

Chapter 19

Cloning the Mammoth: A Complicated Task or Just a Dream?

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Abstract Recently there has been growing interest in applying the most advanced embryological tools, particularly cloning, to bring extinct species back to life, with a particular focus on the woolly mammoth (*Mammuthus primigenius*). Mammoth's bodies found in the permafrost are relatively well preserved, with identifiable nuclei in their tissues. The purpose of this chapter is to review the literature published on the topic, and to present the strategies potentially suitable for a mammoth cloning project, with a frank assessment of their feasibility and the ethical issues involved.

Keywords Somatic cell nuclear transfer (SCNT) • Cloning • Mammoth • Elephant

1 Introduction

Surprisingly, writers and moviemakers have anticipated some of the most extraordinary scientific breakthroughs. In the movie *Sleeper*, directed by Woody Allen in 1973, the protagonists were asked to clone a dictator, killed by a bomb, using a fragment of his nose. Twenty-four years later the transfer of a somatic cell into an enucleated oocyte cloned the first mammal, a sheep named Dolly (Wilmut et al. 1997). In *Jurassic Park*, a 1993 movie by Steven Spielberg based on the 1990 book written by Michael Crichton, DNA extracted from fossil amber was used to generate

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a large proportion of the fauna of that era. This movie was made over 19 years ago, and it still remains science fiction, but for how long?

Bringing back to life extinct species appears to be a common wish among humans. When we step in front of a well-preserved fossil or stuffed specimen, instinctively we start imagining what the living animal looked like.

Cloning by somatic cells nuclear transfer (SCNT) indeed offers the possibility to materialize such a dream. An essential requirement for cloning is the availability of soft, or otherwise well-preserved tissue with identifiable nuclei. Hence, fossil skulls or skeletons are not the ideal material to start with, but frozen mammoth bodies found in the permafrost fulfil the minimum requirements for SCNT. In fact, every time a mammoth body is found, a timely exercise from the mass media is to speculate about cloning it. At the beginning, these forums were confined to the everyday people, but recently even developmental biologists have started considering bringing mammoth back to life through cloning. Whether projects to clone a mammoth have genuine scientific basis, or reflect commercial enterprises we do not know.

In this chapter our effort will be to critically analyse the few published reports on cloning the mammoth, then we propose what might be, in our view at least, a realistic approach to clone a mammoth, using the scientific knowledge currently available. We conclude with a few thoughts on ethical issues involved in such a project.

2 Cloning the Mammoth: What Has Been Done?

Leaving aside abstracts or poster communications in international meetings, only one ISI publication is available on mammoth cloning (Kato et al. 2009); hence, review of the state-of-the-art is a very easy task. So far only one group, led by Akira Iritani, a Japanese scientist, is officially engaged in a mammoth cloning project, but according to some press releases, a second group of South Korean and Russian scientists is also competing for the task ([The Telegraph, UK; 13 March 2012](#)).

In the published data available, Kato et al. (2009) used somatic cells from a 15,000 years old mammoth calf. The source of cells was a leg, from which epithelial and muscle cells were collected. The first surprise was that the tissues had actually maintained their structure over the years, as shown in haematoxylin-eosin stained histological specimen (Fig. 19.1). Nuclei were isolated from these two tissues (Fig. 19.2). The details were not revealed in the paper, but we presume that nuclei were mechanically dissected by micromanipulation from histological sections not mounted on resin and slides. No indications were given on how many nuclei were harvested, however, in our opinion, that must have been a very time consuming and cumbersome effort. The nuclei isolated from muscle and skin tissues were injected into enucleated mouse oocytes, which were activated and monitored throughout the first cell cycle. The two mammoth somatic nuclei sources, muscle and epithelial cells, were not modified by the oocytes, whereas the control oocytes injected with nuclei from frozen mouse bone marrow cells formed well-shaped pronuclei (Fig. 19.3). The message that Kato et al. (2009) paper conveys is very important because single nuclei are identifiable and retrievable from 15,000 years old mammoth sample.

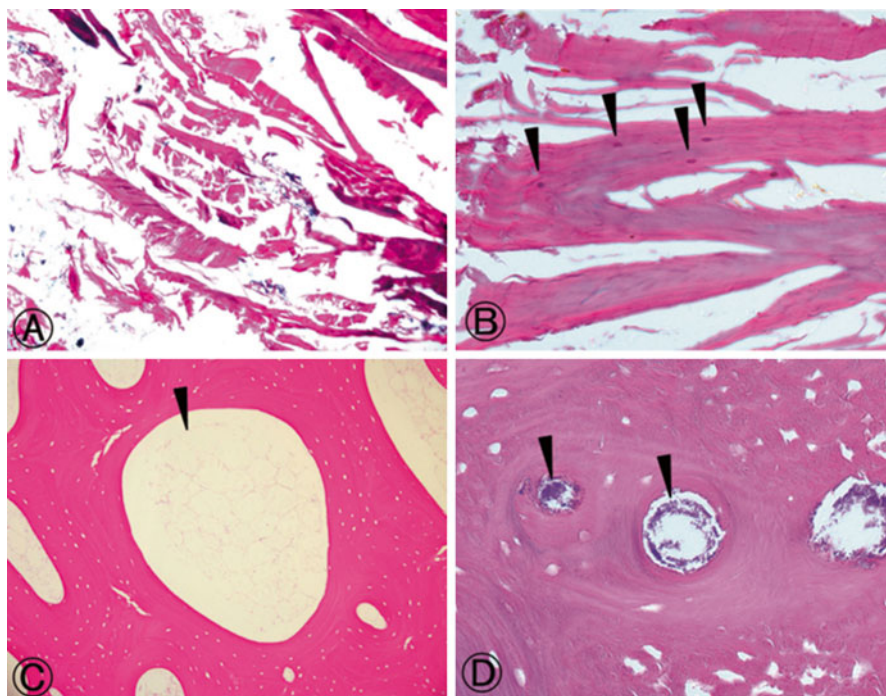


Fig. 19.1 Thin sections of mammoth tissues: (a) skin ($\times 100$), (b) muscle ($\times 400$), (c, d) bone and bone marrow (c $\times 100$ and d $\times 400$) samples stained by hematoxylin-eosin double staining method. There were many cell nuclei in the muscle (Fig. 19.1b). In the medullary cavity of the bone marrow there were many foam shaped structures (Fig. 19.1c) and blood cells or epithelial cells in the bone (Fig. 19.1d) (Reproduced with permission from Kato et al. 2009)

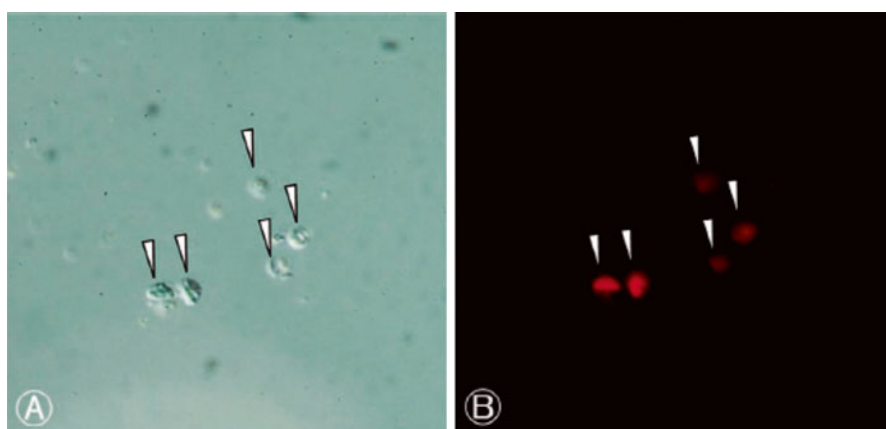


Fig. 19.2 Nuclei isolated from mammoth tissue. A - bright field; B, P.I. fluorescence. From Kato et al., 2009, with permission

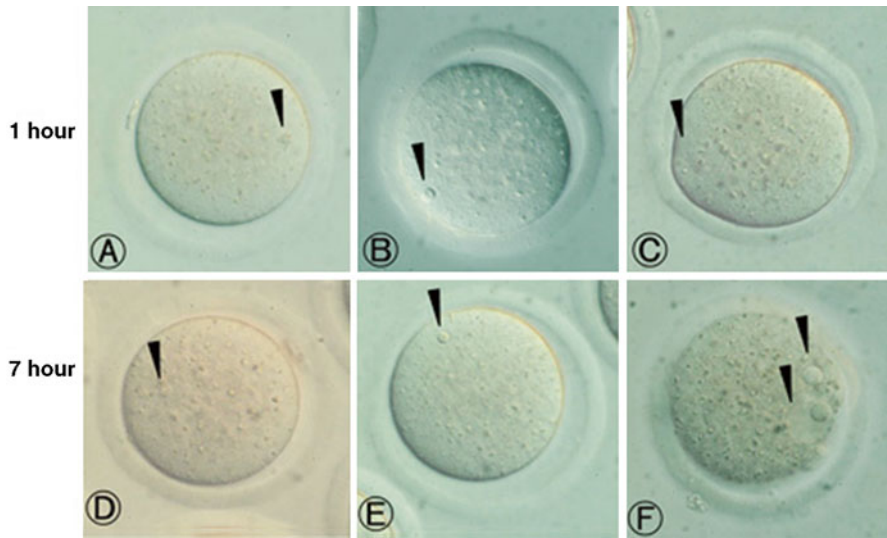


Fig. 19.3 (a–c) Mouse oocytes injected with nuclei derived from mammoth skin (a), mammoth muscle (b) and mouse bone marrow (c) at 1 h after nuclear injection. Injected nuclei were visible (arrows). (d–f) Mouse oocytes injected with nucleus derived from mammoth skin (d), mammoth muscle (e) and mouse bone marrow (f) at 7 h after nuclear injection. In d and e, injected nuclei without any change were still visible (arrows). Meanwhile, oocytes injected with mouse bone marrow derived nucleus transformed into 2 pronuclear-like structure (arrows) (Reproduced with permission from Kato et al. 2009)

This is a remarkable finding indeed. The lack of nuclear remodelling detected could be attributable to the inaccessibility of oocyte DNA remodelling factors to the mammoth’s nuclei caused by structural modification during storage.

This is the present state-of-the-art of mammoth cloning, impressive but not really sound. The Japanese group dealing with the mammoth cloning project is not an off-the-cuff team. This group has a robust reputation in the field of embryo manipulation and is one of the leading Japanese laboratories working on SCNT; hence the project must have some chances for success, as will be discussed in the following pages.

2.1 Cloning the Mammoth

There are two possible strategies to resurrect a mammoth:

1. “Synthetic” genome assembly AND nuclear transfer.
2. “Canonical” Interspecies Somatic Cell Nuclear Transfer (ISCNT).

2.1.1 “Synthetic” Genome Assembly AND Nuclear Transfer

The “*Synthetic*” approach relies on the recent annotation of the mammoth genomic DNA (Miller et al. 2008). Since the application of next generation DNA sequencing technologies (Metzker 2010) to ancient DNA (aDNA), the entire sequences of the mammoth genomic (Miller et al. 2008) and mitochondrial DNA (Gilbert et al. 2008) DNA have become available. Hence, we have the DNA recipe on which a new mammoth genome can be *ex novo* synthesized. The entire procedure has been brilliantly described by Henry Nicholls in a special Nature issue celebrating Darwin’s centenary (Nicholls 2008).

The first step is to synthesize the mammoth genome; secondly, the genome should be allocated into individual chromosomes, using the elephant genome and karyotype as a guide. This in itself is a formidable task given that elephants have 56 chromosomes. Of course the chromosomes must be canonically organized, with telomeres, centrosomes, and all vital sequences required for DNA replication and accurate segregation in mitosis; by all means an overwhelming task.

Let’s imagine we have accomplished the task of arranging the DNA into the respective chromosomes. There is a crucial organelle that is still missing: the centrosome. The centrosome is essential in cell division, therefore a centrosome, presumably obtained from elephant tissues, has to be somehow associated with the chromosome set. Obtaining purified centrosomes with subcellular fractioning through gradient centrifugation is an established procedure (Moritz and Alberts 1998), but the trouble would be to package and hold together the chromosomes and the centrosome in the mammoth-unique structure.

Given that the structure should also allow the transfer of the chromosomes/centriole into an egg, a synthetic lipid monolayer appears to be the most appropriated solution. Artificial membranes have been a reality for many years, and the state-of-the-art is particularly advanced thanks to recent development in the production of artificial cells (Zagnoni 2012). The electro-mediated fusion of the artificial chromosomes along with the centriole into an enucleated elephant egg will finalize the procedure. If all goes well, we will have mammoth/elephant hybrid embryos, with elephant mtDNA, which offers a realistic possibility for development.

In theory, this is an interesting approach. Technical problems such copying errors during DNA synthesis, which could jeopardize the reading of the artificial genome by the oocyte’s transcriptional machinery can occur and will need to be dealt with. An additional problem could be lack of communication between mammoth DNA and elephant mtDNA. The only remaining strategy left would be the generation of synthetic mammoth mtDNA, the sequence of which is available (Gilbert et al. 2008), and packaging it into artificial mitochondria. In the era of synthetic biology and artificial cells (Hammer and Kamat 2012), this might be technically feasible, but will certainly complicate the task. From a nuclear reprogramming point of view, paradoxically this might be an extraordinary possibility to improve the mammoth genome. We can actually confer upon the new DNA a structure compatibility using the reprogramming machinery of the oocyte, thus enabling complete reprogramming and hence normal development.

2.1.2 “Canonical” Interspecific Somatic Cells Nuclear Transfer (ISCNT)

In Interspecific Somatic Cell Nuclear Transfer (ISCNT) a nucleus taken from a target species is transplanted by electro-fusion or direct injection into an enucleated oocyte from a different species. ISCNT is a general term, often misused because in some reports nuclear transfer is accomplished between cell and oocyte donors belonging to different families, orders, or even classes (Loi et al. 2011).

SCNT and its variant ISCNT are potentially powerful tools for the production of unlimited numbers of offspring from a dead animal, in practice a real “asexual” reproduction, which has a tremendous appeal for multiplying endangered animals. The trouble though is that the outcome of the procedure in terms of offspring born is unpredictable and commonly low, about 1–5 %. The reason for the limited efficiency is an incomplete “*nuclear reprogramming*” of the differentiated nucleus. Put simply, the oocyte is unable in most cases to erase completely the differentiation memory accumulated in the form of epigenetic changes on the genome during development. Many excellent reviews are available on the topics, to which the reader is recommended (Gurdon and Wilmut 2011; Maruotti et al. 2010; Loi et al. 2008); in this chapter we would like to address exclusively the technical and biological aspects concerned with the cloning of a mammoth through a “canonical” ISCNT.

Kato’s paper (Kato et al. 2009) has demonstrated that nuclei can be isolated from mammoth tissue. The first step in our opinion would be to verify the state of preservation of mammoth DNA. It is likely that DNA will be mostly degraded after 15,000 years of permanence in the permafrost, probably worsened by cycles of thawing and freezing. The dynamics of DNA degradation over time is a constitutive branch of ancient DNA (aDNA), a relatively new scientific discipline (Hofreiter et al. 2001) launched by Svante Pääbo through his pioneer study on DNA extracted from Egyptian mummies (Pääbo 1985). The temperature and general conditions in permafrost are tolerated relatively well by the DNA, although its structural decay cannot be avoided. DNA degradation starts with deamination and depurination, followed by single and double strand breaks and deletion of large portion of the genome (Briggs et al. 2007). It is plausible that mammoth DNA, although stored in permafrost, has undergone the same kind of damage. Not all tissues however are equally exposed to damaging condition.

The best laboratory practice in aDNA for obtaining good quality DNA for sequencing studies consists of grinding compact bones and extracting DNA from the powder (Briggs et al. 2007). Bone mechanically protects cells and the DNA within from adverse conditions. Kato’s paper (Kato et al. 2009) shows that nuclei might be identified and dissected from mammoth’s tissues; hence we have a starting point for our cloning project. It is possible in fact that some, but not all, cells have a well-preserved nucleus so the first decision to be taken is how to assess genome preservation in isolated nuclei. In our opinion there are no better choices than an empirical approach using a biological assay: the transfer of the mammoth nuclei into enucleated oocytes.

The second decision is the source of oocytes. The long gone mammoth has living relatives, the elephants, particularly the Asian species; hence, our choice should be the use of Asian elephant oocytes as recipients of mammoth nuclei. This is indeed the best option, though not a problem-free route to success. To date there are no reports of

oocyte collection in elephants. Attempts at superstimulation and oocyte recovery in other megaherbivores such as the rhinoceros have been reported (Hermes et al. 2009) so there is good reason to believe that the procedure, at least the oocyte retrieval part, in elephants is not insurmountable. The size of the elephant and the location of the ovaries make such a procedure technically challenging but not impossible. In elephants only one, and rarely two oocytes mature and ovulate at the end of each estrus cycle. With only three to four cycles per year (in the absence of pregnancy), the number of oocytes that each female elephant can “donate” for the mammoth cloning project is very limited (Hildebrandt et al. 2011). If thousands of oocytes will be needed, hundreds of elephants will have to be subjected to repeated oocyte collection procedures. Finding such a large number of female elephants for the procedures is probably going to be an impossible task. The entire world captive elephant population (Asian and African combined) in zoos and circuses is in the range of 1,500–2,000 animals of both sexes and of all age groups (Saragusty 2012). The alternative would be to seek captive elephants in range countries. Although the number of these throughout Southeast Asia is in the range of 15,000 animals, many are inaccessible or unsuitable (including males, immature females, and elephants in temples). Furthermore, by collecting oocytes from an elephant, we will prevent it from the possibility of becoming pregnant, thus putting the female at high risk of developing reproductive pathologies in the future (Hermes et al. 2004) and, by preventing animals from reproducing, putting the entire captive Asian elephant population at higher risk of extinction (Saragusty 2012). Once a procedure for ovarian superstimulation in elephants has been developed, this could be used in an attempt to somewhat reduce the number of elephants needed. The alternative approach would be to search for a different source of elephant oocytes. We think there are two leading options available for consideration.

In some regions of Africa, elephant populations have grown beyond the carrying capacity of the habitat in which they live. One of the measures employed by population managers is culling, often of whole family groups. When culling takes place, the ovaries can be retrieved and oocytes can be harvested. The drawbacks of this option are that, at any point in time, the vast majority of wild female elephants are not cycling either because they are pregnant or because of lactational anoestrus. The ovarian follicular reserve in African elephants is constituted almost entirely of early- and late-primary follicles (Stansfield et al. 2010) so *in vitro* culture and maturation protocols will need to be developed to bring the oocytes to a sufficiently mature stage to be used for the SCNT procedure. These *in vitro* techniques are not yet available. The other drawback of this option is that culling takes place only in Africa, so only African elephant oocytes may become available this way.

The alternative is to collect ovarian tissue slices from deceased Asian elephant cows and either maintain them *in vitro* or transplant them into host animals so that their circulation and hormones will support follicular growth *in vivo*. This has been attempted once when cryopreserved African elephant ovarian tissue slices were transplanted into nude mice (Gunasena et al. 1998). These mice supported the development of antral follicles but oocytes were generally of poor quality. As good quality oocytes will be needed for the SCNT procedures, improvements of the technique, or finding an alternative host that will give better support to follicular development, will be needed. For the number of oocytes required, a large number of

immune-deficient animals will have to be maintained at very high costs. Once harvested, the good quality oocytes will still need to be matured so an *in vitro* maturation protocol will need to be developed. Despite all foreseen and unforeseen difficulties, we think that this approach stands a better chance of success.

Regardless of the approach taken, we think that it would be best to restrict the number of elephant oocytes needed to the very minimum, given the technical difficulties and the high costs involved in collecting and maintaining them. The probability of finding nuclei bearing intact DNA in a mammoth tissue is not very high, so a large number of oocytes, most probably in the order of thousands, will have to be injected with isolated nuclei to find the very few that might be reactivated and start development. As an alternative approach, we propose the use of easily available oocytes for the first round of cloning. A potential candidate for oocyte donation might be the bovine. The state-of-the-art of *in vitro* maturation and culture in cattle is the most advanced amongst all farm animals (Lonergan 2007), and a large number of oocytes can be conveniently collected from ovaries taken from slaughtered cows. Objections, however, can be raised against this option as it clashes with the established concept of ISCNT, namely genomic/mtDNA compatibility and the high probability of Zygotic Genome Activation (ZGA) failure in the “extreme” mammoth/bovine hybrid embryos (Loi et al. 2011).

3 Genomic/mtDNA Incompatibility in ISCNT

Mitochondrial DNA codes for proteins responsible for the production of cellular energy. Given the low fidelity of the mtDNA replication machinery, some of the mitochondrial crucial genes are secured in the nucleus where they are safely duplicated and expressed (Amarnath et al. 2011). Therefore, a coordinated mt/genomic DNA cross talk is necessary for normal embryo development. Mammoth/bovine hybrid cloned embryos have no chance of developing normally, but early cleavages might occur since energy production and mtDNA replication do not occur before the blastocyst stage (Thundathil et al. 2005).

Following our suggested approach, successfully cleaving cloned embryos will be used at the morula or even 4–8-cell stage, before the unavoidable ZGA failure, for a second round of cloning, but this time using elephant oocytes. The role of the first round of nuclear transfer is to probe a large number of mammoth nuclei, and select those with a genome capable of directing embryonic development. The second round of nuclear transfer will transfer the mammoth nuclei into elephant cytoplasm, where an appropriated mt/genomic DNA cross talk, as well as a successful activation of the mammoth “embryonic genome” will probably take place. Technically it is very easy. The blastomeres of the cloned embryo will be disaggregated and electro-fused into enucleated elephant oocytes, essentially, the standard nuclear transfer procedure for embryonic cells.

In our opinion, this is the best approach to the mammoth cloning project, assuming we, the scientists, are in charge. Experienced developmental biologists familiar with nuclear transfer would surely spot further advantages offered by the strategy we pro-

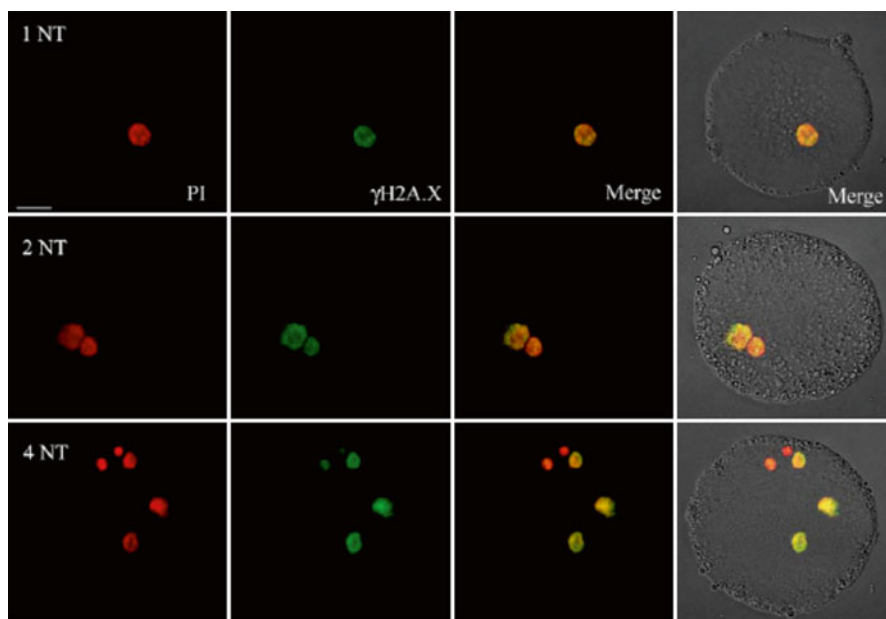


Fig. 19.4 Immuno-localization of histone variant gamma H2AX, which is recruited at sites of DNA repair, in lyophilized cells injected into enucleated sheep oocytes. The DNA repairing activity of the oocyte is not diluted even in case four somatic nuclei are injected (4 NT; the nucleus in the upper part of the oocyte has divided, an event which often occurs in SCNT), suggesting DNA repairing activity of the oocyte is highly redundant; hence it might turn out to be a mighty allied in a mammoth cloning project (Iuso et al. 2013)

pose. First, it has been known since the dawn of cloning that a serial nuclear transfer improves the efficiency of nuclear reprogramming. This is quite logical, if we consider that the nucleus is exposed twice to the oocyte's reprogramming machinery. In case the epigenetic memory of the differentiated cell is partially maintained, the second nuclear transfer will remove it, thus improving the nuclear reprogramming efficiency.

The second advantage comes from the DNA repairing capacity of the oocyte. It is indeed unrealistic to believe that mammoth nuclei will have intact DNA. Single and double strand breaks will probably be scattered throughout the genome. DNA repair is the latest of the oocyte properties revealed, and researchers are still debating its real implications (Ménézo et al. 2010). The redundancy of DNA repairing machinery is by all means unexpected and surprising. In our recent study (Iuso et al. 2013) on nuclear transfer of lyophilized cells, we addressed the issue of DNA lesions caused by the freeze-drying process and whether they are repaired after nuclear transfer. DNA damage was indeed observed in dried lymphocytes, but after nuclear transfer the resulting pronucleus was stuffed with foci of active DNA repair (identified with an antibody raised against modified histone recruited at sites of DNA repair (Podhorecka et al. 2010)). To our surprise, the signal was undiluted even when five somatic nuclei were injected into a single oocyte, indicating redundancy of DNA repairing enzymes in oocytes (Fig. 19.4). Therefore, a second round of nuclear transfer would have the additional advantage of more complete DNA repairing.

Hence, hybrid mammoth/elephant embryos will be allowed to develop to blastocyst stage using the culture conditions most appropriate for elephant embryo. What these culture conditions might be is still a big question mark since any of the *in vitro* techniques related to elephant oocytes and embryos have never been attempted, or at least was not reported on. In the absence of elephant embryo *in vitro* culture protocols, model animals will have to be relied upon to first develop the entire procedure in elephants. Once elephant *in vitro* embryo production and culture protocols have been developed, one can consider attempting to culture mammoth/elephant hybrid embryos. The production of blastocyst stage mammoth/elephant embryos would be already an incredible achievement, but still there is a long way to making the baby mammoth.

4 Transfer of Mammoth/Elephant Cloned Embryos into Elephant Foster Mothers

This might be another leap in the dark. We know that Asian (*Elephas maximus*) and African (*Loxodonta* sp.) elephants can interbreed. These two genera separated about 4.2–9.0 million years ago (mya). About 2.6–5.6 mya the African elephants split into the African savanna elephant (*L. africana*) and the African forest elephant (*L. cyclotis*). During this same time range the mammoth and the Asian elephant split into two genera—*Elephas* and *Mammuthus* (Rohland et al. 2010). When genomic DNA is compared, the Asian elephant is closer to the mammoth than it is to the African elephants. Some still consider the African savannah and forest elephants as being the same species whereas the mammoth and Asian elephant were assigned into different genera, and yet the ratio of genetic divergence of the two African elephants to the Asian elephant-mammoth is close to unity (Rohland et al. 2010). This genetic analysis suggest that it is highly plausible to assume that Asian elephants and mammoths could interbreed, thus increasing the probability of succeeding in transferring hybrid embryos into elephant foster mothers.

Like all other techniques relevant to the handling of oocytes and embryos in elephants, embryo transfer has never been attempted in this species. Assuming all other hurdles have been overcome—oocytes harvested, matured *in vitro*, and injected with mammoth nuclei to produce embryos that have been cultured successfully to the blastocyst stage, performing embryo transfer in elephants would pose primarily a technical problem. Artificial insemination in elephants has been in practice for about 16 years now (Hildebrandt et al. 1998; Hildebrandt et al. 1999). For this procedure, a flexible 3.0-m long customized video chip endoscope is used to place the insemination dose in the vagina, close to the cervical opening. A much longer endoscope will be required to attempt to go through the 15-cm long folded cervix, which in itself is going to be a formidable task, and into the uterus to transfer the embryo.

The alternative would be to conduct embryo transfer by laparoscopy. Elephants, however, do not have a pleural cavity (Brown et al. 1997), so inflation of the abdominal cavity will most likely lead to the collapse of the animal's lungs, resulting in its death. Laparoscopy will thus have to be done without inflating the abdominal cavity,

a technique that is already in practice in human medicine (Paolucci et al. 1995). Due to the enormous weight and size of the elephant's abdominal wall, some of the tools will need to be modified but otherwise, it can be assumed that the technique can be applied to elephants as well.

Twins in captive elephants occur at a rate of about 1 % and experience indicate that when twins do occur, the mother and both fetuses are at high risk of perishing during parturition (Hermes et al. 2008). So, in order not to put the elephants at elevated risk of mortality, only a single embryo will most likely be transferred to each foster elephant cow, thus considerably decreasing the probability of success and increasing the number of animals needed for the project.

Another issue to consider with respect to embryo transfer is the sheer number of elephants that will be needed to conduct such a study. Animals will be needed to first develop oocyte collection and embryo transfer in elephants and then attempt to transfer the product of ISCNT—mammoth/elephant hybrid embryos. To achieve success, a large number of transfers to a large number of elephants will probably be needed before the first offspring will be produced, going through many failures along the way. Elephants are not laboratory mice and no place around the world maintains a large enough number of elephants for this purpose. This means that any such study will either have to rely on a small number of animals, conducting many repeated procedures on each, or recruiting participants from zoos and elephant camps around the world. Either way, the number of facilities that will let their elephants participate will probably not be large. To get the embryos to all these different participating locations, the embryos will need to be transported around the world. This will drastically increase the costs of the project and may compromise the embryos, thus decreasing the rate of success. But, with large enough number of attempts, and after surmounting all the hurdles on the way, pregnancies might be achieved and some might even be carried to term.

5 Some Ethical Questions Associated with Such a Prospective Mammoth Cloning Project

Resurrecting the mammoth is a very attractive and catchy topic but it also brings forth some ethical questions. Suppose this whole endeavour proves successful. With the currently low efficiency of SCNT, and even more so of ISCNT, we would be very fortunate if any research group were successful in producing a single specimen, dedicating much time and huge budgets for the task. Is it really justified to spend all this time and money on resurrecting a single specimen of an extinct species? Given the anticipated success rate, there would probably be many futile attempts to transfer embryos to surrogate mothers, some of which will become pregnant and carry them for different duration of time. The minority of these might carry the pregnancy through its full 22-months length only to deliver a still-born, or a calf that will survive only hours or days. Is it really fair to all these surrogate mothers who will certainly bond with the developing foetus in their uterus?

Assuming the offspring survives, should we leave it with the surrogate mother to raise it? Should we let the family unit (the herd) interact with it? Will we, by doing so, be fair to the mother and herd? Do we really know the needs of mammoth when kept in captivity? After all, it has never been done before. Or will the calf be separated from its surrogate mother and herd and become an isolated research subject, undoubtedly a stressful (and unfair) handling of all animals involved? What will we do with this animal once it matures? Should we put it in a zoo as a tourist attraction? Will it become a reproduction machine—semen “donor” if it is a male or oocyte “donor” if it is a female? Or should it travel around the world to generate more funds? Or maybe we should release it in a place somewhat more suitable for it, such as Siberia, Greenland or Alaska? These are just some of the ethical questions that come to mind when considering the resurrection of the woolly mammoth. Considering all stakes involved, is this scientific endeavour justified?

6 Conclusions

From the two approaches described for cloning a mammoth, the “synthetic” one is, in our opinion, the most advanced approach likely to succeed, but impossible to be implemented at the moment. A newly synthesized genome, bright new and virtually devoid of damage/mutations, would be ideal, far better than the damaged nuclei found in mammoth tissues. Hence, we could transfer the artificial membrane-containing mammoth’s DNA and the centriole directly into elephant oocytes. A further advantage is offered by the possibility to confer upon the naked, “naïve” DNA, an organization that is easily “readable” by the oocyte, thus resulting in an improved nuclear reprogramming, and in turn, development to term. Of course it must be granted that no further complications will arise, such as incompatibility between mammoth and elephant mt/genomic DNA. Likewise, we have to trust that mammoth and elephant are genetically close enough to allow ZGA activation.

This “ideal” approach, however, is still far from our grasp, so the only way to tackle the mammoth cloning project would be a “canonical” Interspecies Somatic Cells Nuclear Transfer approach, as we have described. This approach, we believe, has realistic chances of success. Whether such a project should get under way, knowing the enormous costs and animal welfare issues involved, is still under debate and will probably remain so for many years to come while work in other, more conventional species, aim to improve SCNT and ISCNT efficiency and find solutions to the many pertaining problems involved.

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