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Animal cloning: Art and ethics

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Abstract
Cloning of animals is both fascinating and debatable in this modern world. This is replication of nature's established protocol to generate an organism of similar genetic make-up but with little modifications contributed by humans. Here the creation is trying to act as a creator for replica. Controversies from both ethical and religious point of view have been there regarding animal cloning. Various techniques have been applied to produce clones of different species. Somatic cell nuclear transfer (SCNT) can be considered as a widely accepted technique in the field of farm animals cloning. Though cloning was conceptualized based on conservation of rarest germ plasm and creating an era of replicative life but it is still questionable.

Keywords: Cloning, somatic cell, ethical, controversies

1. Introduction
The creation of Dolly in Roslyn institute by Ian Wilmut, gave us a scope to analyze and recreate the scanty germplasm existing. Approaches targeting preservation of valuable germplasm, creating compatible creatures which can be useful in xenograft transplant and securing the aesthetical emotions of pet lovers who were in a fantasy to buy immortality for their pets. Though it sounds very interesting but creating a clone is just not only limited from its practical applicability but also from ethical point of view. So, we should pay a thorough attention towards the art of cloning and the ethical points before going further.

2. Art of cloning
As we have discussed that the existing cloning method is a mere modification of natural cloning a thorough investigation into it will pave the way to explore the possibilities and technical details of this method.

2.1 Nature: the efficient clone producer
Plants do most often asexually reproduce by cloning. Stem is best characterized for this mode of reproduction. Above ground, stems of strawberry plants produce new plants. Underground stems include rhizomes, bulbs, corms and tubers. In animals, the reproductive process is also diversified. The various forms of asexual reproduction coexist with hermaphroditism and bisexual external and internal copulation (Benagiano and Primiero, 2002) [2]. Various modes of asexual reproduction include budding (jellyfish), fragmentation (worms) and parthenogenesis (some fishes, insects, frogs and lizards). However, most of the animals, that are able to reproduce asexually, reproduce through parthenogenesis during a certain time point. Aphids can be taken as example of organism reproducing by parthenogenesis. Seasonal variation induces this process. During spring when availability of food is more they adopt this mode of reproduction. It is rapid than sexual reproduction and permits quick exploitation of available resources. Fertilized eggs become females, while haploid unfertilized (parthenogenetic) eggs become males in honeybees. But coming to asexual reproduction of mammals, it is not a naturally occurring phenomenon although in mammalian reproduction genetically identical individuals, known as monozygotic twins, do occur.

2.2 Cloning by splitting of embryo
In nineteenth century (1891), Hans Driesch set the basis of animal cloning by separating the blastomeres of a two-cell embryo of sea urchin mechanically by shaking them in seawater. The cells started to grow independently and formed two whole sea urchins, after eleven years the experiment was replicated in salamander by Hans Spemann using a hair from his baby boy to

separate the cells (Spemann, 1902) [23]. Due to lack of technical efficiencies, which are highly needed for such cloning experiments like (1) efficient handling system (2) strictly controlled temperature for development of mammalian oocytes and preimplantation embryos (Bavister, 2002) [1], had hampered the application of the procedure to mammals for more than 8 decades. Then came the first successful embryo splitting which was performed in domestic animals with the purpose of rapid multiplication of valuable individuals (Willadsen, 1979; Ozil *et al*., 1982) [29, 14] but the limitation was that only once or twice an embryo can be splitted thus producing only two to four genetically identical individuals.

2.3 Embryonic cell cloning: another dimension of producing genetically identical individuals

Jacques Loeb in 1894 [11] discovered a new approach to asexual reproduction, while attempting to induce parthenogenesis in sea urchin embryos by use of various salt concentrations; he occasionally observed formation of a large bleb in some of the early embryos. Though rest of the embryo started to develop, this bleb remained unchanged. In certain cases, entry of nucleus into the bleb, started to develop as well and the separated bleb from the original embryo continued to develop independently (Loeb, 1894) [11]. This discovery was a primitive model of all future nuclear transfer experiments providing evidence that embryos could be created by moving the nucleus between cells. By using his more consistent hair-loop technique for creating first transient, later complete separation, Spemann (1914) [24] has repeated the same experiment in vertebrate (salamander) embryos. Illmensee and Hoppe in 1981 [10] reported the first success in mammalian nuclear transfer by using embryonic cells as donors and reported birth of three mice following the transplantation of early embryo cells into enucleated zygotes. But due to inability of this experiment to get repeated in other laboratories, it was declared that mammalian embryos cannot be cloned by this method (McGrath and Solter, 1984) [12]. Fresh trials were done at Cambridge University, Steen Malte Willadsen, a domestic animal embryologist repeated the experiments of Illmensee and Hoppe in sheep. He improvised the technique in many details, but could not get significant success, so he decided to use oocytes instead of zygotes as recipients, though this idea was criticized at that time but it resulted in the first cloned mammals (Willadsen 1986) [30]. Since Willadsen's pioneering work, the same principles have resulted in births of embryonic cell-cloned offspring in other domestic species, including cattle (Prather *et al*. 1987) [16] and pigs (Prather *et al*. 1989) [17].

2.4 Cloning by somatic cell nuclear transfer (SCNT)

Hans Spemann in 1938 described the possibility of reversing the process of cell differentiation and hence, of using more developed cells for nuclear transfer. According to him one could transfer nuclei of four days old (morula stage) embryo "older nuclei of various cells" into enucleated ova. Briggs and King (1952) [3] were the first to perform nuclear transfer based on that hypothesis though they were not aware about that hypothesis at that time. They tried it on a frog. They removed the nuclei of recipient eggs and inserted a donor nucleus. The source of donor nuclei was embryo (morula stage) then subsequently epithelia cells of intestine from tadpoles. The procedure resulted in a considerable success in early development of embryos. However, the more differentiated donor cells was, the less success could be obtained in the advanced stage of development, though researches were further conducted with various degree of success rates, but no one was able to clone an adult frog from adult frog cells (Di Berardino 2001) [8]. But till that time, scientists did not attempt to use adult somatic cells as nucleic donors in the mammalian experiments. There were evidences of using cultured cells from embryos at an advanced stage of development as donors for nuclear transfer (Sims and First, 1993; Campbell *et al*. 1996) [20, 4].

Dolly (Wilmut *et al*. 1997) [31] managed to agree many researchers that it was possible to clone a grown animal by removing the nucleus of a somatic cell from an adult and inserting it into an enucleated ovum. The approach was with minimal technical specification and the only supposed methodological innovation, the serum starvation of somatic cells before transfer was proved to be not indispensable during the subsequent experiments.

It was astonishing and simultaneously a controversial discovery as we got an adult animal successfully but it raised many questions regarding the practice of cloning and SCNT.

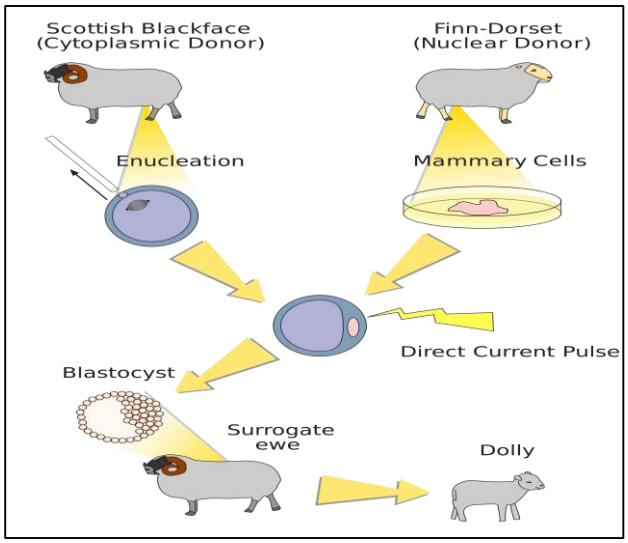

Fig 1: Strategy used for cloning of Dolly (adapted from Dr. Biology, 2009)

2. SCNT: Most promising art of cloning till date

The basic features of the somatic cell nuclear transfer procedure currently used in farm animals can proceed trough following steps. Oocytes from recipients are usually collected from ovaries sourced from slaughter houses or from abattoir. They are matured *in vitro*. Though the quality of oocytes are slightly lower than those derived directly from adult females after *in vivo* maturation induced by hormonal treatment, but the ample amount and low price makes it a rather suitable choice.

Donor somatic cells can be derived from various tissues and from animals of various ages the source can be fetuses, newborn, young and elderly animals and even a deceased animal but within relatively short period after death. Due to some unknown reasons, only a few tissue types (like dozen or so) out of two hundred different tissues present in a mammal can give rise to a clone by this method of SCNT. Tissue specific preferences like those present in the female genital tract (granulose cells, oviductal epithelial cells), seem to be more suitable for generating offspring than others. It can be hypothesized that tissue from young animal may act an excellent source for cloning as compare to older ones but no

concrete evidences are there in support of this hypothesis and various pre-treatments of the donor cells have resulted in limited success so far.

Normally the SCNT consists of three steps:

1. Enucleation of Oocytes
2. Insertion of the donor cells (or nuclei)
3. Activation of the reconstructed embryo.

The cloned embryos are cultured *in vitro* for a while, and after attaining optimal stage for embryo transfer they are transplanted into a "mother" animal. Normally in SCNT the zona pellucida is preserved. This flexible, transparent acellular shell of the egg is regarded as important in supporting further development until day 6–8 as according to different species. To make the necessary delicate manipulations inside the zona pellucida, when the original nucleus is removed and the new one transferred, special and expensive instruments such as micromanipulators are required. The handling of these micromanipulators, as well as the preparation of micropipettes used during micromanipulation, are difficult tasks and require a specially trained, highly skilled workforce and considerable investment. Enucleation starts with fixing the oocytes in the appropriate position and then with the help of the polished end of a holding pipette and application of slight vacuum, chromatin containing part of the oocyte is being aspirated into a sharp enucleation pipette that has been pierced into zona pellucida. There is no concrete method for finding chromatin containing area but careful use of a strategy may result in good overall success rate and decrease damage.

The most common way of introducing somatic cells is by injecting them beneath the zona pellucida followed by an electric impulse to induce cell membrane fusion between the enucleated oocyte and the somatic cell. Alternatively, injection of the cytoplasm-free donor nucleus or the whole somatic cell into the cytoplasm can be practiced. Fusion with chemical and viral agents has been less successful and would now be very rarely used.

When the embryo has been reconstructed, it must be activated. The level of maturation promoting factor (MPF) decreases during normal fertilization, attributed by rise of calcium ion level in the cytoplasm induced by the sperm's penetration. So events causing rise in cytoplasmic calcium levels may be used for activation, which may be in the form of mechanical, electrical and chemical agents. The subsequent drop in MPF level may be maintained by protein synthesis inhibitors for several hours until the reconstructed embryo escapes from developmental arrest. Normally an electric impulse or short exposure to a chemical, triggers activation.

The embryos start to cleave after reconstruction and activation. Appropriate culture conditions lead them to the stage at which they are optimally ready to be transferred. This stage is species specific. In pigs, embryos are usually transferred at one-cell stage, as surgical procedure is unavoidable, and the developmental rates under *in vitro* culture conditions are rather compromised. On the other hand, in cattle non-surgical transfer can be performed at day seven after activation. Standard SCNT (with some modifications) has resulted in the birth of live offspring in 11 species, including cattle, pigs, sheep and goats.

3.1. But Dolly was not a perfect clone!!!!!!

Many biological factors were responsible in making the carbon copy of the genome while cloning

- The mitochondrial DNA comes from the egg and is therefore different from that in the cells of the donor of the nucleus.
- Expression of genes depend not only on the sequence of DNA, but DNA modifications, chromatin structure and the presence of small RNAs contributes a lot towards this. These mechanisms are the basis of epigenetic mechanisms and it is not known how they are reprogrammed in the cloned embryo.
- Multiplication of single cell, more than the destined replicating may introduce unwanted spontaneous mutation and thus may lead to morphological abnormalities.
- Immune system and the brain are not fully developed at the embryonic stage; hence clones may have multiple differences from their nucleus donor.

3.2. SCNT has got a darker side too

After embryo transfer by SCNT there are certain anomalies are seen among the animals which includes low pregnancy rates, an unacceptably high level of losses during early and late pregnancy, stillbirths, early postnatal deaths, short life-span, obesity and malformations. The term most frequently used to describe some of these developmental anomalies is "large offspring syndrome" (LOS) which refers to increased birth weight. LOS is now used to describe a number of malformations and diseases. Increased birth weight is just one of these manifestations. In sheep, cows and mice the following problems were detected:

- Malformations in the urogenital tract (hydronephrosis, testicular hypoplasia).
- Stillbirth, hypoxia, respiratory failure and circulatory problems, lack of post-natal vigour.
- Malformations in liver and brain.
- Immune dysfunction, lymphoid hypoplasia, anaemia, thymic atrophy.
- Bacterial and viral infections
- Placental abnormalities.
- Fetal overgrowth, prolonged gestation.
- Increased body temperature at birth.

4. Handmade cloning (HMC): new approach in cloning

A relatively new approach to nuclear transfer is the so-called handmade cloning technique. This was initially used for embryonic cell nuclear transfer (Peura *et al*., 1998) [15] and then modified for SCNT (Vajta *et al.,* 2001) [26]. With this method, the zona pellucida is removed after maturation and before enucleation. Here micromanipulators are not needed for enucleation and fusion, which reduces the costs associated with investment in laboratory equipment and the employment of highly skilled workforce to operate it. The procedure delivers highly efficient enucleation, fusion and activation results. The *in vitro* culturing of embryos without zona pellucida might seem hazardous, but with modifications to the culture system, appropriate developmental rates can be achieved. This procedure can be standardized and automated (Vajta *et al*., 2005) [25]. HMC and similar zona-free techniques have resulted in the birth of approximately 20–25 healthy calves in Australia, New Zealand and South Africa, and ongoing experiments, now at an advanced stage, suggest possible porcine pregnancies in the foreseeable future.

5. Ethics of cloning

'Consequentialist' was the major outcome of cloning (Rolling, 1981; Singer, 1975) [19, 21]. Cloning is practiced, but

has a wide range of effects on the society. Suffering of the cloned animals comes under narrow effect of cloning but how humans, animals, livestock and also the endangered species are affected by this trial that comes under the broad effects of cloning.

Cloning has both pros and cons associated with it. Due to cloning, it is now being possible to save some endangered species which are on the verge of extinction. Cloned animals are also being used as a dummy for scientific research for testing of medicines, pharmaceuticals etc. Some good germ plasms are also being able to save due to cloning and cloned animals are also being used for food production. But coming to negative consequences of cloning, the foremost consequence is mechanization of reproduction. Cloning disregards, the supreme power, reproduction is a natural phenomenon but due to this cloning in case of human it's now being possible to reproduce asexually as like lower order creatures. By cloning man is now being able to produce his own clone, clone of his lovable ones, so if this trend continues the importance of family will be less realized in the recent future.

Objectification and commodification of animal cloning is also a matter of criticism on various grounds (Regan, 1983) [18]. Due to materialization, the intrinsic values of animals are getting reduced, as animals are being treated as mere objects as they can be produced in an industry and send to market for selling, again if animal becomes older they can be exchanged with their new versions. Animal clones are being used as stuffs which are being sold on certain price to their lovable parents. So, for commercialization, cloning paves the way by making people foolish by emotional blackmailing. A specific type of cloning i.e. pet cloning is always a matter of discussion on the grounds of fraud and false promising. Pet lovers are often do suffer lot due to this as they are motivated to store the DNAs of their beloved pets without knowing the actual cost of cloning. As in cloning the cost involved is quite high which can be used to save the lives of thousands of non-clones.

The percentage of live off-springs from the number of transferred embryos is denoted by the term efficiency and the efficiency rate in animal cloning is only 1-2%, where the failure rate is few less than 100% i.e. 98% (Coleman, 1999) [7]. One more study revealed that the efficiency rate in pig was 5-12% (Walker, 2002) [28].

Cloning is a very complicated process, which results in abortions and suffering of animals. Miscarriage, still births, genetic abnormalities, weak health are some of the common symptoms seen in cloned animals. Suffering during cloning procedure and obstetrical complications in surrogate mothers are also some of the most seen complications in cloning. High birth weight of cloned fetus is also a common feature, which lessens the chance of normal delivery and increases chance of C-section delivery which leads to pain and suffering of surrogate mother. So, it is not morally permissible to let the animal suffer from pain due to this use of biotechnology on animal reproduction.

A study on pig reported 50% mortality rate in the live offspring between 3 to 130 days of old due to chronic diarrhea, congestive heart failure or decreased growth rate (Carter, 2002) [5]. Again, on a study on calves reported only 106 live births, when around 2170 embryos were being implanted among those 106 live births, 24 calves died soon after birth and from those 24 calves, 11 were died due to some physiological abnormalities like digestive problems, disfunctioning of excretory system, respiratory tract problems and skeletal system problems.

Contrary to above findings one study reported no developmental abnormalities were found on 77% cloned animals and the healthy clone percentage ranged between 20-100% Cibelli (2002) [6].

One more negative aspect of cloning is breeding of cloned animals with non-clones. Sometimes due to some deleterious effects of genes expressed in some animals, the food produced from cloned animals not supposed to be healthy. So, the U.S Humane society (HSUS) has requested a ban on products coming from cloned animals. Still today, it is a debatable issue to state whether food produced from cloned animals is safe or not? FDA issued a green signal towards the products from cloned animals on late October 2003 (Neergaard, 2003) [13], but within a very few days they revised their own statement and claimed they lack enough information to state the product from cloned animal was safe (AP, 2003). Till date it is not confirmed by FDA regarding the safety value of products from cloned animals.

To make a 'consequentialist' approach on animal cloning, both the positive and negative aspects of cloning should be studied in detail; the benefits of animal cloning should be weighed against its ill effects like suffering of animals. Animal cloning should be judged on morality basis; public sentiments should be given priority than commercialization of cloning. Uniqueness of every individual in the creation of God is for a specific reason and to honor and glory the Gods' creation proper balancing should be done and cloning should be done only in the case of required.

6. Conclusions

As like other techniques cloning has got both its pros and cons to be dealt with. Complicated approach, highly skilled labor involvement, survival rate and involvement of a large amount of fund still limits cloning procedure. Playing God and breaking nature's rule have to be again addressed. At this stage we can only say that this technique should be applied to such fields where it is an unavoidable necessity like conserving rare germplasm.

7. Summary

Cloning has been a fascinating area for science. Starting from nature various artificial approaches are being made to create a successful clone. Till date SCNT is proved to be an efficient method. though Dolly was cloned after various trials and errors still it was not a complete and perfect clone. Mitochondrial DNA, maternal inheritance is still a problem. Apart from technical inefficiency ethical concerns are still a low lighted area in this field.

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