EMBRYONIC STEM CELLS
THE THEORY OF SUPER COWS

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PASS WITH DISTINCTION

RESEARCH PAPER
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ABSTRACT

This paper has been written to propose how developments in embryonic stem cell research could lead to a new 'generation' of cow, one that does not produce methane and, therefore, does not contribute to the increasingly problematic issue of global warming. In the introduction the paper gives a brief history of embryonic stem cells and how they are used in the research, and outlines the recent developments in medicine and how this could translate to veterinary medicine. Then, in the discussion, the proposition of methane-less cows is discussed in depth. Using the DNA of methanogenic bacteria and via genetic engineering, embryonic stem cells of cows could be altered and then grown into cows able to oxidize methane in the rumen. Considering that each cow produces, on average, 250 litres of methane per day, this would drastically effect climate change for the better; the ethical implications of this new 'generation' is also discussed. The mechanisms involved in the creation of these 'super cows' is discussed in the paper, however it must be considered that limitations in current understanding of anaerobic methanotrophic bacteria have had an impact upon the specificity of the process. Finally, the paper concludes with the limitations of the discussion and summarizes the authors view on embryonic stem cell research.

INTRODUCTION

There has been a lot of publicity recently following the inauguration of President Barack Obama concerning embryonic stem cell research and stem cell therapy: President Obama has lifted federal restrictions, allowing many universities and labs to continue developing and researching this extraordinary therapy. This has been seen as a radical decision for Christian America; for some protestors stem cell research is akin to experimenting on human beings, this 'pro-life' argument being the basis of the restrictions placed upon it during the Bush era. However, the law is changing and already radical new developments are being made, with a potential cure for MS and amazing improvements in those suffering from paralysis being reported, all less than a month into the 'new dawn of medicine'; less than a month since the restrictions were lifted.

2nd February 1963, Ontario Cancer Institute at the Princess Margaret Hospital, downtown Toronto, Ontario, Canada. Ernest McCulloch and James Till first publish 'Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells', a paper that describes an experiment conducted by the scientists during the previous months. This experiment involved injecting bone marrow cells into mice that had been treated with radiation and observing the spleens of these mice. What they had found was extraordinary: small 'lumps' (dubbed 'spleen colonies') grew on the spleens, directly in proportion to the number of bone marrow cells injected. Now, this may not sound as exciting as a cure for MS or the regeneration of organs, but the scientists had made the first, vital step towards this by proving the existence of stem cells, by 'speculating that each lump arose from a single marrow cell', i.e. a cell had grown into other, specialized cells. This cell was a stem cell.

Following this, developments continued in the research of stem cells and their unique differentiation properties, the scientists perhaps unaware of the amazing possibilities resulting from their work. Embryonic stem cells came into the picture in 1981, when two independent research groups, Evans & Kaufman and Martin, discovered stem cells in mouse embryos. It wasn't until 1998 when James Thomson, University of Wisconsin, isolated human embryonic stem cells, the inner cell mass, from the rest of the embryo, that they were finally identified in humans. Thomson found and isolated the part of the embryo with the potential to become any cell in the body (the totipotent cells), creating some exciting new opportunities for medicine. However, before further research could be done, President Bush restricted the funding for research on human embryonic stem cells, halting the progress of the revolutionary research.

Nevertheless, since then there have been several key developments in the use of embryos in stem cell research, the recent ‘miracles’ coming within months of each other. In 2004 Douglas Melton of Harvard created more than 70 embryonic stem cell lines (privately funded), meaning that he took the pluripotent [able to become either endoderm cells (interior stomach lining, gastrointestinal tract, the lungs), mesoderm cells (muscle, bone, blood, urogenital), or ectoderm cells (epidermal tissues and nervous system)] stem cells from inside the blastocyst and grew it in a culture plate, creating more and more stem cells. These were then given to researchers around the world for free, to get around the restrictions on funding in place. This has resulted in multi discoveries and developments in stem cell research, not just with embryos, including the cloning of animals such as Dolly the sheep, but also the creation and development of iPS cells. These are the artificially manufactured pluripotent cells used in embryonic stem cell research; because they are not taken directly from the embryo they avoid the ethical debate; however it remains that only embryonic stem cells are totipotent and can divide into any part of the organism or even the entire organism.

When embryos are used for the research, the process is highly controlled, using embryos donated for the research. When the embryo is about a week old it is a blastocyst, a hollow ball of about 100 cells, containing the inner mass cells (the totipotent cells). Scientists isolate these cells from the rest of the embryo and cultivate them in a culture plate with the required nutrients to manipulate the stem cells into growing into the required organ or organism (figure 1). When the cells are removed, the blastocyst is destroyed, the main reason why the pro-life protestors have called for a ban on the research. The stem cells are then grown until there are many more, an indefinite number, before being treated with different chemicals to encourage them into growing into the desired form.

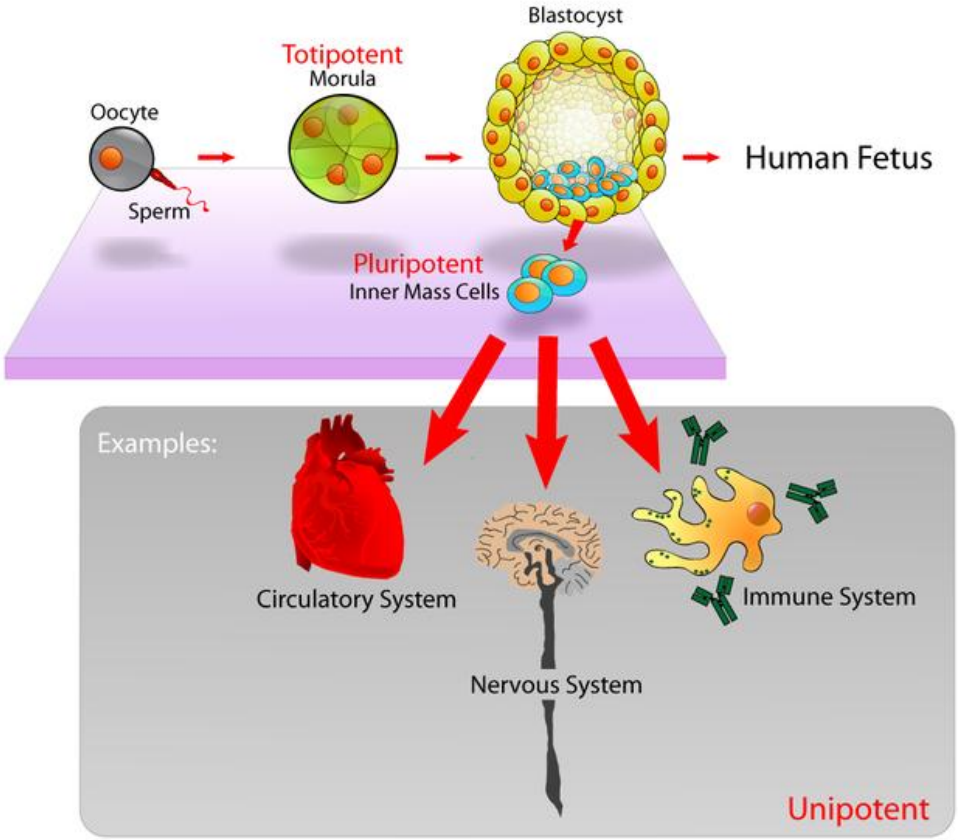

Figure 1

What does this mean for modern medicine? An indefinite amount! The method in which stem cells have been cultivated has shown that organ growth is possible, something that could end the huge demand for transplants. Transplants are often unsuccessful, even if the patient receives the donated organ, because the patient's own body does not recognise the organ and rejects it. If stem cells taken from the patient were used to grow the organ it would be part of the patient, because it would contain their DNA, and the body would accept it. This has already lead to some amazing cases, such as Claudia Castillo's regenerated bronchus, and there is room for development here. Stem cells could also be used for regenerating the eyes of the elderly, the spinal cords and nerves of the paralyzed, or even regenerating the pancreas, this time insulin producing, of the diabetic. There is also the potential to move into cosmetic medicine, using stem cells to regenerate aging facial tissue or creating larger, natural breasts. In terms of veterinary medicine, the bones of dogs could be regenerated when they become arthritic, lessening the cases of euthanasia in old dogs. Another possibility is the regeneration of healthy ligaments and tendons in the legs of horses that damage these, again proving an end to unnecessary euthanasia. Cancer could also be combated using stem cells by expanding our understanding of the disease.

DISCUSSION

The future of embryonic stem cell research is exciting and there are many different medical opportunities to be considered within this quickly expanding field. However, since this project is based upon veterinary lectures I have chosen to consider how it may be used to benefit animals and humans alike; the aim of the veterinary profession is to help both by ensuring the health of animals and I believe my paper should reflect this. A future application of stem cell research could be to tackle the issue of climate change through the genetic modification of cows so that they no longer produce methane (and other livestock ruminants, however cows should be dealt with first because they produce the most, about 250 litres per day), a greenhouse gas contributing to the increasingly worrying problem of global warming.

First of all let us consider how cows (ruminants) produce methane, before discussing how embryonic stem cell research can provide a possible solution to this problem. The digestive process begins, with the majority of vertebrates, in the mouth. The cow has 32 teeth, including 12 premolars and 9 molars, which it uses to chew grass; this chewing process grinds the grass down into a pulp, increasing the surface area for chemical and microbial digestion. This pulp then passes into the Esophagus, and into the first part of the Reticulorumen, the Rumen, this is where millions of protozoa and bacteria break down cellulose and other polysaccharides into fatty acids (volatile fatty acids: VFA), carbon dioxide, ammonia and methane. Cows are dependant on these microbes; without them they would be unable to digest food and survive, and without the cow the microbes would also die: they need the food to digest in order to respire and produce ATP for energy. It is this dependence on each other that makes the cutting down of methane tricky; if the microbes that produce methane, methanogenic bacteria, are destroyed than digestion of grass cannot occur and the cow dies.

Methanosarcina barkeri strain fusaro and *Methanosarcina acetivorans strain C2A* are thought to be the main producers of methane in cows, and are categorized as extreme archaea. This means that they are single-celled organisms without a nucleus and with a membrane different from all other organisms. They survive in an environment practically devoid of oxygen; they gain all their energy for reproduction from anaerobic processes. It is these archaea that could be, using the recent advances in stem cell research, researched, changed or replaced to lessen or eliminate methane production in not just cows but all ruminant livestock. The financial and environmental benefits to reduced methane production in livestock is great: if livestock no longer produced methane as a by-product the environmental advantages would be huge, and a lot of financial gain would be a possibility for the scientists who develop this new super cow! In addition to this, it would probably be used to argue the case for further embryonic stem cell research projects, as once more the possibilities would be proved endless.

Now, let us consider the role of the bacteria within the ruminant stomach. Other bacterium in the rumen, such as *Fibrobacter succinogenes,* break down cellulose by breaking the intermolecular bonds (Hydrogen bonds) between the layers of glucose chains, converting the 3D cellulose structure into amorphous cellulose (with no 3D structure). One of the by-products of this is Hydrogen, which is used, together with

Carbon Dioxide (produced by the digestion of starch by bacteria such as *Bacteriodes ruminocola*) to make methane by the methanogenic bacteria using the following mechanism:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

So, how could embryonic stem cell research be used to stop the production of methane? One possibility is, by using genetic engineering, the DNA of the cow could be altered to code for the production of the enzymes that break down cellulose. The DNA strands in the bacterium that specifically break down cellulose would need to be identified and extracted, then implanted into a cow embryonic stem cell nucleus. The stem cell would then divide and grow, eventually into a fully formed cow, grown in the laboratory. This cow would be able to break down cellulose independently of the bacteria, and would have no need for it; however to design a cow without the bacteria in its rumen would require making the rumen inhospitable to the bacteria. This could be done by changing the pH of the rumen to below 6.0, as the bacterium can only work in conditions that are acidic. The pH could be also be changed by genetic engineering, however this dissertation is not focusing upon this.

The problem with the above method is that it would involve the manipulation of several genes, would be extremely complicated and the cow would still produce methane, as it would still need to dispose of the carbon dioxide and hydrogen produced by the break down of cellulose.

The proposition of this dissertation is not that the cow needs to break down cellulose by its self, as this process would still produce the methane we are trying to halt the production of. Instead, the cow needs to be able to break down methane within the rumen, so that it is never released into the atmosphere. At the moment, little is known about how the microbes can oxidize methane (break it down) without the presence of oxygen, however it does occur and there are several theories as to how.

One such theory is that the opposite reaction of methanogenesis occurs, a hypothesis known as ‘reverse methanogenesis’. However, some research has shown that this would require more energy from the microbe than it would produce, and would therefore not aid the microbe’s survival. Another theory is that the methanotrophic (methane oxidizing) bacteria uses natural sulphates or nitrates, with the methane, to make bicarbonates, water and either hydrogen nitrate ions or hydrogen sulphates ions. At the moment, the exact mechanism of aerobic methane oxidization is unknown, however there are some bacteria that break down methane in this manner, such as *Rhodopseudomonas gelatinosa* (which has been found to oxidize methane anaerobicly, producing cellular components and carbon dioxide).

My theory for one possible use of embryonic stem cell research in the future could be made possible by isolating the gene in the bacteria’s DNA that can anaerobicly oxidize methane and cutting it out. This gene could then be implanted into the embryonic stem cell of a cow, directly into its own DNA. This embryo could then either be placed inside a surrogate mother, as with cloning, or it could be grown in a laboratory (something that has been proven to be relatively easy). Tests on the cow produced would need to be made to ensure that the genetic engineering had been successful and that the cow was no longer producing methane, and that it was healthy. Clones could then be made of this cow, and other cows ‘grown’ in a similar way, to then allow breeding between them to occur. This offspring would again need to be tested to check for methane production and health issues before launching a larger breeding project. The role of the veterinarian throughout this would be to provide the intricate knowledge of the cow’s digestive system needed in order to adjust it, also to ensure the health and care of the animal produced by this process. Success here could lead to a similar process for all other methane producing ruminants.

All embryonic research carries some serious ethical issues, and this is no exception. Research using embryos has always been a controversial area because of a single, vital question: ‘when does life begin?’. The embryonic stem cells used in research, and that would be used to create the modified cows described above, are taken from the blastocyst before it has time to develop into the embryo proper, then into the fetus and finally the fully formed baby. The sperm has already penetrated the egg, and those that believe that life starts at conception have argued that this means that destroying the embryo by removing the stem cells is akin to murder. Extreme pro-life activists claim that by ‘harvesting’ and manipulating embryos, scientists are doing the equivalent of killing unborn children in order to experiment upon them. These are extreme claims, and require a great deal of thought and consideration; scientists cannot disregard the ethical dilemma of this theory, they should be actively trying to counter it or work around it, otherwise resistance to stem cell research will continue.

The following processes described are that of a human (the main controversy behind embryonic stem cell research concerns the use of human embryos. These issues potentially apply to animals such as cows, however due to the usage of animals as food there is less concern in this area). Fertilisation takes place in the Fallopian tubes, one sperm penetrates the egg and the one-cell embryo is formed from the two cells fusing together. The embryo then moves into the uterus (this takes about 5-6 days) where it implants into the lining; the mother is not pregnant until the embryo (by this stage a blastocyst) manages to imbed its self (figure 2). This process has a 20-30% success rate, meaning that 70-80% of fertilized eggs do not ever become babies due to the ‘wasteful nature of nature’.

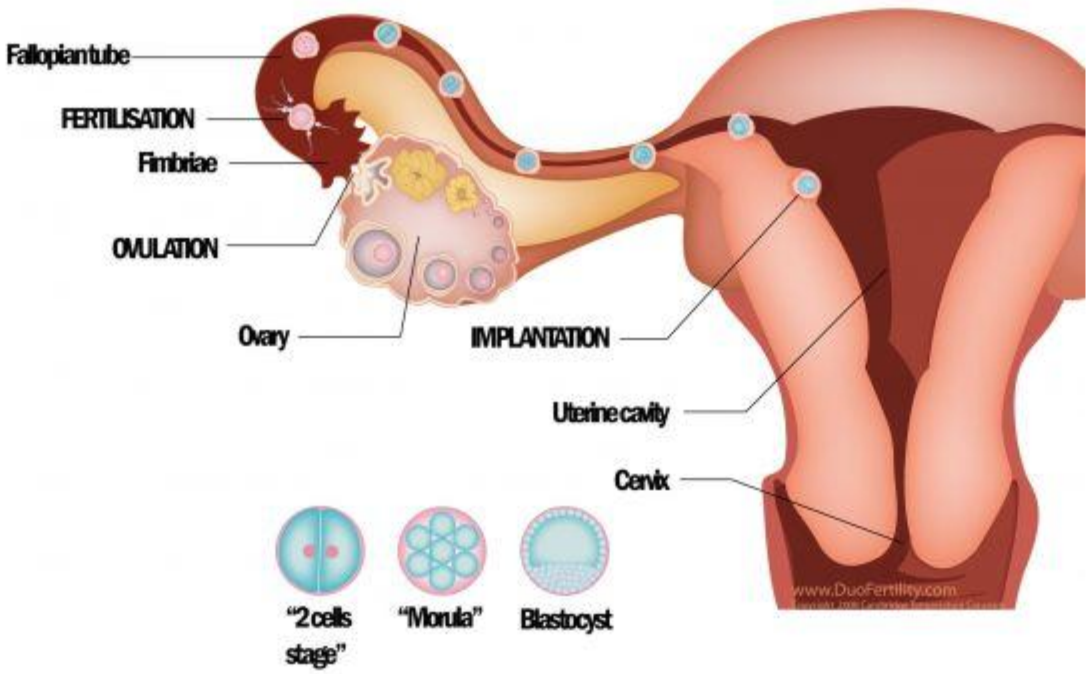

Figure 2

Does this mean that nature herself is a killer of unborn children or does it imply that these fertilized eggs are not unborn children, but instead have the potential to become children: are they alive or do they have the potential for life?

The cells can be considered as alive because, simply, they are not dead. A dead blastocyst could not develop into a child, the cells must be alive to grow and divide, and therefore the termination of life is definitely required to gain access to the stem cells for research. However, how unethical is this considering the natural rate of miscarriage in women: 1 in 36 women will have at least 2 miscarriages at some point in their lives due to the way of nature. 50% of pregnancies end in natural termination, either by the embryo failing to imbed in the uterus lining or for other reasons; nature herself is extremely wasteful of life, does this give the scientist permission to experiment upon embryos? Supposing that embryos donated to scientific research are 'unwanted' by the mother, and their development in her womb would of ended in abortion; it is fair to say that a woman prepared to donate her embryo to science would of otherwise had it destroyed by another means; does it then mean that by conducting research upon the embryo it benefits the greater good of mankind? This utilitarianism argument is that the termination of some 'unwanted' embryos will result in some amazing developments in medicine, and veterinary medicine, that will save a lot more people, and animals, then it will take to 'destroy'.

However, the main flaw for the utilitarianism argument is the fact that the embryo still is alive and has a 50% chance of becoming another life form, and that by scientists experimenting upon this embryo they are using life merely as a 'means to an end'. Life created in order to be destroyed for medical research, and does this process then devalue life its self if scientists can create and manipulate it in laboratory, and so destroy it in order to obtain stem cells so easily?

Applying these ethical ideas to my theory of modified livestock, specifically cows, through stem cell research, I think it can be argued that the pros far out weigh the cons, though this may be a crude way of putting it. The rate at which global warming is developing suggests that something must be done to put a stop to it before the ice caps melt, sea levels rise, temperatures soar and the earth becomes inhabitable. Livestock contribute such an enormous amount of methane that something should be done; in fact would it be unethical to the environment and all those within it if scientists had the means to do something about it but didn't? Although it may take a lot of embryos to create even one specimen that does not produce methane, and a lot of breeding and time to produce enough livestock to have a 'knock on' effect on the progression of global warming, the end result would be worth it, as it would help fight climate change. Climate change has the potential to end all live on planet earth, so surely anything that can be done about it should be done?

There is one more ethical problem to consider, however. This is what to do with the livestock that do produce methane once there is an introduction of livestock that does not. Making these modified animals the majority will only occur if they are the only animals being used for reproduction, as their offspring will also not produce methane. Should then the other animals be castrated/spayed or culled? All would cost farmers money, and the non-methane producing livestock would cost considerably extra to obtain; would the introduction of non-methane animals be fair on farmers and existing methane producers?

CONCLUSION

As I have mentioned, the future of stem cell research is vast, practically limitless. Scientists are reporting amazing advances almost weekly, and it will not be long before this area of medicine is seized upon by the veterinary profession. My proposal for the use of stem cell research to combat climate change by creating a cow with methane oxidizing abilities may be among those advances in the coming years, and the impact upon society would be huge, as discussed in the ethics section of the discussion of this report. At the moment this development is limited, not by our understanding of stem cells, but by our understanding of methanotrophic bacteria. Once the anaerobic methane oxidizing gene is identified and isolated, the developments suggested by this paper can occur, and the 'super cow' can be introduced to society.

During this project I have enjoyed researching embryonic stem cells and learning about how they may impact society, as well as the ethics of the current situation. The field is 'cutting edge', the technology is being developed even as we speak, and the possibilities of regenerative medicine, cancer therapies, cures of paralysis, MS, brain damage etc suggest that there is much to be discovered and many areas of medicine that this research can be applied to. In the words of Christopher Reeve, who would have benefited from the developments in stem cell research to treat paralysis, perhaps to the extent that he may have survived his illness, and who campaigned for further stem cell research until his death in 2004; 'We live in a time when the words impossible and unsolvable are no longer part of the scientific community's vocabulary. Each day we move closer to trials that will not just minimize the symptoms of disease and injury but eliminate them.'

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