Animal and human cloning – the technology and ethics

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The 20th century experienced remarkable progress in our understanding of the living world and this paved the way to harness the unlimited power of biological systems for technological applications. This has given rise to newer fields like Genetic Engineering, Biotechnology, Assisted Reproduction etc, each of which has attracted not only scientists and administrators but also members of general public because of the far-reaching impacts that these applied sciences have on the quality of human life. One of the most awe-inspiring of these developments is the feasibility of human cloning.

What is cloning and why is animal cloning so exciting?

The word "clone" in biology implies all the progeny that can be traced to a single ancestor and, therefore, all of them have identical genetic information. Identical twins in humans are thus clones of each other. Since most of the higher organisms reproduce sexually, i.e., each offspring needs two parents (the male and the female parent), no clones exist for such organisms in nature (except in rare events like identical twins). On the other hand, clones can be easily obtained for those organisms that can reproduce asexually (e.g., most bacteria or simple organisms like amoeba or fungi etc, and those plants that can be vegetatively propagated). In the case of sexual reproduction, the genetic information in the progeny is derived from both the parents and, therefore, the progeny is not identical copy of either of them. In the case of asexually reproducing organisms, on the other hand, the progeny normally carries the same genetic information as the original ancestor and thus all the progeny qualifies to be called clonal descendants.

Embryological and other studies have shown that in almost all higher organisms (including human), only the zygote cell (formed by fusion of the sperm and the egg cells) is totipotent, i.e., has the capability to form a complete individual. All the subsequent division products of the zygote cell lose totipotency and gradually become more and more limited in their capacity to form different kinds of body structures. This happens in spite of the fact that by and large most cells in the body of an organism contain the same genetic information as the zygote cell. It seems that as the cells differentiate, they lose the capacity to utilize certain components of the genetic information and therefore also lose the totipotency.

Studies in Genetics and related fields have suggested that almost all the genetic information that is passed on from one generation to the next is encoded in the chemical structure of the DNA (deoxyri-

bonucleic acid) molecule. The genetic code is built around a language which has only four alphabets with each alphabet corresponding to one of the four nitrogenous bases that polymerise to form the DNA molecule. The ultimate appearance and functioning of an individual (the phenotype) results from a complex interaction between the genotype (the sum total of the genetic information present in the zygote) and the environment. However, it is generally believed that the genotype of the zygote cell has the primary role in defining the basic body pattern which can be modulated within a certain range by the environmental factors.

In light of the above background, cloning would involve reverting the differentiated cells of an organism to regain totipotency so that each of these cells can give rise to a complete individual. All such individuals would be clones of each other and of the individual whose cells were taken in the first step. Since they have identical genetic information, they are expected to develop like the identical twins. The challenge in the act of cloning thus is to make the differentiated cells of body to lose their differentiated status and become totipotent like the zygote cell.

The excitement about cloning comes from the fact that cloning allows generation of a large (theoretically unlimiting) number of individuals with identical genotypes. Such clones may be expected to show similar phenotype. A good breed of any useful animal or plant, if multiplied by cloning, can boost the yield of the useful product by producing many duplicates in a short time. The conventional sexual reproduction is not only time-limiting, but also would not permit production of progeny with identical genotype due to the necessity of fusion of two gamete cells each derived from a different parent. Of course for humans, production of multiple look-likes can be exciting for different reasons.

Why is cloning so challenging ?

In the normal sexual mode of reproduction two gametes (egg and sperm), each of which carries half the genetic information (haploid) of the female and male parent, respectively, fuse with each other at the time of fertilization. The act of fertilization not only restores the full quantum (diploid) of genetic information, but also triggers the egg cell to start the orchestrated programme of cytoplasmic and nuclear activities that culminate in the development of a multicellular organized entity, typical of a given species. Almost all cells of the adult body remain diploid like the zygote.

On the face of it, the process of cloning thus should not appear to be a difficult process since one can get many cells from a living organism without causing any major problem/inconvenience to the donor. And if all these donated cells can be forced to develop like a zygote, one can get as many clones. However, the major hurdle in cloning is the fact that in all higher animals, the egg cell is organized in a unique manner which alone can allow execution of the genetic programme that regulates the development of a single cell into an organized multicellular entity. This stems from the fact that while the cell nucleus has the entire genetic information, its utilization and expression is controlled by signals being received from the surrounding cytoplasm. Only the egg cytoplasm is organized in a manner that will give the appropriate signals to the nucleus to

execute the genetic programme that governs the embryonic development. The unique organization of the egg cytoplasm is achieved during the long period in which the primordial egg cell undergoes maturation within the ovary. Thus, while the body cells are diploid like the zygote and hence do not require the act of fertilization, they lack the unique cytoplasmic organization that an egg cell has. This specific cytoplasmic organization requires the long process of oogenesis during which the precursor of egg cell is not only converted from a diploid to haploid state but every molecule in its cytoplasm is produced and placed in a unique spatial organization. We still do not have any alternative mechanism to create the egg cell cytoplasm. Thus in any cloning experiment, the egg cell remains one of the essential starting material. The other essential component is the nucleus derived from a body cell of the organism whose clone one wants to produce.

The basic steps in cloning

Although there is much talk of cloning in recent years, it is not a recent discovery. Cloning was actually first achieved nearly four decades ago when scientists generated large numbers of clones of some frogs. The basic scheme of manipulative events that were followed for cloning of frogs continues to be followed for cloning of various mammals like mice, sheep, pig, monkeys etc. The steps are to take an egg cell, remove its haploid nucleus by micro-surgery and implant a diploid nucleus derived from a body cell of the animal to be cloned. If things go well, the chimeric egg (the cytoplasm from an egg cell and the nucleus from a body cell of another individual of the same species) develops like a typical totipotent zygote and in due course of time can give rise to a full animal. All cells of this animal will have the same genetic information as the donor animal and is, therefore, considered as its clone.

In the case of mammals, the steps in cloning are a little more elaborate since the embryo develops within the uterus of the mother. Additional complications arise from the fact that a female mammal does not normally produce many eggs at one time. Therefore, the mother whose eggs are to be utilized for cloning has to be treated with a set of hormones to stimulate it to produce many more eggs at one time than usual (superovulation). At the same time, another female (the foster- or surrogate-mother) is treated with another set of hormones so that its uterus or oviduct becomes ready to receive the chimeric embryo prepared outside the body. The steps in preparation of the donor and surrogate mothers are comparable to those involved in production of "test-tube" human babies for assisted reproduction. The eggs (oocytes) taken from the donor mother are eunucleated (i.e., their nucleus is removed by micro-surgery). At the same time the cells from the body of individual to be cloned are prepared for isolation of their nuclei. This preparation involves certain specific steps so that the donor cells stop dividing. One nucleus is either micro-injected into each of the eunucleated egg cells or made to fuse with one egg cell. After culture for some time to let the very early embryonic development begin, these chimeric embryos are implanted into the uterus/oviduct of the foster mother. If these grow to full term, all the new-borns will be clones of the nuclear-donor animal (see figure 1).

***Above*: Cloned sheep "*Dolly*" with her natural born lamb, "*Bonnie*". Dolly is the first cloned mammal, derived by fusion of a mammary gland cell nucleus of an adult ewe with an eunucleated egg of another ewe. Birth of Bonnie shows the full success of cloning of Dolly.**

***Right*: General procedure for production of cloned mice**

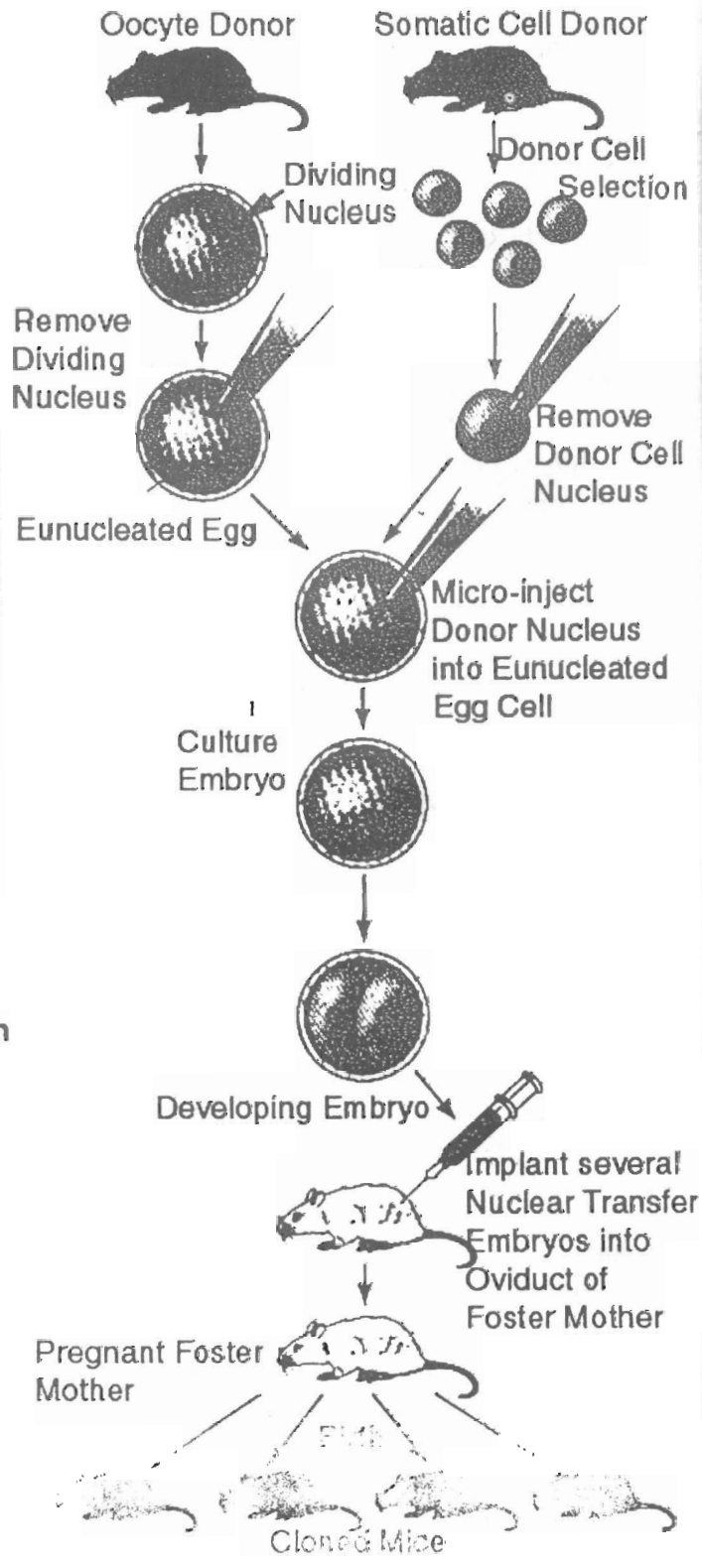

Figure 1

Advantages of cloning of mammals

The first mammal was successfully cloned in 1996 by Dr. Ian Wilmut and his colleagues at Edinburgh when they announced the birth of "Dolly", clone of a 6-years old ewe. To produce this clone, they fused a cell from the udder of the ewe with an egg cell obtained from another sheep and whose own nucleus had been removed. Out of the 434 sheep eggs used for cloning, only one survived to produce the "Dolly". Success of this clone is established by the fact that "Dolly" has since given birth, in the normal sexual manner, to a lamb, the "Bonnie" (Figure 1). Subsequently, clones of cattle, mice and monkeys etc have also been obtained. This has given rise to possibilities of cloning of humans.

Cloning of animals (and humans) is not only a curiosity, but has certain applications. On the basic science side, this allows us to better understand some of the processes that govern our early development. But more important than the improvement in knowledge is the possible commercial gain, specially since it is now possible to produce transgenic animals, which carry specific genes derived from other organisms. The transgenic animals can serve as living factories or bio-reactors for production of certain important pharmaceutical products. For example, if the human gene for a protein like Factor IX (essential for blood-clotting in man and whose absence causes hemophilia) is introduced in the genome of the cow or sheep, the transgenic animal can produce the Facor IX in mammary glands and secrete it with the milk. The Factor IX can subsequently be easily purified and used as drug for treatment. Cloning of such transgenic animals allows their production in large numbers in a short time to facilitate mass production of the pharmaceutical product. Conventional breeding would take a very long time and may also result in loss of the transgene or reduction in its efficacy.

Another potential use of cloning is to have a source of body parts for transplantation. Common problems in tissue/organ transplantation for clinical purposes are the non-availability of donors with immunological matching of donor's tissue with the recipient's body. Since the clones are identical in their genetic makeup, they may be expected to have similar immune properties, and therefore, their body parts may not be rejected because of tissue-type mismatch.

Related to the production of immunologically similar body parts, is the development of "stem-cell" technology. Stem cells are early embryonic cells, which have the potentiality of differentiating into different tissues/organs under special culture conditions. Thus if stem cells are obtained from cloned embryos and made to differentiate into specific tissues/organs in culture, they can provide easy supply of immunologically acceptable materials for transplantation back into the original donor's body as and when needed.

In view of the enormous economic potential of such biotechnological applications, many academic institutions and commercial organizations have invested in a large manner and are hoping that soon it may be possible to routinely clone animals.

Cloning of man: should we or should we not?

All organisms show greater love for their relations and as part of the process of reproduction, every organism wants to

leave as much of his/her genes in the next generation as possible. During the normal course of sexual reproduction, each of the progeny gets only one half of each parent's genes. Compared to this, cloning would permit one hundred percent transfer of genes of one individual. This makes it more exciting and many people would like this to happen.

It has been suggested that cloning may help in "eugenics", i.e., to improve the genetic makeup of the human race. One could select individuals of desired types and clone them to produce many identical copies, as in a factory. Protagonists of such ideas have sought support in the fact that some aspects of behavior or certain special faculties may have a genetic component. And with the sequencing of human genome, the enthusiasts believe that one can make a "judicious" choice based on the genomic information about the individual to be cloned.

Cloning can also help infertile couples to have children of desired sex depending upon whether the wife or the husband is cloned. Another advantage of human cloning is the possibility of using the clones as "banks" for organs that may be required for transplantation if such a need arises. It has also been claimed that since the techniques used for cloning are not very different from those used in "assisted-reproduction", a clone may be taken merely as a "late-born twin".

While cloning of animals has been passively accepted, there has been a serious debate on cloning of humans. At the moment, all countries have put an embargo on any research or experimental work that is directed towards cloning of human, although there are press reports that some enthusiasts are fairly close to "deliver" a human clone. It is difficult to objectively assess the veracity of such claims. A US Biotechnology company recently announced that it succeeded in creating the first cloned human embryos, although none of these survived beyond a few divisions. This has further heightened the debate on human cloning. Following are some of the reasons why different governments and communi-ties have sought to prevent human cloning.

1. **Very low success rate:** The success rate of cloning of various mammalian species is not more than 2-3 % at the best. It is unlikely that in the near future, the success rate of human cloning will be any better since human reproduction is more complex. With such a low rate of success, the risk of spontaneous abortions and/or birth of "clones" showing a variety of developmental defects is very high. Such high risks cannot be acceptable in any civilized society.
2. **Uncertainty about the clones being identical to the donor:** The presumption that clones will remain identical in their physical, behavioural and other mental attributes to the donor, lacks hard evidence. Recent studies clearly show that the physical, emotional and cultural environment available to the growing fetus and child has a very strong effect on development of personality and other physical attributes of the person. Therefore, the presumption that the clones will remain identical in all respects to the original donor and to each other is unfounded. Much more research is still required to determine the roles of genes and environment in shaping of an individual human being. This is notwithstanding the fact that scientists claim to know the

complete human genome and, therefore, the entire genetic blue print that makes a man a man. It must be pointed out that knowing all the alphabets present in the human genome is at best like having all the books in ones' library. Unless all the books are read and understood, the owner does not automatically become wiser than others. So far we seem to have understood only about 2-5% of the genetic information present in our genome. Obviously, to make claims on this little knowledge is boasting beyond one's capabilities. One needs to be very cautious, specially when human matters are concerned.

3. **To have clones as "banks" for human organs is inhuman**: One of the reasons put forward in favour of human clones by some enthusiasts is to create a "bank" of organs which may be used for transplantation in case of need by the donor. This can obviously be of advantage since the waiting period to get a transplant, which is acceptable to our immune system, will be eliminated. However, once the clone is made, it is inhuman to remove a part out of it and thereby make it invalid. The donor cannot claim any right on the clone, just as none of us can claim absolute rights on our progeny! Use of the cloned individuals as "tissue-banks" may push us back to the age of savages. However, considering the potential use of isolated stem cells, the embargo on research on growth and differentiation of human stem cells has been withdrawn in some countries.

4. **Who decides as to who is "better" and therefore, worthy of cloning**: One of the possible "application" of human cloning may be from the "eugenics" point of view, i.e., to improve the genetic makeup of human species. However, the question is who has the authority to define a "better" genetic constitution? Historic facts provide ample evidence that definitions of "genetically better" individuals or populations were highly racially biased and did result in avoidable human misery. Today, the power of genetic engineering and cloning etc is far more strong and therefore, the dangers are also greater.

5. **Cloning may reduce the human diversity**: One of the charms of being human is to live with very diverse groups of people around. However, if in the years to come, the process of cloning can indeed generate many identical individuals, the pleasure of living amidst diversity will be reduced or even eliminated. Family relations between clones will be very uncertain: will a clone be treated as son or daughter or a much younger brother or oneself? Besides the social unacceptability of this monotony, this is undesirable from the evolutionary point of view as well since the survival of a species depends upon genetic diversity and plasticity.

The unique faculties possessed by humans make this species singularly creative unlike any other living organism on this planet. It is because of this creativity that humans have been able to take control of the nature in so many ways. Cloning is one more example of the human success. Animal cloning has some advantages and attractions but may also have serious negative aspects, specially when it concerns human cloning. Therefore, it is necessary that wider cross-sections of population discuss the pros and cons of such powerful techniques, rather

than let some over-enthusiasts go ahead with this without any control. At this point of time, it appears that cloning and other methods to modify the human genome are of advantage only to commercial establishments who have already invested significantly in such activities. It may not be out of place here to remind ourselves of a mythological story which apparently relates to a very efficient system of cloning: this is the story of "Raktabeej", each drop of whose blood could generate an alike (or a clone in current definition) of himself. It is perhaps not insignificant that the mythological story-writer described "Raktabeej" as a demon, rather than a human or god!