

**SESSION 2: PREPARING FOR THE FUTURE OF ART: HFEA'S HORIZON  
SCANNING WORK = DR ROBIN LOVELL-BADGE, HEAD OF STEM CELL  
BIOLOGY AND DEVELOPMENTAL GENETICS DIVISION, NATIONAL  
INSTITUTE FOR MEDICAL RESEARCH = SCIENTIFIC HORIZON AT HFEA –  
AN OVERVIEW AND ASSESSMENT OF KEY THEMES**

Speaker	Transcript
S1	<p>Thank you so much. So, now you have the perfect context for the process. And now we have Robin Lovell-Badge who's Head of the Stem Cell Biology and Developmental Genetics Division, the National Institute for Medical Research, and who is a SCAG member, and who will now take you through some of the Horizon Scanning outcomes of the last little while. Thank you!</p>
S2	<p>Okay, so... As was said I am a co-opted member of SCAG and what I want to do is just give you a flavour of the sort of things we have to do on that Committee and looking at the Horizon Scanning Process, and... I just point out because members of SCAG themselves highlight issues and we welcome anyone to suggest things to us.</p> <p>Uhm, so...this was some of the issues in 2006 and in 2007. I should stress that I cannot possibly go through all the issues that we discussed. Actually, the last round, I think we covered 55 different topics ranging from things like endometrial scratching to spermatogonial transplant. So...well it is an interesting science to talk about but I do not have time for that.</p> <p>So these are some of the sort of things that we...we were looking at 2006/2007. In vitro-growth of ovocytes, embryo selection using metabolomics, embryonic stem cells from blastomeres and some of these topics come up every time. They do not go away and it is important that we, we carry on reviewing this stage of the science and each of this topic. And so the topics on the bottom there, again, these are all things that come up quite frequently. So, <b>(points to projector)</b> in brackets are artificial gametes. I personally would like to make a plea that when we are talking about In vitro derived gametes, we do not refer to them as artificial ever, because if they work and we have children, we do not want to be calling them artificial children. Okay, and then, freeze-drying sperm...that sounds an interesting topic, it is! This works in mice. You can freeze-dry mouse sperm and ship them around the world in an envelope and reconstitute them... Do it till you get live mice on the other end. Yes... <b>(Audience laughed)</b> ... Okay.</p>

So let us talk very briefly about metabolomics. This is a technique that is going to be ...probably quite important as a way of choosing which embryos are best. So there are a number of groups around the world working on these sorts of strategies and the simple way of thinking about it is you just take your embryo growing in culture. Of course, you need each of the embryos to be in a separate little drop of medium, and then you sample the medium probably more than once, during its early growth in vitro and then you analyse the constituents of the medium to see how the embryo is behaving, whether it is metabolising correctly or not. And there were lots of papers getting published on this topic. This is just a very recent one from Henry Leese's Group basically they did amino acid profiling of single embryos as a way of providing a noninvasive marker of DNA damage at the blastocyst stage. So the days that they had were consistent with what they referred to as the quiet embryo hypothesis. Viable embryos with the lowest DNA damage have the lowest amino acid turnover. This is a very nice little study. So it is a noninvasive way of looking at embryo quality that could be important in the future.

There are all sorts of methods that people are trying to develop. There are those that are based on microarrays to analyse either DNA or RNA made in embryos. Now... of course these, these sorts of techniques which basically rely on comparing a normal control with your sample. For example, this is illustrating how you could use this to detect either chromosome with duplications or deletions. These techniques of course will require biopsy of the embryo; although perhaps you could take the polar body and get some information because you need to isolate RNA or DNA from a cell of the embryo, so they are not quite so simple to do, but again could be very important.

This topic has only come up again but in vitro derived gametes, this is an issue that we talked about two years ago, and again this year in great detail. So generally, the idea is that if you have a pluripotent cell type, for example embryonic stem cells, whether you could start off from these pluripotent cells and then use various methods to derive either sperm or eggs entirely in vitro from those blastocyst cell population. Of course, the stem cells could be either chromosomally male, so x y or chromosomally female and this slide is really put up here to illustrate that we know that you should be able to get of course sperms, from x y cells. It is extremely ... well, you can get eggs from x y cells, but it is extremely unlikely and it is much less likely will do so and then you have to content with all sorts of other problems like you can't predict whether the resulting egg is going to be carrying an x y or no sex chromosomes. And so you have likely a set of chromosomes antibody in your embryo. So, that's complicated.

The other way around, female stem cells or xx, you should be able to get eggs from those. Can you get sperm? I think the current statement will say, obviously not because you need genes of the y-chromosomes to allow you to make a sperm. So, that is a little bit of science.

So, as Marybeth mentioned, we have this panel meeting, Horizon's panel meeting annually with ESHRE, et cetera and we look at the output about the ... our SCAG committee meeting. The Horizon's Scanning panel has asked questions, so they are specifically directed to think hard about particular topics and it's partly SCAG who does that, but it's the HFEA in general would do that.

So let us just go back to how this process would work. Say for example in vitro derived gametes, so we have some sort of input into the topic on how this could again be reached, that will of course include research. So, there has been published research suggesting that you can get both male sperm and eggs in... from...by...including in vitro derived methods. Those published researches suggest you can get live offspring from mice in vitro derived sperm. Those mice did not do terribly well. They lived for a few months and then they will have problems and die. They have problems with the imprinting basically so they ... something did not quite go right in the process. So, how do we make decisions based on data or in the mouse like this? Well, it's very hard. You got to...we are saying...we have to try and think or can this basic research done in the mouse, be translated to the human situation?

We do not know what is around the corner because often scientists were able to solve what seems like a major problem by just a simple little trick. And so we always have to be a little optimistic that things will happen faster than they might do so, but we have to be not too optimistic.

We also take on board the view from other experts in the field. Say from conferences and I would just point out in the field of stem cell research there is probably a conference every week of the year and it is actually very hard to go to all of those and keep up to speed with everything.

The other particular groups that discussed specific topics and there was a meeting of the Hinxton Group earlier this year that discussed in vitro derived gametes which included a wide range of experts in the field and their output was very important. So there is consideration by SCAG and by the Horizon Scanning Panel and a

number of conclusions were reached such as human eggs or sperm will be derived in vitro within 5 to 10 years, perhaps sooner, but we do not want to be too ambitious. There is probably a shorter time scale for deriving human sperm than eggs. It is certainly...sperm from female as I mentioned, sperm from female stem cells and eggs from male stem cell are certainly not currently possible and we can think for a large part ignore those possibilities. Of course there are concerns over safety, and these again are big issues to deal with. There are, however, many potential researches, as well as clinical applications and the concerns about safety do not worry so much when we are talking about research. So it is important that this whole field is allowed to go forward.

So, thought the HFEA was likely to receive applications to create an embryo with in vitro derived sperm, perhaps in the near future so they certainly need to be aware of that. However, there were some concern that research is progressing perhaps without proper assessment of gamete formation, that was current research going on in the UK so we need to be always aware that there are research going on in other countries too and there is a very nice research going on particularly in the States and some other countries suggesting that this whole field is going very fast, that we are going to be able to have in vitro derived gametes, probably sperm and eggs in the not too distant future.

The HFEA Ethics in Law Advisory group has to get involved and then of course, because there are issues of for example, scientific unknown consent and parenthood. So, it really works easily and there are ways of getting pluripotent cells not just involving embryonic stem cells but for example using induced pluripotent cells from individuals if it is easy to get a little scraping of skin or something from an individual, turn out into a pluripotent cells and then use those two derived sperms, you know, if you want to, you could choose the father of your babies without the father even knowing, so you have to be...There are always issues that need to be worried about! Okay...

This output from what... from what... how can we consider this... the HFEA position in the event of life and its applications. Criteria that in vitro derived sperm must reach before the HFEA will license research to create an embryo. Of course, it's going to be really important just for research purposes to be able to create the embryos to know whether or not your in vitro derived gametes are functional let alone for clinical applications. Advice to give to Peer Reviews concerning research applications, it is important to brief the Department of Health and to have meetings with Government people before things go ahead.

So this latest Horizon Scanning Cycle, a number of other issues were prioritised. So alternatives to embryonic stem cells, trophoctoderm biopsy for pre-implantation diagnosis, spermatogonial cell freezing, use of 3PN embryos, et cetera, gene transfer into embryos and male germ lines will cover some of those topics.

A number of issues were considered that we had also looked at before; no point in going through all of these. And of course the very important thing that happened, that's been happening all of it going on this year is the current Human Fertilisation and Embryology Bill, this is the part of the Bill that deals with Human Admixed embryos and this is a complex slide **(pointing to projector)**. The only Bill I am going to stress is the bit on the bottom but none of these will be permitted embryos and therefore they cannot be implanted. Also, any embryo produced in these ways cannot be kept intact beyond 14 days. We can simplify that, just by saying the different types of Human Admixed Embryos that is proposed to license. So these are cytoplasmic hybrids...**(pointing to projector)** So, this is nuclear transfer using animal eggs whose nucleus has been removed and human somatic cells or their nuclei; so using the cloning procedure. Two hybrids, so using mixed in sperms for example from humans and eggs from animals; transgenic human embryos, so human embryos containing perhaps animal DNA. Actually, the word animal in this context needs to be interpreted very widely by the HFEA because it could include human, it could include bacteria, including probably, will include things like green protein, which is a jellyfish gene. Could it include plants? That is a question that is going to be thought about. Chimeric human embryos, so those are ways you mix together chimeras, where you mix together cells to embryo or from one embryo and for example, embryonic stem cells. So this is...So we are particularly concerned in this case with using animal cells and human cells. And there is also a "catch all" in case something else crops up that we have not thought of.

So how did this all come about? Actually, the first study was one on Cytoplasmic Hybrids published in 2003 by Chen and Colleague, but there has been a whole range of papers since then and in fact there are probably over 80 papers now published on various forms of cytoplasmic hybrids, some involving attempts to the human-animal mixtures, many on animal-animal mixtures. The HFEA's view, well, three research projects had been licensed to do with cytoplasmic hybrids and that is before the Bill has been passed, but then because these were not actually ruled out under the previous Act.

On the slide, **(pointing to projector)** it says the HFEA is not currently aware of

groups wanting to create other types of Human Admixed Embryos. I am sure there are many, waiting for the Bill to be passed because you actually cannot do a lot of other type of research until that Bill goes through. Indeed, I know that there are scientists out there wanting to do things. So why is there an issue, well, because the Bill is hopefully going to be passed very soon.

So this again, **(pointing to projector)** is the cartoon of how you do cytoplasmic hybrid embryos. An egg from an animal has the nucleus of mitochondria within your sight. You remove the nuclear DNA and then take a human cell, for example from a patient suffering from some disease, perhaps a skin cell. Take either the nucleus or fuse in the whole cell itself into the enucleated animal egg and then allow that to develop. The point of doing this is to be able to try and derive embryonic stem cell lines which can then be used for further study of the nature of the disease.

So this is the first study from Chen and Colleagues. She was head of a group who works in China and this was published in 2003 and has actually taken a little while to have gotten really noticed by the HFEA, I am afraid to say, so they were a bit slow in appreciating that this might become a major topic. So, this was an example of such an embryo after the nuclear transfer developing to blastocysts stages and then embryonic cells were derived and these cells could differentiate into wide range of different tissue. So, this is very promising the use of using of human eggs. In fact, Ms Chen recently tried this because she did not have access to any human eggs, but she had a lot of rabbit colonies, so she used rabbit eggs... Okay...

There has been work subsequently showing that you can do this with the so called therapeutic cloning method to obtain embryonic stem cells from cloned embryos, of course using mice, but also using Rhesus monkey cells. So this gives hope that this procedure might work in humans.

However, there were still lots of questions to be asked, and again in doing this Horizon Scanning, it is very difficult to know exactly, you know, to be clear about exactly where the science is going to go and when you are going to get tangible results to be saying that the technique is going to be working. So this again is very... I just chose one very recent paper to just highlight where some of research to that. So this is taking...**(pointing to projector)** is just doing the cloning procedure using cow eggs and either monkey or cow fibroblasts and... with... In both cases these worked very well to get cleavage stage embryos so they were getting very effective high rates of cleavage stage embryos doing this enucleated

transfer technique. In both cases, the donor cells were engineered to carry particular DNA transgene reporter so this is using this is using green protein driven by the gene called OCT4, which is a marker of embryonic cells and embryonic stem cells, and so they can see if this green protein becomes active, cause it is inactive in the fibroblast but it should be active in the embryo. Its becoming active means it definitely is reprogramming its aspect of the fibroblast, and this is the case, whether they use monkey or bovine fibroblast.

However, in the case of using the monkey fibroblast, the maximum stage they got to was about, the sixteenth cell stage, whereas if they use... Now we get talking about cow in to cow or the bovine fibroblasts, they went all the way to blastocysts. So, for some reasons, the cross species did not work very well in comparison with the intraspecies transfer. Now, this is something that one might say is not going to work, never going to work! But lots of scientists just say, "ah, this is an interesting things to know", and try to understand because this did not work so well. So is it the fact that they are using monkey fibroblast or maybe human ones will work much better? Is it because they are using cow eggs? Maybe using rabbit eggs would be better. So there is lot of issues that can be addressed to try and solve, find out really what is has gone wrong here.

The next on the list is two hybrids. So why on earth would anyone want to mix human sperms with animal eggs? So just to create an embryo in the lab will cost ... There has been the human... the ...hamster... The hamster's egg test for quality of sperm which is not used so much now as certainly not as a clinical practice in assessing quality of the sperm. But there's lot of research that can be done using this sort of technology. So, research into infertility, perhaps into novel contraceptives or something, et cetera.

So you can ask for example what human genes are required for human sperm to fertilise an egg. Now, you do not particularly want to use lots of human eggs to try and find this out but you could use animal eggs and the add or subtract appropriate human genes from the animal eggs using all those sophisticated techniques we have worked for example with mice to do that. To actually find out what is really important for a sperm, human sperm to fertilise an egg. You could use this method to of course test the viability of sperm or new methods of storing sperms, freeze-drying them et cetera. And you can use this method to test the ability of in vitro derived sperm to fertilise eggs rather than wasting valuable human eggs.

I am gonna basically cover the next two together, that is Transgenic and Chimeric

Human Admixed Embryos. So, **(pointing to projector)** this is a typical sort of slide of the information we have about early mammalian development and the various cell types that arise from the fertilised egg going through either giving cell types typical of the placenta or the yolk sac or the embryo itself. All this information comes from work essentially on rodents and mostly on mice. We have almost none of this information, we do not know any of this is really true for normal human embryonic development. We think it might be, but we do not really know, similarly, we know how the mouse embryos developed in terms of their relationship of cell types to each other when particular cell types become committed to a particular cell lineage, whether they are going to be part of the embryo itself or part of the embryonic tissue, for example. But again, for human we do not do that and we do not really know how the particular early embryo develops in terms of structure. So, there is a lot of research that can, and I am sure will be done now if the Bill goes through. Research will now be permitted to allow us to understand the basic questions about how we begin our life and I think that is very important, and of course, all sorts of things go wrong. Again, we may be able to get some information about causes of congenital abnormalities or implantation failure for example due to defect and perfect them.

So lots of applications, clinical applications including research into other human development, reprogramming the origin and fate of the different cells of an embryo. There are a variety of methods that may be used to introduce genes into early embryos. We can sort of recommend that this is likely to be one that is the most efficient, but who knows what is around the corner. There are probably many others that are going to be developed. So again, it is just to say that how you make the transgenic embryo, well, you could introduce the DNA, in fact, at any stage. You can introduce it into the fertilised egg; you can introduce it into blastocyst stage. If you are doing the cloning procedure, you can introduce it into the cells before you do the cloning procedure. So again, the experiment type showed using the GFP put into, say, monkey or cow fibroblast, well you could start off by putting the GFP into human cells and then asking how that gene behaved in the early embryo. Okay...

So, chimeras are again made by mixing cells in two embryos or one animal cell and one human cell and this will cover putting animal cells into human embryo or in fact probably, more likely cases where you put human cells containing an animal gene into human embryo that would have to be classified as making a chimera, and that is very important because you can mark the cell and then you could follow what happens to that particular cell during the early steps of human development,

follow what a cell would become.

What is not on that list, but which will be covered in the Bill is mitochondrial disease treatment or at least research towards it and that is where you either you have a patient who repeatedly has problems giving rise to having normal offspring because of series of mitochondrial disease problems. So, maybe you can solve that by basically transferring the nuclei from the patient's egg into a donor egg from which its nucleus has been removed. And so now you have the patient's DNA surrounded by healthy mitochondria.

The other sort of things that we have been considering on and we have been considering for many years now on SCAG and the Horizon Scanning process are alternative ways to derive embryonic stem cells or similar types of cell. So one suggestion is you can take a single blastomere and derive stem cells from those and clearly that is possible, we know it is possible now. I personally do not think that there is going to be huge demand for this, but it is still something that the HFEA may well receive a license application for, so we need to consider it. Alternative embryonic stem cells and the one that has got everyone very excited is induced pluripotent stem cells where you basically reprogram a human adult cell to behave now like an embryonic stem cell and I will discuss that a little more detail in a second.

A new one that was published last week was Embryonic stem cells like cells from the human testes, so this is relying on the fact that the stem cell population in the testes that may utilise the sperm, this spermatogonial stem cells it appears can be coaxed back into much earlier embryonic state and they resemble very much embryonic stem cells. How useful they will really be, we really do not know. The research is way too early to say, but it is another promising avenue of trying to get cell types. Of course, there will not be any use for women, cause women do not have testes. Research done with them on genetic diseases, for example of course will be relevant to both sexes.

So just a brief word about induced pluripotent stem cells for those who do not yet know about this method. It is very exciting. So it was developed first by Shinya Yamanaka, and Colleagues in Japan. So it was done, of course, initially in the mouse. They took skin fiber off, actually from a tail tip of the mice. They knew, we knew that their whole set of genes which are very important for being pluripotent cell an embryonic stem cell, and I will not go into all the history of this but essentially they ended up using just four genes and by adding these genes using

particular retroviral vector viral vectors into the skin cells over a couple of weeks, they reprogrammed into this pluripotent cells. These are pluripotent cells because we know that they can differentiate in vitro into a wide range of different cell types. They can be assessed by making these particular tumors called teratomas, which compose of a wide range of different cell types and some tissues. And in the mouse, of course, we can also take these cells, reintroduce them back into an early embryo and see if they will contribute to development of the resulting mouse and indeed they do, those mice can even give rise to offspring that were derived from these cells.

So, this is clearly a very powerful thing to make, and it can be done in humans and it works very well. So it is possible to derive these induced pluripotent stem cells from human somatic cells. We know that they will differentiate while in vitro to give a wide range of tissues. You can use this teratoma assay as well, however, what you cannot do is a chimera assay. You can perhaps try in animals, but that is not going to be very successful more certainly, but of course we cannot try it in humans. So, we have to rely on these in vitro assays to say whether or not these cells are normal and doing detailed molecular characterisations within these cells and proper embryo derived embryonic stem cells to see how good these cells are likely to be, plus work in the mouse. Now, although this is very exciting there are still lots of unknowns about these cells and it is not clear that they really are truly identical to embryonic stem cells, and that is a question for future research. But anyway, that is why we absolutely still need to have the ability to derive embryonic stem cells from human embryos left over from IVF treatments.

So I am going to finish with a couple of slides. One, which I have labeled Risks and Problems Associated with the Horizon Scanning Process. So to quote by famous quotation, "It is tough to make predictions especially when they are about the future." I do not know who said this, but in fact it has been attributed to at least 10 different people as far as I could tell. Science progresses very fast, in fact so fast we just really do not know what is around the corner. In fact this whole story I just told you about induced pluripotent stem cells is one such very dramatic example. It was not possible, people were thinking, "would it not be wonderful if this could happen", but we all thought it is gonna be so difficult and the fact that it just required four genes to do this was remarkable. Other examples like RNA interference, that was unpredictable and it is now a huge field. So new discoveries can lead to novel approaches and more powerful methods. All that can challenge previously accepted dogma and leave old methods going out of flavour.

	<p>Another risk or issue that we have to worry about is time scales of predictions. Whatever we say on SCAG or in the Horizon Scanning Process, this of course is going to be balanced with the optimism of the scientist and actual reality. So a scientific result can come quite fast, but it can take an awful long time to translate that into something practical and to which the Regulatory Body will accept is now usable. And finally, the process can give a distorted view as it is not quantitative. So a method needs to be considered by the HFEA even if only one person might ever want a license to do that. And so it is, although we may say that something gets high priority, it is because we expect one person is going to get the license or a hundred people is going to get the license. So it does not... It should not distinguish between that.</p> <p>And then of course the other problems we have to worry about are changing political and public opinion. Ah, so it is not only the science that changes, it is the politics and social views on particular techniques or applications. And just to finish, this process is quite high in some of the risks and problems. It is very valuable way to warn the HFEA of issues that may have an impact on its activities. You will often involve new discoveries at the cutting edge which can be a challenge to explain to non-scientists by considering them early on before the implications have reached the public. This should put the HFEA in a better position to respond to inquiries from the public or to scientist seeking a license. In turn, it makes the members of the Horizon Scanning Panel and of SCAG consider the potential applications and new technologies in more depth than they would probably otherwise do so. So, even if something is amiss, without such a process the Authority will always be reactive rather than proactive and it is always better to be proactive. So I will stop there. <b>(round of applause)</b></p>
S3	
S2	