Hybrids and Chimeras

A report on the findings of the consultation

October 2007

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

Chair's foreword

In January 2007 the Authority considered the question of creating human-animal embryos for research. Recognising that this is a complex and sensitive issue, on which there is a wide range of views, we designed a public consultation which provided a forum for the public to engage in an informed debate.

After careful consideration of the evidence gathered through the consultation, the Authority decided that cytoplasmic hybrid research should be allowed to move forward, with caution and careful scrutiny. Research teams wishing to pursue a licence for this type of research will have to demonstrate, to the satisfaction of an HFEA licence committee, that their planned research project is both necessary and desirable. They must also meet the standards required by the HFEA for embryo research.

During the consultation it emerged that people are clearly interested in understanding much more about what researchers are doing now and what their plans are for the future. We are committed to maintaining an open dialogue with the public on issues such as this. Over the next few months we will be looking at how we can continue to engage with the public on issues of science and research.

We have gained a valuable insight into public opinion as a result of this consultation and this has enabled us to make a policy decision based on robust evidence. We are extremely grateful to all those who participated and who helped us to understand public opinion on this complex issue.

Shirley Harrison
HFEA Chair

sciencewise

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

1. Introduction

1.1. In November 2006, the Human Fertilisation and Embryology Authority (the Authority) received two research licence applications to derive stem cells from embryos created by Somatic Cell Nuclear Transfer (cloning) using animal eggs.

1.2. At its meeting on 10 January 2006, the Authority concluded that in the light of current scientific opinion the regulation of research using human-animal embryos is probably within its scope. In addition the Authority decided that a full public consultation should be held on the ethical and social implications of creating such entities.

1.3. A public consultation was held to examine the full range of issues arising from the creation of human-animal embryos. One aspect of this has been the exploration of the social and ethical issues, the other being the examination of the scientific background. The consultation ran for three months, from 26 April to 20 July 2007.

1.4. This report summarises the findings from the consultation under themes which emerged. Detailed findings from all strands of the consultation can be found in the appendices at the back of this document.

2. Scientific context

History of animal-human constructs in research

2.1. The mixing of human and animal genetic material has a long history in science and has been used in a number of different ways to greatly progress medical research. The fusion of human and animal cells (to create somatic cell hybrids) is extensively used in research and was a technique first used in 1970s/80s in the mapping of the human genome and to investigate the interactions between the nuclear and mitochondrial genomes.

2.2. The HFEA has previously licensed the creation of true hybrids, with hamster eggs and human sperm, as a diagnostic test for the quality of human sperm. However, the Human Fertilisation and Embryology Act (1990), prohibits any such embryos from developing further than the two cell stage.

2.3. The creation of transgenic animals, in which a human gene is introduced into the germline of an animal and therefore transmitted to all cells in the offspring, is a long established technique used for production of pharmaceutical products and as a model for human disease. The production of growth hormone in the serum of transgenic mice in 1982 was the first example of the production of a human therapeutic protein from an animal. The introduction of gene sequences into mice has allowed scientists to identify and understand the role of particular genes in a large number of diseases e.g. mouse strain with the gene for Alzheimer's disease. Further examples are outlined in section 1.1 of Appendix B.

2.4. Animal chimeras, which are created by the transfer of human cells to animal embryos (or at later stages of development), have proven to be a useful tool to test for the pluripotency of human stem cells.

2.5. Scientists have been creating cytoplasmic hybrid embryos, of various animal species, for over a century. This technique has been used to investigate interactions between nuclear mitochondrial genomes and to attempt to clone endangered species. Details of the various types of cytoplasmic hybrids which have been created, and the stages of development which they reached, are outlined in section 1.2 of Appendix B.

Why scientists propose to create interspecies cytoplasmic hybrids

2.6. The creation of embryos using the technique of somatic cell nuclear transfer (SCNT), and development of these embryos to blastocyst stage will, in theory, allow the production of embryonic stem cells which are genetically related to the donor cell. This technique holds the key to potentially significant advances in medicine as it could be used to produce disease specific embryonic stem (ES) cell lines in order to model diseases and screening for drug therapies. Also, ES cells produced in this way could be differentiated into most cell types and in theory used as a source of patient specific cells to replace damaged tissue (the concept known as therapeutic cloning). There is already evidence that human ES cells, derived from IVF embryos, have the potential to develop into a vast array of cell types (see Appendix B).

2.7. It has also been suggested that embryos created in this way could be used to investigate the mechanism used to reprogram DNA to a pluripotent embryonic state and this knowledge could potentially be used to create methods to produce stem cells from somatic cells (therefore avoiding the use of human eggs and embryos). In addition as cytoplasmic hybrids will contain animal derived, and possibly some human derived mitochondria, they could be a useful tool to study mitochondrial disease and the relationship between the mitochondria and the nucleus.

2.8. However, the technique of SCNT to produce ES cells still needs investigating as, although there has been success in animals (see section 2 of Appendix B), it has not been proven to work with human eggs. To date there is only one example of this technique being used to create a human embryo, which developed to blastocyst stage but did not lead to the derivation of stem cells.

2.9. The availability of human eggs and embryos is a major limiting factor for investigating and utilising this technique in humans. Therefore scientists have suggested that one alternative is to use eggs from another species which are accessible in abundance. There has already been a report, from China, of pluripotent ES cell lines, with many properties of conventional human ES cells, being derived from human-rabbit cytoplasmic hybrid embryos (see section 1.2 of Appendix B).

3. International perspective

3.1. Most countries have not formed specific legislation to cover the creation of human-animal hybrid embryos. Countries that already prohibit the creation of human embryos for research, including many in Europe, may not feel the need to review their legislation. Some countries with more permissive policies, such as China, Japan and South Korea, already allow the creation of embryos for research through SCNT. The majority of these do not specifically prohibit human-animal embryos, which is why studies that have created cytoplasmic hybrid embryos have been able to go ahead in China.

3.2. To date only Australia, Canada and the USA have passed legislation on human-animal embryos. Australia allows embryos to be created for research but the Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006 prohibits creating human-nonhuman chimeric and hybrid embryos. The only exception is that researchers can apply for a licence to create a hybrid embryo for the purpose of testing human sperm quality.

3.3. The Canadian Assisted Human Reproduction Act (2004) prohibits the creation of human chimera embryos. In addition the Canadian Institutes of Health Research (CIHR) and the Tri-Council Policy Statement (TCPS), which covers Canadian stem cell research, prohibits the creation of human-animal hybrid embryos.

3.4. Currently in the USA, federal funds can only be used for human ES cell research using specified pre-existing stem cell lines and no federal funds can be used to create new human ES cell lines. Specifically, the USA Draft Human Chimera Prohibition Act of 2005 (S.1373) prohibits creating or attempting to create a human chimera. In this draft legislation, some human-nonhuman hybrids would come under the definition of a chimera.

3.5. Appendix C gives further information on the legislation in Australia, Canada and the US, and details the general policies on human embryo research of other countries.

4. Consultation: the approach taken

4.1. The consultation was structured in two distinct parts. The first being a consultation document and public dialogue work, designed to gain an insight into the views of members of the public. The second being a scientific consultation and literature review, intended to build a picture of the scientific context to the consultation.

4.2. At its widest point, the consultation sought the views of members of the public through an opinion poll. This provided an indication of the views of the UK population by the sampling of a representative group. The deliberative work helped to interpret the findings of the opinion poll, focusing on how people's views change and develop when introduced to different information. The written consultation and the public meeting provided an insight into those with a specific interest in the issues, however as the participants were self-selecting the findings from these strands of the consultation were not necessarily representative.

4.3. The consultation provided a flavour of public opinion, from which it has been possible to identify key themes. This helped to categorise some of the areas of concern and gauge the levels of acceptability for creating human-animal embryos for the purpose of research. In carrying out the consultation, efforts were made to ensure that a representative group of the public was engaged and their voices heard.

4.4. To ensure the consultation was effective in gauging public opinion and attitudes, it was undertaken with the support of Sciencewise, a programme run by the Office of Science and Innovation (part of the Department for Business, Enterprise and Regulatory Reform) which helps policy maker's commission and carry out public dialogue activities. Sciencewise provided the HFEA with a grant of £60,000 and helped to ensure that the consultation was run in line with the Government's Guiding Principles for Public Dialogue on Science and Technology.

Consultation document

4.5. As a basis for the consultation, the HFEA wrote a consultation document which explained the science involved in creating different types of human-animal embryos for research. This document also explained some of the social and ethical arguments for and against the research and great care was taken to ensure the document was accessible to all audiences. The following questions were posed in the document, with responses gathered via an online questionnaire:

1. The following types of embryo research are legally permitted and licensed in the UK. Which of them in your view are acceptable?

- Research using human embryos donated by IVF patients
- Research using human embryos created specifically for research from donated eggs and sperm
- Research using cloned embryos created specifically for research through cell nuclear replacement (CNR)
- No research using human embryos is acceptable
- Not sure/undecided

2. Do you think that the HFEA should issue licences to allow research using cytoplasmic hybrid embryos?

3. Do you think that the law should in future permit the creation of true hybrid embryos for licensed research purposes?

4. **Do you think that the HFEA should in future issue licences to allow research using human chimera embryos?**

5. **If you have answered yes to questions 2 to 4, what limits do you think should be placed upon human embryos research?**

4.6. Respondents to the written consultation included both organisations and individuals. Of the 810 that responded via the online questionnaire, 74 (9%) responded on behalf of an organisation and 736 (91%) responded as an individual representing their own opinion. The findings from the written consultation can be found at Appendix D.

Public Dialogue: Deliberative work

4.7. The HFEA commissioned Opinion Leader (a research based consultancy) to undertake a public dialogue on the issues raised in the consultation document. There were three distinct strands to the public dialogue; deliberative work, an opinion poll, and a public meeting. The development of these strands was assisted by a Stakeholder Advisory Group, who advised and commented on the plans for this work and the development of materials to be used with members of the public. Advisory Group members represented a range of organisations that have a special interest in stem cell research.

4.8. The main focus of the public dialogue work was the deliberative work, undertaken to explore and understand various public perceptions, motivations and attitudes to creating human-animal embryos for research. The first stage of this work involved establishing deliberative groups. In these groups participants were taken through the different types of human-animal embryos and the science behind them, and initial reactions were also gathered. 104 people took part in this first stage, which consisted of 12 groups held in London, Manchester, Newcastle, Belfast, Glasgow and Swansea.

4.9. The second part of the deliberative work consisted of a full day workshop held in the first week of June. 44 of those that participated in the deliberative groups attended this meeting; participants were selected at random to ensure a representative mix. The aim of this second stage was to explore how the views and opinions of participants changed when exposed to different information. Expert speakers were used to illustrate the different issues and arguments relating to the consultation, thereby stimulating questions and debate. The workshop was recorded and a short film of the day was shown to the audience at the public meeting. This film was also made available for viewing via the HFEA website. The detailed findings of the deliberative work, including both the group work and the workshop, can be found at Appendix E.

Public Dialogue: Opinion Poll

4.10. In early July 2007 an opinion poll was conducted to gauge the views of 2,000 residents of Great Britain and 60 residents of Northern Ireland. Participants were selected at random, with quotas set on age, sex, geographical regions, and housing tenure. To ensure a representative sample, data was weighted against the profile of the United Kingdom.

4.11. The questions for the poll were developed with the assistance of the Stakeholder Advisory Group and built on the early findings of the deliberative work. The full results of the opinion poll can be found at Appendix F.

Public Dialogue: Public meeting

4.12. A key aim of the consultation was to engage with the public in a meaningful way, informing the debate by ensuring that the public are aware of the various arguments for and against the creation of human-animal embryos.

4.13. 153 people attended the meeting and all participants were self selecting and therefore not representative of the general public. 37% of participants described themselves as members of the public, 36% attended as a representative from an organisation with an interest in the area and 27% were from a scientific or academic background. No other information was gathered about participants.

4.14. To encourage debate of the issues a panel of speakers, holding various views, were asked questions by the audience and a lively debate between the panel and the floor ensued. An audio recording was made of the debate which was then made available on the HFEA website. Electronic voting was used during the meeting to capture the views of those who attended. A full account of the meeting, including the results of the electronic voting, can be found at Appendix G.

Scientific literature review

4.15. A comprehensive literature review of the scientific context and the issues surrounding the creation of human-animal embryos for research was undertaken. The review outlines the history of interspecies constructs in research, the reasons why scientists propose to create cytoplasmic hybrids and explores whether this is a feasible technique. It investigates the potential biological issues with creating cytoplasmic hybrids including nuclear reprogramming, interaction of the nuclear and mitochondrial genome and mixing human and animal mitochondria. Alternative avenues of research and sources of stem cells have also been outlined. This review can be found at Appendix B.

Scientific consultation

4.16. In addition to the literature review, a small number of stakeholders were consulted on specific scientific questions. Responses were gathered from external stakeholders, the HFEA's Scientific and Clinical Advances Group (SCAG) and the HFEA Horizon Scanning Expert Panel (HHSEP). The external stakeholders included the British Fertility Society (BFS), Human Genetics Alert, Scottish Stem Cell Network and the Motor Neurone Disease Association.

4.17. The purpose of this exercise was to gain an understanding of the scientific issues surrounding human-animal embryos. The findings of the scientific consultation can be found at Appendix H.

5. Themes emerging from the consultation

The use of human embryos in research

5.1. During the course of the consultation it quickly became clear that there were a large number of respondents who are against any type of embryo research. This view was overwhelmingly represented in the responses submitted to the written consultation and was also dominant at the public meeting. It was also evident in the deliberative work and the opinion poll, although to a significantly lesser extent.

5.2. As research using human embryos is currently licensable by the HFEA, the purpose of the consultation was to gauge public opinion of embryo research in general. However, in the context of the consultation it is useful to be able to distinguish those objecting to the fundamental notion of using human embryos in research, from other respondents, to explore where others might impose limits.

5.3. Those not against the use of human embryos in research were generally supportive of research using spare embryos donated from patients undergoing fertility treatment and, to a slightly lesser extent, research using human embryos created from donated gametes.

> *"I think its better to donate them than just leave them, put them in the freezer, and argue over it when you get divorced."*
>
> Swansea man, participant in the deliberative work

> *"Why would you object to donating your embryos if it goes to a good cause? Abortion goes to nothing."*
>
> Glasgow man, participant in the deliberative work

5.4. At the public meeting the majority considered that it was not acceptable to use animal eggs as an alternative to human eggs. Whilst in the deliberative work a more permissive view was expressed. Indeed it was deemed a necessary option rather than a preferable one. There were also some respondents who appeared concerned about the risks associated with the donation of human eggs.

> *"Given the difficulty and potential risks to women who donate eggs this would be a safer and potentially richer source of eggs."*
>
> James King, in response to the written consultation

5.5. In 2006 the HFEA consulted on whether women should be allowed to donate their eggs to research projects and, if so, how to ensure their interests are best protected. As part of this consultation the HFEA hosted a meeting of scientists involved in stem cell and embryo research. The meeting raised issues regarding alternative sources of eggs and embryos for research and some experts expressed views on the creation of hybrids.

5.6. Some of the researchers felt it was too soon to be carrying out somatic cell nuclear transfer research with human materials as human eggs are in such sort supply and there is still a great deal that could be learnt using animal studies. Those that held this opinion fell into two groups. The first group felt that the field could benefit from further research using only animal materials (not using human eggs or nuclear material). One researcher commented that creating hybrid embryos would result in a confusion of information and that it would not tell us what we need to know.

The other group felt that it was too soon to use human eggs for this research but it was appropriate to use hybrids. One researcher commented that although there could be complications in using animal eggs and human nuclear material it would still be possible to obtain high quality data. There were also those who felt that stem cell research could benefit from work using human eggs.

Creating human-animal embryos

5.7. As mentioned in the scientific context, the mixing of human and animal material is not new. However, for many people this is the first time they have been aware of the intention to create embryos with a mix human and animal material. It is therefore perhaps unsurprising that some initially viewed this idea with disgust.

5.8. Certainly at the outset of the deliberative work, many of the participants expressed an initial repugnance in reaction to the suggestion of mixing human and animal material. Associations were drawn with incidents such as the Northwick Park drug trials, myths and legends, and the elephant man. However, when further factual information was provided and further discussion took place, the majority of participants became more at ease with the idea, although as one participant observed, *"The gut reaction is hard to overcome"*.

5.9. There were some suggestions of a compromise approach in the deliberative work, at the public meeting and in the responses written consultation. Some supporting the research if it had the potential to lead to a better understanding of the biological processes, but expecting further work to then be undertaken with human eggs.

> *"I think they should use these eggs to understand better how it works – they'll use human eggs after that won't they."*
>
> Glasgow woman, participant in the deliberative work

> *"It may be necessary to do it for a short time in order to see how cells re-programme, and you can't possibly do that without looking at these kinds of stem cells from embryos."*
>
> Speaker at the public meeting

5.10. In the deliberative work, opinion poll and written consultation there was more support for the creation of cytoplasmic hybrids than for other types of human-animal embryos. Of those who did feel differently, some felt unconvinced by the need for creating other types of embryo, whilst others questioned whether there would ever be any benefits in creating any of the other types of human-animal embryos where there was more than 0.1% animal present.

> *"People need to know what it's for rather than research for research's sake, there has to be an end in sight."*
>
> Manchester man, participant in the deliberative work

Citing the benefits

5.11. After expressing their initial reactions, participants in the deliberative work were intrigued to understand why scientists would want to create human-animal embryos.

> *"If I thought it would have some benefit I would go for it."*
> London man, participant in the deliberative work

Throughout the deliberative work it was made clear that there are no guarantees that the research will lead to any significant advances. However, in both the deliberative work and from the opinion poll it emerged that the *potential* benefits of the research had a significant impact on opinion. The key issue for most was whether there is a clear rationale for the research. Some felt it was acceptable if the research could yield results to further our understanding of disease, whilst others considered that the potential applicability of the research to human diseases was the key to whether the research should take place.

5.12. This shift in opinion was not replicated at the public meeting, where the majority of participants felt that the potential benefits failed to outweigh their ethical concerns. This may have been because the audience were self-selecting, having already formed a view. One participant suggested that citing potential benefits is misleading, particularly as there is no guarantee that the research will result in any.

> *"I think it's fraudulent to tell people with diseases that you will generate useful date."*
> Audience member at the public meeting

This highlighted what was found in the deliberative work: the importance of communicating the complete factual picture, explaining the science alongside a realistic explanation of the potential benefits.

Scientific worth: Views from the consultation

5.13. Introducing information about the potential benefits of the research in the second part of the deliberative work, the full day workshop, also prompted some questioning of the scientific worth of using animal material.

> "*I personally think that if it is humans they're trying to cure then it is human they should be trying to do it with, not animals.*"
> Glasgow woman, participant in the deliberative work
>
> *"We could go through it all and decide that it is never going to work anyway because it is not going to be the same as getting it from the humans."*
> Swansea woman, participant in the deliberative work

This concern was reflected at the public meeting, with audience and panel members questioning whether the research is in fact safe and how applicable any findings would be to human beings.

"We do not know whether such hybrids will lead to diseases and genetic illnesses being transmitted from the animal species to the human species, for example." In response to the written consultation

"Can you really guarantee that there will be no abnormality in the stem cells that are produced combining humans and animals?" Audience member at the public meeting

"It seems unsafe to carry out procedures that are unnatural in the sense of being impossible by natural processes. It seems risky to do something that nature prevents." In response to the written consultation

5.14. In the written consultation, those against the creation of cytoplasmic embryos were largely against the proposal for ethical reasons. Some respondents raised the issue of safety, the majority citing cross species contamination as the basis of their concern.

Scientific worth: Evidence from the scientific literature review

5.15. As outlined in the scientific literature review, in order for this technique to result in the creation of embryos, the somatic genome of the donor cell must be reprogrammed to allow the correct expression of genes for embryonic development. This is a hurdle for the successful creation of all embryos by the process of somatic cell nuclear transfer (SCNT), not just interspecies hybrids. This process is likely to be more problematic in interspecies hybrids as different species may have different mechanisms for reprogramming.

5.16. There has been only one credible report of a human embryo being created, following SCNT. This embryo developed to the blastocyst stage but did not result in the derivation of stem cell lines. There are limited reports of the creation of human-animal cytoplasmic embryos but studies from the US and Korea have reported successful development of human-cow embryos to blastocyst stage. Analysis of these embryos by one US group demonstrated that the embryos contained human genomic DNA specific for the individual DNA profile of the donor cells.

5.17. A study from China reported the creation of human-rabbit embryos which developed to blastocyst stage and lead to the derivation of stem cell lines (see section 1.2 of Appendix B for more details). The use of this technique in animals has shown mixed success. Examples of the animal-animal hybrids, and the stages of development they reached, are outlined in section 1.2 of Appendix B. Few studies have demonstrated the establishment of ES cell lines from animal-animal embryos although recently mouse ES cell lines have been derived from embryos created with mouse somatic cells and cow eggs.

5.18. As hybrid embryos develop towards the blastocyst stage the gene products (proteins and RNA (ribonucleic acid: single stranded molecule transcribed from DNA in the cell nucleus and mitochondria, the structure and base sequence of which determines protein synthesis)) will gradually become more human derived. By 14 days the embryo will be entirely human with respect to protein and RNA apart from 13 proteins encoded by the animal mitochondria.

5.19. Animal mitochondria will be present in the cytoplasm of the enucleated recipient egg, so cytoplasmic hybrids will contain at least some animal mitochondria, and therefore animal mitochondrial DNA (see section 3.1 of Appendix B for background information on mitochondria). It is also likely that some human cytoplasm, containing human mitochondria, will be transferred with the nucleus during the creation of hybrids. As outlined in section 3.2.2 of Appendix B the amount of human mitochondria transferred is likely to depend on the technique used for transfer of the nucleus.

5.20. A number of mechanisms need to be effective for hybrid embryos to develop successfully and for cells derived from these embryos to be viable:

1. A particular number of mitochondria must be present
2. Mitochondria must be capable of replicating and expressing their proteins
3. Proteins encoded by the nuclear and mitochondrial genomes must interact together in order to allow the cell to produce energy

5.21. There is a risk that in hybrid embryos humans may be too distant in evolutionary terms from other mammalian species, such as rabbits and cows, for the genomes to be compatible. Animal-animal cytoplasmic hybrid studies indicate that the energy production mechanisms (oxidative phosphorylation function and ATP production, for more information see section 3.3 of Appendix B) of these embryos are compromised and that these mechanisms will become less functional when the evolutionary distance between the two species is increased.

5.22. However, survival of human-rabbit and human-cow embryos to the blastocyst stage suggests that this is not always problematic. This may be due to the human nucleus preferentially replicating the human mitochondria present. Human mitochondria have been found to be present in human-cow embryos up to blastocyst stage, however, they are unlikely to account for the majority of mitochondria present.

5.23. These issues are investigated in more detail in Appendix B. However, there is little literature investigating the interaction of the nuclear and mitochondrial genomes in inter-species embryos and therefore it is hard to reach any certain conclusions about development of human-animal hybrids and functionality of any cells derived from them.

5.24. Some scientists have suggested that human-animal hybrid embryos, or any cells derived from them, may only be functional if they are inserted with supplementary human mitochondria.

The alternatives to using human-animal embryos

5.25. In all strands of the consultation, a key theme was the alternatives and whether creating human-animal embryos for research purposes was justifiable when other sources of stem cells are available. In the course of the deliberative work participants debated the issue of alternative methods of research, with many of the participants concluding they were content with the creation of human-animal embryos alongside alternative research methodologies, with the proviso that such research was conducted under strict regulation.

> *"If there was another way of doing it (e.g. a skin cell) I would much prefer this route. However I still feel that we should try it both ways."*
>
> Participant in the deliberative work

5.26. The majority of participants in the deliberative work felt that using other sources of stem cells avoided the ethical dilemmas. However, it was generally felt that all avenues of research should be pursued if there is potential for greater understanding of disease.

5.27. Some respondents to the written consultation held the view that it would be better to invest more energy in other types of research, believing that promising advances were being made through alternative research methodologies.

> *"More funding should be given to researchers who are getting good results from using adult stem cells, and women who have given birth should be asked to donate the umbilical cord for stem cell work."*
>
> In response to the written consultation
>
> *"Is it not true that New York Scientists have produced the equivalent of embryonic stem cells in mice without destruction of embryos."*
>
> In response to the written consultation

5.28. There are two main alternative research options to creating human-animal embryos. The first option is to use an alternative source of stem cells, such as adult or cord blood stem cells. Adult stem cells are found in many tissues and can develop into a range of cell types related to the tissue they are derived from. They are involved in tissue renewal and repair, and established treatments include bone marrow, skin and corneal transplants. Animal models and clinical trials using adult stem cells are being developed for the treatment of heart disease, type 1 diabetes, spinal cord injury, stroke, Parkinson's disease and Huntington's disease. Cord blood cells, isolated from the blood of the umbilical cord, have been successful in the treatment of leukaemia and other blood disorders, especially in children.

5.29. The second option is to directly reprogram somatic cells to produce embryonic-like stem cells. Recent studies in mice have reprogrammed fibroblast cells without transferring the cells into an egg or creating an embryo. The resulting cells are termed induced pluripotent stem (iPS) cells and have similar properties to embryonic stem cells. Another alternative technique uses fertilised eggs as hosts for SCNT, instead of unfertilised eggs. Fertilised human eggs that have extra sets of chromosomes are automatically discarded from IVF treatment. In mice, these have been successfully used as hosts for SCNT, and the resulting embryos could potentially be used to derive embryonic stem (ES) cell lines. These, and further alternative research options, are explored in more detail in Section 5 of Appendix B.

5.30. Although a very important avenue of research, adult stem cells are limited in the types of cell or tissue they can give rise to. Not all tissues contain stem cells whilst others are inaccessible, such as stem cells from the central nervous system. Populations of adult stem cells are also highly heterogeneous, making them hard to isolate and purify. Some studies have tried to induce adult stem cells to broaden the range of potential tissues they can form. However, though some stem cells appear more flexible than previously thought, the mechanisms controlling this process are poorly understood. At present there is only a very limited range of diseases that can be treated using adult stem cells. Cord blood stem cells are also limited in the disorders they can treat and although there are some claims that these cells have wider potential, these have not been substantiated.

5.31. Adult and cord blood stem cell research is significant; however ES cells may offer a potentially more flexible range of research options if the different differentiation pathways can be directed. Research on other sources of stem cells, and alternative ways of deriving embryonic stem cells without destroying viable embryos (see Section 5, Appendix B), is at a very preliminary stage and does not currently offer a viable alternative to human-animal embryos.

5.32. The process of reprogramming somatic cells into iPS cells has been achieved by three separate groups, which shows positive support for the results. However research is still at a very early stage and the reprogramming process is inefficient. Different factors may be involved for humans than those identified for mice. The process of using fertilised eggs for SCNT has only had one successful study published to date and similarly the technique has not been attempted in humans.

5.33. Members of the scientific community are of the opinion that all avenues of research, including adult stem cells, human-animal embryos and direct reprogramming of somatic cells, should be explored.

Concern for the future: The boundaries to research

5.34. The findings of the deliberative work and the opinion poll highlighted that there is concern that a slippery slope would be embarked upon if the creation of human-animal embryos were to be permitted.

> *"It is human nature; you always want to push the boundaries to see what is going to happen if you just go a little bit further."*
>
> Swansea man, participant in the deliberative work

Another view expressed was that the risks associated with the slippery slope argument are outweighed by the potential benefits to be gained. In the deliberative work, some felt that their concerns about starting on a slippery slope were lessened by the fact the research would be tightly regulated. However, caution was still called for by some, as regulation can only control what is done within the UK and consequently the slide down the slippery slope maybe embarked upon elsewhere.

> *"I think this is a dangerous direction for research to go, especially since scientists in other countries may take the information gained here and use it to create hybrids that will not be destroyed at 14 days."*
>
> In response to the written consultation

5.35. A proportion of those concerned by what the research may lead to, cited situations which occurred in the past, revealing a level of distrust for scientists and their work.

> *"This surely follows on from Nazi experiments during World War II."*
>
> In response to the written consultation
>
> *"For instance we have allowed abortion - now murders of children are almost daily events. … If this research on human-animal embryos is permitted, what is to say that in a few years laws will be passed to legalise bestiality."*
>
> In response to the written consultation

Many of the participants felt quite far removed from medical research and considered there to be a lack of communication about scientific and medical advancements.

> *"It seems to be secretive. I don't think that we the general public feel as though we are in touch with it, or we're being informed."*
> Manchester man, participant in the deliberative work

Some participants were concerned that there are a small number of scientists who are irresponsible in their pursuit of knowledge, regardless of the controls in place.

> *"I'm sure they've done it already (mix of human and animal material)."*
> London woman, participant in the deliberative work
>
> *"How do you control illegal research by people that are not applying for licenses?"*
> Participant in the deliberative work

However, others expressed great trust in the work undertaken in by scientists and medics.

Regulation: Limits and controls

5.36. Those who supported research involving the creation of human-animal embryos appeared to agree that such research should only be undertaken in a regulated environment.

5.37. A small number of respondents to the written consultation considered that such research should be completely unregulated.

> *"We should remove the time limit for all research and allow unfettered scientific exploration. It is only the fear of an imaginary being that makes some people claim that we should not investigate ourselves. If 'moral' objections apply, it should be up to the producer of the egg to decide whether experimentation is allowed, not the 'authority' vested in some religious leader by a fairy tale."*
> In response to the written consultation
>
> *"You have to rely on the people with expert knowledge in the field. We cannot limit the researchers as the future of medical health for me and my children may well depend on these people being able to work without fear of restriction."*
> In response to the written consultation

5.38. During the course of the deliberative work the issue of regulation arose frequently and was often cited as a proviso when support was given to the creation of cytoplasmic hybrids. Throughout the consultation the current regulatory framework was considered to be appropriate, although some felt that those who breeched the standards imposed should be subject to penalties.

"But what would be the punishment if they did keep it longer [than 14 days]? They'd have to take away the licence then they couldn't work."
Newcastle man, participant in the deliberative work

Levels of understanding

5.39. The majority of those that attended the public meeting appeared to know about the debate around human-animal embryos, however this is hardly surprising given that the audience was self-selecting. The results of the opinion poll however indicated that the general public know only a little about using human embryos for research, stem cell research or the possibility of creating cytoplasmic hybrid embryos.

5.40. Throughout the consultation it was clear that a number of misunderstandings are held by the public. During the course of the deliberative work, comments were made about the lack of information, and even misinformation about medical research including the benefits that had been achieved. Again this raises the need for full and accurate information to be made available to the public.

5.41. Nearly all of those that attended the public meeting thought that it was important to consult the public on issues such as this and the majority of participants went on to say that they would be responding to the consultation, or had already done so.

6. Conclusions

6.1. The general view of the organisations we consulted, and the view expressed in the Academy of Medical Sciences' recent report on inter-species embryos[7], was that currently there is no reason why scientists would want to create human transgenic embryos, true hybrids or human chimera embryos. However, although there is not currently a demand for the creation of these entities it is always difficult to predict how scientific research may develop in the future. There is evidence for success of these techniques in animal studies, so in theory it could be technically possible to create such entities using animal material. The Academy of Medical Sciences suggested that researchers will at some stage have good reasons to conduct research involving the creation of human-human transgenic embryos. These techniques could facilitate the investigation of gene function in early embryo development or, for example a gene could be introduced in a human embryo to increase the efficiency of the derivation of stem cells.

6.2. As during the consultation it was not possible to provide the public with a comprehensive account of the scientific need for creating all types of human-animal embryos, the debate very much focused on the topic of cytoplasmic hybrids.

6.3. Throughout the consultation there was some questioning, mostly by members of the public, of the scientific worth of creating human-animal embryos. However, the scientific community appears to feel confident that the creation of cytoplasmic hybrids is an avenue of research worth exploring and, in particular, it could be a viable alternative to using human eggs, to investigate the mechanisms of creating patient matched embryonic stem cells. As this research has not been undertaken in this country yet and it is still in the very early stages of development elsewhere, it is not possible to make any firm conclusions on the potential of this research. Despite this, in all strands of the consultation, there were calls for all avenues of research to be pursued, which is the approach that has generally been taken in the UK to date.

6.4. The potential benefits of creating cytoplasmic hybrids had a significant affect on public opinion. Many appeared to view a clear rationale for the research as the key to determining whether it is acceptable or not. The potential benefits of creating cytoplasmic hybrids are outlined in section 2 of this report.

6.5. In all strands of the consultation there was discussion of alternatives. The use of adult or cord blood stem cells has been suggested as a viable alternative to the derivation of ES cells from human-animal embryos, and was cited throughout the course of the consultation. Although the use of adult and cord blood stem cells is already established in a number of treatments, including bone marrow, skin and corneal transplants, unlike ES cells they are limited in the types of cell or tissue they can give rise to. Research into expanding the types of cells that adult and cord blood stem cells can give rise to is at a preliminary stage and the mechanisms involved are poorly understood. The technique of directly reprogramming somatic cells to produce embryonic-like stem cells was also identified as an alternative option to creating human-animal embryos. Recent success has been achieved with this technique in mice, however, research is still at a very early stage and there has been no success in humans.

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[7] 'Interspecies embryos: A report by the Academy of Medical Sciences'. June 2007

6.6. During the course of the public dialogue work the participants showed an interest in the issues and were keen to understand the complete picture for research involving the creation of human-animal embryos. Not only did the public want to understand the science, but also why the research needs to take place and the proposed benefits. Furthermore, this information appeared to be significant to those forming their opinion on the issue for the first time. So whilst some members of the public initially reacted with disgust, after hearing more information and discussing the issues with others, their opinion often shifted significantly.

6.7. From the public dialogue work it also appeared that explaining the regulatory controls (i.e. the 14 day rule) is crucial in helping the public to understand that the research being discussed would take place at a cellular level. Whilst some people still view the creation of any human-animal embryos as the start of a slippery slope, the regulatory context reassured many people who initially held this concern. Those that registered support for the use of human embryos in research were generally in favour of the creation of human-animal embryos, with the proviso that there are good reasons for undertaking the research and that it is carried out in a tightly regulated environment.

6.8. The consultation highlighted the need for increased communication with the public. There was great appreciation from participants in the deliberative work and the public meeting for being consulted and a strong desire from people to continue to learn about issues such as this. The distrust and suspicion around scientists, also indicates a need for the HFEA and scientists undertaking high profile research to establish ongoing communication with the public. In the course of the consultation there was a great deal of support for the current regulatory structure, with emphasis placed on the need to regulate such research tightly and with high levels of scrutiny. Furthermore, the suspicion surrounding medical research and scientists supports the need for the HFEA to communicate its role in regulating research and to be clear about the limits and the controls that it exerts. This links in with the recommendation made by the parliamentary scrutiny committee that the HFEA should 'improve and inform public understanding'[8].

[8] Joint Committee on the Human Tissue and Embryos (Draft) Bill 1 August 2007. 'Human Tissue and Embryos (Draft) Bill Volume 1: Report'

7. The Authority's Decision

7.1. On 5th September 2007 the Authority considered how they should approach the licensing of human - animal hybrids and chimera research.

7.2. The Authority decided that such research legally falls within the HFEA's remit, and having looked at all the evidence, decided that there was no fundamental reason to prevent cytoplasmic hybrid research. The Authority acknowledged that public opinion is very finely divided with people generally opposed to this research unless it is tightly regulated and likely to lead to scientific or medical advancements.

7.3. It was decided that individual research teams should be able to undertake research projects involving the creation of cytoplasmic hybrid embryos if they can demonstrate, to the satisfaction of an HFEA licence committee, that their planned research project is both necessary and desirable. They must also meet the overall standards required by the HFEA for any embryo research.

7.4. The Authority also agreed to look at what improvements can be made to how science and research is communicated by the HFEA to the public in a wider context.

Glossary

Adult (or tissue-specific) stem cells
Cells found in many tissues, e.g. bone marrow, that have the potential to form a range of cells related to the tissue they are from

Animal chimeras
Animal embryos which have human cells added to them during early development

Blastocyst
An early stage embryo (day 5-6 after fertilisation)

Chromosomes
Threadlike structures carrying genetic information found in the nucleus of every cell

Cord blood stem cells
Cells found in the blood of the umbilical cord that have the potential to form different cell types

Cytoplasm
The gel-like substance enclosed in the main body of the cell

Cytoplasmic hybrid embryos
Embryos created by removing the nucleus of an animal egg and inserting the nucleus of an adult cell from a different individual (and possibly of a different species)

Derivation of stem cells
The process of obtaining stem cells from a source such as embryos, bone marrow, or cord blood

DNA profile
The unique genetic make-up of a cell

Embryonic stem cells
Cells taken from an early stage embryo that have the potential to form a wide range of other cell types

Embryonic stem cell lines
Cells from an embryo that can continuously divide to produce identical cells and can also produce cells that have formed (differentiated) into other cell types

Genes
Units of hereditary information that are made up of DNA and determine specific characteristics in offspring. Genes are carried on chromosomes

Gene sequences
The combination of DNA molecules that make up specific genes

Germline
Cells which develop into sperm or eggs

Growth hormone
A substance that stimulates the growth of almost all cells and tissues of an animal or human

Heterogeneous
Composed of various cell types

Human chimeras
Human embryos which have animal cells added to them during early development

Human genome
The complete set of genetic material for an individual human

Hybrid embryos
Embryos which are created by mixing human sperm and animal eggs, or human eggs and animal sperm

Induced pluripotent stem (iPS) cells
Adult cells that have been directly reprogrammed to behave like embryonic stem cells.

Mitochondria
Structures in the cytoplasm of the cell that make the energy for the cell and contain a small amount of genetic material (DNA)

Mitochondrial diseases
A group of disorders relating to the mitochondria in a cell

Mitochondrial genome
The genetic material contained within mitochondria

Multipotent
The ability of cells (e.g. adult stem cells) to form a variety of cells closely related to the tissues they are found in

Nuclear genome
The genetic material contained within the nucleus i.e. the chromosomes

Nucleus
The part of a cell that contains the majority of the cell's genetic material (DNA)

Pluripotent
The ability of cells (e.g. embryonic stem cells) to develop into a wide range of cells and tissues including all three embryonic tissue layers

Somatic Cell Nuclear Transfer (SCNT)
The transfer of the nucleus from an adult somatic cell (any cell forming the body of an organism) into an egg from which the nucleus has been removed

Stem cells
Cells that can continuously divide to produce identical cells and also have the ability to produce cells that have different, more specialised properties

Therapeutic cloning
The process of creating embryos through SCNT (above) to produce embryonic stem cells that are genetically matched to a particular person, for the treatment of disease.

Transgenic human embryos
Human embryos which have animal genes inserted into them during early development

Appendix A – Human-animal embryos: Chronology

2000: The Sir Liam Donaldson's report, Stem Cell Research: *Medical Progress with Responsibility,* recommended, among other things, that "mixing of human adult (somatic cells) with the live eggs of any animal species should not be permitted". However, the report did not discuss the thinking behind this recommendation.

2002: The House of Lords Select Committee report on *Stem Cell Research*, took issue with the recommendation of Sir Liam Donaldson's expert group that there was a need for an outright ban on research involving inter-species embryos:

"We are aware of reports of experiments in other countries involving the replacement of a nucleus of an animal egg with the nucleus of an adult human cell. These developments raise important issues. It would clearly be totally unacceptable to implant such an entity in a woman with a view to bringing it to term……. For any possible therapeutic applications there would also be significant concerns relating to safety, on which reassurance would be needed. However, if placing a human nucleus in an animal egg provided a way of creating human ES cells for research, some might argue that it was more acceptable to use such an entity for research, the creation of which involves no human gametes, than an embryo created by CNR."

September 2004: Roger Pedersen gave a presentation to the HFEA Scientific and Clinical Advances Group (SCAG) on chimeras and the role they play in stem cell biology. SCAG also considered a scoping paper on chimeras which fed into subsequent consideration by the Group on definition of an embryo.

March 2005: The House of Commons Science & Technology Committee report on *Human Reproductive Technologies and the Law* recommended that new legislation was required to define the nature of inter-species embryos and make their creation legal for research purposes subject to the 14 day rule and the prohibition on implantation in a woman.

September 2005: Human-animal hybrids were identified by SCAG's 2004-5 horizon scanning process, as a medium priority issue. SCAG was informed that this issue was now being considered by DH as part of the Review of the HFE Act and that the HFEA would consider it further, as necessary, following the report of the consultation of the Act.

November 2005: The HFEA responded to the Department of Health's Review of the Act consultation. The HFEA stated:

"The creation of human-animal hybrids is permitted until the two cell stage under the current Act and the HFEA considers that research within the constraints outlined by the Government should be permitted. As long as it can be ensured that such entities would never be implanted into a woman or allowed to develop beyond the 14 day stage, and as long as the research would fall under current research purposes, it could be argued that the ethical justification for the creation of such entities is consistent with research as it is currently allowed. Nevertheless, we recommend that the Government has proper consideration to the diversity of views on this issue. The HFEA would recommend that hybrids and chimeras are defined in the new Act."

February 2006: T he HFEA Ethics and Law Committee (ELC) and SCAG considered a scoping paper for further decision on the creation of the use of hybrid embryos in research. Scientists in the UK had publicly stated that they may wish to create hybrid embryos by fusing human cells wit rabbit eggs.

The Committees agreed that, in order to advise the Authority, SCAG would be asked to look at the evidence and give a view on the scientific aspects of creating human-animal hybrids

on 26th April 2006 and ELC would be asked to examine and provide a view on the legal and ethical aspects of creating human-animal hybrids.

April 2006: SCAG was asked to review the role that mitochondrial DNA plays in the development of embryos and whether embryos containing human nuclear DNA and both human and animal mitochondrial DNA would be a human embryo. If so, whether the creation of these embryos would be necessary for one of the purposes set out in Schedule 2 to the Human Fertilisation and Embryology Act 1990 as amended by the Human Fertilisation and Embryology (Research Purposes) Regulations 2001.

SCAG considered (1) any human mitochondria present would probably have a replicational advantage as they were more compatible with the genome; (ii) any egg/embryo with a human genome falls under the remit of the HFE Act; (iii) the proportion of human derived and rabbit derived proteins should be considered when deciding whether the hybrid embryos should be classed as human.

SCAG's general opinion was that these hybrids should be classed as human and the creation of these hybrids was necessary for research projects due to the lack of availability of human eggs.

May 2006: The ELC was asked to examine and provide a view on the legal and ethical aspects of creating human-animal hybrids and to consider to main questions: (i) the significance of the word 'human' in section 1(1)(a) of the Human Fertilisation and Embryology Act 190 ('Meaning of "embryo"') (ii) how the HFEA should respond in practice should a scientist intend to create embryos artificially from human and non-human components.

The Committee agreed that an embryo containing human nuclear DNA and both human and animal mitochondrial DNA should be regarded as an 'embryo' for the purposes of the 1990 Act. The Committee agreed that the creation, keeping or use of such an embryo is capable of being regarded as necessary or desirable for one of more purposes set out in Schedule 2 of the HFEA Act (as amended), and therefore a licence committee would have the discretion whether to authorise these activities in the context of an individual licence application.

July 2006: The HFEA sought Counsel's opinion on whether a cytoplasmic hybrid is regarded as a 'human embryo' for the purposes of the Human Fertilisation and Embryology Act 1990 and whether the creation and use of such an embryo would be prohibited or licensable under the Act.

November 2006: The HFEA received two applications for research licenses for derivation of embryonic stem cells from hybrid embryos.

The HFEA's Horizon Scanning Expert Panel was asked a number of questions regarding hybrids to inform further opinion from Counsel. Respondents agreed that the hybrid embryo would contain a complete human genome, however there was no consensus on whether a hybrid embryo would be capable of implantation.

The Authority received a briefing paper in preparation for a full discussion in January 2007.

December 2006: The Government's *White Paper on Review of the Human Fertilisation and Embryology Act* stated that:

"The extent to which the law and regulation would apply to embryos created in these circumstances is not sufficiently clear, although the law would clearly prevent such embryos being placed in a woman. In some circumstances the embryos created could be, genetically

speaking, almost entirely human and therefore fall within the regulatory controls applicable to human embryos".

The White Paper went on to propose that "revised legislation will clarify the extent to which the law and regulation applies to embryos combining human and animal material", adding that:

"The Government will propose that the creation of hybrid and chimera embryos in vitro should not be allowed. However….the law will contain a power enabling regulations to set out the circumstances in which the creation of hybrid and chimera embryos in vitro may in future be allowed under licence for research purposes only".

January 2007: The HFEA sought an updated opinion from Counsel on whether hybrid embryos would fall under the remit of the HFEA. At its meeting on 10 January 2007 the Authority was advised that:

"If the embryo contains a complete human genome and it cannot be shown definitively that the embryo does not have the normal potential to develop, it is most likely that the Court would find that this constitutes a live human embryo for the purposes of the Act. The Courts are likely to see the "hybrid" embryo in this way to ensure that this type of research falls under the scope of regulation rather than to allow it to be unregulated".

Presented with this opinion the Authority concluded that hybrid embryos are probably within its scope and decided to hold a full consultation on human-animal embryos to gauge public opinion on the issue.

March 2007: The House of Commons Science & Technology report on Government proposals for the regulation of hybrid and chimera embryos found that the Government's White Paper proposals were *"too prohibitive and that the promise of future regulation was insufficient".* Instead the Committee called for permissive legislation which would allow research using animal-human hybrid and chimera embryos through licensing, stating that:

"In general, the creation of all types of human-animal chimera or hybrid embryos should be allowed for research purposes under licence by the regulator".

The Committee's intention was that this would include true hybrids

In addressing the role of the HFEA in regulating research, the Committee said that:

We support the decisions of the HFEA Science and Clinical Advances Group, Ethics and Law Committee and Horizon Scanning Group that an embryo containing human nuclear DNA and mitochondria of animal origin should be regarded as a human embryo for the purposes of the 1990 HFE Act."

On the issue of public understanding, the Committee said:

"We welcome the HFEA proposed consultation on general principles and commend steps taken by the Authority to ensure appropriate drafting. We also commend the Government for allowing funding to be allocated towards education in this area".

May 2007: The Government published the *Human Tissue and Embryos (Draft) Bill*. Although the draft Bill follows the model outlined in the December 2007 White Paper, the Government issued a statement announcing its intention to accept in part the Science and Technology Committee's recommendation of March 2007 and allow in legislation, under licence, certain categories of inter-species embryo. However, 'true' hybrids would remain proscribed unless permitted by regulations made by the Secretary of State.

August 2007: This issue was addressed in the report of the Joint Parliamentary Committee on the Bill. The Joint Committee recognised this as a very sensitive area and recommended that *"the creation and use of inter-species embryos for research purposes should be put to a free vote in both Houses".* The Joint Committee recommended an alternative definition of inter-species embryos and proposed that authority should be give to the regulator:

"To interpret and apply that definition to individual research applications, based on the principles set out in legislation".

Appendix B - Scientific Literature Review

1. Use of interspecies constructs in research

1.1. The history of interspecies constructs in research

1.1.1. The mixing of human and animal genetic material has a long history in science and has been used in a number of different ways to greatly progress medical research.

1.1.2. The fusion of human and animal cells is extensively used in research and was a technique used in the mapping of the human genome in the 1970s1. Before the introduction of ICSI and other assisted reproduction technologies the 'hamster test' was used to examine the quality of human sperm as a diagnostic procedure in clinical studies of male infertility. This test was established in the 1960s and involves mixing hamster eggs with human sperm and observing the percentage of eggs that are penetrated by the sperm. A number of centres in the UK were licensed to carry out this technique.

1.1.3. The creation of transgenic animals, in which a human gene is introduced into the germline of an animal and therefore transmitted to all cells in the offspring, is a long established technique which is used for the production of pharmaceutical products and for modelling human disease.

1.1.4. The production of growth hormone in the serum of transgenic mice, in 1982, was the first successful example that transgenic animals could produce human products for therapeutic use[2]. Since then this technique has been successfully used to produce a variety of human therapeutic proteins in the milk, blood serum, urine and semen of mouse, rabbit, sheep, goat and pig[3]. For example, the production of human alpha-1-antitrypsin[4], a protein used to treat the rare genetic disorder of alpha-1-antitrypsin deficiency.

1.1.5. The introduction of human gene sequences into mice has allowed scientists to identify and understand the role of a particular gene in a large number of diseases. For example, the identification of human oncogenes by creating a mouse strain that promotes the development of various human cancers[5]. Other examples include the creation of a mouse strain with the gene for Alzheimer's disease, which exhibited brain lesions and memory loss, used to test therapies for the disease and the creation of a mouse strain with an extra copy of chromosome 21, used to facilitate Down's syndrome research[6].

1.1.6. In addition, transgenic animals are being developed by some companies to provide organs for transplantation such as kidneys, livers and hearts. For example, pigs with human histo-compatibility genes may provide organs for transplantation which are less likely to be rejected by a patient's immune system.[7]

Summary: Mixing human and animal genetic material has a long history in medical research. Fusing human and animal cells has been used since the 1970s/80s to map the human genome. The HFEA has previously licensed the creation of true hybrids, with hamster eggs and human sperm, as a diagnostic test for the quality of human sperm. The creation of transgenic animals has long been used for the production of pharmaceutical products and to model human disease and animal chimeras have proven a useful tool for understanding the role of specific genes in diseases.

1.2. Cytoplasmic hybrids

1.2.1. The technique of nuclear transfer involves introducing a nucleus from a cell into an enucleated oocyte, followed by parthenogenetic activation to form an embryo. The use of interspecies nuclear transfer was first attempted 120 years ago in order to investigate the roles of the nucleus and cytoplasm in heredity[8]. This attempt involved the transfer of zygotic nuclei between a frog egg and toad egg, which resulted in the development arrest of both eggs. The creation of mouse-human cytoplasmic hybrids, using two somatic cells, was reported in the late 1970's/early 1980's. This technique was used to investigate the interactions between nuclear and mitochondrial genomes[92 93 94]. The technique has also been used more recently to attempt to clone endangered species where oocytes of that species are not readily available.

1.2.2. In 2003 xenopus-human embryos were created by transferring several human somatic cells into frog eggs. These embryos were used to investigate the reprogramming process, and were not allowed to develop. This study demonstrated that human somatic cells can be reprogrammed to a pluripotent state (as indicated by the expression of oct-4, the most diagnostic mammalian stem cell marker) by the nucleus of amphibian oocytes[9].

1.2.3. A recent article[20] by a group at Monash University, Australia provides an extensive analysis of literature regarding the developmental competence of inter-species nuclear transfer embryos according to their donor cell, embryo development, pregnancy and offspring. The pregnancies and offspring reported mostly involve animals of the same genus. For example intra-genus bovine, ovine, equine and feline species.

1.2.4. The following are examples of interspecies embryos which have been reported to develop to blastocyst stage: horse donor cell and cow oocyte[20], monkey donor cell and rabbit oocyte[10], various mammalian species and cow oocyte[11], mountain bongo antelope donor cell and cow oocyte[12], buffalo donor cell and cow oocyte[13], dog donor cell and yak oocyte[14], panda or cat donor cell and rabbit oocyte[15], takin donor cell and yak or cow oocyte[16].

1.2.5. The following are examples of interspecies embryos which ceased developing at stages earlier that blastocyst: Antarctic minke whale donor cell and cow or pig oocyte[17], rabbit donor cell and cow oocyte[18].

1.2.6. One group demonstrated that in an embryo created with the somatic cell of a macaque and a rabbit oocyte, mitochondria from both animals was present from the one cell stage to the morula stage. The number of mitochondria derived from the macaque decreases dramatically at the blastocyst stage[19].

1.2.7. In a recent study equine-bovine embryos (with the donor nucleus being from the horse) and control equine-equine embryos had a relatively high rate of survival to cleavage stage. The number of embryos which reached the blastocyst stage was lower. The embryo development rate to blastocyst stage for aggregated embryo culture and single embryo culture was about 2.2% and 2.3% respectively. One putative embryonic stem (ES) cell line was established from these embryos. However, it could not be maintained after passage 12[20].

1.2.8. Few studies have demonstrated the establishment of embryonic stem cell lines from interspecies cytoplasmic hybrid embryos. However, recently mouse embryonic

stem cell lines have been derived from embryos created with mouse somatic cells and bovine oocytes. These cells differentiated into various typical embryonic germ tissue types and contributed to chimeric offspring when transferred to mouse blastocysts[21].

1.2.9. There are reports of the creation of human-cow and human-rabbit cytoplasmic hybrid embryos by groups from the US, Korea and China. ES or ES-like cell lines have been successfully derived from these embryos.

1.2.10. In 1999 an American group at the company Advanced Cell Technologies was reported to have created a cytoplasmic hybrid by inserting the nucleus of an adult human cell (from human lymphocytes or oral mucosal epithelial cells) into an enucleated cow's egg[22]. The group claim that a colony of cells, which looked like ES cells, were derived from this embryo and were allowed to develop to 12 days before being destroyed. However, their data did not provide specific information about fusion and activation of the bovine oocytes or characteristics of human somatic donor cells. Also, there was no information on the obtained embryos or their origin from the donor cell's genome.

1.2.11. The creation of human-cow cytoplasmic hybrid embryos has also been reported by Professor Zavos's team (based in the USA and Cyprus) in 2003 and 2006[23]. In their most recent study enucleated bovine oocytes were fused via somatic cell nuclear transfer (SCNT) with either human granulosa or fibroblast cells and cultured *in vitro*. This resulted in 31.3% and 29.3% embryonic development respectively, some of which progressed to blastocysts (compared to 46.8% and 64.9% for the control non-manipulated parthogenetically activated oocytes). About half of the fused and activated bovine oocytes did not show signs of development. The group considered this to be due to the following factors: 1) inferior cell cycle stage of the donor cells during fusion; 2) aberrant reprogramming of the donor cell's genome after fusion; and 3) nonefficient ooplasmic environment following maturation, enucleation, or activation of the bovine oocytes.

1.2.12. Overall, from a total of 37 SCNT embryos, 11 reached morula stage and 3 reached blastocyst stage. The group demonstrated that, through PCR amplification and DNA sequencing the interspecies embryos contained human genomic DNA specific for the individual DNA profile of the donor cells. In addition both bovine and human specific mitochondrial DNA was detectable up to the blastocyst stage.

1.2.13. In 2003 Chen *et al* at the Shanghai Second Medical University announced the creation of human-rabbit cytoplasmic hybrids (from the insertion of human somatic cell nuclei into enucleated rabbit oocytes), which developed to blastocyst stage, and the derivation of stem cell lines from these blastocysts[24]. Approximately two thirds of the blastocysts gave rise to stem cell lines and the group demonstrated that these cells possess the properties and phenotypes of conventional ES cells, that they retain normal karyotype, and that they are capable of multi lineage differentiation.

1.2.14. In situ hybridization showed that the nuclear material in the blastocysts was from the human and the mitochondrial DNA was rabbit. Microsatellite analysis on the differentiated embryonic stem cells confirmed that they were encoded by the genome of the nuclear donor cell lines. It was also shown that both human and rabbit mitochondrial DNA co-exists in these embryonic stem cells.

1.2.15. A group at Seoul National University reported that the introduction of single human fibroblasts from the umbilical cord of neonatal offspring into enucleated cow

oocytes gave rise to embryos which survived to the blastocyst stage at a success rate of 4%[25].

Summary: Scientists have been creating cytoplasmic hybrid embryos with various animal species for over a century. This technique has been used to investigate interactions between nuclear mitochondrial genomes and to attempt to clone endangered species. Two groups have reported creating human-cow cytoplasmic hybrid embryos, which developed to blastocyst, and one group has reported creating human-rabbit cytoplasmic hybrid embryos, which developed to blastocyst and resulted in the derivation of stem cell lines.

1.3. True hybrid embryos, human chimera embryos and transgenic human embryos

1.3.1. The view expressed by the Academy of Medical Sciences, in their recent report on inter-species embryos, is that there are presently no proposals to create true hybrid embryos or to transfer animal DNA or cells into human embryos to create either transgenic or chimeric human embryos. However, they suggested that researchers will at some stage have good reasons to conduct research involving the creation of human-human transgenic embryos[26]. These techniques could facilitate the investigation of gene function in early embryogenesis or, for example, a gene could be introduced in a human embryo to increase the efficiency of the derivation of stem cells.

1.3.2. Although there is not currently a demand for the creation of these entities it is always difficult to predict how scientific research may develop in the future. There is evidence for success of these techniques in animal studies.

1.3.3. The creation of animal chimeras by the insertion of animal or human cells into non-human embryos and animals at later stages of development has been demonstrated as a useful technique to test the potential of stem cells. Mouse blastocysts have been used to demonstrate pluripotency of human ES cells. A recent study by a US group claimed that human ES cells could engraft into mouse blastocysts, where they proliferate and differentiate *in vitro* and persist in mouse/human embryonic chimeras that implant and develop in the uterus of pseudopregnant foster mice. The group propose that mouse embryos can be used as a surrogate for human ES cell differentiation. However, there was a poor contribution of human cells to these embryos[27].

1.3.4. A number of groups have combined blastomeres from goat and sheep blastocysts to create chimeric blastocysts. These are viable and give rise to sheep-goat chimeras, know as 'geep' [28] [29] [30]. It has been demonstrated that during early development of the sheep-goat blastocyst chimeras, increasing the proportion of transplanted cells in the inner cell mass can influence the presence of donor or host specific characteristics[31].

1.3.5. Successful development of interspecies chimeras through gestation and to adulthood has also been reported in the following species: house mouse-Ryukyu mouse[32], cow-zebu[33] [34] and sheep-cow[35].

1.3.6. The transfer of human cells to animals at later stages of development is already widespread in research and used to study the pluripotency and tissue specificity of stem cells.

Summary: There are currently no proposals to create true hybrids, transgenic or human chimeric embryos. However at some stage researchers may want to create human-human transgenic embryos to investigate the genes involved in embryo and stem cell development. Animal chimeras, which are created by the transfer of human cells to animal embryos and at later stages of development, have proven a useful tool to test for pluripotency of human stem cells. Successful development has been reported in some interspecies animal-animal chimeras.

2. Why create interspecies cytoplasmic hybrids?

2.1. The technique of somatic cell nuclear transfer (SCNT) involves introducing a nucleus from a somatic cell into an enucleated oocyte, followed by parthenogenetic activation to form an embryo. Development of these embryos to the blastocyst stage will, in theory, allow the production of embryonic stem (ES) cells which are genetically identical to the donor cell. This technique could result in significant advances in medicine. It could be used to produce disease specific ES cell lines in order to model diseases, by observing molecular changes, and screen for drug therapies. This could be particularly important for the study of diseases which are known to have a genetic basis but for which the mutation has not yet been identified.

2.2. ES cells produced in this way could also be differentiated into most cell types and in theory used as a source of patient specific cells to replace disease damaged tissue (the concept known as therapeutic cloning).

2.3. There is already evidence from a number of groups that human ES cells, derived from IVF embryos, are pluripotent and can maintain the potential to develop into cells of all three germ layers[36]. ES cells have been differentiated to produce a vast array of cell types including insulin producing cells, cardiomyocytes, smooth muscle cells and neuronal cells. Cells created through SCNT would be genetically identical to the donor cell, so will be immunologically compatible with the donor organism (i.e. the patient) and could be used in the treatment of disease (e.g. Parkinson's disease, diabetes, motor neurone disease) without the use of immunosuppressants.

2.4. In theory therapeutic cloning could be used to treat human conditions where there is a defined genetic defect. ES cell lines created using donor cells of patients with this defect could be repaired (through replacement of the defective gene by homologous recombination), differentiated into a particular cell type and replaced back into the patient. This method has been successfully used to correct a gene defect in mice[37].

2.5. It is also possible that this technique could be used to investigate the mechanisms used to reprogram DNA to an embryonic state and this knowledge could potentially be utilised to devise methods to produce ES cells from somatic cells, without the use of oocytes.

2.6. In addition, as cytoplasmic hybrids will contain animal derived, and possibly some human derived mitochondria, they could be a useful tool to study mitochondrial disease and the relationship between the mitochondria and the nucleus.

2.7. The availability of human eggs and embryos is a major limiting factor for investigating and utilising this technique in humans. Therefore scientists have suggested that one alternative is to use oocytes from another species which are accessible in abundance.

Summary: Creating embryos by somatic cell nuclear transfer (SCNT) will, in theory, allow the production of embryonic stem cells that are genetically related to the donor cell. This technique could be used to produce disease specific embryonic stem (ES) cell lines in order to model diseases and screen for drug therapies. Cell types could be differentiated from the ES cell lines and used in therapeutic cloning. Embryos created in this way could also be used to investigate reprogramming mechanisms in somatic cells and nucleo-mitochondria interactions. The lack of human eggs and embryos is a major limiting factor for investigating and utilising this technique in humans, which is why some scientists have suggested using eggs from another species.

3. Is the technique of creating human-animal cytoplasmic hybrids feasible?

3.1. The technique of somatic cell nuclear transfer (SCNT) to produce embryonic stem (ES) cells still needs investigating. Although there has been success with animals, it has not been proven to work with human eggs and there is a question as to whether somatic genes can be completely reprogrammed, and therefore generate embryonic stem cells, in this way.

3.2. ES cells have been successfully derived following SCNT in the same species, for example in cows (injecting bovine eggs with granulosa or cumulus cells, which yielded success rates of 69%[38] and 38%[39]), in rabbits (success rate of 61%[40]) and mice (success rate of 56%[41]). However, generally this technique yields poor developmental success rates and a high rate of abnormalities in the embryos, foetuses and offspring. This is thought to be due to epigenetic abnormalities and irregular patterns of gene expression[42 43]. To date, only one group has successfully used SCNT in humans. The embryo survived to blastocyst stage but did not result in the derivation of embryonic stem cells[44].

3.3. The only report of ES cell lines derived from SCNT, which contain a human genome, is from the creation of human-rabbit cytoplasmic hybrid embryos[24]. Analysis of these cells demonstrated that ES cells created by interspecies nuclear transfer possess many properties of human embryonic stem cells, including the origin from the inner cell mass (ICM), expression of surface markers, special growth requirements (such as dependence in feeders and independence in leukaemia inhibiting factors), capabilities of self renewal and differentiation into cells of three germ layers. The cells were capable of self renewal and could differentiate into a wide range of cell types *in vitro*. However, they failed to form teratomas when transplanted *in vivo*[45] and further experiments are necessary to prove whether these cells have the same developmental potential as conventional human ES cells.

Summary: ES cells have been successfully derived from animal SCNT embryos, but not from human SCNT embryos. There has been one report of human embryos created by SCNT. These developed to blastocyst stage but no ES cell lines were derived. Human-rabbit cytoplasmic hybrid embryos, created by SCNT, produced an ES cell line that contained a human genome. The ES cells produced had some, but not all, the properties of human ES cells.

4. Biological issues concerning interspecies cytoplasmic hybrid embryos

4.1. Introduction

4.1.1. There are several interlinking factors which contribute to the success rate of somatic cell nuclear transfer. For example, abnormalities in gene expression following SCNT have been linked with an inability of the oocyte cytoplasm to sufficiently epigenetically reprogram the nucleus[46]. In cytoplasmic hybrid embryos the nucleus of the donor (i.e. human) nucleus is transferred into the recipient animal oocyte. Animal mitochondria will be present in the cytoplasm of the enucleated recipient oocyte, so cytoplasmic hybrids will contain at least some animal mitochondrial DNA (mtDNA). Embryos can possess mtDNA from the recipient oocyte only (homoplasmy) or from the donor cell and recipient oocyte (heteroplasmy). The replication of mtDNA could be very important in determining the early developmental potential of these embryos. This has also raised questions over whether the genome of the resulting embryo will be human.

4.2. Background information on mitochondrial DNA (mtDNA)

4.2.1. Mitochondria contain DNA that encodes 13 essential genes associated with the electron transfer chain (ETC). The ETC generates the cell's energy – ATP – through the process of oxidative phosphorylation (OXPHOS). The remaining genes are encoded for by the nuclear genome[47]. This is the only entity in mammalian cells that is encoded by two genomes. Mitochondria also have a role in steroid synthesis[48] and apoptosis (programmed cell death)[49], required for normal embryonic development.

4.2.2. Mitochondria replication is not strictly tied to cell division as the numbers can vary according to the cell and its environment. The number of mitochondria in the germline varies from about 10 in a mouse primordial germ cell, to 1000 in a blastocyst cell, to about 100,000 in an oocyte before fertilisation[50]. There is a threshold of about 100,000 mtDNA copy numbers that has to be exceeded for fertilisation and subsequent embryo development to take place[51]. Oocyte mitochondria contain only a single copy of mtDNA, whereas somatic cells contain between 1-15 copies[52].

4.2.3. In normal embryonic development (by fertilisation), there are high numbers of mitochondria during the final stage of oocyte growth because there is a high energy requirement for fertilisation. MtDNA replication ceases prior to fertilisation and is not reinitiated until late blastocyst stage[53]. MtDNA replication is controlled by nuclear-encoded replication factors, such as the mitochondrial specific DNA Polymerase Gamma (POLG) and mitochondrial transcription factor A (TFAM). Replication initiates at a start site within the D-loop of mtDNA.

4.2.4. However, nuclear transfer (NT) contravenes the strict mechanisms that normally regulate mtDNA transmission after fertilisation and NT embryos are unable to tightly regulate mtDNA replication factor expression[54].

4.3. Source of genome in human-animal cytoplasmic hybrid embryos

4.3.1. Nuclear DNA content

4.3.1.1. In creating cytoplasmic hybrids, the nucleus from a human donor somatic cell is transferred into an enucleated animal oocyte. Therefore the embryo should contain a complete complement of human nuclear DNA (46 chromosomes). Analysis of embryos created from enucleated bovine oocytes fused with human granulosa or human fibroblast cells showed that the embryos contained human genomic DNA specific to the individual DNA profile of the donor cells used[23]. Karyotypic analysis of embryos created by fusing enucleated bovine oocytes with human cord fibroblasts showed that 56% of the embryos evaluated had the same number of human chromosomes as their respective donor cells[25]. Karyotypic analysis of NT embryos created from enucleated rabbit oocytes and human somatic nuclei showed apparent normal human chromosomes[24].

Summary: Analysis of human-animal cytoplasmic hybrid embryos showed that the nuclear DNA is human and specific to the donor cell used.

4.3.2. MtDNA content

4.3.2.1. The process of natural fertilisation or IVF usually results in the presence of a single identical population of mtDNA in the embryos, which is inherited from the mother i.e. homoplasmy. Creating interspecies cytoplasmic hybrids involves transferring the nucleus from a somatic cell into an enucleated oocyte. The process of nuclear transfer (NT) affects the replication, transcription and transmission of mtDNA. It is likely that some cytoplasm from the donor cell, containing mitochondria, is transferred with the nucleus.

4.3.2.2. The presence and persistence of donor mtDNA in NT embryos is variable. Embryos and offspring can exhibit mtDNA from the recipient oocyte only (homoplasmy) or varying degrees of mtDNA from both the donor cell and recipient oocyte (heteroplasmy). Donor mtDNA has been detected in some cases of interspecific bovine NT embryos, though not others[55 56], in interspecific caprine NT embryos[57] and cross-species NT embryos[10 25].

4.3.2.3. Homoplasmy may result from a failure of donor mitochondria to enter the ooplasm following donor cell fusion[58]. Heteroplasmy may be influenced by the starting numbers of donor and recipient mitochondria, the ability of donor mtDNA to replicate and persist, and the ability of products encoded by the mitochondrial and nuclear genomes to interact and function properly to support embryonic development. These factors are affected by the evolutionary diversity of the fusion partners. Differences in NT technique may also have an effect[20].

Transfer of donor mtDNA

4.3.2.4. MtDNA copy number may influence embryo development[46]. If a large amount of cytoplasm is removed from the oocyte at enucleation, then mtDNA levels may drop below the threshold level for viable embryo development. The addition of donor

mtDNA could increase the mtDNA levels above the threshold to allow development to continue, however this may be detrimental[59].

4.3.2.5. There is some literature on pronuclear transfer studies that indicate that approximately 20% of donor mtDNA is transferred[60]. However this is not comparable to SCNT in cytoplasmic hybrids because the mitochondria are in a different state in the pronuclei stage and they cluster round the pronucleus, suggesting transfer will be higher.

4.3.2.6. It has been suggested that the site of donor mtDNA can affect transmission[61] and that perinucleur mtDNA (those close to the nucleus) are selectively replicated compared to those that are more dispersed in the cytoplasm[62]. Another study indicates that the perceived preferential replication is only due to large numbers of mitochondria surrounding the nucleus[63].

4.3.2.7. One study reported that even residual amounts of donor mtDNA resulted in the replication and transmission of donor mtDNA in some NT embryos[64].

4.3.2.8. The creation of somatic cell cybrids, by fusing enucleated human myoblasts (containing a mixture of mutant and wild type mitochondria) with a human cell line devoid of mitochondria demonstrated that the nuclear genetic background of the recipient cell can influence the shift in proportion of mutant and wild type mitochondrial genomes[65]. This suggests that the type of somatic cell used as the donor cell in the creation of interspecies cytoplasmic hybrids could influence the proportion of donor or recipient (i.e. human or animal) derived mitochondria in the embryo. One study detected a greater level of donor mtDNA transmission in mice SCNT offspring when using adult fibroblasts instead of immature Sertoli cells or cumulus cells as nuclear donors[66]. There are also a variety of NT techniques and these may affect the amount on donor mtDNA transferred and how it is subsequently replicated[54].

Summary: There is a threshold level of mtDNA copy number needed for embryo development. The amount of donor mtDNA transferred may be affected by the position and numbers of mtDNA in relation to the nucleus, the type of donor somatic cell used and the nuclear transfer technique employed. One study revealed that even residual amounts of donor mtDNA were replicated in some of the resulting NT embryos.

Replication of donor mtDNA

4.3.2.9. MtDNA transcription and replication are regulated by mt-specific factors encoded by the nucleus. Therefore the interactions involved between the nuclear and mitochondrial genomes are crucial, particularly the ability of the nuclear products to interact with the D-loop of the mtDNA genome.

4.3.2.10. A mismatch between the human nuclear factors and the animal D-loop sequence of the mitochondrial genome may result in defective transcription and/or replication of the animal mtDNA. However studies that fused somatic nuclei from one species with somatic mtDNA from another did not always result in compromised mtDNA transcription and replication[67].

4.3.2.11. In a somatic donor cell the mechanisms regulating mtDNA transcription and replication are active. A study in primate-human cybrids suggests that the donor nucleus may preferentially select its own mtDNA at the expense of the recipient mtDNA[68]. Evidence that donor mtDNA can persist to blastocyst even when only

residual levels are transferred suggests that an active mtDNA replication mechanism can also persist after NT[64]. Donor cells are therefore still programmed to drive mtDNA replication and express replication factors in preimplantation NT embryos, unlike embryos created by fertilisation. In one study, though the majority of NT embryos did not replicate their donor mtDNA population, donor mtDNA was detected at considerably increased levels in a few embryos[54].

4.3.2.12. This suggests that in some cases nuclear encoded mtDNA replication factors are interacting with the donor mtDNA more efficiently and thus preferentially replicating its own mtDNA. This has been linked to increased expression of the replication factors TFAM and POLG at the 16-cell stage, which promotes the persistence of residual levels of donor mtDNA[54]. Abnormal expression of factors such as TFAM and POLG may prematurely drive mtDNA replication and impact on early embryonic development[54].

Summary: Transcription and replication of mtDNA relies on the interaction of human nuclear factors with the animal D-loop section of the mitochondrial genome. Following SCNT the mechanisms regulating mtDNA transcription and replication are still active in the donor nucleus, unlike in fertilised embryos. In one study, donor mtDNA was preferentially replicated in some of the NT embryos, though the majority of NT embryos did not replicate donor mtDNA. Preferential replication of donor mtDNA has been linked to increased expression of replication factors.

Persistence of donor mtDNA

4.3.2.13. Evidence from interspecies NT experiments indicates that the cytoplasm of one species can support some embryonic development with nuclei of another species. However the persistence of donor mtDNA and the level of development reached is very variable[54].

4.3.2.14. A study that analysed the origin of the mitochondria in interspecies embryos derived from SCNT of human cord fibroblasts into enucleated bovine oocytes found that both human and bovine mtDNA was present in the interspecies embryos up to the 16 cell stage[25]. However, only bovine mtDNA was detectable beyond the morula stage. Another study which fused enucleated bovine oocytes and human granulosa or fibroblast cells found the presence of both bovine and human mtDNA was detectable in almost all embryos up to the blastocyst stage[23]. This was similar to the presence of donor mtDNA reported in blastocysts derived from rabbit-monkey SCNT[10].

4.3.2.15. It has been reported that preservation of donor mtDNA following NT might occur to a greater extent when the donor nucleus and recipient oocyte are from more diverse genetic backgrounds[54]. In genetically close species the mtDNA arises primarily from the oocyte[69 70 71]. In more unrelated species mtDNA may be derived from both the somatic donor cell and the recipient oocyte[10 19 24 25]. However the donor cell mtDNA in studies does not account for the majority of mtDNA in cells by the blastocyst stage[20] and interspecies NT leads to further potential problems for the generation of ATP.

4.3.2.16. Evolutionary distance can affect development to blastocyst and, in many cases, result in the elimination of donor cell mtDNA[54]. Studies that fused human cells without mtDNA with enucleated primate cells, suggested that there is an evolutionary barrier that is reached with increasing evolutionary divergence where animal mtDNA cannot be maintained. Chimpanzee and gorilla mtDNA were

replicated and transcribed in human cells, but mtDNA from orang-utan and more evolutionary distant species were not[72].

Summary: The persistence of donor mtDNA is very variable. Some studies have reported donor mtDNA persisting in embryos up to the blastocyst stage, others up to the 16 cell stage. Evolutionary distance between fusion partners appears to affect the preservation of donor mtDNA.

4.3.3. Protein and RNA content

4.3.3.1. The protein and RNA content of the cytoplasmic hybrid will shift from being mostly oocyte-derived to more donor-derived as it develops towards blastocyst. Studies have shown this transition to begin at the 2-cell stage in mice[73 74], the 4-cell stage in humans[75], nearer the 8-cell stage in cows[76] and more gradually during the cleavage stage in rabbits[77]. From this point the donor (human-derived) gene products will accumulate and many of the oocyte (animal-derived) products will be degraded[78].

Summary: Apart from 13 proteins (and 2rRNAs and 22tRNAs) encoded by animal mitochondria, the embryo will be entirely human with respect to protein and RNA by 14 days50.

4.4. Embryonic development and functionality of cytoplasmic hybrids

4.4.1. Introduction

4.4.1.1. Experiments creating rabbit and human[24], cow and human[23 25], and rabbit and monkey[10] cytoplasmic hybrids have survived until blastocyst stage. This shows the potential of non-human mammalian oocytes to sufficiently reprogram the human somatic nucleus and support development to blastocyst.

4.4.1.2. However other inter-species NT experiments have not reached blastocyst stage[25 54 57 79]. Alan Trounson's group's recent paper on inter-species cell nuclear transfer[20] gives a comprehensive summary of the developmental competence of a range of inter-species NT embryos. The use of bovine oocytes has shown to be successful for a number of donor cells to blastocyst. However the overall low development to blastocyst stage in cross-species SCNT embryos may be a result of inefficient nuclear reprogramming, mitochondrial heteroplasmy and incompatibilities between the donor nucleus and recipient cytoplasm[20].

4.4.2. Nuclear reprogramming

4.4.2.1. For the process of SCNT to be successful the somatic genome of the donor cell must be reprogrammed to allow the correct expression of genes for embryonic development. Formerly inactive genes needed for embryonic development need to be upregulated and the gene expression of the donor cell must be repressed. Failure of the recipient oocyte to completely reprogram the donor nucleus results in incomplete reactivation of genes associated with pluripotency, such as Oct4[80].

4.4.2.2. Information on the genome of cells in a pluripotent state is more broadly available than in somatic cells. The oocyte cytoplasm would need to reset the epigenetic state of the somatic cell genome and histone proteins (which determine the structure of the DNA) to ensure that the correct areas of DNA are open to

transcription (euchromatic regions) to enable pluripotency. This process is possible by demethylating DNA and either methylating or demethylating histone proteins.

4.4.2.3. However, it may be hard for the oocyte cytoplasm to completely reprogram the donor cell genome as it is in a very different epigenetic state compared to gamete cells (which are reprogrammed following natural and *in vitro* fertilisation) and it is possible that genes specific to the donor cell are still expressed in the early embryo[81]. It is possible that these deficiencies could be transmitted to embryonic stem cell lines derived from NT blastocysts but the process of ES cell derivation may select for the cells without these defects[82].

4.4.2.4. It is likely that any abnormalities in epigenetic reprogramming, following SCNT, will only be exacerbated by the fact that, in interspecies cytoplasmic embryos, the nuclear and cytoplasmic components are from genetically distant species. It has been suggested that there are significant differences in the conservation of epigenetic reprogramming (by demethylation) between mammalian species. Although, it has been suggested that the role of demethylation in reprogramming embryos to a totipotent state may not be significant as once thought. One study shows the dynamics of this process are not conserved between sheep and human[83]. This study suggested that only a subset of the genome may be required for epigenetic reprogramming as, in normal development of sheep embryos, the genome retains at least 57% of the methylation of the 1 and 2 cell stages.

4.4.2.5. As outlined by Ilmensee *et al* in their report of the creation of human-mouse embryos[4] a number of groups are investigating nucleocytoplasmic interactions and reprogramming of transferred somatic cell nuclei by the cytoplasm of recipient oocytes[84], chromatin remodelling of somatic nuclei by oocyte factors[85], rebuilding of telomere length of somatic chromosomes[86 87 88 89], and cell cycle coordination between donor nucleus and cytoplasm of the recipient oocyte during the cloning procedure[90 91].

Summary: In order to create embryos using SCNT, the somatic genome of the donor cell must be reprogrammed to allow the correct expression of genes for embryonic development. Though this is an issue for all embryos created by SCNT, the process is likely to be more problematic in interspecies hybrids as different species may have different mechanisms for reprogramming.

4.4.3. Interaction of nuclear and mitochondrial genome

4.4.3.1. The interactions between the nuclear and mitochondrial genomes have been studied using mouse-human hybrids, created by fusing two somatic cells[92 93 94]. These studies reported that the mitochondrial DNA of the parent whose chromosomes were segregated from the nucleus was undetectable or present in marginal amounts in the hybrid constructs created.

4.4.3.2. The nucleo-mitochondrial interaction of NT embryos is out of sequence compared to embryos generated through IVF. Two key factors for mtDNA transcription and regulation (transcription factor A and mitochondrial specific DNA polymerase Gamma), encoded by the chromosomal genome, have been found to be expressed early in preimplantation ovine nuclear transfer embryos, compared to IVF embryos. Unregulated nuclear-mitochondrial cross-talk, and the closer nucleo-mitochondrial genetic compatibility between the donor cell and its own mtDNA population, may lead to premature and preferential replication of the donor cell mtDNA prior to blastocyst stage[64].

4.4.3.3. The products encoded by the nuclear and mitochondrial genomes need to be able to interact together and allow the mitochondria to generate appropriate levels of ATP. Interspecies cybrids studies showed that the process of oxidative phosphorylation (OXPHOS) that generates ATP was compromised[67 95 96]. A genetic divergence between gene products encoded by nuclear and mitochondrial genomes may affect ATP output. The study of hybrids created by inserting mitochondria from primate species into human cell lines that lack mitochondria has also facilitated the study of nucleo-mitochondrial interactions. The use of mitochondria from closely related primate species, such as chimpanzee, pigmy chimpanzee or gorilla, restored OXPHOS function of the human cell lines. However, mitochondria from more divergent primate species, such as orang-utan, African green monkey, squirrel monkey and Lemur failed to do so[72].

4.4.3.4. This suggests that mitochondrial and nuclear genomes can only functionally interact in closely related species and that incompatibility of these genomes and the subsequent decline in OXPHOS function, and consequently ATP production, may be the reason for the developmental arrest of distantly related interspecies embryos[67]. It is likely that nucleo-mitochondrial interactions become sub-optimal once a certain evolutionary distance is exceeded, resulting in elimination of donor cell mtDNA[54], or the developmental arrest in embryos, but the existing data in the literature are too few to draw valid conclusions[20].

Summary: The interaction between the nuclear and mitochondrial genome is not regulated in the same way as normally fertilised embryos. Humans may be too evolutionary distant from other mammalian species, such as rabbit and cows, for the nuclear and mitochondrial genomes to be compatible. ATP production of these embryos is compromised and these mechanisms appear to become less functional when the evolutionary distance between the two species is increased. This may be the reason for developmental arrest of interspecies embryos. However, survival of human-rabbit and human-cow embryos to the blastocyst stage suggests that this is not always problematic. This may be due to the human nucleus preferentially replicating the human mitochondria present.

4.4.4. MtDNA heteroplasmy

4.4.4.1. Heteroplasmy of mitochondrial DNA may be involved with incompatibilities between the nucleus and the cytoplasm, which may inhibit the development of cytoplasmic hybrid embryos. In mouse embryos created by nuclear transfer and parthenogenesis, mitochondrial heteroplasmy is associated with a reduced ability to develop to the blastocyst stage[59 97].

4.4.4.2. Within the same species the presence of two or more mtDNA genotypes, due to mutations or deletions, can result in a mitochondrial disease. The phenotype of the disease is dependent on the proportion of mutated mtDNA to wild type. It has been reported that, in the case of some mitochondrial diseases a high percentage of mutant mtDNA is required before the phenotype presents (e.g. Leber's Hereditary Optic Neuropathy (LHON), >60%[98] and Myoclonic Epilepsy with Ragged Red Fibres (MERRF), >85%[99]). This demonstrates that some interspecies cytoplasmic hybrids with a high degree of heteroplasmy may be phenotypically normal.

4.4.4.3. Mixing mtDNA from different sources may also result in some electron transfer chains (ETCs) being more functional than others. The sequence differences of the

mitochondrial populations may give rise to proteins with slightly altered amino acid sequence, as demonstrated in pigs[100] and cattle[101]. Therefore components of the ETC may have abnormal protein conformation. In addition, both the mitochondrial and chromosomal genes contribute proteins to the electron transport chain so if they are not compatible this will lead to inadequate interaction between separate sub units of the ETC resulting in potentially serious affects on ATP production[67 102]. Many abnormalities observed in NT embryos may be caused by deficiencies in OXPHOS, possibly due to heteroplasmic mtDNA populations. However, these deficiencies may not be obvious in cell culture as the embryo and ES cells will not be dependent on normal mitochondrial function (they will be glycolytic) due to the high glucose concentration of typical culture media.

4.4.4.4. Mitochondria from an oocyte are in a different state to mitochondria from a somatic cell. One study demonstrated that somatic cell mtDNA can impair embryonic development[59]. It has been suggested that specific mtDNA haplotypes may influence the development potential for NT embryos[54]. It is also possible that mtDNA from the oocyte could be a source of immunologic incompatibility. Potential differences in mtDNA encoded proteins of the different populations of mitochondria could stimulate a T-cell response specific for mtDNA encoded minor histocompatibility antigens.

4.4.4.5. However, there are a number of examples of heteroplasmic interspecies nuclear transfer embryos which have survived to the blastocyst stage, including the creation of human-cow and macaque-rabbit embryos[19 25 103].

4.4.4.6. It has been suggested that the ability to replace recipient oocyte mitochondria with donor cell mitochondria in interspecies somatic cell nuclear transfer embryos will have significant impact on future reproductive and stem cell technologies[20].

Summary: Some cytoplasm containing donor mitochondria is likely to be transferred into the recipient oocyte along with the nucleus, resulting in heteroplasmy. Heteroplasmy has been associated with reduced embryonic development. The sequence differences of the animal and human mitochondrial DNA may give rise to proteins with slightly altered amino acid sequence. This may mean that abnormal proteins are produced which impair ATP production and cell function.

4.5. Embryonic stem cells derived from cytoplasmic hybrids

4.5.1. If the cytoplasm of an oocyte has the required potential to reprogram the somatic cell nucleus of another species and re-establish embryonic gene expression, the embryo could develop to blastocyst and embryonic stem (ES) cell lines could be derived that match the nuclear genome of the donor.

4.5.2. Embryos formed from enucleated rabbit oocytes and human fibroblasts formed blastocysts from which embryonic stem cells were derived that could self-renew and differentiate into all three germ layers[24]. The ES cells had many (although not all) properties of conventional human ES cells, however the analysis of mtDNA of the ES cells was not presented. Mouse ES cells have been derived from cross-species SCNT using bovine recipient oocytes and mouse somatic donor cells[21]. Equine-bovine cross species SCNT embryos resulted in a pututative embryonic stem cell colony and one trophectoderm stem cell line was established, although this could not be maintained[20].

4.5.3. Any mismatch between human nuclear factors and animal mtDNA will not become apparent until there is a demand for high levels of ATP and therefore a need for mitochondrial function. This will be after the stage that embryonic stem cells will be derived and any mismatch may not become apparent until cells are derived which require high energy levels[50]. For cell lines to be functional and of use in disease modelling, they need to have functional mitochondria and produce their own ATP, rather than rely on glycolysis.

Summary: ES cell lines can potentially be derived from interspecies cytoplasmic hybrid embryos. However for these to be functional cell lines need to produce their own ATP and this requires effective nucleo-mitochondrial interactions.

5. Alternatives to human/animal hybrids and chimeras

5.1. Alternative sources of stem cells

5.1.1. Adult stem cells

5.1.1.1. This review uses the term adult stem cells to refer to tissue-specific stem cells. Cord blood stem cells and other 'adult' stem cells are reviewed separately below. Adult stem cells are often multipotent and are found in many tissues including bone marrow, muscle, liver and skin. They have variable potency and can develop into a range of cell types related to the tissue they are derived from. For example, bone marrow contains haematopoietic stem cells which produce blood cells, and mesenchymal or stromal stem cells which support the haematopoietic and other cells in the marrow and may be involved in tissue repair[104]. Adult stem cells are often thought to occupy special micro-environments or 'niches' in tissue, which influence the stem cell's development[105].

5.1.1.2. Adult stem cells are involved in tissue renewal and repair, and established treatments include bone marrow, skin and corneal transplants. These work by transplanting patient specific stem cells to avoid the problems of graft rejection. Animal models and clinical trials using adult stem cells are being developed for the treatment of heart disease, type 1 diabetes, spinal cord injury, stroke, Parkinson's disease and Huntington's disease[107].

5.1.1.3. However adult stem cells are limited in their applications for a number of reasons. Not all tissues contain stem cells, and those that do may be limited in the types of cell or tissue they can give rise too. For example, adult stem cells may not be able to give rise to dopamine neurone cells for the treatment of neurological diseases such as Parkinson's disease[106]. Some stem cells are inaccessible, for example Central Nervous System (CNS) stem cells. It is hard to isolate and purify well-characterised differentiated cells for transplantation because most adult stem cell populations are highly heterogeneous[107]. Some patient specific stem cells would contain a genetic defect or the patient may have a disease that involves the loss of stem cells. At present there is only a very limited range of diseases that may be treatable using adult stem cells. Embryonic stem (ES) cells may offer a potentially more flexible range of research options, if the different differentiation pathways can be directed.

5.1.1.4. Some studies have tried to transdifferentiate adult stem cells away from the tissue they originated from, to try and broaden the potential of these cells. Researchers have attempted to induce this 'plasticity' through culture conditions or by

transplanting cells into different organs. Haematopoietic stem cells, bone marrow mesenchymal (or stromal) cells and neural stem cells have shown substantial plasticity[107]. However, though some adult stem cells appear to be more flexible than previously thought, the mechanisms controlling this process are not well understood. It has been suggested that transdifferentiation may occur because of other cell mechanisms, such as cell fusion[108]. The validity and reproducibility of these studies has not been confirmed and the technique is also very inefficient, limiting its practical applications.

Summary: Although a very important avenue of research, adult stem cells are limited in the types of cell or tissue they can give rise to. Some studies have tried to induce adult stem cells to broaden the range of potential tissues they can form. However, though some stem cells appear more flexible than previously thought, the mechanisms controlling this process are poorly understood. At present there is only a very limited range of diseases that can be treated using adult stem cells.

5.1.2. Cord blood and foetal stem cells

5.1.2.1. Cord blood stem cells can be isolated from the blood of the umbilical cord at birth and then stored. Cord blood stem cells have replaced bone marrow and blood cells in the treatment of leukaemia and other haematopoietic disorders, especially in children[109]. The International Bone Marrow Transplantation Registry (IBMTR) estimated that since 1998, a fifth of stem cell transplants in young patients are cord blood transplants, mostly for acute lymphoblastic leukaemia and acute myeloblastic leukaemia[110].

5.1.2.2. Cord blood transplants from both related and unrelated donors have been successful in treatment[111]. There are several advantages of using cord blood as a source of stem cells over bone marrow[112]. Cord blood transplantation tolerates a greater mismatch of tissue types between donor and recipient than bone marrow or peripheral blood transplants. There is a low incidence and severity of graft versus host disease and lower incidence of viral transmission. Transplants are also available faster than conventional bone marrow grafts and donors are less likely to change their mind. However there are lower numbers of haemopoietic progenitor cells and stem cells, which may cause delayed engraftment, and there is a lack of available subsequent donations of stem cells and/or lymphocytes in case of graft failure or disease relapse.

5.1.2.3. There are claims that cord blood cells have a wider potential. However these have not been substantiated and robust methods are needed to culture and expand cells *in vitro*. There is some evidence that foetal-derived stem cells can be used to treat neurological disease[113] and animal models have been developed that use umbilical cord stem cells to improve cardiac function in acute myocardial infarction[114 115 116]. Cord blood stem cells have also been used in cases of spinal injury[117].

5.1.2.4. There have been recent reports that mesenchymal stem cells can be harvested from human umbilical cord perivascular cells, which are isolated from tissue normally discarded after birth[118]. This may provide a more readily available source of mesenchymal stem cells than from bone marrow, and may potentially have use in therapies for the regeneration of the musculo-skeletal system. However this research is at a very preliminary stage.

5.1.2.5. Foetal stem cells can be derived from a range of tissues following a pregnancy termination, such as the foetal nervous system. These cells can potentially be

used in treatment of diseases of the brain, such as Huntington's disease, Batten Disease and stroke. Trials are currently underway or seeking approval. Germ cell derived stem cells can also be isolated from the gonads of an early foetus. These are pluripotent and are similar to ES cells. However they are difficult to derive and maintain in culture.

Summary: Cord blood cells have been successful in the treatment of leukaemia and other blood disorders, especially in children. However they are limited in the disorders they can treat and although there are some claims that these cells have wider potential, these have not been substantiated.

5.1.3. Stem cells from amniotic fluid

5.1.3.1. Recently it has been claimed that cells isolated from amniotic fluid show a high degree of multipotentiality. In one study, human and rodent stem cells were derived from amniotic fluid that expressed embryonic and adult stem cell markers. The cells were broadly mulitpotent[119]. Human stem cell lines could differentiate into cell types representing each germ layer. However there is still uncertainty about the true nature of these cells and their physiological relevance. Additionally amniocentesis is needed to obtain these cells, which raises safety issues.

5.1.4. Stem cells from testicular tissue

5.1.4.1. A study claimed to have derived pluripotent stem cells from the adult testis of mice[120]. The group isolated spermatogonial stem cells (SSCs), which are responsible for maintaining spermatogenesis throughout life in the male, from adult mouse testis. These isolated SSCs acquired ES cell properties in culture. The cells were able to differentiate into derivatives of the three embryonic germ layers, could generate teratomas and showed germline transmission. However research is still preliminary and the results have not yet been substantiated.

5.1.5. Mouse epiblast stem cells

5.1.5.1. Mouse ES cells are typically used as a model for human ES cells but, despite their apparent common origin and similar pluripotency, they have different signalling pathways to maintain their pluripotent status. Recent studies have derived pluripotent stem cell lines from the epiblast layer of post-implantation mouse embryos[121]. These epiblast stem cells (EpiSCs) have a different epigenetic state and different signals controlling their differentiation to mouse ES cells but are similar to human ES cells with respect to patterns of gene expression and signalling responses. EpiSCs could serve as an improved model for human ES cells and explore the differences between human and mouse ES cells, however research is at an early stage.

Embryonic stem cells that do not involve destroying viable embryos

5.1.6. Stem cells derived from individual blastomeres

5.1.6.1. The derivation of stem cells from blastomeres has been suggested as an alternative source of ES cells that avoids destroying an embryo in the process of deriving a stem cell line. A cell could be removed from an embryo at the 8-cell

stage and then cultured *in vitro* under conditions that allow a stem cell line to develop. The embryo from which the cell was removed would continue to develop and could be transferred back to the woman in the hope of establishing a pregnancy. This technique is similar to that used in pre-implantation genetic diagnosis (PGD), which does not appear to interfere with the embryo's developmental potential, though this is not known for certain. The production of a stem cell line from a single mouse blastomere was reported in 2005[122]. In 2006 the group derived human embryonic stem cell lines from individual blastomeres[123]. However this study derived blastomeres from disaggregated embryos, not from biopsy. The procedure is still inefficient and the growth conditions are complicated because the biopsied blastomere requires co-culture with a previously derived human ES cell line.

5.1.7. Stem cells derived from abnormal and arrested embryos

5.1.7.1. This technique involves deriving embryonic stem cells from embryos which have arrested before they reach blastocyst stage. A study has shown that arrested human embryos have viable blastomeres that can proliferate and form primary outgrowth and human ES cell-like colonies[124]. Poor grade embryos were also shown to be capable of producing stem cell lines. Arrested embryos, which never reach the morula or blastocyst stage, have generally been regarded as being 'dead'. However they still possess some viable blastomeres. The method is a potential way of deriving human ES cell lines without destroying viable human embryos. Arrested embryos derived after nuclear transfer (NT) could be a source of patient-specific stem cells. Currently the derivation process is inefficient, and the mechanisms and factors involved need to be identified.

5.1.8. Stem cells derived from parthenotes

5.1.8.1. Parthenogenesis is the biological phenomenon by which embryonic development of an oocyte is activated without the presence of sperm. Although it is common in lower organisms, the mammalian parthenote does not result in a successful pregnancy. Parthenogenesis of monkey eggs has resulted in the development of embryos to blastocyst stage and their use to create pluripotent stem cell lines[125]. Parthenogenetic ESCs (pESCs) have been shown to have the properties of self-renewal and the capacity to generate cell derivatives from the three germ layers[126]. Genetic material is derived exclusively from the female oocyte donor so could therefore provide a potential source of autologous cell therapy in the female that bypasses the need to create and destroy a viable embryo. However a source of donor oocytes is still required and this is severely limited for human eggs.

Embryonic stem cells that have an alternative source of oocytes

5.1.9. Donated human oocytes

5.1.9.1. The primary source of oocytes for research is through women donating spare oocytes from IVF programmes. However the supply is severely limited and treatment to retrieve oocytes exposes women to a variety of risks, the most serious being Ovarian Hyper Stimulation Syndrome (OHSS). Altering the IVF procedure to retrieve more oocytes is unethical. The donation of oocytes is unlikely to meet the numbers needed by future research requirements.

5.1.10. *In vitro* growth and *in vitro* maturation of oocytes

5.1.10.1. Preliminary research has shown it is possible to grow and mature immature mammalian oocytes in culture. *In vitro* growth (IVG) refers to the development in culture of oocytes from immature (preantral) follicles. *In vitro* maturation (IVM) refers to the maturation of oocytes from the germinal vesicle stage – the final stage of maturation of an oocyte. Immature human oocytes could be harvested from removed adult ovaries, or from fetal ovaries obtained from pregnancy terminations, or potentially derived *in vitro* from embryonic stem cells (see section 5.1.11 below). IVG of oocytes has been carried out in various animal species and live offspring have been produced from mouse eggs that were grown *in vitro*[127] [128]. There are groups working on developing this technique in humans but at present success has been limited and more research needs to be carried out before this is a viable alternative.

5.1.11. *In vitro* derived gametes

5.1.11.1. ES cells appear to be able to differentiate into germ cells of various stages spontaneously and quickly. This could be due to either the inherent nature of ES cells or the micro-environment of the culture conditions. Studies in mice have derived primordial germ cells (PGCs) *in vitro* from ES cells that may form oocyte-like cells and develop into blastocyst-like structures. Research is more advanced for male gametes and one group managed to produce viable transgenic offspring from ES cell-derived male gametes, though these exhibited abnormalities and died prematurely[129]. The equivalent level of research has not been achieved for deriving oocytes. Research into human ES cells is preliminary. There have been indications that human ES cells can differentiate into PGCs and occasionally early spermatid cells, though not oocytes[130] [131]. If techniques could be developed to derive human oocytes, then this could provide a potential alternative source of oocytes. However research is at an early stage, in particular with regards for deriving oocytes, and there is currently a lack of data for humans.

Summary: Adult and cord blood stem cell research is significant; however ES cells may offer a potentially more flexible range of research options if the different differentiation pathways can be directed. Research on other sources of stem cells, and alternative ways of deriving embryonic stem cells without destroying viable embryos, is at a very preliminary stage and does not currently offer a viable alternative to human-animal embryos.

5.2. Reprogramming of somatic cells

5.2.1. 'Direct' reprogramming of somatic cells into induced pluripotent stem (iPS) cells

5.2.1.1. One area of research has concentrated on re-programming adult somatic cell nuclei directly, without transferring them to oocytes. Recently there have been some successful studies published on re-programming mice fibroblasts into cells that behave like ES cells, known as induced pluripotent stem (iPS) cells. The first group to achieve this identified four transcription factors, expressed using retroviral vectors and known to be required for pluripotency, to reprogram fibroblasts to iPS cells[132]. Subsequently three studies[133 134 135] have improved on this method by using a better marker gene to select the relatively rare cells that have been reprogrammed. The iPS cells obtained by these three groups resemble ES cells more closely than the original study. The iPS cells appear to be equivalent to ES cells based on morphology, proliferation, teratoma formation, gene expression, methylation and chimera formation. One group achieved germline transmission, where chimeras produced offspring that must have come from sperm originating from the iPS cells. The research is significant because it shows that the method is reproducible and that the technique is improving. Reprogramming is still slow and inefficient which indicates other factors could be identified to improve the process. In the future similar methods could be applied to human somatic cells. However this is still a long way off and human ES cells are different in a number of respects to mouse ES cells. The same four transcription factors may not work for human cells. There are also safety aspects as one of the retroviral vectors, cMyc, is an oncogene and can result in tumours.

5.2.2. Using fertilised supernumerary oocytes discarded from IVF

5.2.2.1. Earlier this year a group carried out SCNT experiments in mice using fertilised eggs as hosts for somatic cell nuclei instead of unfertilised oocytes[136]. Previous similar attempts have been unsuccessful because the DNA from the somatic cell has not been sufficiently reprogrammed. This group used inhibitors to temporarily arrest cells in mitosis. At this stage the chromosomes are not surrounded by a nuclear membrane and it appears that the re-programming factors are dispersed in the cytoplasm. The resulting embryos supported somatic cell reprogramming, the production of embryonic stem cell lines and the full-term development of cloned offspring. The research is significant because human fertilised eggs are more available than unfertilised oocytes. It is estimated that 3-5% of fertilized human zygotes contain supernumerary sets of chromosomes[137]. These are automatically excluded from use in IVF and discarded because they do not develop. These fertilised eggs could potentially be used to produce ES cell lines using the technique demonstrated. However this has been the only successful study published to date and the technique has not been attempted in humans.

5.2.3. Stembrids

5.2.3.1. The creation of stembrids (stem cell hybrids) involves a donor somatic cell being fused to an enucleated already-established human ES cell. Components of the cytoplasm of the ES cell reprogram the somatic DNA from the donor cell and a new donor-specific stem cell line is established. If the process is effective it could be an alternative to producing patient-specific ES cells without having to use oocytes or produce embryos. However it does rely on previously established human ES cell

lines. There is a patent application on this work and as such published work is limited[138139]. Research is at a very early stage and stembrids have not yet been shown to be an alternative to ES cells derived from an embryo. Differences in cell types may arise because of the limited reprogramming ability of embryonic stem cells compared to an oocyte.

Summary: Three separate groups have directly reprogrammed mice fibroblast cells into cells similar to ES cells, without transferring the cells into an egg or creating an embryo. However research is still at a very early stage and the reprogramming process is inefficient. Different factors may be involved for humans than those identified for mice. Another technique uses fertilised eggs, which are more available than unfertilised eggs, as hosts for SCNT. The process of using fertilised eggs for SCNT has only had one successful study published to date and the technique has not been attempted in humans.

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Appendix C – International Perspective

1 Introduction

1.1 Most countries have not formed specific legislation to cover the creation of human-animal hybrids. Australia, Canada and the USA have all considered the issue, and a summary of their legislation is given in Section 2.

1.2 A table summarising countries' general policies towards human embryo research is given in section 3. Countries that already do not allow the creation of embryos for research may not feel the need to review their legislation. Those with permissive policies, which allow the creation of embryos for research through Somatic Cell Nuclear Transfer (SCNT), may be more likely to consider reviewing their legislation to cover human-animal embryos. The current legislation on embryo and stem cell research for these permissive countries is outlined in Section 4.

NB. All legislation prohibits human reproductive cloning.

2 Specific legislation on human-animal embryos

Australia

2.1 In December 2005 a government-appointed commission published a list of recommendations for new legislation on embryonic stem cell research, in the Lockhart Review[1]. The recommendations included permitting the creation of hybrid embryos by introducing the nucleus of a human cell into an animal egg under licence.

2.2 In late 2006 the Australian Government adopted most of the Lockhart Review's recommendations but did not pass legislation that would permit the creation of human-animal hybrid or chimera embryos. It only permitted under licence, creating a hybrid embryo for the purpose of testing human sperm quality through the fertilisation of an animal egg up to the first mitotic division.

2.3 The Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006 prohibits:

- Placing a human embryo clone in the body of a human or the body of an animal
- Creating a human-nonhuman chimeric embryo.
- Creating a human-nonhuman hybrid embryo without a licence.
  - The only licence that can be obtained is a licence to fertilise a human egg by a human sperm up to, but not including, the first mitotic division, outside the body of a woman for the purposes of research or training in Assisted Reproduction Technology.
- Placing a human embryo in an animal.
- Placing an animal embryo in the body of a human for any period of gestation.

Canada

2.4 The Assisted Human Reproduction Act (2004) prohibits:

[1] www.lockhartreview.com.au/

- Creating a human clone by using any technique, or transplant a human clone into a human being or into any non-human life form or artificial device.
- Altering the genome of a cell of a human being or in vitro embryo such that the alteration is capable of being transmitted to descendants.
- Transplanting a sperm, ovum, embryo or foetus of a non-human life form into a human being.
- For the purpose of creating a human being, making use of any human reproductive material or an in vitro embryo that is or was transplanted into a non-human life form.
- Creating a chimera, or transplanting a chimera into either a human being or a non-human life form.
- Creating a hybrid for the purpose of reproduction, or transplanting a hybrid into either a human being or a non-human life form.

2.5 The prohibition only applies to human chimera embryos. Research involving the creation of an animal chimera embryo (transplanting human stem cells into nonhuman embryos) is not prohibited in law.

2.6 The Canadian Institutes of Health Research (CIHR) and the Tri-Council Policy Statement (TCPS) "Ethical Conduct for Research Involving Humans" have issued guidelines that apply to all Canadian researchers and research institutions that receive funding from CIHR and other federal funding agencies. Since, as far as is known, there are no private researchers functioning in private research facilities that would be exempt from these rules, the CIHR stem cell Guidelines and the TCPS cover all stem cell research in Canada.

2.7 The CIHR stem guidelines, published in 2002 and updated in 2005 and 2006, expressly prohibit the creation of animal chimera embryos for research. Since 2002, this prohibition has included:

- Research in which human or non-human ES cells, embryonic germ (EG) cells or other cells that are likely to be pluripotent are combined with a human embryo.
- Research in which human ES cells, EG cells or other cells that are likely to be pluripotent are combined with a non-human embryo.

2.8 The TCPS, adopted in 1998, expressly prohibits the creation of hybrid embryos and more generally prohibits the creation of human embryos for research. This states that:

- It is not ethically acceptable to create, or intend to create, hybrid individuals by such means as mixing human and animal gametes, or transferring somatic or germ cell nuclei between cells of humans and other species.
- It is not ethically acceptable to create human embryos specifically for research purposes. However in those cases where human embryos are created for reproductive purposes, and subsequently are no longer required for such purposes, research involving human embryos may be considered to be ethically acceptable.
- It is not ethically acceptable to undertake research that involves ectogenesis, cloning human beings by any means including somatic cell nuclear transfer, formation of animal/human hybrids, or the transfer of embryos between humans and other species.

USA

2.9 The *Draft Human Chimera Prohibition Act of 2005 (S.1373)* prohibits:

- Creating or attempting to create a human chimera
- Transferring or attempting to transfer a human embryo into a nonhuman womb
- Transferring or attempting to transfer a nonhuman embryo into a human womb
- Transporting or receiving for any purpose a human chimera

In this draft legislation, some human-nonhuman hybrids would come under the definition of a chimera.

3 General policies towards human embryo research

Policy Type	Asia & Oceania	Europe	Middle East & Africa	The Americas
Permissive (e.g. SCNT is specifically permitted under certain conditions)	Australia, China, India, Japan, Singapore, South Korea	Belgium, Finland*, Sweden, UK	Israel, South Africa	California (US), Connecticut (US), Illinois (US), Maryland (US), Massachusetts (US), Missouri (US)¤, New Jersey (US), Rhode Island (US)
Permissive Compromise (e.g. SCNT is prohibited; hESC research using supernumerary IVF embryos is specifically permitted or not prohibited)	Hong Kong, New Zealand, Taiwan	Bulgaria. Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland*, France, Georgia, Greece, Hungary, Iceland, Latvia, Moldova, Netherlands, Portugal, Romania, Russia, San Marino, Slovenia, Spain, Switzerland, Turkey	Iran	Argentina, Arkansas (US), Brazil, Canada, Indiana (US), Iowa (US), New Hampshire (US), Virginia (US)
Restrictive Compromise (e.g. hESC research only permitted using cell lines created before a certain date)		Germany, Italy		
Prohibitive (e.g. research using embryos or cell products derived from embryos is prohibited)		Austria, Lithuania, Norway, Poland, Slovakia	Tunisia	Colombia°, Costa Rica°, Ecuador°, El Salvador°, Florida (US), Louisiana (US), Maine (US), Michigan (US), Minnesota (US), North Dakota (US), Panama°, Pennsylvania (US), Peru°, South Dakota (US)

* Finland is categorized between Permissive and Permissive Compromise because the relevant law does not consider the product of SCNT to be an embryo. It is understood that SCNT – as it is not prohibited – is permitted in the country.

¤ Missouri is partly Permissive: The Missouri Stem Cell Research and Cures Initiative is very supportive of stem cell research, and while it prohibits human reproductive cloning and fertilization solely for the purposes of research, it allows researchers to conduct any research permitted under federal law. U.S. Federal law does not currently prohibit SCNT.

°These categorisations have been based on national policies extending a right to life to conceived or unborn persons. It is unclear whether the constitutional language would prohibit the destruction of embryos for any purpose, including research.

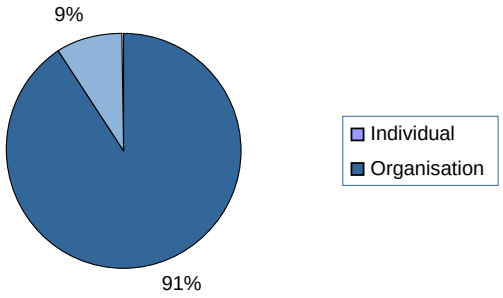
4 Summary of legislation of countries with a permissive policy towards human embryo research

Australia	**Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006** • Therapeutic cloning for the production of stem cells is allowed with a license • Creating a human-non human embryo for the purposes of testing human sperm quality up to, but not including, the first mitotic division is allowed with a license • A license cannot be obtained for creating a hybrid embryo by introducing the nucleus of a human cell into an animal egg • Creating a human-nonhuman chimeric embryo is prohibited
China	**Ethical Guidelines for Research on Human Embryonic Stem Cells (2003)** • Human embryonic stem cells used for research purposes can be derived from: - Spared gamete or blastocyst after in vitro fertilization; - Blastocyst obtained either by parthenogenetic split or by somatic cell nuclear transfer technology
India	**Draft Guidelines for Stem Cell Research/Regulation** • Embryos should not be generated for the sole purpose of obtaining stem cells • However, in special situations where cloning is for therapeutic purposes with regard to cells, tissues or organs, the Committee [National Apex Committee (NAC) for cell based research & therapy] will examine them on a case to case basis
Japan	**The Law Concerning Regulation Relating to Human Cloning Techniques and Other Similar Techniques (Law No. 146, 2000)** • Embryos can be created through SCNT for research • Transfer of a human somatic clone embryo, a human-animal amphimictic embryo, a human-animal hybrid embryo or a human-animal chimeric embryo into a uterus of a human or an animal is prohibited
Singapore	**The Human Cloning and Other Prohibited Practices Act 2004** • Only prohibits reproductive cloning
South Korea	**Bioethics and Biosafety Act, effective on January 1, 2005 (Act. No. 7150)** • SCNT allowed only for conducting research aimed at curing rare or currently incurable diseases
Belgium	**Law on Research in Embryos In Vitro (11 May 2003)** • Does not prohibit therapeutic cloning • Creating embryos for research is forbidden unless research goal cannot be achieved by research using supernumerary embryos
Finland	**No. 488/1999 Medical Research Ac** • Creation of embryos exclusively for research is forbidden • The law does not consider the product of SCNT to be an embryo so therefore SCNT is not prohibited
Sweden	**Government Bill 2003/04:148** • SCNT is permitted in the context of research

Israel	**Prohibition of Genetic Intervention (Human Cloning and Genetic Manipulation of Reproductive Cells) Law, 5759-1999** • Only prohibits reproductive cloning and germ line gene therapy
South Africa	**National Health Bill** • Genetic material of human gametes, zygotes or embryos cannot be manipulated • Therapeutic cloning may be permitted utilising adult or umbilical cord stem cells
Certain USA states	**California – California constitution Article 35: Medical Research – Section 5** • Pluripotent stem cells may be derived from somatic cell nuclear transfer or from surplus products of in vitro fertilization treatments when such products are donated under appropriate informed consent procedures **Connecticut – Bill No. 934** • Research involving embryonic stem cells is permitted if the ethical and medical implications are considered, subject to an institutional review **Illinois – Executive Order Creating the Illinois Regenerative Institute for Stem Cell Research, 2005-6** • The IRMI program shall provide funding for stem cell research that involves adult stem cells, cord blood stem cells, pluripotent stem cells, totipotent stem cells, progenitor cells, the product of somatic cell nuclear transfer or any combination of those cells **Maryland – Maryland Stem Cell Research Act of 2006 5-2B-02** • State-funded stem cell research shall be conducted in a manner that considers the ethical and medical implications of the research **Massachusetts – Chapter 27 of the Acts of 2005** • Research and clinical applications involving the derivation and use of human embryonic stem cells, including somatic cell nuclear transfer, human adult stem cells from any source, umbilical cord cells, parthenotes and placental cells shall be permitted with written approval by an institutional review board **Missouri – The Missouri Stem Cell Research and Cures Initiative** • Any stem cell research permitted under federal law may be conducted in Missouri, and any stem cell therapies and cures permitted under federal law may be provided to patients in Missouri, subject to the requirements of federal law **New Jersey –Senate Bill No. 1909** • Research involving the derivation and use of human embryonic stem cells, human embryonic germ cells and human adult stem cells [from any source], including somatic cell nuclear transplantation, shall be permitted with full consideration for the ethical and medical implications of the research and subject to an institutional review board operating in accordance with applicable federal regulations **Rhode Island - 23-16.4-2** • Nothing in this section shall be construed to restrict areas of biomedical, microbiological, and agricultural research or practices not expressly prohibited in this section, including research or practices that involve the use of: (i) Somatic cell nuclear transfer or other cloning technologies to clone molecules, DNA, cells, and tissues; (ii) Mitochondrial, cytoplasmic, or gene therapy; or (iii) Somatic cell nuclear transfer techniques to create animals

Appendix D – Written Consultation: Summary of responses

810 people responded to the consultation, the majority of which were individuals.

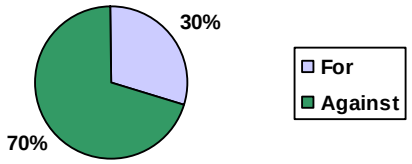


Question 1:

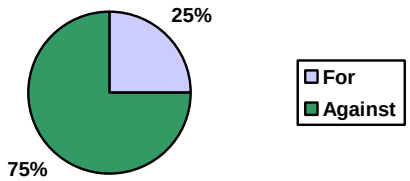
The following types of embryo research are already legally permitted in the UK. Which of them, in your view, are acceptable?

This initial question was asked to establish respondents' views on different types of embryo research already licensed by the HFEA. This provides a useful context to the answers given in response to questions about the creation human-animal embryos.

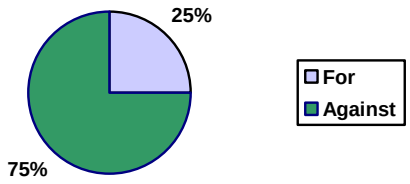
Research using human embryos donated by IVF patients



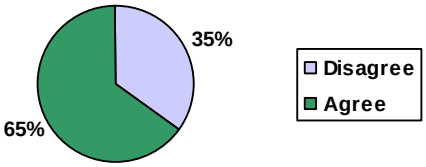
Research using human embryos created specifically for research using donated egg and sperm



Research using cloned human embryos created specifically for research through cell nuclear replacement (CNR)

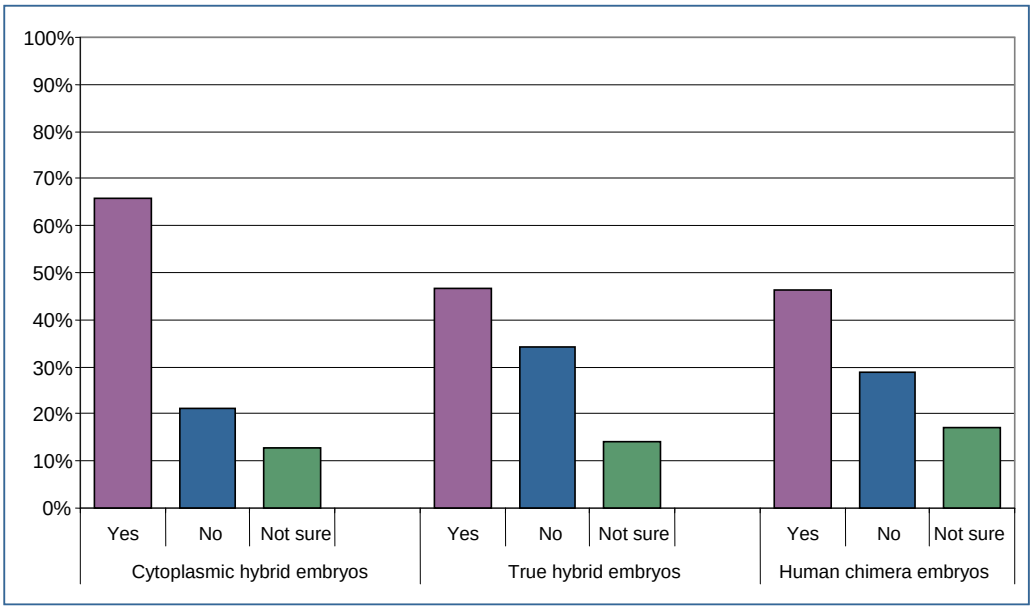


No research using human embryos is acceptable



Of the 35% of respondents that disagreed with the statement 'no research using human embryos is acceptable', the majority were supportive of the proposal to create cytoplasmic hybrid embryos for the purpose of research.

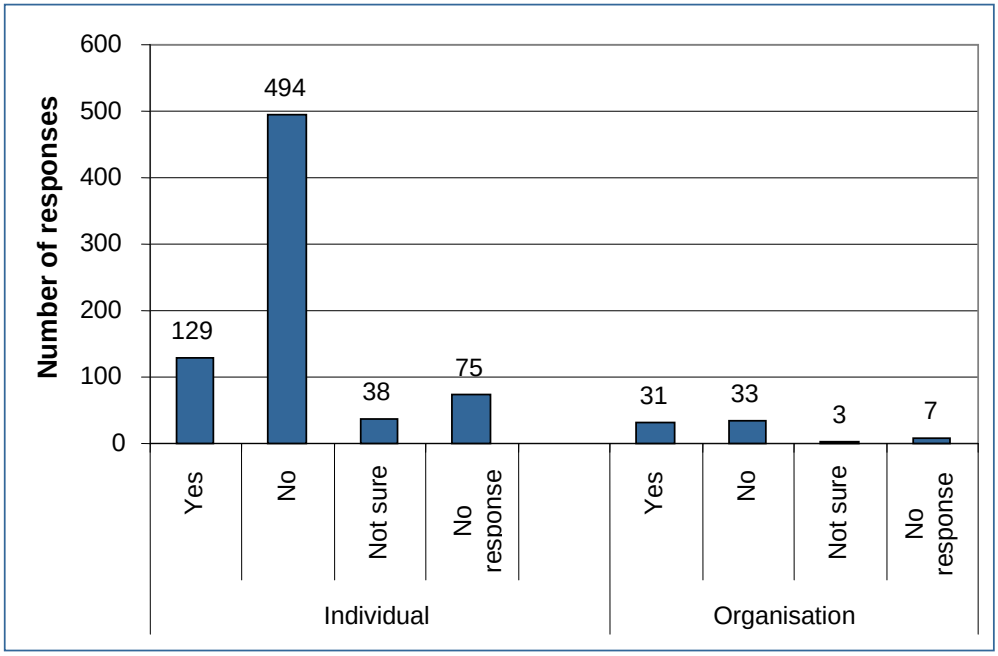
The chart below shows how those not opposed to embryo research responded to the question of whether the HFEA should licence the creation of cytoplasmic hybrid embryos, true hybrid embryos or human chimera embryos.



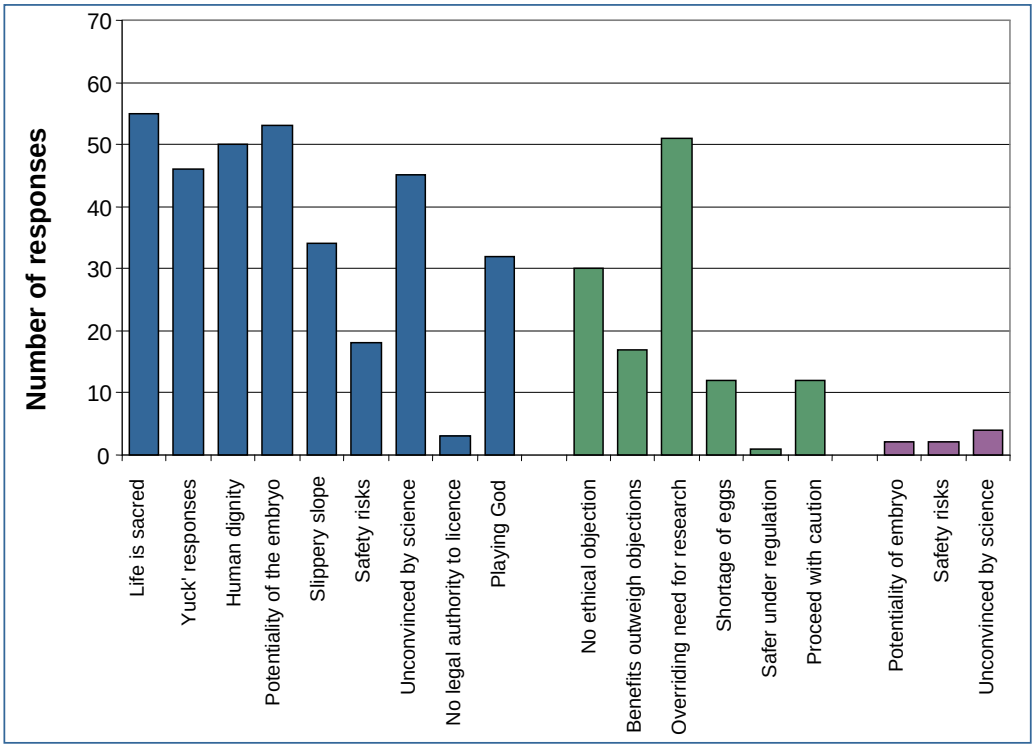
Question 2:

Do you think that the HFEA should issue licences to allow research using cytoplasmic embryos?

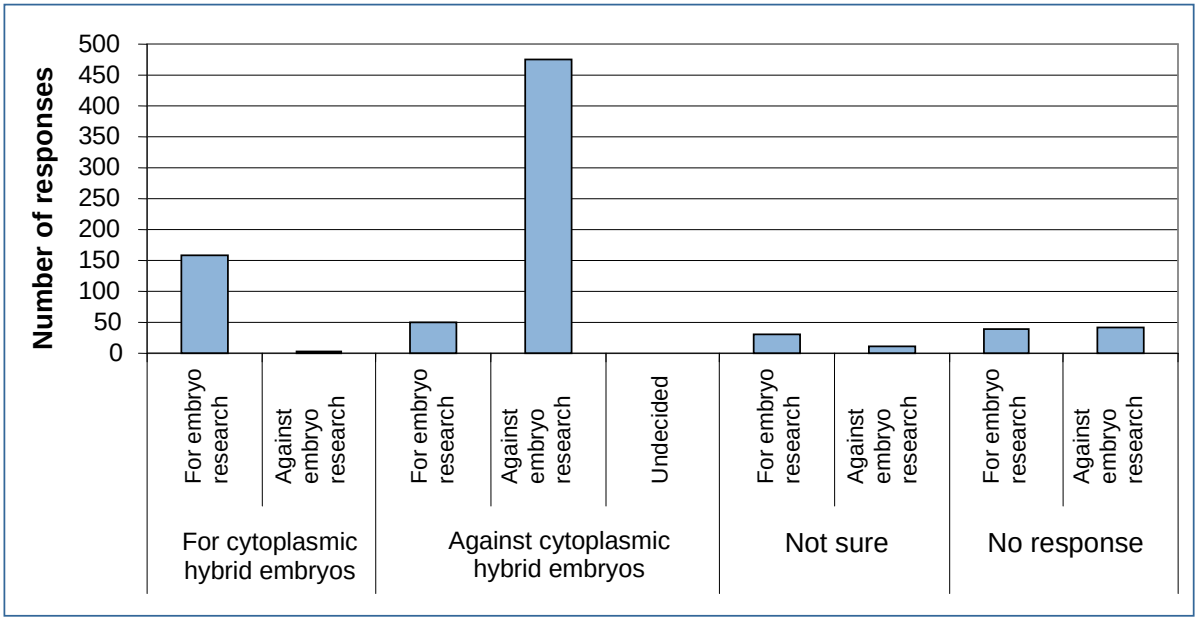
Out of 810 respondents, the chart below illustrates how individuals and organisations responded to question two of the written consultation.



Out of the 810 respondents, 728 gave reasons for their response; 336 gave a reason why they were against the use of cytoplasmic hybrid embryos in research (columns in blue), 123 gave a reason why they were in favour of their use in research (columns in green) and 8 gave reason for feeling unsure (columns in purple).



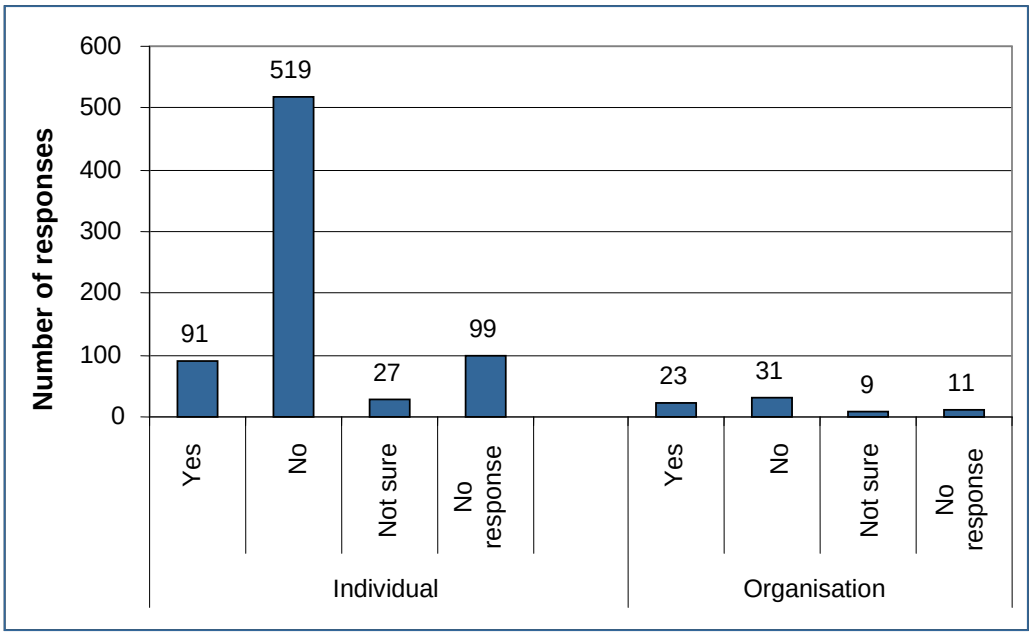
The chart below cross references respondents views on whether the HFEA should licence research involving the creation of cytoplasmic hybrid embryos, with their views on embryos research in general. The chart indicates that the majority of those against research involving cytoplasmic hybrid embryos are opposed to research using human embryos.



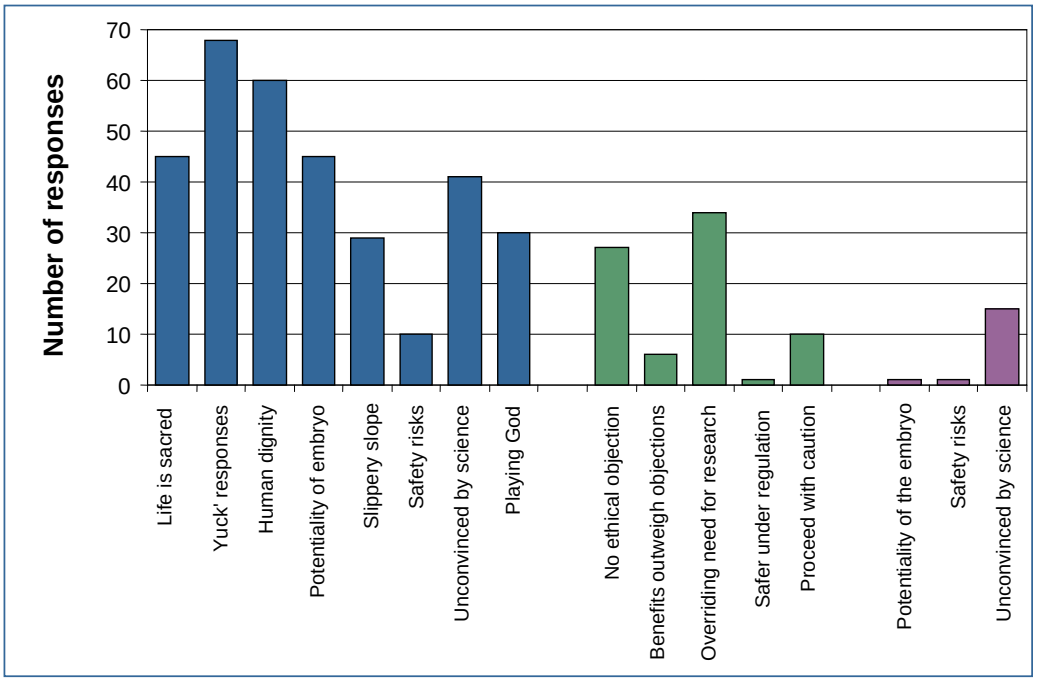
Question 3:

Do you think that the law should in future permit the creation of true hybrid embryos for licensed research purposes?

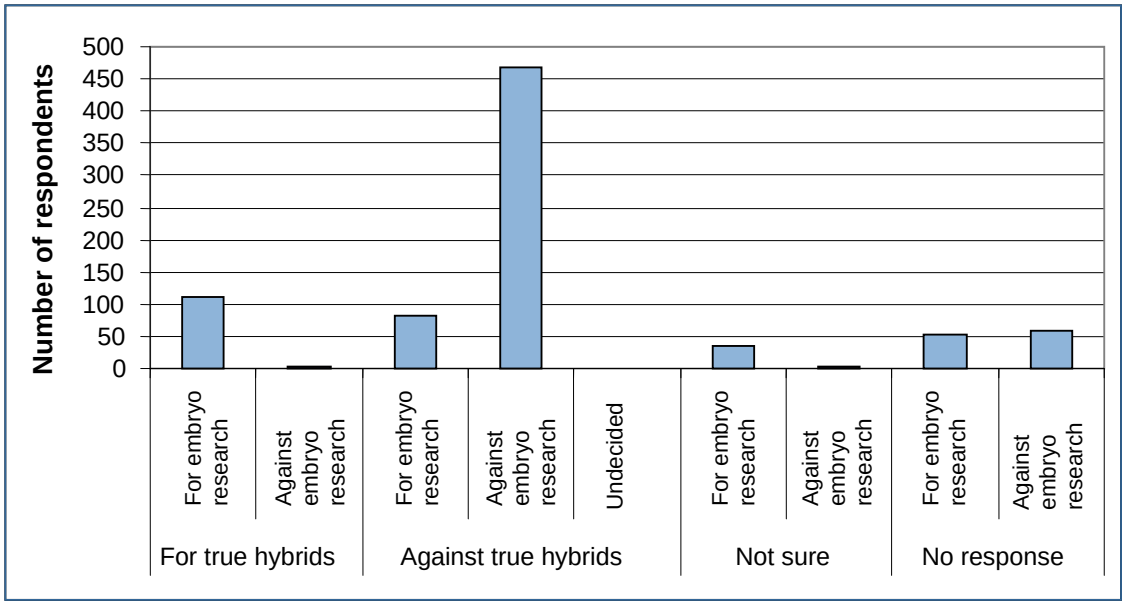
Out of 810 respondents, the chart below illustrates how individuals and organisations responded to question three of the written consultation.



Out of the 810 respondents, 423 gave reasons for their response; 328 gave a reason why they were against the use of true hybrid embryos in research (columns in blue), 78 gave a reason why they were in favour of their use in research (columns in green) and 17 gave reason for feeling unsure (columns in purple).



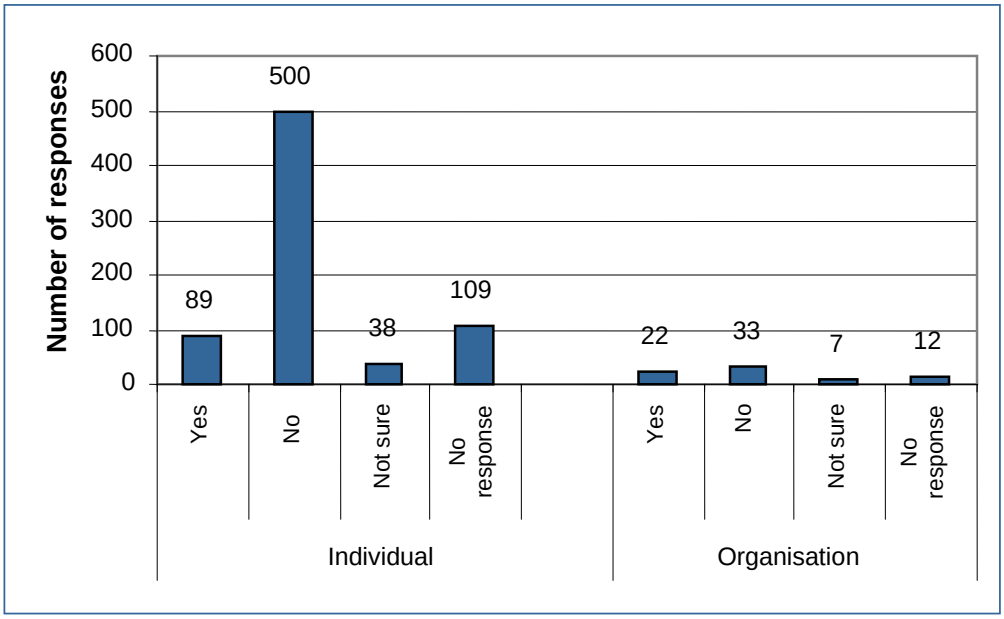
The chart below cross references respondents views on whether the HFEA should licence research involving the creation of true hybrid embryos, with their views on embryos research in general. Again the majority of those against research using true hybrids are opposed to research involving human embryos.



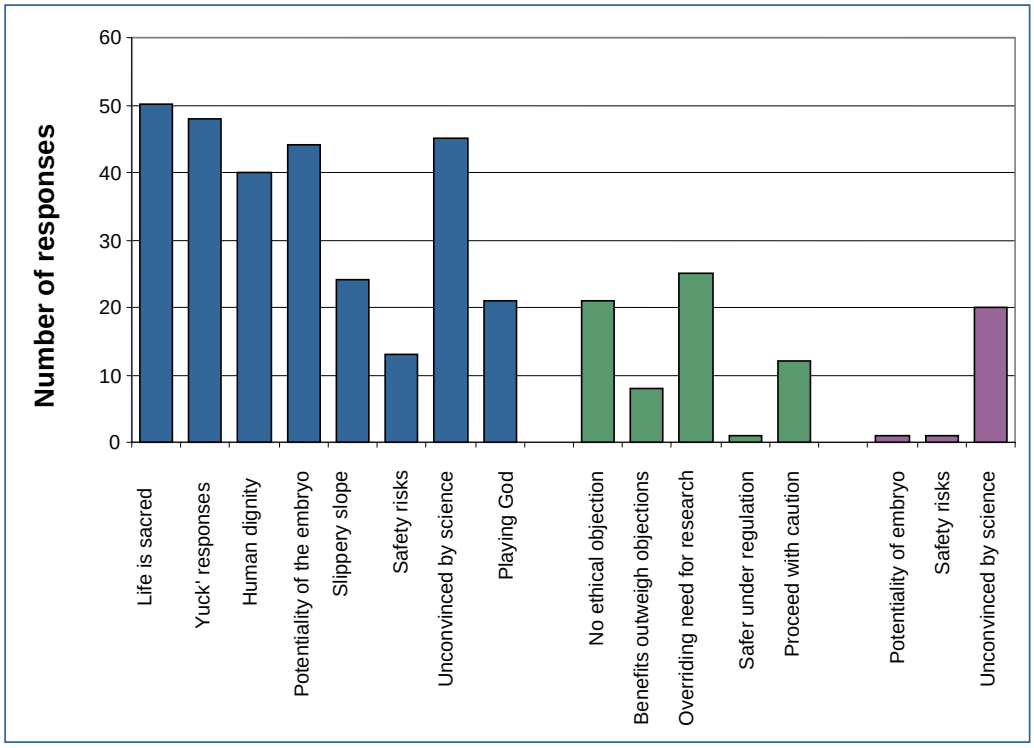
Question 4:

Do you think that the HFEA should in future issue licences to allow research using chimera embryos?

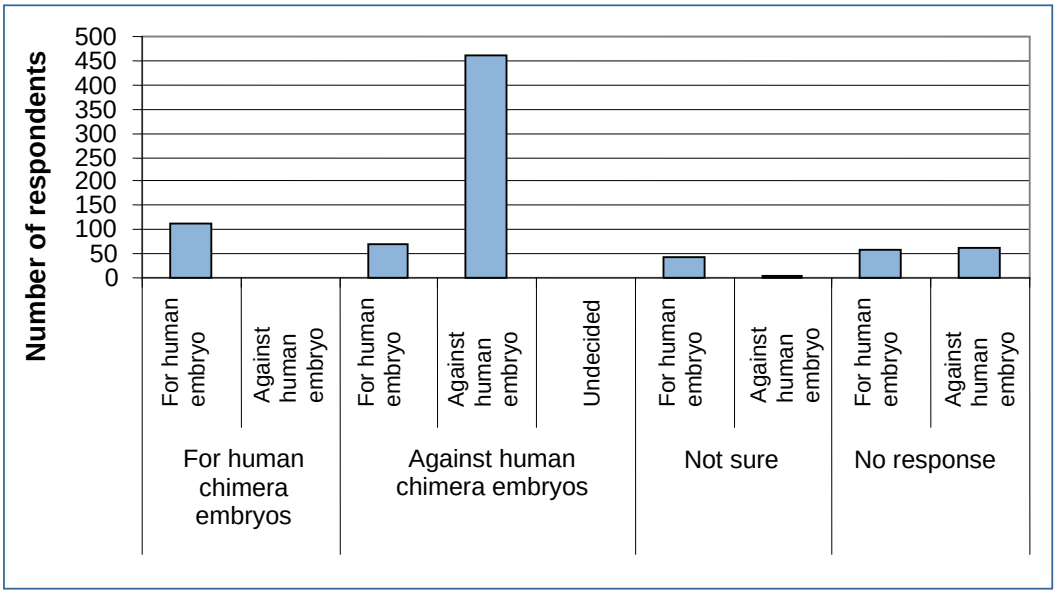
Out of 810 respondents, the chart below illustrates how individuals and organisations responded to question four of the written consultation.



Out of the 810 respondents, 374 gave reasons for their response; 285 gave a reason why they were against the use of human chimera embryos in research (columns in blue), 67 gave a reason why they were in favour of their use in research (columns in green) and 44 gave reason for feeling unsure (columns in purple).



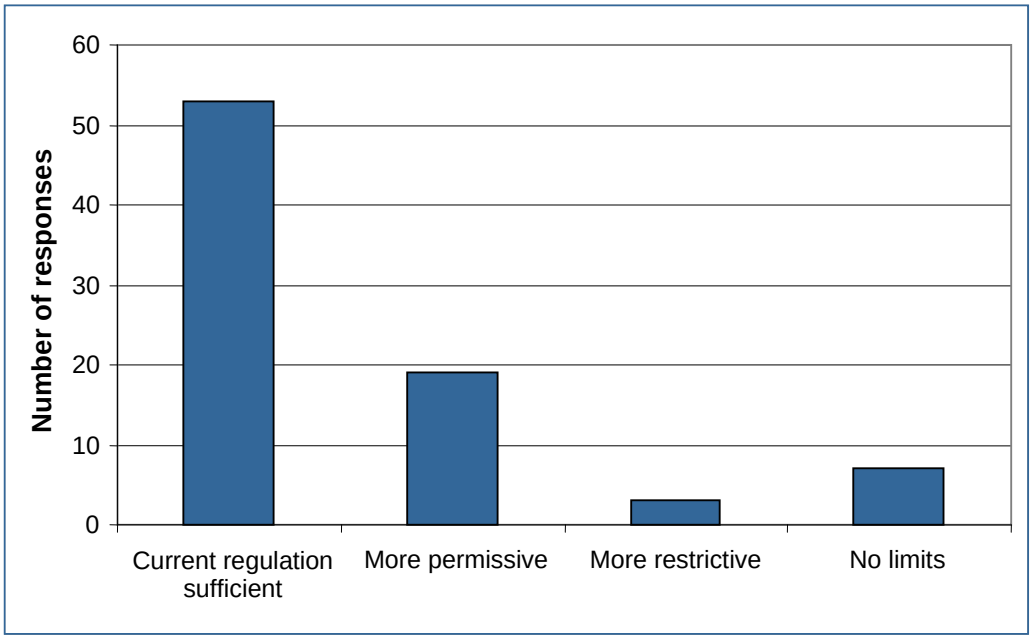
The chart below cross references respondents views on whether the HFEA should licence research involving the creation of human chimera embryos, with their views on embryos research in general. Once again the majority of those against research using human chimera embryos are opposed to research involving human embryos.



Question 5:

What limits do you think should be placed upon human embryo research?

Out of 810 respondents, the chart below illustrates the views of the 82 that responded to question five.



Appendix E – Public Dialogue: Deliberative Work

1. 104 people participated in the 12 discussion groups, held as first part of the public dialogue deliberative work. Participants were recruited using a team of recruiters across the UK. The groups lasted for 2 hours and participants were presented with the basic scientific background about cells, sources of human embryos and descriptions of cloned human embryos, cytoplasmic hybrid embryos, transgenic human embryos and true hybrid embryos. At the end of the discussion groups participants were given the full consultation document to take away.

2. At the reconvened workshop 44 of the participants from the discussion groups (51 were recruited) were gathered together. On the day, participants worked in mixed breakout groups and plenary to explore the evidence and the arguments for and against hybrid research. Briefing notes, presentations from speakers and a Q&A session with experts enabled participants to formulate a more informed viewpoint on the topic.

3. Speakers at the reconvened event were:

- **Dr Sue Kimber** - Reader in Early Development in the Faculty of Life Sciences, University of Manchester
- **Dr David King** - Founder and Director of Human Genetics Alert
- **Professor Peter Lipton** - Head of the Department of History and Philosophy of Science at Cambridge University
- **Josephine Quintavalle** - Co-founder and Director of Comment on Reproductive Ethics (CORE)
- **Professor Christopher Shaw** - Professor of Neurology and Neurogenetics at the Institute of Psychiatry, King's College London

Spontaneous knowledge and understanding of medical research

4. The research indicates that the participants do not readily recollect many of the developments in medical research over the past 20 years. Few are able to think of specific advances, but there is some acknowledgement of improvements in the treatment of various long term conditions such as cancer, diabetes and heart disease. A number of personal experiences were referred to in which medical research had proved beneficial – and therefore in these cases initial thoughts were more positive.

5. Many of the more negative spontaneous associations with medical science were about rare but extreme occurrences in the industry. For example, there were many spontaneous mentions of the Northwick Park Drug trials, with participants citing ‘the elephant man’.

6. Some would like to know more about medical research; they currently perceive it to be a complicated area of science that is not accessible to them. Others say it is something that they do not really think about but say that to people with certain medical conditions it is a vital resource, and could be for them in the future.

Knowledge and understanding of stem cell research

7. In this context it is perhaps unsurprising that people have some awareness of stem cells and stem cell research but do not really know what they are or what they do.

8. Some recall celebrities such as Michael J Fox, Mohammed Ali and Christopher Reeve, who have allegedly had stem cell treatments for diseases. However, few know the details about how such treatment is carried out.

9. Upon further exploration of the topic, with the aid of briefing materials (see appendix), the majority believe that it is right to investigate the opportunities offered by stem cells in more depth. However, many expressed concerns that they did not know that scientists conducted such research.

10. They had further key questions which were addressed over the course of the research process.

    These key questions were;

    - What are the differences between embryonic stem cells, adult stem cells and cord blood stem cells and can each of them be used in the same way?
    - What evidence is there to indicate that stem cell research will be useful?
    - What happens to the embryos that are used / can the embryos be put into a woman?
    - Where do embryos for the research come from? (With a minority concerned that embryos are removed from women in utero)

11. A minority across the group discussions were against the use of embryos in research altogether. Their view was clear cut, that using and destroying embryos is destroying life and that is against their moral / religious beliefs. The pursuit of further understanding of diseases, by this means, did not justify the cost of life.

Research using human embryos

12. As noted in the key themes, the majority were in favour of using human embryos in research if a clear benefit was stated and the research was subject to strict regulatory controls. There was a difference of opinion regarding the use of embryos from different sources, as illustrated below.

Research using human embryos donated by IVF patients

13. A minority of people in the group discussions were aware that embryos donated by IVF patients could be used for research purposes. To many others this was a surprise.

14. The majority believed that these embryos should be utilised. A minority believe that these embryos should not be used for research because the original purpose of creating the embryo was to produce a child, and as such the use of these embryos would be emotionally charged.

15. Some stress that the embryos should be offered to other people in fertility treatment where possible, or stored safely until the couple themselves know they will not need it to complete their fertility treatment, before they are used and destroyed in the research process.

16. In any case, the public are only in support of using these embryos for specified research purposes if fully informed consent is gained and strict regulatory controls adhered to.

17. Views towards using these embryos in research show the least dramatic change across the deliberative process with 39 – 40 out of 44 agreeing that they should be used for stem cell research.

Appendix F – Public Dialogue: Opinion Poll

1. Four questions were formulated with input from the advisory group and from the deliberative and public meeting findings. The questions were put on an omnibus survey run by ICM research between July 11th and July 16th. A sample of 2073 residents of the UK were interviewed during this period. All participants were adults aged 18+. Quotas were set on age, sex, standard geographical regions and housing tenure. The data was weighted against the profile of the UK to provide a representative sample. Random digit dialling was used to recruit participants for the interviews.

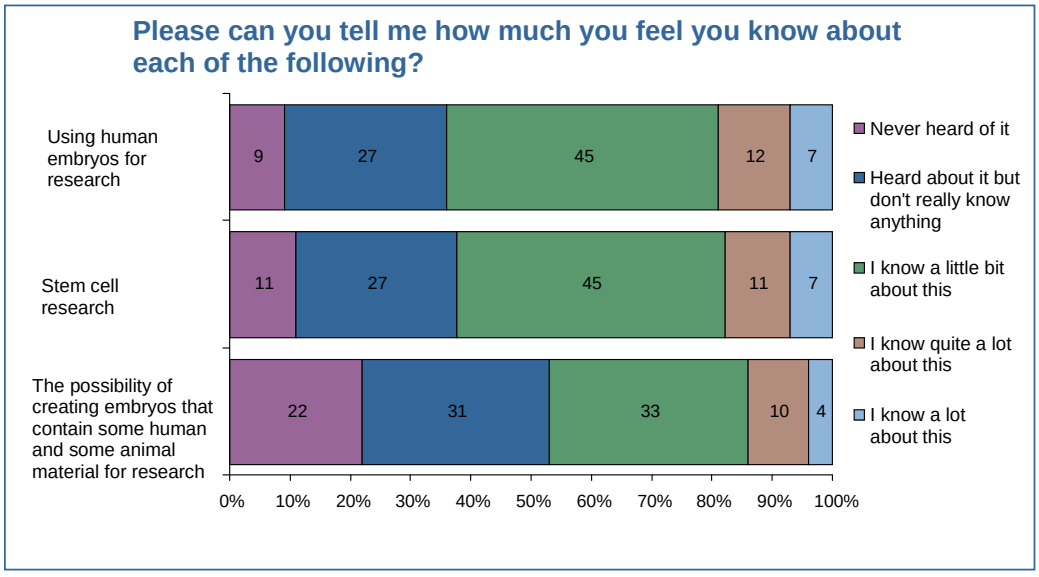
Knowledge of stem cells and the usage of embryos in research

2. There is a mixed level of knowledge amongst the general public about the usage of human embryos in research. Figure 1 illustrates that less than one in ten say they know a lot about using human embryos in research (7%). A similar proportion say they have never heard of it (9%).

3. The same pattern emerges for knowledge of stem cells with less than one in ten saying they know a lot about stem cells and just over one in ten saying they have never heard of it (7% and 11% respectively)[2].

4. Knowledge about the possibility of creating embryos that contain some human and some animal material for research is significantly lower. Less than one in twenty claim to know a lot about this (4%). Over one in five say they have never heard of the possibility of creating embryos that contain some human and some animal material for research (22%).

Figure 1: Knowledge about research
Base All: 2073



Where knowledge of research has come from

5. Over two thirds of those people who knew a lot or quite a lot about the research areas gleaned their knowledge from the news on television/radio (68%). Just under two thirds of people said their knowledge came from newspapers (60%).

[2] In previous research 5% of the UK public rated themselves as very familiar with stem cell research (Gaskell et al. 2006)

Almost one in three had gained their knowledge from documentaries on the television or radio (32%). One in ten gained their knowledge from specific websites/journals that they looked at for general interest (10%). One in twenty knew about these research areas through their line of work (5%) or their studies (5%).

6. 12% of the people who said they knew a lot or quite a lot got their knowledge from their line of work and 12% got it from their studies, compared to 4% for those who knew a little, 2% for those who had heard about it but didn't know anything and 2% for those who had never heard of it.

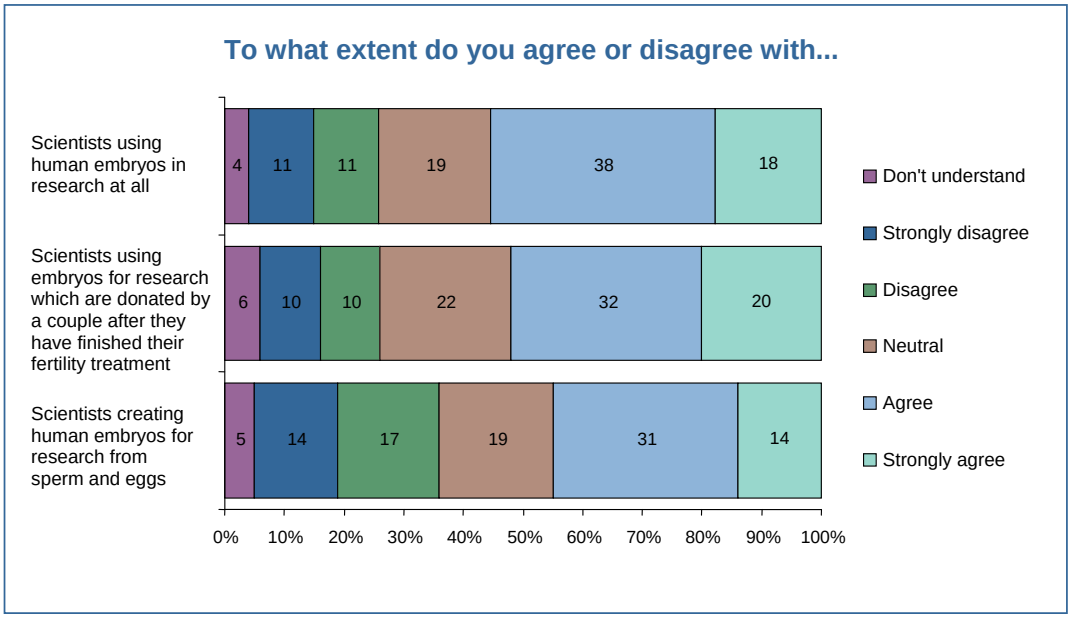
The use of human embryos in research

7. Most people agree with using human embryos in research. Over half of the UK population agrees with scientists using human embryos in research at all (56%). Just over one in five disagrees (22%).

8. Over half agree with scientists using embryos for research which are donated by a couple after they have finished their fertility treatment (52%)[3]. One in five (20%) disagrees.

9. Almost half agree with scientists creating human embryos for research from sperm and eggs (45%)[4]. 30% disagree.

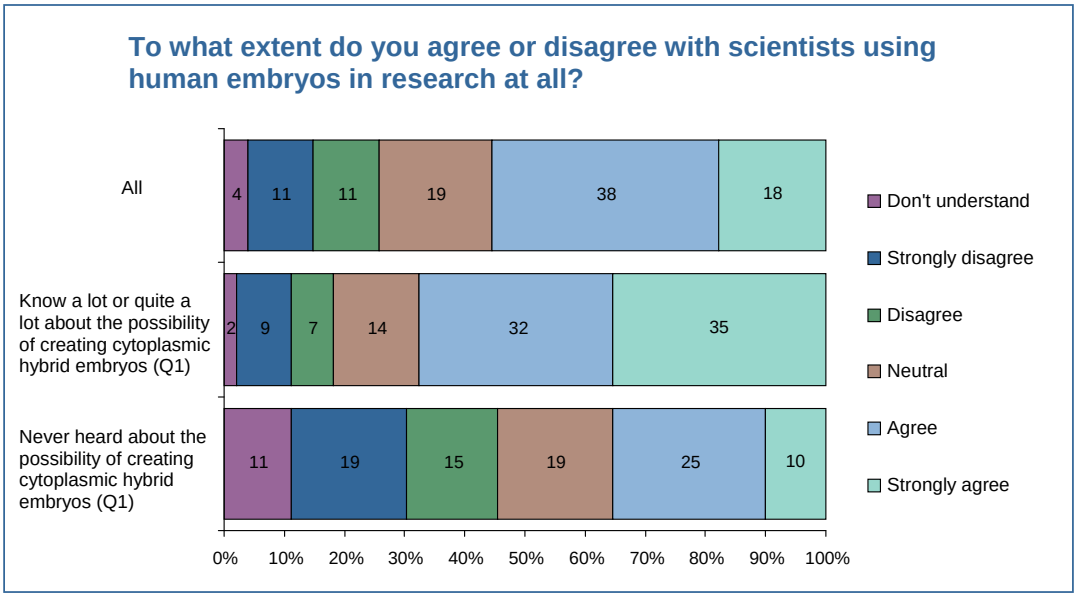
Figure 2: Opinions on the creation and use of human embryos
Base All: 2073



[3] In previous research 68% of respondents felt that it was "acceptable to use 'spare' early embryos left over from fertility treatment, such as IVF, for the purposes of medical research" (YouGov, 2005 for the Daily Telegraph)
[4] 41% of respondents felt it was "acceptable to create human embryos deliberately solely for the purposes of medical research" (YouGov, 2005 for the Daily Telegraph)

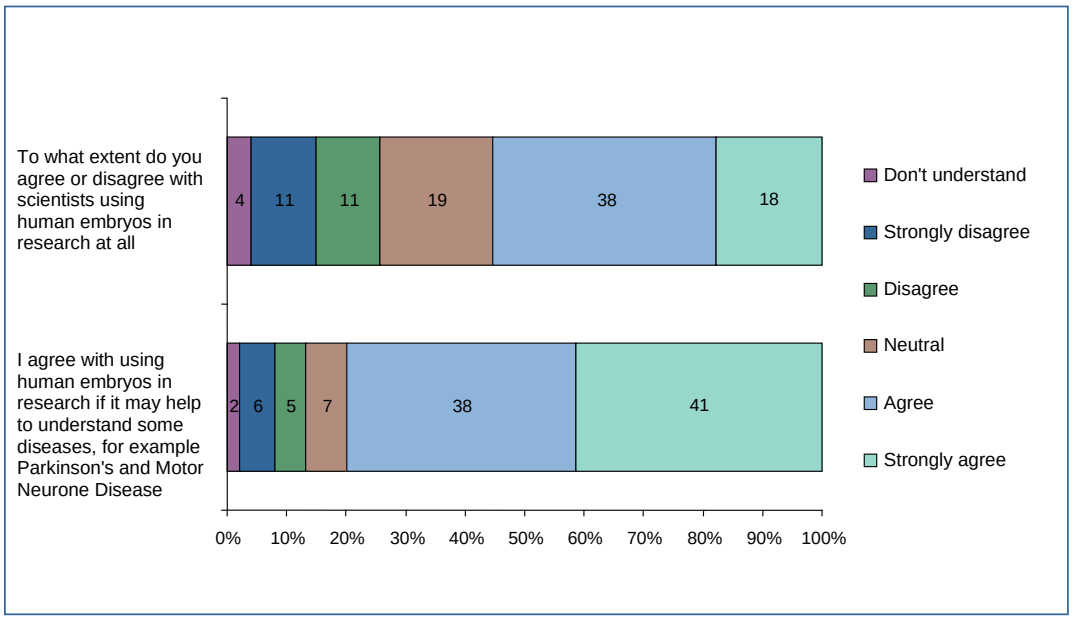
Figure 3: The effects of knowledge on opinions on the use of human embryos
Base all: 2073

(Total 2073, knew a lot or quite a lot about using human embryos in research: 390, never heard of using human embryos for research: 186)



10. There is a strong increase in agreement with the use of human embryos when people are given a rationale for doing so. Nearly four fifths agree with using human embryos in research if it may help to understand some diseases, for example Parkinson's and Motor Neurone Disease compared to under three fifths agreeing to using embryos in research at all without a rationale (79% vs. 56% respectively)[5]

Figure 4: Changing attitudes to the use of human embryos with a rationale
Base All: 2073



[5] Previous research found that 70% of the British public support the use of human embryos for medical research to find treatments for serious diseases and for fertility research (MORI 2003).

The use of embryos which contain mostly human with a small amount of animal genetic material (cytoplasmic hybrid embryos)

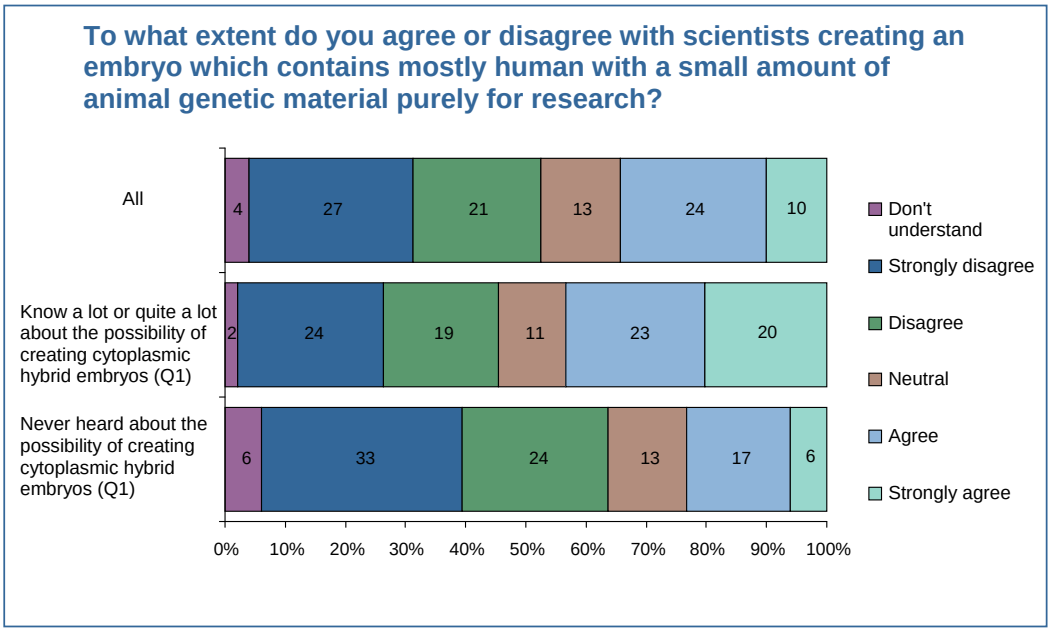
11. Just over a third of people agree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research (35%); just under half disagree (48%).

12. Again, levels of agreement were higher amongst those that know something about the possibility of creating embryos that contain some human and some animal material for research, compared to those that have never heard of it: 43% of people who know a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research at Q1 agree with creating cytoplasmic hybrid embryos compared to 41% of people who know a little, 32% of people who have heard about it but don't know anything and 24% of those who have never heard of it.

Figure 5: The effects of knowledge on perceptions of use of cytoplasmic hybrid embryos

Base all: 2073

(Total 2073, Knew a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research: 287, Never heard of the possibility of creating embryos that contain some human and some animal material for research: 460)

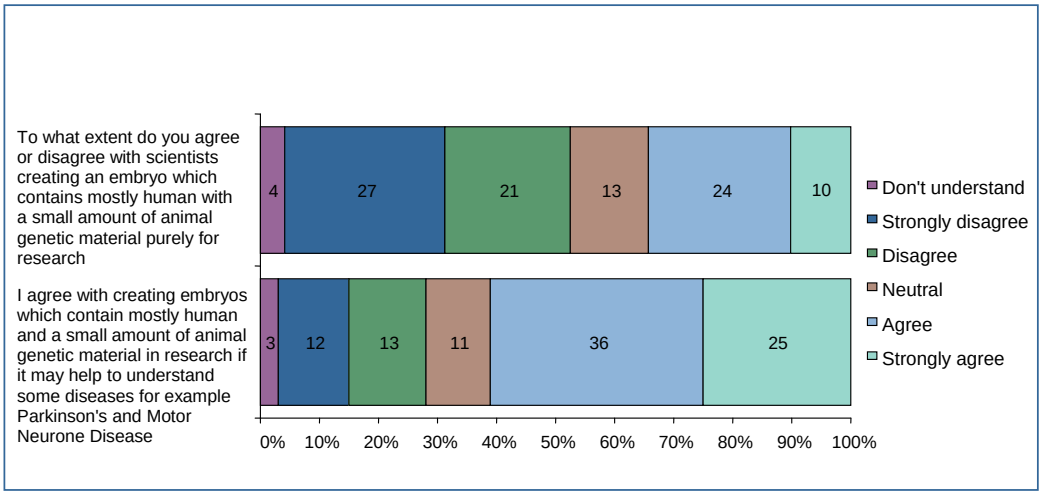


13. A similar pattern emerges when people are given a reason for conducting the research. Again there is a strong increase in agreement with creating embryos which contain mostly human and a small amount of animal genetic material in research if it may help to understand some diseases, for example Parkinson's and Motor Neurone Disease (61% agree compared to 35% who agree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research).

14. Again, levels of agreement were higher amongst those that know something about the possibility of creating embryos that contain some human and some animal material for research, compared to those that have never heard of it: 67% of people who know a lot or quite a lot about the possibility of creating embryos that contain some human

and some animal material for research at Q1 agree with creating embryos which contain mostly human and a small amount of animal genetic material in research if it may help to understand some diseases, for example Parkinson's and Motor Neurone Disease compared to 66% of people who know a little, 58% of people who have heard about it but don't know anything and 53% of those that have never heard of it.

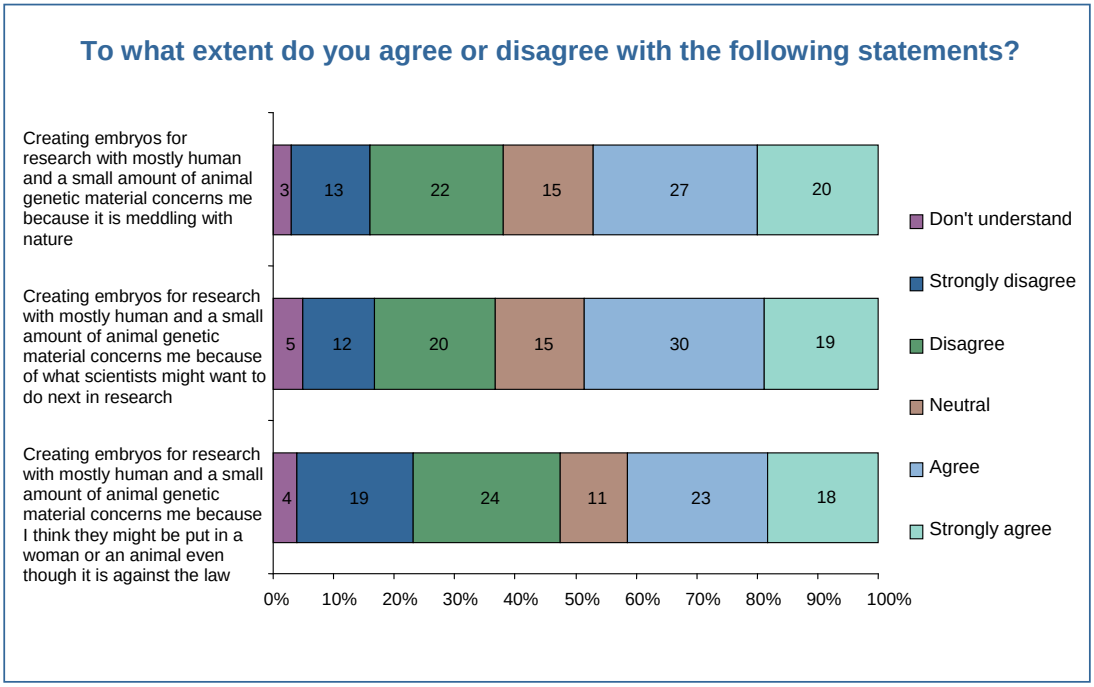
Figure 6: Changing attitudes to embryos that contain mostly human with a small amount of animal genetic material
Base All: 2073



Concerns

15. People did have some concerns about creating embryos with a mix of human and animal genetic material. Overall nearly half agree that creating embryos for research with mostly human and a small amount of animal genetic material concerns them because it is meddling with nature (47%). Nearly half agree that creating embryos for research with mostly human and a small amount of animal genetic material concerns them because of what scientists might want to do next in research (49%) and just over two fifths agree that creating embryos for research with mostly human and a small amount of animal genetic material concerns them because they think they might be put into a woman or an animal even though it is against the law (41%).

Figure 7: Concerns with mixing genetic material
Base All: 2073



16. Those that know something about the possibility of creating embryos, that contain some human and some animal material, for research were less likely to have concerns:

- 42% of people who know a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research at Q1 disagree with the statement “creating embryos for research with mostly human and a small amount of animal genetic materials concerns me because it is meddling with nature”, compared to 38% of people who know a little, 28% of people who have heard about it but don’t know anything and 33% of those that have never heard of it.

- 36% of people who know a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research at Q1 disagree with the statement “creating embryos for research with mostly human and a small amount of animal genetic materials concerns me because of what scientists might want to do next in research”, compared to 28% of those that have never heard of it.

Figure 8: How knowledge affects the concern of meddling with nature

Base all: 2073

(Total 2073, Knew a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research: 287, Never heard of the possibility of creating embryos that contain some human and some animal material for research: 460)

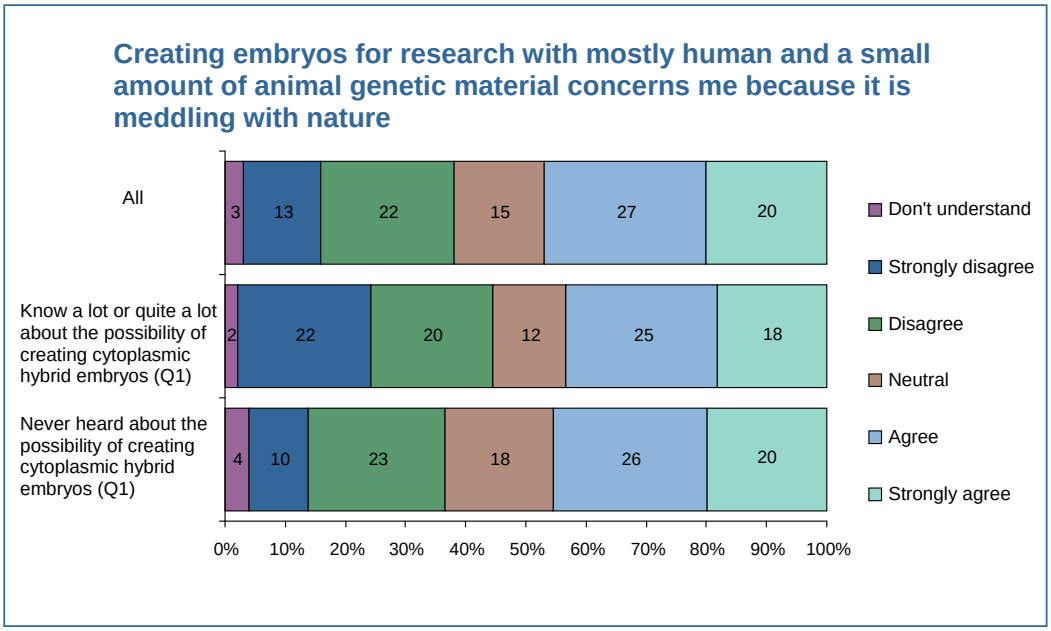


Figure 9: How knowledge affects concerns of a slippery slope

Base all: 2073

(Total 2073, Knew a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research: 287, Never heard of the possibility of creating embryos that contain some human and some animal material for research: 460)

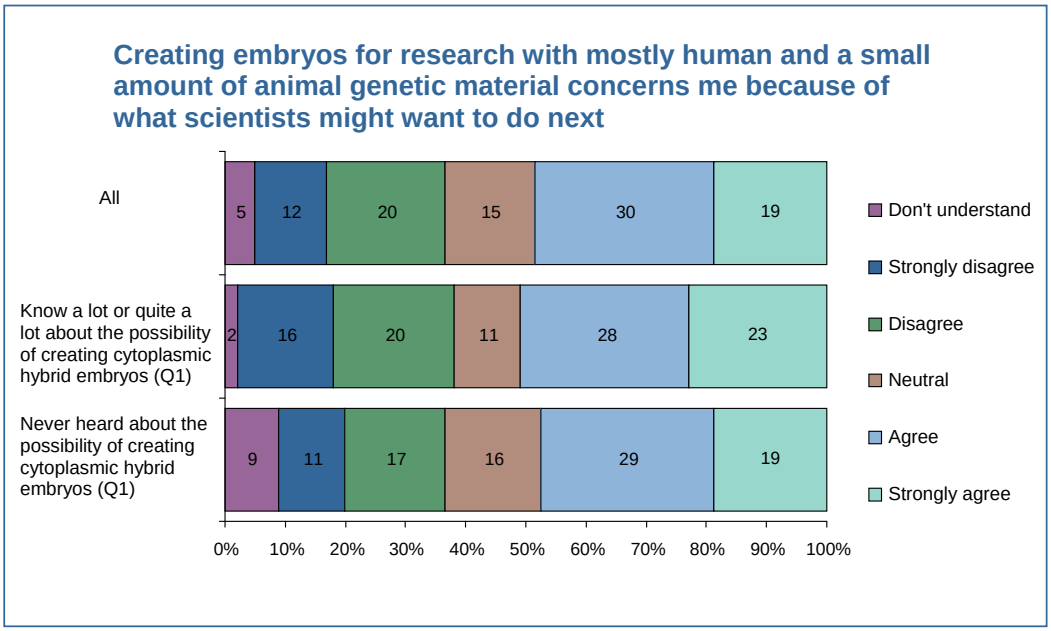
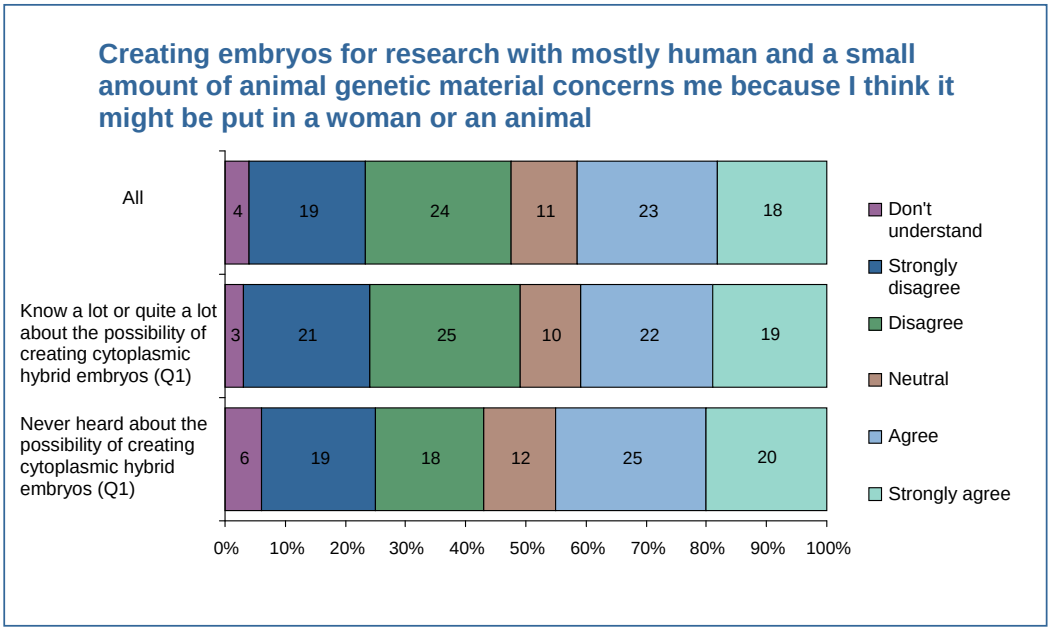


Figure 10: How knowledge affects concerns about implantation of hybrid embryos
Base all: 2073

(Total 2073, Knew a lot or quite a lot about the possibility of creating embryos that contain some human and some animal material for research: 287, Never heard of the possibility of creating embryos that contain some human and some animal material for research: 460)



17. Some of the people who agree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research (Q4) still had concerns about the research:

- 31% of people who agree with scientists creating cytoplasmic hybrid embryos also agree that creating embryos for research with mostly human and a small amount of animal genetic material concerns them because it is meddling with nature.
- 33% of people who agree with scientists creating cytoplasmic hybrid embryos also agree that creating embryos for research with mostly human and a small amount of animal genetic material concerns them because of what scientists might want to do next in research.
- 34% of people who agree with scientists creating cytoplasmic hybrid embryos also agree that creating embryos for research with mostly human and a small amount of animal genetic material concerns them because they think that they might be put in a woman or an animal even though it is against the law.

Figure 11: Concern of meddling with nature crossed by agreement with creating cytoplasmic hybrid embryos (Q4)

Base all: 2073

(Total 2073, Agree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 718, Neutral about scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 276, Disagree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 986)

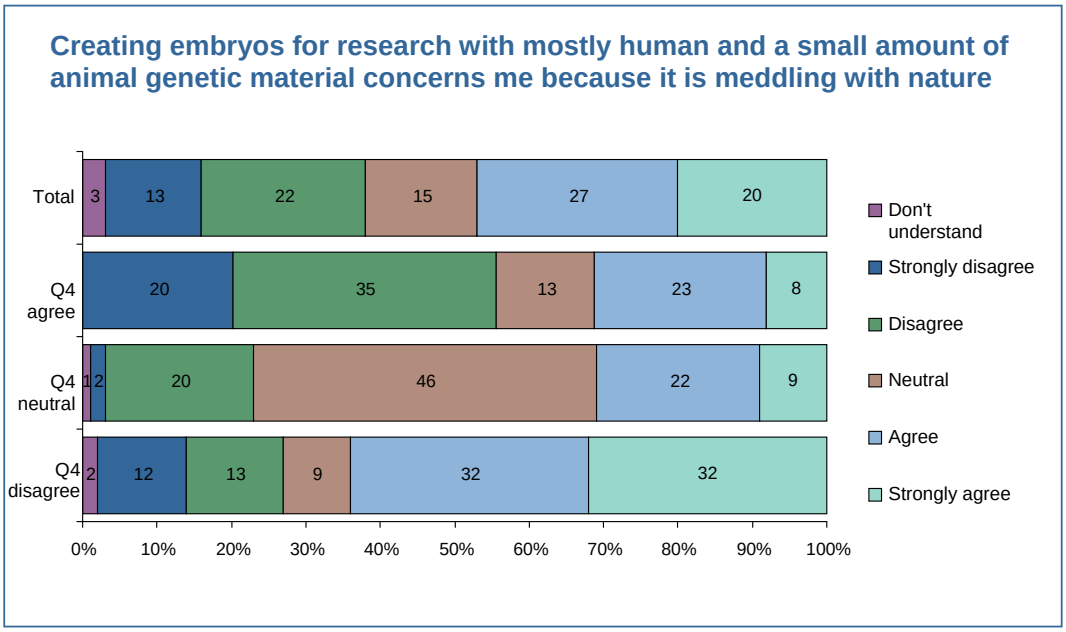


Figure 12: Concern of what scientists might want to do next in research crossed by agreement with creating cytoplasmic hybrid embryos (Q4)

Base all: 2073

(Total 2073, Agree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 718, Neutral about scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 276, Disagree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 986)

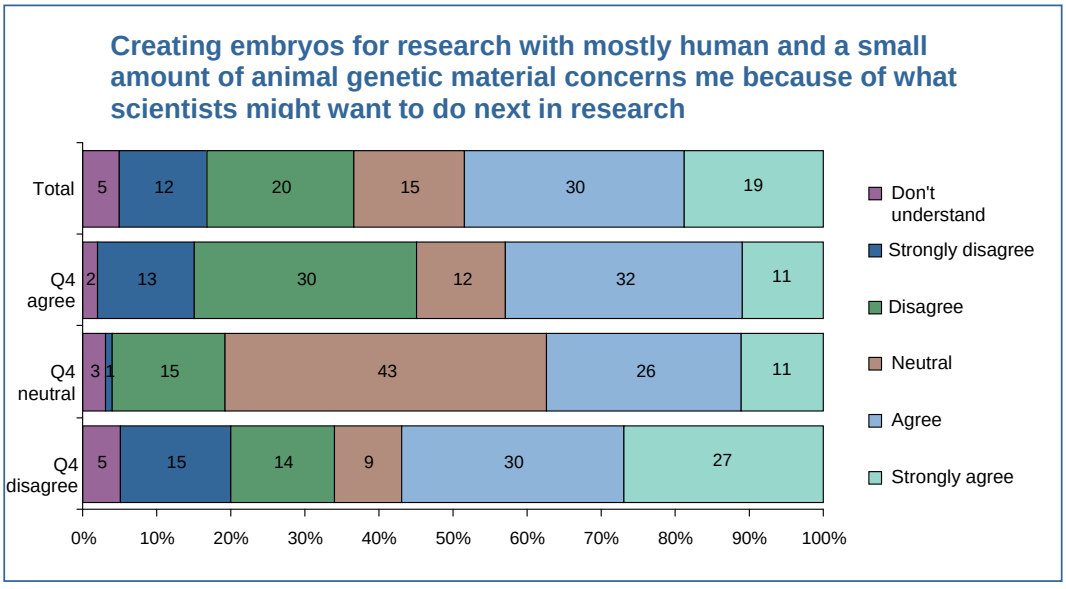
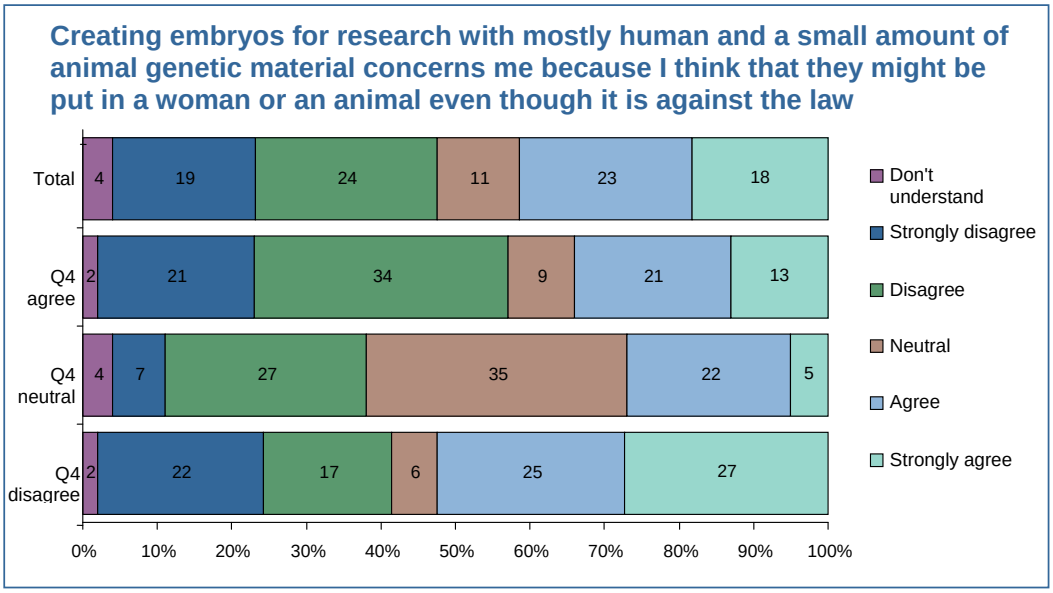


Figure 13 Concern of implantation crossed by agreement with creating cytoplasmic hybrid embryos (Q4)

Base all: 2073

(Total 2073, Agree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 718, Neutral about scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 276, Disagree with scientists creating an embryo which contains mostly human with a small amount of animal genetic material purely for research 986)

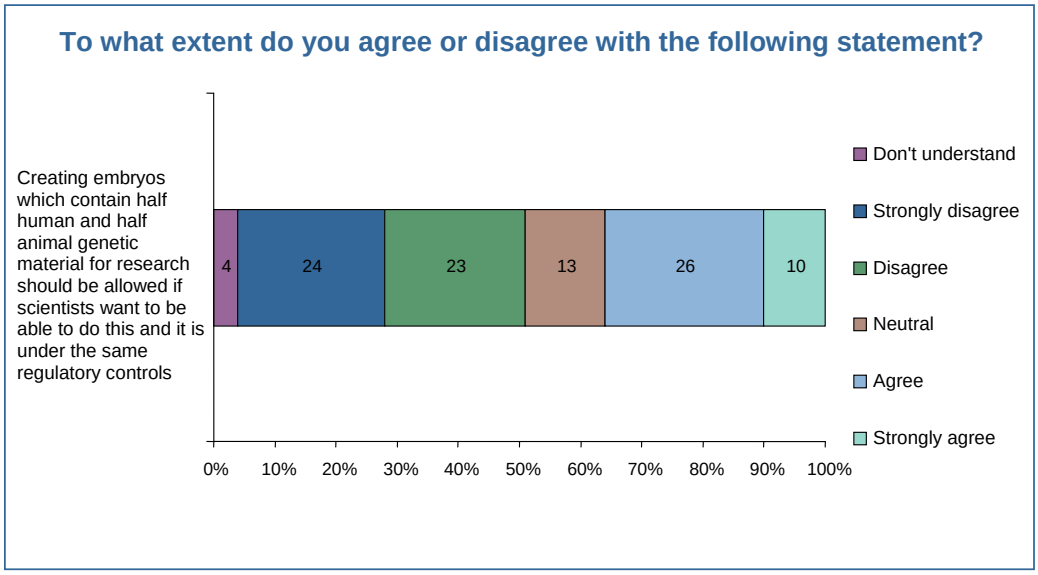


True Hybrid Embryos

18. Nearly half disagree that creating embryos which contain half human and half genetic materials for research should be allowed if scientists want to be able to do this and it is under the same regulatory controls (47%) and over a third agree (36%).

Figure 14: Views on half human and half animal embryos

Base All: 2073



19. People who know more about the possibility of creating embryos that contain some human and some animal material are more likely to agree.

20. Over four in ten of those who know about the possibility of creating embryos that contain some human and some animal material for research agree that creating embryos which contain half human and half animal genetic material for research should be allowed if scientists want to be able to do this and it is under the same regulatory controls (43%). This compares to less that three in ten of those who have never heard of such research agreeing that it should be allowed (26%).

21. There is strong consistency between views on creating mostly human with a small amount of animal embryos and the half human half animal embryos. Almost three quarters of people who agree with scientists creating an embryo which contains mostly human and a small amount of animal genetic material purely for research (Q4) also agree that creating embryos which contain half human and half animal genetic material for research should be allowed if scientists want to be able to do this and it is under the same regulatory controls (72% agree).

Appendix G – Public Dialogue: Public Meeting

1. The HFEA held an open public meeting in London on the 26th June 2007 as part of their ongoing consultation on the creation and use of human/animal hybrids for research. 153 members of the public attended to debate the issues, chaired by Nick Ross and supported by 5 expert panel members;

- **Dr Lyle Armstrong,** Lecturer in Stem Cell biology, University of Newcastle
- **Rev. Dr. Stephen Bellamy,** The Mission and Public Affairs Council of the Church of England
- **Josephine Quintavalle,** Co-founder and Director of Comment on Reproductive Ethics (CORE)
- **Christine Young**, Carer and patient representative, Special Parkinson's Research Interest Group
- **John Cornwell,** Director of the Science and Human Dimension Project at Jesus College, Cambridge and regular writer for the Tablet

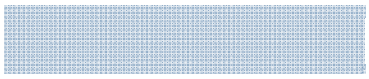
2. The discussion was wide and varied with a mix of questions from the floor and polling questions. Some of the key issues raised were;

- Whether or not the research is necessary given that the therapeutic advances made in stem cell research to date have been from adult and cord blood stem cells.
- The efficacy of the proposed research, exploring if it is indeed possible, and if so how applicable research findings from hybrid embryos would be to human beings.
- Whether or not this research is desirable, e.g. whether or not this type of research crosses any important ethical boundaries and whether the moral and ethical reasons not to pursue the research outweigh the potential benefits it might bring.

Results of the electronic voting

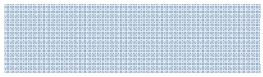
Are you here today as…

1. **A representative from an organisation with an interest in this area?**



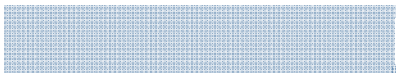
36%

2. **A scientist/academic?**



27%

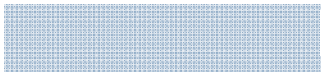
3. **A member of the public?**



37%

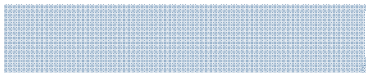
Have you heard/seen much in the media in the last 6 months about the issue of using embryos that are a mix of animal and human genetic material for research?

1. **Heard/seen a lot about this**



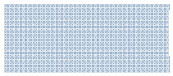
30%

2. **Heard/seen a bit about this**



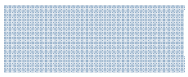
36%

3. **Heard/seen little**



16%

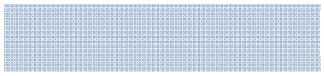
4. **Heard/seen very little**



18%

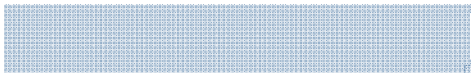
How much would you say you know about the issue of using embryos that are a mix of animal and human genetic material for research?

1. **Know a lot about this**



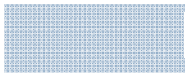
30%

2. **Know a bit about this**



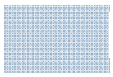
45%

3. **Know little**



16%

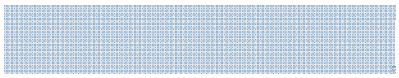
4. **Know very little**



9%

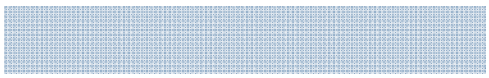
Is using animal eggs to create embryos an acceptable alternative to using human eggs?

1. **Yes**



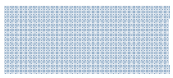
39%

2. **No**



47%

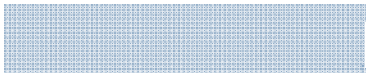
2. **Don’t know**



14%

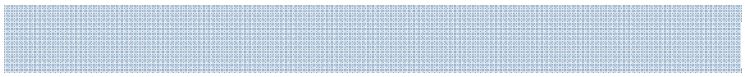
Do the potential benefits outweigh any ethical concerns?

1. **Yes**



34%

2. **No**



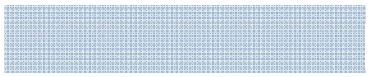
63%

3. **Don’t know**

3%

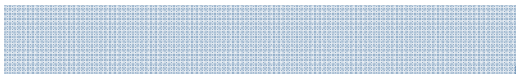
Would you be happy to receive therapies derived from human/animal embryos?

1. **Yes**



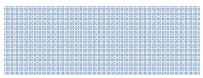
34%

2. **No**



48%

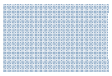
3. **Don’t know**



18%

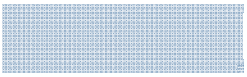
What, if any, concerns or issues do you have with creating and using cytoplasmic hybrid embryos for research?

1. **Safety**



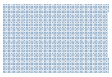
11%

2. **Ethical issues**



21%

3. **Whether it will be effective**



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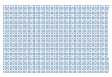
4. **Applicability of results**



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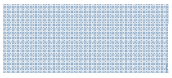
5. **Slippery slopes – once scientists are allowed to do this they will want to do something more extreme**



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6. **Time it will take to get results**

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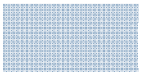
7. **Using embryos in research in general**



13%

8. **Other**

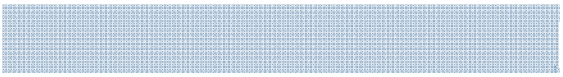
6%

9. **None**

7%

How important do you think it is to consult the public on issues such as this?

1. **Very important**



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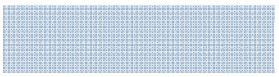
2. **Quite important**



30%

3. **Not very important**

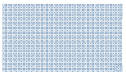
6%

4. **Not important at all**

4%

Are you going to respond to the HFEA online consultation on this issue?

1. **I have already responded**



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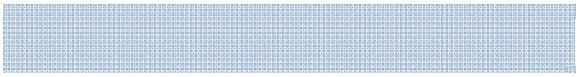
2. **I will be responding**

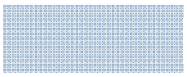


62%

3. **I won't be responding**

8%

4. **Don't know yet**



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Appendix H: Summary of scientific consultation responses

1 Overview

1.1 The scientific consultation was carried out to gain a greater understanding of the scientific issues surrounding human-animal hybrid embryos and to determine whether or not they can be classed as live human embryos, and therefore whether their creation falls within the remit of the HFEA. Firstly there is the question whether the entities will contain a complete human genome. Secondly, it needs to be considered whether the embryos would have the potential to develop if replaced into a woman (NB: this is banned by the Human Reproductive Cloning Act 2001). Relevant stakeholders (15 scientific organisations, funding bodies and others) were asked for their views on a number of scientific questions regarding hybrids, as outlined in section 3.

1.2 Respondents agreed that cytoplasmic hybrid embryos contain a complete human nuclear genome i.e. 46 chromosomes. However the entities will also contain animal mitochondrial DNA (mtDNA). If human mitochondria are transferred with the nucleus then the entities will contain human mtDNA as well.

1.3 Respondents agreed that there was no way to test whether cytoplasmic hybrid embryos have the normal potential to develop. The presence of animal mitochondria was identified as likely to have a detrimental effect on development. There was general agreement that the embryo would be unlikely to be viable beyond early development and would be unlikely to develop if implanted in a woman. This view was supported by the Royal Society, the British Fertility Society and key researchers in the fields of mammalian embryology, developmental biology and reproductive genetics. Somatic cell nuclear replacement (SCNT) was seen as being highly complex and inefficient in the same species and respondents thought that problems were likely to be increased in experiments between different species. It was thought that the larger the evolutionary difference between species, the less likely it is that there will be normal development.

1.4 Views were also gathered from the HFEA's Scientific and Clinical Advances Group (SCAG), at their May 2006 and June 2007 meetings, and the Horizon Scanning Expert Panel (HHSEP), via a questionnaire in November 2006 and at their annual meeting in July 2007.

1.5 In summary, the broad conclusions of SCAG were:

- Cytoplasmic hybrids would contain a full human genome and that any egg/embryo with a human genome falls within the remit of the HFE Act (e.g. parthenotes and embryos created as a result of cloning)
- Transferred/implanted cytoplasmic hybrid embryos may not survive, but were they to do so, the human mitochondria were likely to have a replicational advantage
- Creation of cytoplasmic hybrids could be justified for research projects due to lack of availability of human eggs but the technique should be demonstrated to be effective in animal models first

The broad conclusions of the HHSEP were:

- Cytoplasmic hybrids would contain a complete human genome, with the exception of human mitochondrial DNA

- These entities were unlikely to be viable beyond the early developmental stages
- At some stage after embryonic genome activation all proteins produced in cytoplasmic hybrids embryos (with the exception of those coded by the animal mitochondrial genes) would be human
- Cytoplasmic hybrid embryos would contain a mixture of human and animal mitochondrial DNA which would have a negative effect on their development, reducing their viability

2 Relevant stakeholders

2.1 Stakeholders who responded to the scientific consultation:

- Medical Research Council (MRC)
- Wellcome Trust
- The Royal Society
- Association of Medical Research Charities
- Motor Neurone Disease (MND) Association
- Human Genetics Alert
- Association for Clinical Embryologists (ACE)
- Royal College of Obstetrics and Gynaecologists (RCOG)
- British Fertility Society (BFS)
- Scottish Stem Cell Network (SSCN)

3 Summary of responses to the scientific consultation

Question 1: Do you think creating embryos by cell nuclear replacement (CNR) into animal eggs will be beneficial to research? Please give reasons for your answer.

3.1 Human Genetics Alert expressed the view that creating hybrid embryos would not be useful for research. However, this organisation is opposed to the creation of any type of embryos purely for research. They thought that due to the limited success of creating cloned human embryonic stem cell lines it may not be a useful strategy to make interspecies embryos in which there is a risk that the cytoplasmic factors and nuclear components will be mismatched. They were of the view that these entities will have mitochondria with mixed species proteins and it is likely that human proteins will not interact properly with animal proteins, thereby rapidly killing off mitochondria which produce the cell's energy.

3.2 The majority of organisations expressed the view that although it is unknown whether cell nuclear replacement (CNR) will prove to be a viable method for generating stem

cells, all avenues of research, which may lead to greater understanding of and treatments for diseases, should be explored. They felt that although there is a wide range of views and ethical issues on this subject, on balance the creation of hybrid and chimera embryos offers important opportunities for research into a wide range of important medical conditions while not harming any existing person or human embryo, and should therefore be allowed. The technique was seen to provide a valuable experimental tool and may ultimately lead to therapeutic benefits.

3.3 In their response the Motor Neurone Disease Association stated that:

"The possibility of programming human embryonic stem cells genetically identical to someone affected by MND, into human motor neurones offers the potential of the most accurate model of human MND to date….There is no source of eggs from women living with MND, due to the progressive nature of the disease, and the potentially harmful effects of the IVF hormones and procedures on their MND. Thus the use of animal eggs offers an alternative method of developing human motor neurones."

3.4 If animals eggs from abattoir material were used this technique would support the Royal Society's principle of the three R's. This means every effort must be made to: replace the use of live animals by non-animal alternatives; reduce the number of animals used in research to the minimum required for meaningful results; and to refine the procedures so that the degree of suffering is kept to a minimum.

3.5 In summary the following benefits of the technique were suggested:

- The use of animal eggs will provide the necessary large number of oocytes for this research to progress whilst avoiding the complex situation of IVF patients donating eggs to research. This will allow scientists to improve the technical efficiency of CNR so that a much smaller number of human eggs could subsequently be used to generate stem cells.

- The basic biology of stem cells created using this technique could be investigated. This technique may provide valuable experimental models of reprogramming of gene expression, facilitating further understanding of the mechanisms of reprogramming and of the factors required to establish pluripotency.

- This technique could be used to investigate the inheritance of mitochondrial DNA and investigate ways to reduce heteroplasmy with the aim of enhancing the reproductive success of 'older' oocytes or developing therapies for mitochondrial diseases.

- Research using this technique may subsequently inform the development of alternative methods to derive embryonic stem cells directly from somatic cells, without the need for oocytes or early embryos.

- This technique could provide invaluable models of cellular disease, for example, motor neurone disease, Parkinson's disease, diabetes and Alzheimer's disease and may eventually lead to the development of therapies for these diseases.

Question 2: The applications that we have received relate to a very specific aspect of 'hybrids and chimeras' (the creation of cytoplasmic hybrid embryos). Can you think of any reasons why scientists or researchers may wish to create other embryos where there is a mix of human and animal cells or DNA?

3.6 The general view of organisations consulted was that currently there is no reason why scientists would want to create human transgenic embryos, true hybrids or human chimera embryos.

3.7 It was suggested by two organisations that there is likely to be more of a case for the creation of human-human transgenic embryos for research than human-animal. One organisation referred to The Academy of Medical Sciences report on interspecies embryos which stated that researchers will at some stage have good reasons to conduct research involving the creation of human-human transgenic embryos. These techniques could facilitate the investigation of gene function in early embryogenesis or, for example, a gene could be introduced in a human embryo to increase the efficiency of the derivation of stem cells.

3.8 A number of the responses pointed out that the creation of transgenic animals, by introducing human genes into animal embryos has been standard scientific practice for over 20 years for investigating functions of genes and their mechanisms of regulation e.g. a number of transgenic animal models of motor neurone disease exist. Also, animal chimeras (animal embryos with human cells) are useful for a number of research purposes e.g. models of human disease, identifying signals that determine early stages of differentiation and testing developmental potential of human embryonic stem cells.

3.9 Therefore there is evidence that these techniques are successful in animal studies, so in theory they could be technically possible on human embryos. However, one organisation pointed out that the fact that technology for genetic modification of embryos has existed for so long without any demand for its use on human embryos suggests that it is not currently useful or practicable.

3.10 Although there is not currently a demand for the creation of these entities it was generally thought that it is always difficult to predict how scientific research may develop in the future.

Question 3: Can you anticipate any biological problems with embryos, or stem cells derived from embryos, created by CNR using animal oocytes that will limit their use in research?

3.11 The general view of organisations consulted is that it is still unknown whether this technique will prove to be a viable method of generating stem cells. Somatic nuclear reprogramming is highly complex and it has been shown to have a low success rate and give rise to abnormalities in same species models.

3.12 The British Fertility Society gave the view that problems are likely to arise from: mitochondrial heteroplasmy, epigenetics (incorrect remethylation of the genome) and possibly incorrect activation of the human embryonic genome in response to animal rather than human cytoplasmic factors. This view was reflected in the majority of responses received and it was generally felt that there are likely to be problems with interactions between the human derived nucleus and predominantly animal derived mitochondria e.g. improper interaction between human and animal derived proteins.

3.13 In their response Human Genetics Alert stated that:

"In the proposed experiments, the scientists are hoping for thousands of cross species molecular interactions, between both the mitochondria and the cytoplasm of the egg and the nuclear genes and proteins to work perfectly, in order to produce a normal cell. Different mammalian species have differences in the programmes of gene expression in early development, so it is optimistic in the extreme to expect this to work."

3.14 The Scottish Stem Cell Network pointed out that stem cell lines derived from cytoplasmic embryos are unlikely to be useful models for diseases involving abnormal function of mitochondria due to the likely mixture of human and animal mitochondria.

3.15 The Royal Society suggested that, as it is possible to grow ES cells in culture conditions where mitochondrial function is not required and as most of the proposed research on ES cells would be conducted *in vitro*, this suggests that any problems with mitochondrial function may largely be overcome. However, *in vivo* experiments with the cells might be compromised.

3.16 A number of organisations also suggested that there is a risk of animal disease transmission to embryos created with animal eggs, which will mean that ES cells derived from cytoplasmic hybrid embryos are unlikely to ever be used in clinical therapies.

Question 4: Are you aware of any data or information that would indicate that embryos created by CNR using animal eggs would not have the normal potential to develop if replaced into a woman? NB: this is banned by the Human Reproductive Cloning Act 2001.

3.17 The general view was that the question of whether cytoplasmic hybrids would have the normal potential to develop could ultimately only be answered by carrying out illegal experiments. However, there is a large amount of information from animal cloning which shows that animal embryos produced by somatic cell nuclear transfer have a very reduced potential for development, and those animals that develop are likely to be abnormal.

3.18 The Royal Society pointed out that:

"Successful implantation requires a highly co-ordinated series of cell and tissue interactions and, to date, there has been little success with animal interspecies embryo transfer. For example, mice into vole and vice versa fail at implantation because the embryo and uterine tissues do not recognise one another, whilst interspecific transfers between the more closely related sheep and goat usually implant successfully but fail in mid-gestation for immunological reasons …Whether implantation would be affected by differential display of animal proteins on the developing embryo and the human host is unknown. There is the possibility that relevant proteins would be replaced by human proteins once transcription of nuclear genomes has begun, however, while this is very likely, details with respect to timing and extent are unknown. If implantation was to occur, but there were problems with mitochondrial replication or function it is likely that the embryo would fail at gastrulation stages."

Question 5: Do you consider a cytoplasmic hybrid embryo to contain a complete human genome?

3.19 The majority of organisations are of the view that for cytoplasmic hybrid embryos to be classed as having a complete human genome they would need to contain the complete human mitochondrial, as well as nuclear, genome.

3.20 Cytoplasmic hybrids would contain a complete human nuclear genome but the presence of a human mitochondrial genome would depend on the number of human mitochondria transferred with the nucleus and whether they are replicated. If no human mitochondria are transferred in the process of SCNT then the cytoplasmic hybrid will be missing the 0.3% of genes which are mitochondrial.

3.21 One organisation gave the view that as the normal procedure for creation of cytoplasmic hybrids is to insert the entire human somatic cell into the animal egg both the nuclear and mitochondrial DNA will be included.

4 Non-respondents

4.1 Responses were received from 10 of the 15 organisations the scientific questions were posed to. Three of these organisations did not specifically answer the questions in their responses.

4.2 Out of the 5 non-respondents, one funding body did not respond because they did not expect to fund the creation of human-animal embryos as the research is unlikely to fall within their remit. One organisation thought that it would not be appropriate to respond as individual scientists within the organisation would be providing the HFEA with information.

5 Scientific and Clinical Advances Group (SCAG)

5.1 Members of SCAG

- **Professor Neva Haites** - Professor in Medical Genetics, University of Aberdeen
- **Professor Chris Barratt** - Scientific Director, Birmingham Women's Health Care
- **Mr Roger Neuberg** - Consultant Gynaecologist, Leicester Royal Infirmary
- **Dr Maybeth Jamieson** - Consultant Embryologist, Glasgow Royal Infirmary
- **Professor Peter Braude** - Professor of Obstetrics & Gynaecology, King's College London
- **Lord Harries of Pentregarth** - House of Lords and former Bishop of Oxford
- **Ms Clare Brown** - Chief Executive, Infertility Network UK
- **Professor Lorraine Young** - School of Human Development, University of Nottingham
- **Miss Melanie Davies** - Consultant Gynaecologist, University College London Hospital
- **Professor Richard Gardner** - Department of Zoology, University of Oxford
- **Dr Daniel Brison** - Scientific Director, Department of Reproductive Medicine, University of Manchester
- **Professor David Barlow** - Executive Dean of Medicine, University of Glasgow
- **Dr Robin Lovell-Badge** - Division of Stem Cell Biology and Developmental Genetics, The National Institute for Medical Research

5.2 In May 2006 SCAG was asked to give a view on whether an interspecies cytoplasmic hybrid embryo would be human.

Responses

5.3 Members were of the view that particular consideration needs to be given to the role of mitochondria, as it is unknown what the proportion of contribution from human and animal mitochondria will be in these hybrid embryos. Members were of the view that if hybrid embryos were transferred/implanted they may not survive, but if they do then human mitochondria are likely to have a replicational advantage as they are more compatible with the genome. The group expected that if cell lines were derived from these embryos and cultured, then it is likely that the human mitochondria will dominate over the animal mitochondria, although this has not been proven.

5.4 One issue raised was whether the hybrid embryo would be human from the two cell stage, or only become gradually human after a number of days development. One SCAG member was of the opinion that for the first 5 or 6 days of development the entity would initially be predominantly animal because it would contain animal proteins (proteins coded for by animal DNA). It would then become gradually humanised, becoming predominantly human by 8 or 9 days of development.

5.5 The general opinion of the group was that interspecies cytoplasmic hybrid embryos should be classed as human.

5.6 In June 2007 SCAG were asked for their views on the questions posed to stakeholders, as outlined in section 1.

Responses

5.7 Members were of the view that the creation of hybrids is necessary for research projects due to lack of availability of human eggs. These research projects could include investigating the interaction of mitochondrial and nuclear DNA in order to study mitochondrial diseases. These hybrids could also be used for many of the same research purposes that have been proposed for SCNT using human oocytes.

5.8 Members agreed that all avenues of research should be explored. One member noted that cell nuclear transfer is poorly understood and that research on nuclei and cytoplasm interactions need to be carried out from human to animal, animal to human and animal to animal. Another member suggested that animal-animal models should be carried out first. Literature on the use of animal-animal hybrids for the conservation of rare species was highlighted as a potentially useful resource. The Group were of the view that creating cytoplasmic hybrid embryos would involve less genetic manipulation than other models, such as reprogramming fibroblasts.

5.9 The Group were of the opinion that there is no scientific case for true interspecies hybrids.

5.10 Members thought it was impossible to tell if embryos created in this way would have the normal potential to develop if replaced in a woman, and there was no way of testing it. One member was of the opinion that mitochondrial function would be severely compromised in a significant proportion of cells around the gastrulation stage and that embryo development beyond this would be very abnormal. It was noted that although the embryo may fail, this would not prevent embryonic stem cell derivation, because this only requires one or a few cells and because they have little requirement

for mitochondrial function. One member pointed out that the HFEA already regulates research on types of embryo that have little or no normal potential to develop if they are replaced in a woman, e.g. embryos carrying chromosomal or severe genetic abnormalities and parthenogenetic embryos.

5.11 The group thought that the mitochondrial element of the genome had to be taken seriously and that epigenetics were important. One member was of the opinion that a cytoplasmic hybrid would not contain a complete human genome, but would contain 46 chromosomes. Another member thought that it would contain a complete human genome because it will contain both nuclear and mitochondrial genomes from the human donor somatic cell. It was suggested that some human mitochondria would have to be transferred with the nuclear DNA. The group thought that at different stages different proportions of the human genome would be present and there was some concern that using eggs from a different species would change the gene expression because the nucleus will be surrounded by proteins from the host egg.

6 HFEA Horizon Scanning Expert Panel (HHSEP)

6.1 Members of HHSEP who responded:

- **Professor David Edgar**, School of Biomedical Sciences, University of Liverpool (research interests - development biology, human embryology)
- **Dr Maureen Wood**, Research Embryologist, Assisted Reproduction Unit, University of Aberdeen
- **Professor Peter Andrews**, Department of Biomedical Sciences and the Centre for Stem Cell Biology, University of Sheffield
- **Professor Alan Trounson**, Monash Institute of Reproduction and Development, Monash University, Australia
- **Professor Henry Leese**, Department of Biology, Univeristy of York (research interests - biochemistry and physiology of early mammalian embryos)

6.2 The advice of the HFEA's Horizon Scanning Expert Panel was initially sought on the issues of hybrids, by sending out the following questionnaire, in November 2006:

1. Would any entity created by activating a human somatic cell nucleus within an enucleated animal (e.g. cow or rabbit) oocyte:

   a) be viable, or, at least, possibly viable?
   b) contain a complete human genome?
   c) be a human embryo?
   d) ever have the potential to develop and result in a live birth, if implanted? (N.B. the HFE Act 1990 prohibits this)

2. Given that the proteins present would be predominantly animal, would the entity created be human from the moment of activation? If not, at what stage, in

your opinion, would the entity become human? How long would the animal proteins be present?

3. What would be the significance and likely effect of the presence of animal mitochondrial DNA on any such entity's development?

Also, at their annual meeting on 2nd July 2007 the Panel discussed whether the creation of hybrids and chimeras would be beneficial for research.

Responses

6.3 Panel members agreed that the entity would contain a complete human genome with the exception of human mitochondrial DNA (mtDNA). One panel member was of the opinion that the entity may contain human mitochondrial DNA, as well as nuclear DNA, as mitochondria transferred with the donor nucleus may be preferentially replicated. It was pointed out that the mitochondrial genome is very small and only encodes a few mitochondrial proteins relating to oxidative phosphorylation.

6.4 There were mixed views on the potential of these entities to develop, but it was generally thought that they were unlikely to be viable beyond the early developmental stages. One member suggested that data from animal models suggests that large species differences make it unlikely. It was noted that some cross-species cloning has produced offspring but this tends to be between closely-related species. The interspecies problems of mitochondrial and nuclear compatibility were raised. One member thought that entity would be capable of normal developmental behaviour, at least in the initial stages, but would expect increasing problems as development proceeds. Another member stated that the entity cannot develop to term and that there is no evidence for this at all.

6.5 The general view of Panel members was that at some stage after embryonic genome activation all proteins produced (with the exception of those coded by the animal mitochondrial genes) would be human. One member thought that most proteins would be human within a few rounds of cell replication and certainly by the time of implantation. It was noted that in humans, embryonic genome activation does not happen until between the four and eight cell stage. Therefore, until this stage, the embryo is relying on proteins and genetic messages that were present in the oocyte (i.e. from the animal) and that the entity may not be regarded as 'fully human' during this early period. One member thought that any stem cells formed would be almost entirely human.

6.6 Members who felt able to answer question 3 were mostly of the view that the mixture of human and animal mitochondrial DNA would have a negative effect on the development of this entity, reducing its viability. These entities may be more viable if the animal mitochondrial DNA is eliminated, although it was noted that some papers have argued that somatic mtDNA hinders embryonic development. However one member thought that the persistence of a few animal mitochondrial genes would not have much significance for the behaviour of the resulting entity. One member thought that there is a high risk of epigenetic change and disruption of development. Members felt that the work on animal cybrids (the fusion of an enucleated somatic cell with a somatic cell) would be worth reviewing. It was noted that experiments creating human and primate cybrid cell lines resulted in slower growth and respiratory rates. When the evolutionary distance was too diverse, effective cellular function could not be sustained.

6.7 At their annual meeting on 2nd July 2007 the Panel discussed whether the creation of hybrids and chimeras would be beneficial for research. The Panel members expressed mixed views as to whether creating interspecies cytoplasmic hybrid embryos would be

beneficial for research. One panel member was of the view that the limited work that has been carried out on animal-animal interspecies cytoplasmic hybrid embryos has shown that stem cell lines derived from these entities show slow cell replication, that there is no connection between the mitochondrial and nuclear components of the embryo and the method is currently inefficient. It was also suggested that there are a number of other sources of embryos and methods to create stem cell lines before the creation of interspecies hybrid embryos should be considered.

These sources/methods include:

- Mitotically arrested zygotes
- Triploid embryos
- Tri or mono pronuclear eggs
- Reprogramming somatic cells

Another panel member was of the view that scientists in the UK should be allowed to create interspecies cytoplasmic hybrid embryos in order to demonstrate whether or not it is possible to repeat the results of the groups who have reported the creation of human-rabbit and human-cow entities. This panel member felt that every avenue of research should be explored and that the creation of hybrids should be permitted as they will never be transferred to a woman and allowed to implant, as there is regulation in place to prevent this happening.