Cloning the Woolly Mammoth

Will the Mammoth return?

A Term Paper by

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3b

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1. Preface

Cloning in general is a fascinating topic. The idea of having two genetically identical organisms seems impossible. The complexity of cloning processes is very intriguing, how these processes work and are used is what especially interests us.

One particular use of cloning that we will explore in this paper is the revival of previously extinct species. Looking at pictures and researching extinct animals who lived many thousands of years ago, transports you to another world. It is surreal to think of those giant beasts of the ice ages, for example the Irish elk, the woolly rhinoceros or of course the woolly mammoth living today. Trying to imagine any of these beasts living today is a very mind-numbing thought. Do humans really have the ability to revive such fascinating, mysterious creatures?

Scientists have said time and time again, that it would be feasible to revive the mammoth, but not for a while, as the technical limits are not yet ready for such an undertaking. The moral and/or ethical concerns, as well as the technical and physiological requirements for such a feat are enormous and seemingly impossible. After a closer look, however, it is surprising how comprehensible and possible such a quest seems. That is in fact why we are so interested in this topic and want to share with others how captivating it is. To do that, however, we raised questions involving the possibilities and the concerns of this project:

Will we be seeing mammoths roaming free within a few decades, or are researchers still too far from that point?

Will a revived mammoth be a genetic copy of the mammoths which lived thousands of years ago?

What are the challenges which arise in the revival process, especially those which may be particular to mammoths?

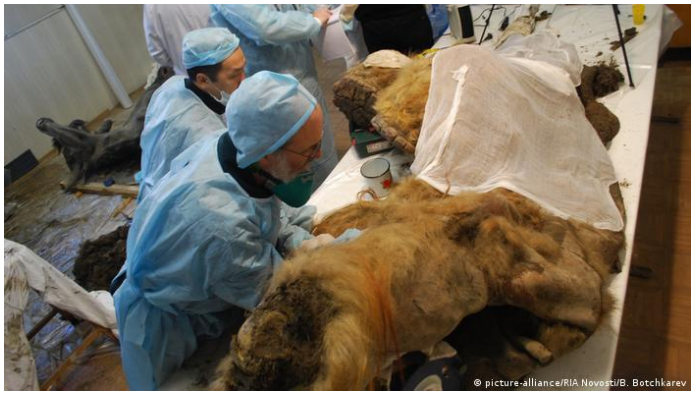

Fig. 1: Japanese scientists working on mammoth carcass

Would revived mammoths be able to survive or even thrive on their own in the wild?

One of the biggest concerns with this project, much like other cloning projects, is the ethical concern. Not only would the life of the organism most suitable to birth the clone be at high risk, but is it even advisable to revive a species that went extinct many millennia ago? Although it doesn't seem too long ago, mammoths lived at a time different in every way to today. The planet offered many suitable habitats as it was colder and most of the planet was covered in ice. It seems, that the only way a mammoth could live today is in captivity with constant monitoring, which again begs the question if it's even advisable to revive the mammoth? There is, after all, a reason they are extinct.

2. Introduction

2.1. Context – Rediscovering the Mammoth

Fig. 2: Yuka, a recovered Mammoth specimen

Cloning the mammoth is a project which really took off in the last few years, with millions of dollars in funding and many different research groups working on the subject. But why is this project only now becoming mainstream? Since 1799, only 12 significant mammoth remains have been found (all but 1 of which were found in Siberia). As more intact and well-preserved mammoth carcasses are uncovered (most recently in 2012), the genome of the mammoth becomes more and more clear, because the DNA is able to be sequenced. After it's sequenced, fragments of DNA from mammoths can be repaired, which leads to more options as to how it will be cloned. Basically, it looks like it is becoming more and more possible to clone a mammoth, but what is the main technique needed for cloning and how was it discovered?

2.2. History – How did SCNT Develop?

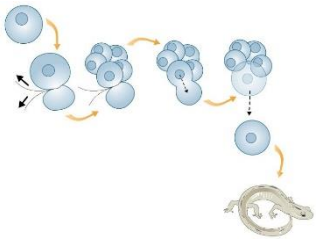

Fig. 3: Procedure of first Salamander clone

1928: A simple precursor to Somatic Cell Nuclear Transfer (SCNT) was successfully done by Hans Spemann. By squeezing a fertilized salamander egg, the cell divided, whereby only one of the new cells included a nucleus. After 16 cells where present, the squeezing was loosened allowing the nucleus from the original cell to slide into the previously empty cell. After separating this new cell with the identical genetic information, an Identical salamander grew from it.

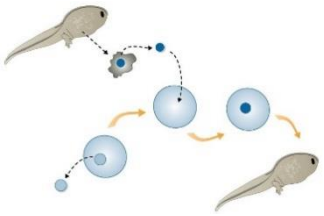

Fig. 4: Procedure of first SCNT

1958: John Gurdon successfully transplanted the nucleus from a somatic tadpole cell (a cell not needed for reproduction) into an enucleated frog egg cell (cell without a nucleus). The resulting individual was a clone of a tadpole from which the somatic cell came. This was the first successful Somatic Nuclear Cell Transfer.

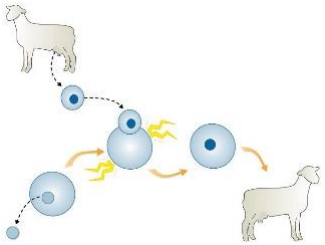

Fig. 5: Procedure of first Mammalian SCNT

1996: Ian Wilmut and Keith Campbell created the first mammal formed by SCNT. The nucleus of a somatic cell (udder cell) from an adult sheep was transferred into an enucleated sheep egg and a sheep was successfully born from it. This proved that it was possible to see embryonic development in mammals with the use of somatic nuclei.

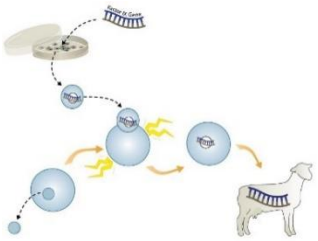

Fig. 6: Procedure of first SCNT with engineered cells

1997: Ian Wilmut, Keith Campbell and Angelika Schnieke successfully formed a sheep clone through nuclear transfer by using genetically engineered laboratory cells and an enucleated egg. They used the nucleus from a skin cell grown in a laboratory dish and transferred it into the donated egg.

2013: Shoukhrat Mitalipov and colleagues successfully created a cloned human blastocyst made from a skin cell (somatic cell) and a donated enucleated egg from a mother. This again reflects the ethical concerns of cloning, as this experiment was stopped after a certain cell count was reached, due to those concerns.

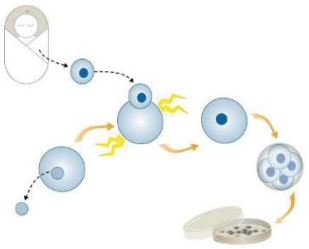

Fig. 7: Procedure of first human clone

2.3. Why SCNT?

Why is SCNT the best technique for this project? SCNT is a process which is highly adaptable, meaning there are many adaptations of the technique allowing for the best possible result in a clone. For example, somatic nuclei can either be directly fused with an unfertilized egg, or the somatic cells could be deprived of nutrients causing them to fall into a dormant stage, allowing a shock to fuse them with an enucleated egg cell. As seen in 1997, this technique also allows for genetically engineered clones, meaning that a fully intact somatic cell from a mammoth is not necessary, if the DNA has been sequenced.

2.4. Artificial Twinning – Why doesn't it work for the Mammoth?

Another common cloning technique is artificial twinning. This involves the fertilization of an egg as normal cross reproduction would. In the early stages of development, however, the cells are separated from each other and begin growing genetically identical individuals independent from each other. This method obviously can't be used for mammoths, as there are no mammoths present to provide male and female gametes.

3. Genetical Engineering Techniques

3.1. SCNT – How does it work?

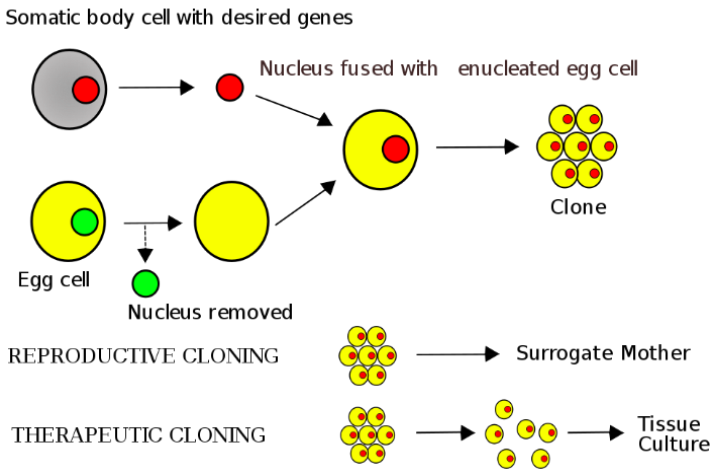

Fig. 8: Graph of Procedure of SCNT

The process of Somatic Cell Nuclear Transfer is quite simple to understand. In SCNT, two different cells are required. First, an unfertilized egg cell needs to be harvested or donated from a mother. The other type of cell needed is called a somatic cell, hence the name Somatic Cell Nuclear Transfer. A somatic cell is a cell from a living organism excluding those used for reproduction. Examples of somatic cells include, liver cells, skin cells, etc...

Then the genetic material is removed from the female gamete, making it an enucleated egg or a deprogrammed egg. The nucleus of the somatic cell chosen is then fused with the deprogrammed egg. Because of the fusion, the nucleus is reprogrammed by the host egg cell. The new zygote (a fertilized egg) is electrically shocked to begin the dividing process. After this, the zygote can undergo mitotic divisions as a cross fertilized egg could, forming a blastocyst with an identical genome to the original organism. For further development the embryo can be implanted into a surrogate mother's (host mother's) uterus, until giving birth to it at term.

The Defining factor of this process is that the Somatic cell and egg cell chosen must come from the same species.

3.2. ISCNT – Not much different from SCNT!

Interspecific Somatic Cell Nuclear Transfer is mostly used to help restore endangered species or resurrect an extinct species. The only difference here is that the genetic information comes from an organism within the same genus (not necessarily the same species) as the organism which donates an unfertilized egg cell.

This is the technique used to clone the mammoth.

3.3. Technical Challenges – Context for Modern Techniques

3.3.1. Collecting DNA

To be able to revive mammoths, first their DNA must be discovered. But how is it possible to find intact, readable DNA from animals which died many millennia ago? The survivability of DNA depends on many factors and can vary greatly depending on the influence of these factors.

The first important factor is exposure to heat. Mammoth carcasses are frozen in permafrost, slowing down all activity, including the decomposition of DNA. The carcasses are also not exposed to sunlight (UV light) or oxygen, also allowing the DNA molecules to degrade more slowly. One of the hardest factors to overcome however, and one of the reasons why research is taking so long, is the fact that these carcasses are extremely rare and hard to find.

Since 1799 only 12 significant mammoth remains have been found.

But does a point come where the DNA is unrecoverable? To answer this question, the half-life of DNA must be considered. Ten years ago, it was estimated, that under ideal conditions a DNA molecule has the half-life of around 521 years (after which half of the bonds in the DNA would be broken). In theory, this allows DNA to survive up to 6.8 million years with intact bonds, but at that point it is completely unreadable, as there is no order to the structure. DNA still has enough bonds to be readable after up to 1.5 million years, in perfect conditions. This makes sense when compared to the oldest recorded DNA, which was found in ice in Greenland and was between 450-800 thousand years old.

3.3.2. Artificial Uterus

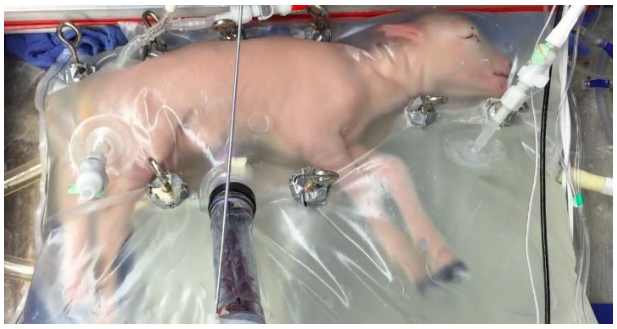

Fig. 9: Artificial Uterus used to grow lamb embryo

The technology of an artificial uterus is very modern and not very common. This technology is, however, very relevant and perhaps an untapped reservoir for the revival of species. The use of an artificial uterus to grow a mammoth clone is an idea that came from the molecular biologist, Dr. George Church, who has gathered millions of dollars in funding.

An artificial uterus would allow extracorporeal pregnancy, bypassing the implantation of a blastocyst into the uterus of a suitable (and willing) mother, which in the case of a mammoth would likely be the Asian elephant.

The Asian elephant is within the same Genus, Elephas, as the woolly mammoth allowing ISCNT.

As the Asian elephant is closely related to the mammoth, it would likely be chosen as a surrogate mother. This would mean suffering for the mother chosen. This is a main reason for the use of an artificial uterus. An artificial uterus would allow exact physiological measurements, suitable for a mammoth embryo, the correct amount of nutrients and the ability to tend to its every need, whether that be a temperature change, oxygen variation or even the addition of amniotic fluid, allowing the embryo to move freely.

3.3.3. Transforming the Asian Elephant Chromosome

An interesting technique was pointed out to us during our interview. This is another technique, that Dr. George Church has investigated and is a variation of ISCNT. In this process, an intact, live, somatic cell from an Asian elephant is taken. The chromosomes in the cell are exchanged with certain synthesized, sequenced, known genetic fragments from the mammoth. Most of the genetic information now belongs to the mammoth, although the Asian elephant chromosome was used as a foundation. All cells do however contain mitochondrial DNA, which can't be changed, so the mitochondrial DNA would still belong to an Asian elephant and not a mammoth. The cell can then be further developed as a SCNT process would.

A mammoth born from this process would not be a genetic mammoth clone, but an Asian elephant, with mostly mammoth DNA and exhibiting phenotypes of the mammoth.

4. Interview

Professor Doctor Patrick Tschopp:

Since 2016, Patrick Tschopp is an assistant professor for zoology at the University of Basel (laboratory of regulatory evolution). He is an expert in embryology and post-embryotic development. Additionally, he did research on evolutionary and developmental biology (EvoDevo). In 2011 he graduated at Harvard Medical School in Boston USA.

Fig. 10: Professor Doctor Patrick Tschopp

What are the steps of cloning? How does the DNA of an organism get into a parental animal in general?

"You have to distinguish between animals that still exist and animals that got extinct. It all starts with the DNA. Traditional cloning works by transferring a nucleus of a somatic cell (of a mammal) into an egg cell, which doesn't contain any form of DNA anymore. The diploid chromosomal DNA of the nucleus is fused with the egg cell by electroporation. If the transfer was successful, an embryo develops in the Petri dish. The embryo can then be implanted into a mother animal by the use of invitro fertilising techniques.

The problem with extinct species being, that you don't have any DNA. For the Mammoth you have preserved skin cells in the permafrost of Siberia, but even there it is not possible to find a nucleus with a 100% intact DNA.

The company of George Church follows a different approach. They take the fragments of the DNA of a nucleus found in the permafrost and sequence them. By synthesis of these fragments, they produce lager fragments of mammoth DNA and splice them into the DNA of an Asian elephant, the closets relative of mammoths nowadays. The splicing process takes several steps and is achieved with Crispr cas9 in a cell culture of Asian elephant cells in a petri dish. The result is not really a mammoth nucleus but, rather an elephant nucleus in which a larger amount of DNA is replaced by mammoth DNA. This hybrid nucleus would then be inserted into an egg cell of an Asian elephant.

The resulting animal will never be a mammoth. Parts of the DNA are still from the Asian elephant. In the step of somatic cell nuclear transfer, the transfer of mitochondria is not possible. But because the mitochondria also contain a mitochondrial genome, this genome always originate from the Asian elephant. In the nucleus we have a Patchwork of mammoth and elephant DNA but in the mitochondria, we only have elephant genome. De facto it's an Asian elephant that contains parts of mammoth DNA."

What are the major challenges of cloning, especially in consideration to the mammoth?

"The main problem of cloning is the loss of heterozygosity. If you cross two almost identical cloned animals you will often get homozygotic animals which are less resistant to diseases, parasites, or fungi, than heterozygotic individuals. But theoretically they are capable of reproducing within their newly cloned species if the fertility isn't affected, such as in mules or other hybrids.

Another problem in consideration to the mammoth is, that, other than Dolly, which was a sheep carried out by a sheep mother, a mammoth must be carried out by an elephant mother. It is questionable if a mammoth, which is significantly larger than an Asian elephant, can be carried out by an elephant. Not only the size difference but also possible immune reactions

harbour a great risk for the birth. An idea would be to create an artificial uterus for the elephant mother, to control the birth of the mammoth. The team of Dr. George Church is currently working on such an ex vivo uterus."

What's the difference between Somatic cell nuclear transfer (SCNT) and interspecific SCNT (ISCNT)?

"A somatic nuclear cell transfer (SNCT) is a cell transfer within the same species. An interspecific somatic nuclear cell transfer (ISNCT) is the cell transfer between two different species. The cloning of the mammoth goes a step further, as not even the nucleus is from the mammoth, but from an elephant.

ISNCT was applied to the preservation program of endangered ferrets. They took a nucleus of the endangered species and implanted it into an egg cell of a not endangered ferret species. The cloned ferret was then crossed with animals from a breeding program to increase the genetic variety of the species."

What are the criteria for a compatible cloning parental animal?

"*There are genetic, anatomical and physiological factors that limit the ISNCT. First you look at the genetic distance, how different the species are evolutionally speaking. However, genetic factors are very hard to foresee, anatomically you can't breed a very small animal with a large animal, even if they are genetically closely related. For all species that can produce hybrid offspring, it's most likely that they can undergo ISNCT. But to be able to tell what comes out or what the challenges will be is only possible to a certain degree. "*

The bucardo goat was the first extinct animal to be re-introduced. As this species didn't go extinct long ago, it wasn't a challenge do preserve the DNA. What are the major differences between the cloning process of a mammoth and the bucardo goat?

"The cloning of the bucardo goat was much easier. You had the DNA, as the species didn't go extinct long ago. Even there you had the two drawbacks of mitochondrial DNA, which will not originate from the extinct species and the drawback of the genetic bottleneck, the strongly decreased genetic diversity.

Speaking of genetic variety, the cloning of a mammoth would only bring a few individuals which are more identical than siblings. This extremely low diversity would drive the species immediately into larger problems, such as a disease. Interbreeding of cloned mammoth most likely won't even be possible, because hybrids such as the mule, are often sexually sterile."

Do you think it is possible to revive the mammoth, and what are the ethical concerns?

"I have the feeling that it's possible to create such an animal, but not now. Genome engineering and nuclear transfer are already advanced enough to do so. The main technological problem is the creation of an artificial uterus, which must be engineered first. But even this is not impossible. But I doubt that there will be a population of mammoth someday, because they are not genetically diverse enough. It's not realistic at all that in the future herds of mammoths will walk across the Siberian tundra, but it's a selling point of Mrger. Church's project to get the money from investors.

Another question is if you should clone mammoths. There are ethical concerns, and it is more important to take care of the existing species and to preserve them before they go extinct. If you ask me, it's complete nonsense to clone extinct animals because they went extinct due to a reason such as environmental or ecological factors. Cloning does not give security that we can bring back every animal, and before we put money and effort into cloning of these animals,

we should take care of the existing wildlife. Considering that elephants are very socially active and live in herds, the cloning of only a few mammoths, in regards to their well-being, is probably a very bad idea."

Fig. 11: Harvard NOCERA LAB-FAS: Working place of Dr. George Church and once of Dr. Patrick Tschopp

5. Discussion

5.1. Progress made – Dr. George Church and his Research Team

Dr. George Church and his Harvard-based team have actually made a stunning amount of progress when it comes to resurrecting the mammoth. They have been able to find enough mammoth DNA to sequence it. The team have published many fully sequenced, complete mammoth genomes. As they knew what the DNA looked like and how it was built after sequencing, they were able to artificially synthesize the DNA in a lab.

After taking a somatic cell out of an Asian elephant, the team successfully rewrote parts of the elephant's chromosomes with the synthesized DNA from that of the mammoth. The research team is making Asian elephant cells more and more mammoth like, with each mutation that they add. Some of the successfully transferred mutations include: Mammoth haemoglobin, extra hair growth, fat production, altered sodium ion channels in cell membranes.

Note, that these added mutations are actual mammoth DNA and not alterations of the Asian elephants DNA.

The team is using an advanced adaptation of SCNT. In this process, not a whole nucleus is taken out of a somatic cell (in this case from the Asian elephant), but rather parts of the chromosomes within the nucleus are replaced.

5.2. Future Steps

5.2.1. Analysis of Genes – Regulating Gene Expression

Some notable future steps for the team in Harvard lab, is the analysing of genes responsible for gene expression. These genes are vital to clone the mammoth, as they are important mutations to be able to adapt to colder climates, so they need to find the correct positions and orders for these genes.

The Asian elephant genome is actually a 99.96% match to that of the mammoth, but it is dire for the team to get as close to the mammoth genome as possible, as their goal isn't just to resurrect a mammoth or a mammoth resembling creature, but to resurrect multiple mammoths suitable for the wild to release them.

5.2.2. Artificial Uterus – Still far from Use!

To birth a developed mammoth infant, the lab wants to use the previously mentioned technique of an artificial uterus. As mentioned before, this eliminates the need for a surrogate Asian elephant as a mother, who would not be safe during the process. However, this artificial uterus still needs to be built suitably for a mammoth and is still far from being suitable to grow a mammoth clone.

5.2.3. First Generation – In Captivity!

If the first generation of mammoth is eventually born, nearly 8 millennia after they went extinct, it would most certainly have to be kept in captivity. The mammoths will have to be kept in zoos with Asian elephants. Asian elephants exhibit similar herding behaviour as mammoths are thought to have. This will help the mammoths get ready to potentially be released into the wild and to form herds of their own. Unfortunately for the mammoths, Asian elephants live in much warmer climates, meaning the mammoths may need more time to adapt to the colder climates before being released. Restoration parks in the arctic is however the end goal for these mammoths.

5.3. Ethical Aspects

5.3.1. Why should we clone the Mammoth?

Mammoths are fascinating creatures. Having a live specimen will help researchers study their behaviours and tendencies. Completing this project would be a validation and a proof for the restoration and conservation of other large mammalian species. This includes the restoration of species hunted to extinction by humans.

One reason many people may not know, is their potential ability to restore grass lands around the world. Many taigas and tundras around the world were once “mammoth steppe” which was a habitat for many herds of different species which were herbivories. With the extinction of the mammoth, these habitats slowly turned to tundra filled with dead plant material, unsuitable for animals. The addition of the mammoth, however, could reintroduce a nutrient cycle, that allow grasses to grow. This would support biodiversity and help permafrost last longer.

5.3.2. Why shouldn’t we clone the Mammoth?

The main problems which arise from cloning the mammoth are the suffering of the mammoth itself and the surrogate mother. Since Dr. George Church wants to use an artificial uterus, the surrogate mother will not be mentioned further.

A mammoth in an artificial uterus is not the same as it would be in the wild. It is the assumption, that a certain amount of suffering would come to the mammoth in the artificial uterus, as it is unnatural. Once the mammoth is born, it comes out to a new world, more than 10 thousand years older than the one it is used to. The world is much different now than 10 thousand years ago, there are less habitats for mammoths and it’s a lot warmer. They went extinct for a reason. Do humans then have the right to just resurrect every species that goes extinct, without considering how the animal might suffer in new conditions?

A cloned mammoth born today would be threatened and endangered by many things. New viruses could affect it, the temperature could be too hot, their diets will have to change, they would have habitats, which are too small, and humans could hunt and kill them, as they once did, too extinction!

6. Summary

The project of cloning a mammoth has become bigger in the past few years, with the discovery of some important mammoth specimen in Siberia. DNA from some of these specimens were preserved in permafrost, shielded from heat, UV light and oxygen. The DNA was then able to be sequenced and the entire mammoth genome became known and was published.

The technique that the leading research team in this project wants to and has already used, is an adaptation of ISCNT. In this process, certain lengths of DNA chains (from the mammoth) replace the corresponding genes on the chromosome in the nucleus of the somatic cell. This is not always possible, as a closely related foundation of genetic material is needed. This is luckily provided by the Asian elephant, which is more closely related to the mammoth, than to the African elephant.

The somatic cell, containing mostly mammoth genome, would then be inserted into an artificial uterus, which avoids the need and suffering of a surrogate mother and allows for close examination of the organism.

For Dr. George Church and his team, the limiting factor is surprisingly not the lack of intact mammoth DNA, but rather the technology needed for further development of a mammoth blastocyst, such as an artificial uterus, which has so far only been used to grow a lamb.

The ability to resurrect species which went extinct long ago was once thought to be impossible, but this project could help shed light on the rapid improvements made by leading researchers. The implications of resurrecting such species could benefit our planet and help save us from environmental crises. On the other hand, humans are a factor leading to many extinctions, so why not work on the problems we already and prevent species from going extinct in the first place.

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