine, resveratrol

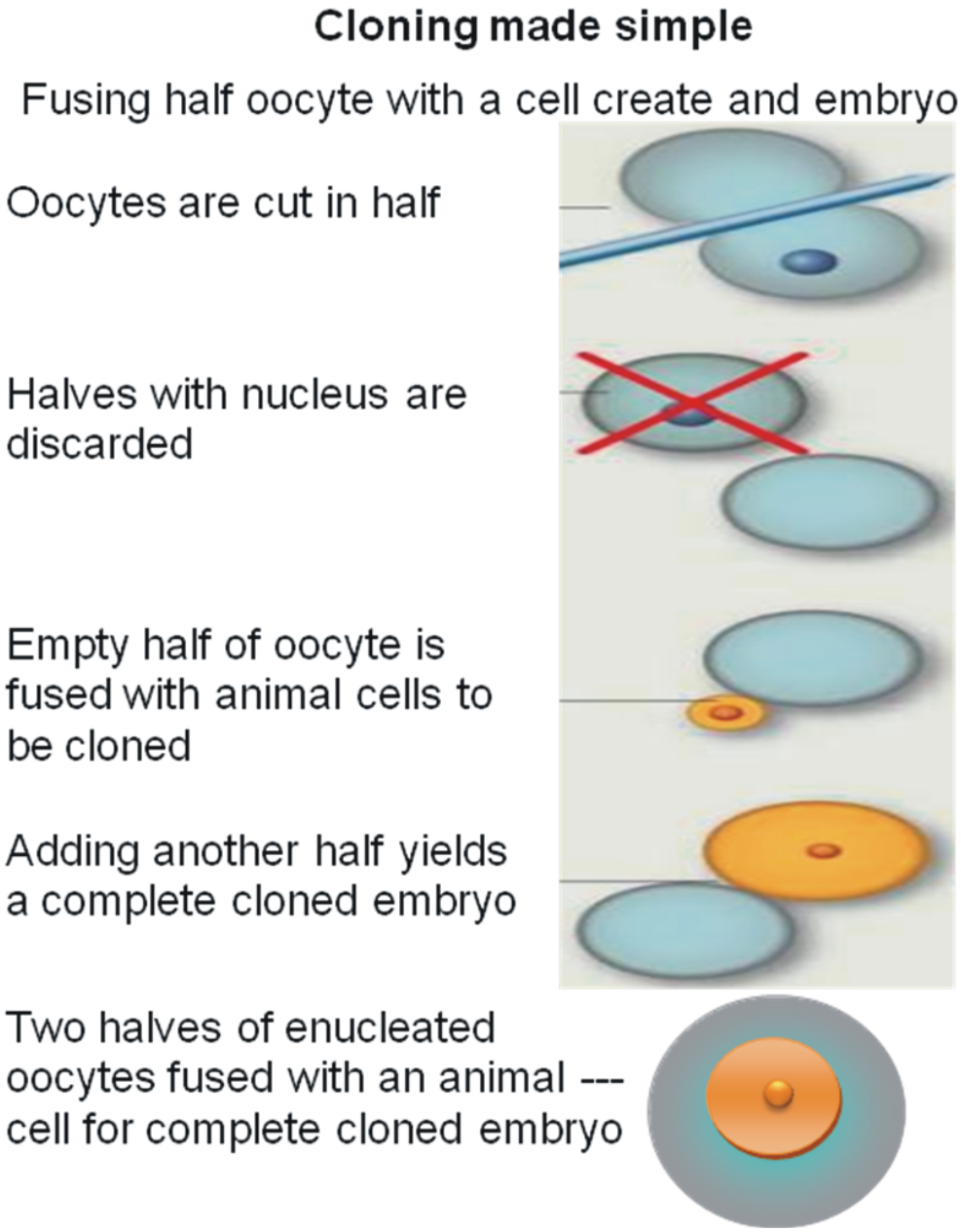

Fig 1. Steps in Hand-made cloning for cloned embryo production (Westphal, 2002)

and cytochalasin (Das *et al*., 2003; Huan *et al*., 2013 and Mukherjee *et al*., 2013). Electric pulses were also applied for the production of cloned embryos using traditional method (Shen *et al*., 2006) in goats. Shen *et al.* (2006) suggested that 2.33 kV/cm electric pulses were more effective than 1.67 kV/cm to increase cloned embryos and kid production. He further reported that no need of Ca ionophore for activation in cloned embryo production while using 2.33 kV/cm electric pulses. Combined electrical and 6-DMAP treatments were used for nuclear transfer in cattle (Cibelli *et al*., 1998) and rabbits (Mitalipov *et al*., 1999). Because these zona-free embryos were found to be more sensitive to chemical activation (Booth *et al*., 2001) in two-step chemical activation procedures, the concentration of the Ca ionophore was decreased to 2 m M (Vajta *et al*., 2003). Prolonged exposure to 6-DMAP resulted in chromosomal abnormalities in cattle (Van-de-Velde *et al*., 1999). The incubation period between reconstruction, activation and during exposure to DMAP was prolonged from 3 to 4 h and from 4 to 6 h, respectively (Kasinathan *et al*., 2001ab). The activation of reconstituted nuclear transfer embryos with 7% ethanol followed by culture in the combination of 1.9 mM 6-DMAP + 1.25 mg/ml cytochalasin B + 10 mg/mL cycloheximide was found to give higher morula and blastocysts yield than individual or two of any of the activating agents (Parnpai and Tasripoo, 2003). In hand-made cloning (HMC) in goat was found a significant increase in cleavage rate and blastocyst yield of 78.57% and 21.43% when electrical pulse used for activation of the reconstructed oocytes. But cleavage rate and blastocyst yield were lower 62.63% and 10.61% when calcium ionophore used for activating agent (Akshey *et al*., 2010a).

Culture of reconstructed Embryos:

One of the most challenging steps of the zona free work is the *in vitro* embryo culture. The simplest and commonly used method after zona removal is to culture embryos individually in small droplets to avoid aggregation problem (Oback and Wells, 2003). An alternative possibility is the glass oviduct (GO) system developed by Thouas *et al*. (2001). Several culture systems like Well-of-the wells (Vajta *et al*., 2001), agarose gels (Peura, 2003), glass oviduct (Thouas *et al*., 2001) and microdrops have been successfully developed for zona free cloned embryo culture. Zona digested, *in vitro* fertilized embryos have been cultured on a flat surface in a well of four well dish (4WD) (Vajta *et al*., 2004). The highest blastocyst rates after zona free individual culture of either *in vitro* produced or nuclear transferred bovine embryos were reported in the Well of wells (WOW) system (Vajta *et al*., 2003 and Shah *et al*., 2008) using a modified synthetic oviduct fluid supplemented with 5% cattle serum as a culture medium (Holm *et al*., 1999). In case of goat HMC, flat system was found best

for culture of zona free cloned embryos (Akshey *et al*., 2010b, 2011a). There was a significant ($p<0.05$) increase in hand-made cloned blastocyst production when cultured in Flat surface (FS) (23.78 ± 3.33 %) than Well of wells (WOW) (15.84 ± 2.12 %) or microdrops (MD) (0.7 ± 0.7%). Culturing of cloned embryos on flat surface of 4 well dish was advantageous as lesser trophoblast adhesion of blastocyst to the plastic surface was observed (Shah *et al*., 2009 and Akshey *et al*., 2010a).

Over all in vitro efficiency of hand-made cloned embryos:

In cattle, the HMC system based on random bisection is capable of producing approximately 50% blastocyst rates using the WOW system, among the highest described for somatic cell cloning (Vajta *et al*., 2004). The reported fusion rate in the two cytoplast plus one somatic cell sandwich system was 94% (Tecirlioglu *et al*., 2005; Shah *et al*., 2009 and Akshey *et al*., 2010b, 2011b) compared to the 67% that has been achieved with fusion of one cytoplast to a somatic cell (Oback and Wells, 2003). In porcine somatic cell nuclear transfer, the development rate to blastocysts is 1 to 11% which is comparable 5 to 6% achieved in zona free cloning by Booth *et al*. (2001); Kragh *et al*. (2004) and Bartolac *et al*. (2015). Regarding blastocyst quality, the limited number of observations indicates that the only difference between zona free and traditional cloning may be the slightly higher cell number in the embryos derived from the zona free system for cattle, goat and pig (Vajta *et al*., 2003; Kragh *et al*., 2004; Akshey *et al*., 2010b, 2011a and Onishi *et al*., 2000).

Pregnancy and calving rates of cloned embryos:

A comparative study did not find differences between the rate of live offspring achieved with HMC and traditional nuclear transferred embryos (7% vs 6.3% respectively) (Vajta *et al*., 2004). Zona free nuclear transfer resulted in 15% of transferred embryos developing to term and 8% to weaning and beyond (Oback and Wells, 2003). The overall efficiency of the HMC procedure in term of enucleation, fusion, blastocyst, and pregnancy rate was comparable with traditional micromanipulation technique (Dominko *et al*., 1999). In case of fusion of two cytoplasts derived from two oocytes, the overall efficiency is 50% per oocyte. However, the technique does not require specialized equipments, even though it is more efficient than traditional somatic cell nuclear transfer to produce cloned embryos. This makes the technique amenable to large-scale applications of cloning in laboratories with limited experience and/ or resources and the efficient production of large number of constructed embryos (100-200 embryos /day/person).

This technique is now used almost exclusively in all laboratories due to

disadvantages of traditional cloning, including expensive equipment, time-consuming work, the need for highly qualified and skilled personnel, and the generally low efficiency of cloned embryo production (Lewis *et al*., 2001). The ability to vitrify cloned blastocyst without loss in viability on day when recipient were not available or suitable, offers greater flexibility by preventing embryonic wastage. Separate experiments were performed to optimize the chemical environment for oocyte bisection, timing of fusion, cleavage and embryo development for requirement of protein sources in culture condition for cloned embryos (Tecirlioglu *et al*., 2005).

Factors affecting cloning efficiency

Cloning efficiency associated with the development of embryos to offspring remains the major obstacle to the widespread use of this technology. Dinnyes *et al*. (2002) suggested that the extensive variability in developmental rates of cloned embryos and low rates of development to offspring requires improvements in both the procedures and the biological material used to produce the cloned embryos.

Cloning of mammals by nuclear transfer can lead to birth of healthy adult animals but more often compromises the development of reconstructed embryos. One of the most difficult challenges faced, is low efficiency in cloning, high developmental abnormalities including various clinical and pathological abnormalities and cloning syndromes (Kubota *et al*., 2000 and Dominko *et al*., 1999). Currently, the efficiency is in nuclear transfer between 1–10%.

Micromanipulation based oocyte nucleation technique was used by Willadsen (1986) for embryonic cell cloning, then slightly modified by Westhusin *et al*. (1992) and same technique was used for creation of 'Dolly' (Wilmut *et al*.,1997). A remarkable modification, the direct injection of the donor nucleus into the cytoplast, has been successful in different species (Wakayama *et al*., 1998; Oback, and Wells, 2003; Choi *et al*., 2003; Galli *et al*., 2003 and Jena *et al*., 2010, 2012).

There are various factors present in oocyte cytoplasm, which reprogram the donor cell nucleus after nuclear transfer. The volume of oocyte cytoplasm is affected by micromanipulation and it decreases up to 39-50% of total cell volume of oocyte. This affects proper nuclear reprogramming of donor cell nuclei. It also affects the pregnancy rate due to improper placentation and various anomalies in cloned offspring generally called as "Cloning Syndrome".

The cumulative damage acquired by each step during NT may reduce the developmental potential of NT embryos and could ultimately cause embryonic and fetal

death at any time point in development (Dominko *et al*., 1999). Success rate is also dependent on operator skills and speed of manipulation (Wakayama *et al*., 1998). Based on a recent method established for embryonic cell nuclear transfer a group of zona free manipulation procedures have been developed for SCNT in domestic animals (Vajta *et al*., 2001). Some of these methods still require micromanipulators for enucleation, but other can be performed entirely by hand under a stereomicroscope (Kragh *et al*., 2004) and were recently named hand-made cloning (HMC) (Vajta *et al*., 2003). The in vitro efficiency of the HMC in cattle is high, with a 50% blastocyst rate per reconstructed embryo being obtained.

In HMC higher cloning efficiency was found in addition to the low cost of equipment (no micromanipulator or related tools such as grinder, microforge, and capillary puller are required) makes this technology very economical and affordable even for laboratory with a limited budget. With increased interest in cloning in livestock, new approaches have been developed. These include methods for zona free nuclear transfer that can be performed with or without the use of micromanipulator. Micromanipulation based somatic cell nuclear transfer was used in creation of various species. However, without micromanipulation using zona free or handmade cloning procedure cloned animals like cattle (Vajta *et al*., 2003 and Tecirlioglu *et al*., 2005), horse (Lagutina *et al*., 2005), buffalo (Shah *et al*., 2009) pig (Du *et al*., 2005) have been produced.

Technical factors associated with somatic cell cloning efficiency

Nuclear transfer is a technically demanding process, particularly when large numbers of oocytes are to be processed to achieve large-scale production of cloned animals. The most cumbersome procedures such as enucleation and nuclear transfer by traditional nuclear transfer using micromanipulator require skilled personnel, it is time consuming and its efficiency is very low. Some alternative methods are available like (1) Gradient centrifugation enucleation (Tatham *et al*., 1995), (2) Chemical enucleation (Fulka *et al*., 2001), (3) Enucleation by pressure (Singla *et al*., 1997), (4) Telophase enucleation (Bordingnon and Smith, 1998) and (5) Hand-made cloning (Vajta *et al*., 2001).

Application of cloning technology

One great advantage of cloning from somatic cells is that specific types of somatic cells can be easily propagated in culture to many millions of cells that can be used either to produce large numbers of identical offspring or for genetic modification of cells. The second advantage is that somatic cell can be recovered from adult animals and can be successfully used to make genetically identical copies of existing animals (Campbell *et al*., 1996a and Kasinathan *et al*., 2001a).

SCNT has proven to be a more efficient method of production of transgenic animals than pronuclear DNA- microinjection and hand-made cloning (Cibelli *et al*., 1998; McCreath *et al*., 2000; Vajta and Callesen, 2012 and Verma *et al*., 2015). This is comparatively easy technique and gives 100 % transgenic animal without any further analysis.

This technology opened a new area of research. A number of applications of SCNT have been described beneficial to animal, agriculture and human. SCNT provides a novel tool for investigating nuclear and/or cytoplasmic components involved in embryonic development and loss.

Application of cloned animals in agriculture

1 This technology can be used for production of genetically elite /superior animals, with desired traits that can be used in breeding programme (Lewis *et al*., 2001 and Paterson *et al*., 2003)

2. Production of diseases resistant animals to mastitis (Wall *et al*., 2005), BSE and Scrapie (Paterson *et al*., 2003).

3. Obtaining high quality meat, which is desirable for human consumption (Paterson *et al*., 2003).

4. Milk quality improvement by altering b-casein and k-vasein ratio (Brophy *et al*., 2003).

5. Cloning can be used to increase the number of desired male/female offspring in beef/dairy industry, like transgenic bulls that produce only female or only male offspring (Faber *et al*., 2003)

6. There is also increasing interest to utilize SCNT to restore endangered or even extinct species like gaur, mammoth, dinosaurs and tiger and many more (Wells *et al*., 1999 and Solti *et al*., 2000).

7. Interspecies SCNT can be used to increase the number of endangered species (*Bos gaurus*) which subsequently help in conservation (Lanza *et al*., 2000). Interspecies nuclear transfer was used for production of embryos reconstructed from cat somatic cells and bovine ooplasm (Thongphakdee *et al*., 2008). Few year back production of cloned Asian elephant embryos using an interspecies somatic cell nuclear transfer (iSCNT) technique was reported (Sathanawongs *et al*., 2010).

8. Eco-friendly animals like pig which digest plant phytate, leading to less phosphate in the manure from the animal and thus less environmental pollution such Enviropig ™ can be procuced.

Application of cloned animals in medicine

SCNT can be use for generation of histocompatible tissue, which suit to the patient and avoid rejection which is the major challenge in transplantation

medicine. This can be use in treatment of burn, wound, organ transplantation and blood transfer (Lanza *et al*., 2000).
The use of nuclear transfer techniques in farm animals to efficiently generate cloned transgenic offspring capable of producing valuable proteins could have a marked impact on the pharmaceutical industry (Echelard, 1996). The mammary gland is well suited for the production and expression of human recombinant proteins (Moura *et al*., 2011), alpha-1-antitripsin (Wright *et al*., 1991), lactoferrin (Meng *et al*., 2013), human clotting factor IX and antithrombin III (Baguisi *et al*., 1999) and antibodies (Pollock *et al*., 1999 and Peng *et al*., 2015).

Obvious benefits of using this technique for production of transgenic animals to provide such human pharmaceuticals include:

1) High product yield,
2) Low capital investment compared to cell culture techniques,
3) The ability to perform complex post translational modifications (e. g., glycosylation and gamma-carboxylation) and
4) Elimination of reliance on products derived from human blood, which may contain pathogens

These genetically modified cloned animals also act as organ donor animal for xeno-transplantation or allo-transplantation (Bondioli *et al*., 2001). Dairy goats are ideal for the transgenic production of therapeutic recombinant proteins because of their high yield of purified product and relatively short generation interval (Yoisungnern and Paul, 2014). One of the limitations of SCNT in sheep and cattle is very low success rate, with a high proportion of fetal loss (Campbell *et al*., 1996a; Well *et al*., 2000 and Wilmut *et al*., 1997), and an increase in perinatal morbidity/mortality (Cibelli *et al*., 1998; McCreath *et al.*, 2000 and Wolf *et al*., 2004).

Application of cloned animals in research

In addition to its practical applications, cloned animals has become an essential tool for studying gene function (Capecchi, 2000), genomic imprinting (Solter, 1998), genomic re-programming (De Sausa *et al*., 1999 and Winger *et al*., 2000), regulation of development, genetic diseases, and gene therapy, as well as many other burning topics.
An area of major interest not only to cloning but also to aging and cancer research is the regulation of cell proliferation and senescence, stimulated by the report on telomere length in Dolly (Shiels *et al*., 1999). However, telomere length does not seem to be a problem in other studies (Lanza *et al*., 2000 and Xu and Yang, 2001), nor is telomere length the only component affecting the inherent ability of cells to continue to proliferate.

In spite of wide range of applications of cloned animals, there are many reports on production of cloned animals with lower

efficiency like cattle, sheep, goat, buffalo, pig, horse, mice, rabbit, cat, dog and other species. Many factors are involved which hampers the success of somatic cell nuclear transfer. Many biological factors are being modified for improvement of cloning efficiency as reported by various workers. Further efforts and new paradigms are needed to make this technology perfect and extend it to its fullest potential.

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