mentioned, of course, is the fact that with increased maternal age the frequency of aneuploidy, the frequency of chromosomal dysfunctions, as well as dysfunctions in the organization of the spindle to which these chromosomes are attached actually increases substantially. So, a large number of oocytes that exist in women of advanced reproductive age will not be rescued by any means because the chromosomal abnormalities, and spindles, structural abnormalities exist and they will not be fixable.

But in other cases, especially for eggs of older women, they look entirely normal and they really are indistinguishable from oocytes of younger women. But in large measure, whatever has happened prior to this egg meeting sperm, which this particular egg has not, things have happened to this egg which largely determine its competence, and it is the question of whether cytoplasm transfer or other procedures actually will be able to rescue whatever insults have been imposed on an egg prior to its meeting with the sperm.

This slide just shows examples of an egg. Here is a two-cell embryo, starting off perfectly normal except this has multiple nuclei. One of the

features that might be of interest to some in this case, because the issues of chromosomal segregation and additional chromosomal additions from cytoplasmic transfer came up, is the fact that the early human embryo, especially at the one- and two-cell stage, has unique capacity actually to encapsulate individual chromosomes in a nuclear membrane. So, you can get multiple nuclei that occur in these embryos, some of which you can show have one or two chromosomes and others have more, but these eggs tend to be developmentally lethal. So the ectopic transmission of the chromosome may or may not be an important issue in cytoplasmic transfer.

This slide shows an example of an embryo which, as you have heard, is fragmented. You can see some fragments here. The severity differs between embryos within the same cohort so you can have 12 or 15 embryos. Some of them have much more extensive fragmentation, some have none. So, it is an embryo-specific event. Some patients have all their embryos fragment like this, which is relatively rare, but it is common to see fragmentation of this sort.

The problem is, is this rescuable? Is it

a problem in terms of the ability of the embryo to implant? Here again, as Jacques mentioned and others have shown, in fact the fragmentation patterns seen at a static image such as a four-cell stage can change.

So, embryos that had this fragmentation that looked relatively severe early, in fact, go to the blastocyst and hatch and, in fact, implant. I don't show baby pictures but I do show embryo pictures, and this is a little girl that was born about two years ago.

So, we have the situation where we can see dysmorphologies and some may be of clinical significance and others are not. There is recent work that shows that some types of patterns of fragmentation are transient; that they exist in one stage and later on in development seem to disappear. So, it is not clear whether subjective criteria looking at embryos is actually predictive of competence. In some cases, obviously, if there are no cells left that is a problem.

So, you have to look in terms of sort of the molecular mechanisms that take place in eggs where their competence may be affected by influences that they have experienced.

This slide tells us a little something about eggs in terms of mitochondria. What you see here is a pronuclear human egg. These are the two nuclei and these little dots here are mitochondria. I always grew up with the notion that, in fact, the number of mitochondria in human eggs was about 150,000, although that is not based on any morphometric analysis but that is about the right number. All these dots here are, in fact, what we are talking about, mitochondria.

One of the interesting things about mitochondria both in the human and in the mouse, and in other systems as well, is the fact that their distribution is not static, that during different stages of oocyte maturation, especially before the egg comes out of the follicle as well as during embryogenesis, there is a lot of spatial remodeling of the cytoplasm. These mitochondria can move around and have different locations and different positions based on what the cell is doing.

If we look at this slide, it gives you an example of mitochondria in human at the electron microscope level. These guys here, the little dots are at the surface of the cell. It is upside down

but here are the mitochondria. They are fairly unusual when compared to somatic cell mitochondria. These are relatively undeveloped. They are not in a dormant state but developmentally and in terms of their differentiation they are in a more primitive state. But they do move around. During oogenesis for example, in the mouse and other rodents they migrate around the nucleus. You can barely see that here but they do. They form interesting patterns that extend from the plasma membrane down to the nuclear membrane, shown here, almost arrays which we and others have suggested may be important in certain signal transduction pathways. So, they are unusual. Their distribution is not static, and they undergo remodeling as the embryo and oocyte progress.

This slide shows this a little more here, mitochondria at higher magnifications, a two-cell embryo in the human. Their spherical structure is about half a micron in diameter, and they remain this way in a pretty undeveloped state until fairly late in the pre-implantation period as the embryo becomes a blastocyst. Some of these then will start to change into the more orthodox configuration that one sees in somatic cells, but

these are really unique structures.

This slide just shows some rearrangements that occur fairly rapidly during oocyte maturation. This is a mouse oocyte that is stained, the mitochondria are stained with a mitochondrial specific fluorescent probe, and what happens is that as the oocyte matures, in this case in vitro, mitochondria translocate and move around towards the center of the cell around the nuclear region to form a very compact structure here. Then, during the first miosis they start to redistribute themselves and they go back to a more or less uniform distribution at metaphase II, which is when the oocyte is ovulated.

These are dynamic structures. They are dynamic in their orientation and organization, and they undergo spatial remodeling as eggs and embryos divide. This is maybe actually an important feature in determinants of the oocyte's competence while the oocyte is still in the ovary. In other words, how these organelles are located and distributed may actually be fairly important.

Their distribution, shown in this slide, is directed by microtubules in many species, in this case the mouse, and you can see this is the

central region where chromosomes are maturing, oocytes are forming. These are mitochondria that have translocated from around the cytoplasm towards this rim or ring of mitochondria around the nuclear region. Here are microtubules and the mitochondria, we think, migrate as in other cells and are translocated along microtubular paths. So, the organization of the cytoplasm in terms of its microtubular organization may, in fact, be a very important determinant of how mitochondria are distributed, and whether the distribution of mitochondria in space and time, in fact, turns out to be determinant of competence.

This slide shows another example of presumed mitochondrial function, and this has to do with energy. We have heard, and it is true, that energy may not be a critical component of competence because it is clear that while you have mutations in respiratory mitochondria the embryos develop quite normally, otherwise they wouldn't be individuals that carry this particular respiratory mutations in their mitochondria.

In this type of experiment, what we did in the mouse was to knock down mitochondrial respiration substantially and we found that you

could reduce mitochondrial respiration by about 60 percent and still get the eggs to mature normally. They fertilize in vitro, but what is interesting about this particular experiment is the fact that when these embryos reach pre-implantation stages they start to die off. This may or may not be a mitochondrial effect. It may be a downstream toxic effect of this treatment which was done days before at the oocyte level. But the point is that we were able to establish here that, in fact, there was a downstream consequence during embryogenesis, early embryogenesis of knocking down respiration at the beginnings of maturation in vitro which is, in this case the germinal vesicle stage.

This experiment showed that at zero hours in culture knocking down mitochondrial respiration actually had no effect on maturation, which is what would occur in the ovary prior to ovulation, fertilization cleavage but did progressively have effects on the embryo's ability to develop to the blastocyst stage and implant. So, it was an effect that was actually seen four or five days later.

In the case of the human, one of the proposed effects of mitochondria and why would mitochondrial transfer or cytoplasmic transfer if

it involves mitochondria be beneficial? One is ATP generation during pre-compaction stages seems to be respiratorily driven rather than driven by glycolysis. So, the early stages seem to be requiring some level of mitochondrial input. It is not clear in the human whether glycolysis in the presence of mitochondrial defects that affect respiration can be up-regulated to supply enough ATP.

Of course, mitochondrial replication begins after implantation. So the putative effects of mitochondrial dysfunctions that have been suggested, not proven yet but suggested for early human development which may be rescuable is cytochrome C release if perhaps the mitochondria are damaged resulting in apoptosis; reactive oxygen species generation which may be a toxic effect from mitochondrial dysfunction of some sort that hasn't been identified; or low ATP production from metabolically incompetent mitochondria. These have been proposed but not clearly identified.

This slide suggests something that is really quite interesting. This asks the basic question. As I said, I always grew up with the notion that there were about 150,000 mitochondria

and a number of years ago we approached this problem for actually completely different reasons, looking at the question of how many mitochondrial DNA copies were present and we looked at a particular mitochondrial gene at that time using PCR, and we had quite a few oocytes from gift procedures that were left over. One of the things that we saw and tried to quantitate is that the number of copies of this participant mitochondrial gene, in fact, ranged from about 30,000 upwards to--I don't remember the actual number but something like 400,000 or 500,000. We were seeing variations in the number of mitochondrial DNA copies per oocyte within the same patient.

In that particular situation, what we were seeing is almost an order of magnitude difference in the number of mitochondrial DNA copies in oocytes from the same patient. We never did anything with this data because, actually, I simply didn't believe it. I didn't believe that you could get that variability.

But recently work has come out from a number of groups, including Jacques Cohen and others, who have looked at the number of mitochondrial DNA copies, and the number is about

20,000 to over 600,000, 700,000. Now, does that mean that an oocyte that looks the same, that you cannot distinguish at the light microscope level, one from the other, that in one case you have 20,000 mitochondria if there is one mitochondrial DNA copy per egg all the way up to 800,000? Which is a problem because if that is the case, then if there is one mitochondrial DNA copy per mitochondria you are dealing with eggs that look identical at the light microscope level from the same patient, whether it is a patient or a donor, where the number of mitochondria can differ by an order of magnitude?

If that is the case, then going into an egg with a pipet and removing cytoplasm could be problematic because you cannot make the assumption that the number of mitochondria that are being transferred are the same. In other words, from egg to egg or from patient to patient. That is a real issue and that has to be addressed.

So, it looks like the number of mitochondria, in fact, seem to vary, at least mitochondrial DNA copy number almost by an order of magnitude and that is not predictable by any morphology or by any light microscopic inspection.

So, this is a problem, potentially. It is surprising in the human, but it may not be so surprising as I will show you in the next slide.

This slide basically shows a picture of an egg, and this is just stained for mitochondria and, again, here we see some interesting differences. In this particular egg, and these eggs are from the same patient, pretty much the fluorescence is uniformly distributed, very little in this case in the polar body but pretty well uniformly distributed, and we can quantitate and do all sorts of interesting measurements about the fluorescence intensity and correlate this with mitochondrial numbers, the point being that here is one egg that is stained.

The next egg from that same patient shows something a little bit different. This is not an artifact of the procedure or the staining. These are live eggs. What has happened here is that, in fact, there are regions of this particular cytoplasm where mitochondria are absent. This is something that we consistently see looking at eggs, that you have regional differentiation and regional specialization of mitochondrial distributions that are not predicted by any other means, other than

this. So, you cannot say that going into this particular region of the cytoplasm will produce an equivalent number going into this region of the cytoplasm. So, now we have the further complexity of having perhaps a difference in an order of magnitude or certainly mitochondrial DNA numbers and now we have regional specializations in terms of distribution within the cytoplasm that is not predicted by just looking at morphology.

This slide shows another example of this where, in fact, the relative fluorescence intensity is quite reduced. So, there may be something to correlating fluorescence intensity by this method and mitochondrial DNA numbers, except that in order to do that you have to destroy the egg, which means it is not very useful other than for experimental purposes.

This slide shows something about energy distributions in human eggs. This is some old data that we published a number of years ago. It simply asks a basic question, what is the ATP content of eggs in the same cohort? A very simple-minded question. Just look at the distribution. It is quite remarkable. These are eggs that were gotten by stimulation for IVF in the same way we normally

do it, and the distribution was over an order of magnitude. Again, this was one of these puzzling findings, except that in terms of outcome, when we had eggs that were left over, excess donated, that were in the high range of ATP content, those tended to be the women that got pregnant from embryos that were transferred in their cycles. Those that had a preponderance of low ATP content eggs, when we transferred their embryos, even though they were morphologically identical to ones from high ATP cohorts, in fact they rarely got pregnant.

So, here you have a spectrum of an order of magnitude difference in ATP content, and both differences within cohorts and between cohorts of patients. So, in this case we now have the complexity of saying we now know that not only do we have a huge variability in mitochondrial DNA content, we may have a mitochondrial numbers variability in terms of how actual mitochondria are in an egg, which is not detectable just by looking at it, and now we have energy differences that may be related either to mitochondria numbers or to something else that is going on in these particular cells.

So, it is not just simple to say that, in

fact, when you have a mitochondrial basis for certain types of infertility that, in fact, it is related strictly to mitochondria because there are too many complex, confounding issues with mitochondria alone that are important.

This slide just sort of summarizes this. The size of the mitochondrial complement, how many mitochondria really are there? We really don't know how mitochondria there are in human oocyte. The variability in mitochondrial DNA content is important, but how does it relate to the size of the complement? And, is the size of the complement actually important? Differential spatial distribution at the pronuclear state, and I will talk a little bit about that, and disproportionate inheritance during cleavage, which is another issue in terms of how we understand the relationship between mitochondria, if any, and development.

This is shown on this slide. I think I will just pass this one up. Here, we started looking at how mitochondria are spatial distributed within the egg and in the early embryo. This is one of the earlier pictures that we have seen from looking at an analysis of the mitochondrial distribution. Here are the two pronuclei, one here

and one there. Here is the mitochondria around it. What you see here is the relative intensity of how many mitochondria are present, but what is particularly interesting about this guy is the fact that the mitochondria are asymmetrically distributed in the pronuclear stage. This is just before cell division.

So, we followed this along in quite a few embryos from the pronuclear stage onward. I will just summarize the results. This basically says that you have symmetrical and asymmetrical distributions. Here are mitochondria around the pronuclei from the one-cell stage, in a cross section. The point being that the segregation, at least the inheritance of the mitochondria at the one-cell stage, the pattern or the spatial distribution at the one-cell stage determines in large measure the proportion of mitochondria that are distributed at the first cell division in the human.

So, we follow this along and we see embryos that have fairly good and equivalent segregation, others where the segregation is disproportionate. We can do this both by looking at mitochondrial DNA copy numbers as well as by

metabolism.

Just to show you some examples of that, here you have relatively unusual segregation. Again, all of these were first examined at the pronuclear stage, the one-cell stage, and then subsequently. What we found is that you can have different distributions. For example, a normal appearing embryo, absolutely normal appearing, can have some cells where you have relatively high, relatively moderate and relatively low inheritance. We can, again, quantify this in a number of ways reflect the intensity of fluorescence. At the eight-cell stage in perfectly normal embryo you have some cells that have relatively few mitochondria, others that have inherited quite a few.

The consequence of this is that cells that have under-representation of mitochondria tend to die. They tend to divide more slowly, which may be what Jacques Cohen described as the slowly dividing embryos but, nevertheless, if there are enough cells that have inherited a fairly reasonable amount or close to normal amounts, the embryo is still competent.

So here, just the organization of

mitochondria and their distribution can have profound effects on embryo development and competence, and that is shown on this slide, where you have, for example, blastomeres of an eight-cell stage, where you have mitochondria that are relatively evenly distributed. So, here we have relatively normal, even distribution.

This slide shows examples where that distribution actually is quite asymmetric, again, traceable back to the one-cell stage leaving several cells that are deficient. These cells eventually lyse and disappear. Other cells that are deficient, such as this one, simply don't divide again and remain in that position.

So, not only do we have the situation where we have differences in mitochondrial number initially present in the oocyte, but now we also have the complexity of how these mitochondria are distributed at cell division, which is not necessarily uniform. It is not an equivalent distribution.

This slide just simply shows the basis of this, and we think a lot of this has to do with microtubules. These are mitochondria that you can see. Most eggs and embryos will slide along

microtubular tracks. It is the position of the microtubules and their organization, both at the one-cell level and multi-cell level, that we think determines the proportion or uniqueness of segregation whether it is even or disproportionate among blastomeres.

This slide is an example of what is called a central zonal defect. What has happened here is that you normally see mitochondrial clustering around microtubules. In this case there are no microtubules because he has a central zonal defect and there is no migration of the mitochondria.

This comes to another point, that I will end with, and that has to do with the notion of cytoplasmic transfer. We have talked about and published work on mitochondrial transfusions, going from one oocyte to another and I just want to show you some of the complications that come in with this type of approach to cytoplasmic transfer, something that needs also to be considered.

In this method what we have done, we have segregated pretty much all the mitochondria into one compartment. This was an original oocyte where you can see one compartment here. This contains DNA and here are the mitochondria.

This slide shows a different method. Here is a cytoblast. Here is the nucleoblast which is very, very efficient in mitochondria. This is very heavy. So, we did a number of experiments, taking by micropipet, mitochondria from this enriched fraction and asking a very simple question, what happened to it.

That is shown in the next series of slides. Here what you see is the case of putting mitochondria that are labeled into a germinal vesicle stage oocyte, and this cloud material, here, is about five to ten hours after mitochondria were injected as a bolus, right around here. This is stained both for mitochondria and nuclear DNA. This is the nucleus of the germinal vesicle and this was, with think, the injected mitochondria.

This is shown in other slides. This slide shows different variations. Here is an oocyte injected at an earlier stage of maturation, after the germinal vesicle. These are the labeled mitochondria and, in fact, some of those mitochondria have gotten quite heavily into the first polar body. So, this shows that, yes, you can inject mitochondria and many hours later you can detect them and they seem to be pretty well

segregated or at least spatially oriented in a sort of uniform manner, except it again is egg specific.

So, if we look at this slide, it just shows another example where mitochondria were placed in the center of the egg. These are stained mitochondria so the resident mitochondria are not visible. In this case, here is a polar body but there was virtually no detectable segregation of mitochondria into this polar body. So, sometimes they are lost; sometimes they are not. But in most cases they seem to sort of evenly distribute when injected early in the maturation phase, that is, well before the time that we would consider doing this in the human, which is after ovulation where the egg is mature.

Now, if we inject mature eggs, here is the issue. These are metaphase II eggs. In this case, what has been done is to inject mitochondria in different places, here, here and here, and watch what happens. In fact, in some cases the mitochondria simply stay in one position. There is no spatial remodeling or redistribution. If we activate these eggs, not by fertilization but so that they divide, in fact, the segregation is entirely asymmetric. One cell will have a fairly

substantial, disproportionately high distribution of the injected mitochondria, others will not.

Here is another example of this. Here you can see three zones of mitochondria that were injected at the metaphase II stage and they stayed in place. They did not move in this particular egg.

This shows another example where actually they did move. Here we put mitochondria in the center and a little bit later there were, in fact, a lot in the center but they had actually migrated to the cortex of the egg as well.

This slide shows another pattern where they were injected in the subcortical location and pretty much stayed there. Again, when you activate these eggs you get unequal segregation. We have not yet seen in any of our eggs that we have examined by this method of injection equivalent segregation. It is all asymmetrical, which is a problem in terms of how mitochondria may, in fact, find their way into one-cell lineage or placenta or perhaps different tissues in the fetus.

I just want to end, if I have two more minutes, and I just want to talk about one potential other function of mitochondria early in

development that has very little to do with metabolism. This has to do with the notion of involvement in calcium signaling or in ionic signaling.

This is a human egg that is stained with a probe that picks up mitochondria that are high polarized. These are mitochondria that have a high membrane potential. We think these are actively involved in other cells in calcium signaling. What you are seeing here are these little dots or the high polarized mitochondria. They are at the cortex. What we think happens is that at fertilization these mitochondria participate in an important way in calcium modulation.

Shown in this slide is that when we actually activate these eggs, in fact you get a very early calcium discharge which we have now been able to show comes from those mitochondria. We think this discharge is actually very important in terms of subsequent signal transduction pathways that occur later on in development, which are required for normal gene activation and normal development.

This slide shows an example--well, you can't see it but there are very few asymmetric high

polarized mitochondria. When we activate this egg, we see the following, which simply shows that, in fact, the signaling is restricted to one part of the egg.

This slide shows where, in fact, there are no detectable, in the egg, high polarized mitochondria. They are only found in the polar body. When we activate these eggs we get nothing.

So, in addition to metabolic and in addition to other functions that these mitochondria may have, they also appear to be involved in early events in calcium signaling which we think actually turn out to be important in setting up the right signaling transduction pathways in the cytoplasm as the egg and embryo develops. It is an influence on the normality of development. So, there are a number of different functions that these organelles are involved in, other perhaps than metabolic, which are important in competence determination. Thank you.

Question and Answer

DR. SALOMON: Thank you very much for a very interesting topic. We should have a discussion of sort of mitochondria per se for a few minutes. As a scientist, I have fifty questions

here that are just about mitochondria, but you don't need to waste your valuable time answering those. It is obviously a fascinating area.

There are a number of questions that specifically relate to the issues on the table today. So, just to kind of start, one of the things I heard was that this is pretty safe because there is a very high threshold for dysfunctional mitochondria and that would be a safety feature. I am just trying to get little key things here, but that is something I got.

DR. SHOUBRIDGE: Yes, I think that is true. The other safety feature in the animal experiments that we have, we really haven't seen any evidence that the animals are sick in any way. We haven't done careful studies in histopathological things, behavioral tests or anything, but we have had this colony since 1995, a colony of heteroplasmic animals, and done all these kinds of different genetic experiments and different back crosses or half a dozen other nuclear backgrounds and we have really never seen anything unusual. I mean, we haven't been looking for it either so we haven't done a careful analysis but the mice look pretty normal.

DR. SALOMON: Good.

DR. CASPER: From the other point of view then, it was suggested earlier this morning that you might be able to treat mitochondrial diseases by mitochondrial transfer. In view of the stochastic segregation that happens, is that possible to actually happen from generation to generation, or would it only be feasible in the actual injected offspring?

DR. SHOUBRIDGE: I am not quite sure I understand the question. The transmission is stochastic so if you look, for instance, at the distribution of mutant mitochondrial DNA in the ovary of the woman where about 50 or 60 oocytes are available, some of them have no mitochondrial DNA mutations at all. So, in that case, I think if you were going to treat the disease, the best option would be to look for an oocyte that didn't have any mitochondrial DNA mutations at all. If you were to completely remove that cytoplasm and then put in donor cytoplasm, the prediction would be that to the extent that you left the recipient cytoplasm in there you would get the same kind of stochastic transmission to the next generation. But having taken out most of it, the chances are that the

child, if it developed in that egg, would have mostly donor mitochondrial DNA and not very many from mom, but in the next generation in the female that would segregate.

DR. CASPER: In other words, if you had an embryo that would be heteroplasmic with a mutation as well as normal mitochondrial DNA, you could change the threshold by putting in more normal mitochondria. Is that right?

DR. SHOUBRIDGE: Probably, yes. It is simply stochastic; it is a numbers game so you can treat this as a bowl of marbles, black and white marbles. The sample size with determine the rate of segregation. So, if you actually do the statistic, you can calculate in the mice under a particular model the effective number of segregating units as about 200 in the next generation, and you can figure out, given the sample size of 200, that you would get distributions like I showed you.

DR. MULLIGAN: On that point, if you had diseased mitochondria that behaves like whatever the mouse strain, wouldn't that be a way of actually promoting disease because you would actually over a time course--I mean, if you were so

unlucky that the diseased mitochondria also had the same property that is the property that allows you to selectively reconstitute cells, wouldn't that be a way to amplify that?

DR. SHOUBRIDGE: Absolutely, and that is probably what happens in many of the diseases, probably not all, but there is pretty good evidence for directional increases in the mutant mitochondrial DNAs in many diseases, in muscle tissue for instance. The idea there is that the muscle cell is continuously reading out the oxidative phosphorylation capacity. So, if you decided you wanted to be a marathon runner tomorrow--maybe you are today, I don't know--but if you wanted to be, you can up-regulate the number of mitochondria in your post-mitotic muscle cells. We don't really understand the nature of the signals that are involved in that pathway. Then, if you decide you don't want to run a race it will go down.

The thinking is, at least my thinking on this is that the selection of that occurs at the level of organelles. So, some signals are given at the organelles and that somehow feeds back to the nucleus. Factors are produced to give you more

mitochondria. If you now have an organelle that has mutant mitochondrial DNA the same signals go back. It looks like overworked mitochondria. It looks like it is running a marathon. In fact, it is just the mutation. The nucleus doesn't know that. What it does is make more of those guys and so it makes more of the bad ones. So, there is kind of a positive feedback loop. It doesn't seem to happen in the context of every mutation so it isn't a completely general phenomenon, but it certainly could be a problem.

DR. MULLIGAN: In this concept of loading with an excess of certain type, what is the role of the decay of the existing mitochondria? That is, in principle, there is a competition and there is a fixed number of mitochondria that should be in this particular kind of cell, then whatever determines that number presumably influences the decay characteristics of the mitochondria. So, if the cell only usually has X number and you put in 10 X, presumably for it to refix itself there has to be loss of some mitochondria.

DR. SHOUBRIDGE: Nothing, virtually nothing is known about mitochondrial turnover.

DR. SCHON: Maybe the definition of the

word mitochondria needs to be expanded a little. What we do know is that cells control the mass of mitochondrial DNA. That is what is being regulated, and it is controlled rather well. The number of organelles that enclose those DNAs is what we don't know. But since it is a completely dynamic system, at two o'clock in the afternoon you can have a thousand organelles and at 3:30 you could have two hundred merely because they are fusing and then they are repartitioning. So, it may not be that useful for this discussion to talk about organelles per se, although I agree 100 percent, I think selection is at the level of the organelle, not at the level of the DNA.

I would like to amplify a little bit about the tissue specificity. Bioenergetics probably play some role in the distribution and the amplification but it can't be everything, and I will give you two examples.

There is a disease caused by deletions of mitochondrial DNA. Invariably the deletions pile up, among other places besides muscle, in the choroid plexus of the brain and in the dentate nucleus of the cerebellum more than they do in, let's say, in the epithelium of the ventricles, and

we have no idea why that is but there is predilection. There is some signal that is going back and forth that is operating. It is hard to see how it is operating at the level of the genome but it is a genome-specific effect.

DR. SHOUBRIDGE: There is one thing to add. Even though it is true in cells in culture that the regulating mitochondrial DNA mass seems to be the signal, but obviously it is not happening in pathology because there is dysregulation in muscle cells. There can be 50 or 100 times more mitochondrial DNAs in a segment of a muscle fiber than normal. So, there is some feedback that is due to the presence of the mutation, presumably, which dysregulates that.

DR. MULLIGAN: When the DNA replicates, what is the organelle's status?

DR. SCHON: I am not understanding the question really.

DR. MULLIGAN: Does the DNA replicate within an otherwise intact organelle? Or, is it compromised or changed in shape in some fashion?

DR. SCHON: We don't know anything about it. It just happens.

DR. SALOMON: Remember, what I want to

focus you guys on is what about all of this relates back to the safety and to the kinds of biological questions that these guys in the IVF field are going to face in developing an IND? I think they will be happy to say that they will screen patient donors for mitochondrial disease, which they have admitted they haven't done up until now, but if they do that, then one is assuming we are transferring normal mitochondria and, therefore, if they add that one little piece it seems like they will substantially remove this as a safety issue, and the fact that there is this high threshold anyway would seem to even enhance that.

So, that is all good news for them in terms of safety issues. What I want to make sure though is, as we go around here, that there aren't other issues that they need to address.

DR. VAN BLERKOM: So, maybe the first question is, from what we have heard so far, is there any evidence that mitochondria are rescuing these eggs to begin with?

DR. SALOMON: Right. I was listening to you and the one question I wrote down, and I am going to put it to you now--I wrote down first any specific measure of oocyte mitochondrial function

that compares good or normal oocytes to those from infertile females. You then launched into an ATP content slide and made one comment, which I thought was at least partially addressing this, and that is that there seems to be some correlation. Now, how much of that was hand waving and how much of that was stuff that would really stand up to statistical analysis?

DR. VAN BLERKOM: First of all, that was published stuff and it was actually statistically analyzed so it wasn't hand waving. But the point is that at the time it was done there was a relatively limited number of patients. We had 30 or 40 in that group. But there was no explanation for why those differences existed because, again, these were analyzed at the same time, from the same patients, so there was no culture artifact or anything of that sort.

Now, with the notion that you have differences in mitochondrial DNA copy numbers that can be an order of magnitude, the question then comes are the ones that are the low ATP producers low ATP because they had, for some reason, inherited a low number of mitochondria? What the metabolic experiment showed was that, in fact, to

make an egg and to make an embryo you don't need a lot of mitochondria, functional mitochondria. It may be that at later stages at some point you do, but the number of mitochondria that are being injected is so small, and since they are not replicating, it is hard to imagine that if you are starting out with an egg that is below a certain threshold to get a normal embryo through the first four or five days of development you need 150,000 and you put an extra 10,000 in, it is hard to imagine that that is going to make a difference.

So, numerically it doesn't make sense. I think there are eggs that fall away in terms of natural developmental failure, perhaps their inheritance of mitochondria is very low for whatever reason. But, you see, those are gone anyway. They are not going to be rescued.

DR. SALOMON: Can I follow-up on that? Actually, another question I wrote down was just what you said. I am still confused here. So, the question I wrote down is why are there so many mitochondria in an oocyte, 100,000, as compared to a somatic cell--

DR. VAN BLERKOM: Because they are replicating until after implantation.

DR. SALOMON: So you think they need all these in order to survive?

DR. VAN BLERKOM: I mean, that is it. I mean, all mitochondria come from that. All the mitochondria that are present as the cell divides and parceled out come from that initial population.

DR. SALOMON: So, you think there has to be this big reservoir of mitochondria and then, as you go to eight and twelve and so many cells, you start distributing around and you get down to what a normal somatic cell has. So, that is a real simple explanation like that. Does anyone know what the function of the 100,000 mitochondria--it is obviously not 100,000 times the ATP reservoir of a somatic cell. Is that right?

DR. VAN BLERKOM: Well, they are involved in APTP product. They seem also to be involved in calcium signaling in the cell. They also seem to be involved in other functions that are not necessarily metabolic. They redistribute themselves, as I showed, in terms of spatial remodeling, presumably for ionic purposes or energy purposes, early in the division. But you are dealing with a very big cell. I mean, this is a 100 micron cell. So, I don't know why whoever put

in 100,000 or whatever mitochondria decided that was an important number, but it probably was an important number in terms of the reservoir that exists for later on. It is probably an over-capacity or redundancy in terms of development because you can knock out function for a fairly substantial proportion of those mitochondria and the egg still divides.

DR. SCHON: We shouldn't have tunnel vision here. The mitochondria is not synonymous for ATP production. There are TCA cycles, steroid oogenesis, beta oxidation, amino acid synthesis, and on and on and on, especially steroids for oocytes. You might need 100,000 just to partition out little molecules that are important for this egg, and that could be the end of it, and the ATP goes to sleep because you don't need it until down the road, and that is the simple answer.

DR. SALOMON: I guess you guys see where I am going with this. I am asking the question how can you construct a rational series of experiments even to test the hypothesis that injecting the extra mitochondria from the good eggs into the bad eggs, if you will allow me to be that simplistic, is doing anything here? You are injecting 10,000

to 20,000, but the point is that if you don't know what it is about the function of 100,000, what do you measure? So, can we even think of a way to compare these, or is this really possible right now?

DR. SCHON: I don't think this is the venue for experimental design. Having said that, if you want to test whether ATP production had an impact, there is a line of cells that make no ATP; they are otherwise normal and you can inject those. It is not an easy experiment but it can be done. I am not sure what you would learn from such a thing however, to be honest. It goes back to the issue of what I said before. This is a multi-level interacting system and checking one at a time may or may not give an answer, and I don't know how to interpret it.

DR. SHOUBRIDGE: There is an experiment you could do but it is an inhibitor experiment, and they are all inherently dirty, but there are some dyes that irreversibly knock out mitochondria, like rhodamine 6G for instance, so you could treat your extract with rhodamine 6G, kill the mito's and inject the ooplasm and see if you got the same rescue. So, I mean, it can be approached this way.

I prefer to do things genetically because I think it is a little tidier, but there are ways to do that genetically--not ways to do that experiment but I can think of a lot of genetic experiments that would test the notion that you need that many.

I personally think, and this may be an extreme view, that you just need them to parcel them out. So, if you look at the mitochondria at the egg level, morphologically the look like mitochondria in the rozero cells that Eric was talking about that have no mitochondrial DNA. They look like inactive or dead mitochondria and I think it is just a mechanism to hand them out to the descendants in a system, for whatever reason, where there is no mitochondrial replication.

DR. MOOS: A couple of things, just a quick, offhand comment although we are not going to get into details of experimental design, if we generate some good ideas for experiments that we should all be thinking about, that is a great outcome for this meeting.

I too was struck by the ATP slide, not necessarily because it might all by itself be definitive but there is a hint there perhaps of something that we can use. So, I am curious

whether what was done was simply to measure total ATP content, or whether P31 NMR to look at energy charge, or techniques to look at metabolism either have been or might be considered because the other thing that needs to be kept in mind is that the oocyte is not a bag of stuff that is mixed isotopically and, indeed, there might be extremely rapid turnover of nucleotides tightly localized in particular regions that, you know, some high-powered analytical biochemistry might be used to address. That would then give us the beginnings of something that we can use to look at the process and keep it characterized and controlled.

DR. VAN BLERKOM: Can I answer that? That was total ATP measurements, but you are right about micro-compartmentalization of ATP. It turns out to be really important in terms of cell function, and I don't know how you would actually study that--oh, he does; he is smarter!

The issue is that you want to keep these things alive and actually do something to them functionally afterwards rather than just looking at them in static.

DR. MOOS: Sure. There are two tiers. There is the investigative tier and that is

separate from a QA sort of tier.

DR. VAN BLERKOM: Right.

DR. SALOMON: Dr. Casper?

DR. CASPER: Coming back to the point of why maybe just injecting 10,000 mitochondria would be helpful, from the clinical point of view, we have been discussing patients who make fragmented embryos and trying to rescue those fragmented embryos, there is some data that embryo fragmentation may be related to apoptosis or programmed cell death sort of issue. We have actually shown that cell death gene transcription does increase with increasing embryo fragmentation.

Nobody has mentioned so far that mitochondria actually have Bcl-2 family member proteins associated with them. So, one of the issues may well be that we are injecting enough mitochondria that we are adding some cell death suppressors, enough to sort of inhibit or antagonize cell death genes that could be turned on abnormally in some of these embryos.

DR. SALOMON: That is really interesting. The problem with that is that at least our current understanding of this is that these are occurring at the mitochondrial cell surface itself. It would

be an interesting concept to set up competition with controlling caspase activation at the native mitochondria by injecting new mitochondria because these proteins are not necessarily translocating to new mitochondria in the process.

DR. CASPER: No, but they wouldn't translocate. You are putting them in right at the time of fertilization, so very early on in the process. It could be controlled by the nucleus of the cell. You may just have to get the embryo past a certain stage so mitochondria can replicate and make more of its own protective proteins.

DR. SALOMON: Dr. Naviaux and then Dr. Rao.

DR. NAVIAUX: There is a dynamic interplay in bioenergetics. There are two ways that the cell can produce ATP and, because of the interplay where we started to get some understanding of that, actually in the last century when Pasteur, you know, defined the suppression of glycolysis by oxygen and later, around 1927 a biochemist, Crabtree, defined the suppression of oxidase phosphorylation by glucose. Traditionally, when you try to measure the contributions of glycolytic and ox phos pathways to overall ATP synthesis, you

do it under laboratory conditions of ambient oxygen, let's say, at 20 percent. But the female reproductive tract, of course, is one of the most anaerobic environments in the human body and low oxygen tension actually does alter the relative contributions of bioenergetics available to the egg, particularly before implantation and the blood supply is established.

There are some early experiments that look at radiolabeled glucose and its oxidation to either lactate of 14-labeled CO2, and in early embryos a very large proportion, exceeding 80 percent of the carbon, can come out as 14C-labeled lactate as opposed to 14C-labeled CO2, emphasizing the importance of glycolysis in bioenergetics of embryos at least at an early stage.

DR. SALOMON: Dr. Rao and then Dr. Murray.

DR. RAO: I want to try and take off from what you just said about rather than looking at experiments to see what we can take home from here in terms of application, and there are two issues that struck me from the points you made. Does this tell us anything about the reproducibility of taking ooplasm at any site? Should one suggest a particular site, or does it tell you that there is

going to be so much variability that you have no predictive power at all?

The second thing is does this tell you about selection of the donor oocyte or the recipient oocyte in any fashion in terms of doing this?

Lastly, if one assumes that mitochondria can play an important role in signaling, then does this tell us that even the small number that you place, because of patterns of signaling which are critical in terms of dynamism in this thing, that small number can be quite critical and, therefore, where you place them might be very important as well? If anybody can comment on the specifications?

DR. WILLADSEN: I am Steen Willadsen, from St. Barnabas. First of all, I think I should tell you a little bit about the historical start of this. We weren't concerned about mitochondria specifically, and I think that in a way we are now barking up the wrong tree with the wrong dog.

Obviously, this committee is concerned because there is DNA being transferred. That was not our primary concern. It would be very easy, I think, to design experiments where no mitochondria

were transferred. In fact, we don't even know that the mitochondria that are in the egg have any particular function at the time. As was pointed out by one of the speakers, they are probably useful for making the egg, which is a very specialized cell. So, I think the real issue with the mitochondria in this context is are they dangerous and how the egg otherwise gets along. I think it is wrong to focus so completely on the mitochondria because they can very easily be brought out of the picture. Then, where would the FDA be?

The second thing is that obviously when you look at these risks, and I think I will say at this point if you look at the risks, I can only speak from the basis of the evidence that I have some insight into, the major risk if you enter as a patient into this program is that you could get pregnant. That is the major risk. Whether you would like to say that this because it is a treatment or whether you say it is because of the place, it is a big risk if you go into the program because 40 percent of the patients got pregnant. Thank you.

DR. RAO: Can I respond to that?

DR. SALOMON: Okay, but I think what we have to realize here is that what we are doing right this second is focusing on the mitochondria. It doesn't mean that we will end the day focusing on it, it is just that we are following a discussion of two very, you know, high level professors telling us about mitochondria. So, I think it is very appropriate right this minute to be focusing on the mitochondria. But I think that to think of this in context, to be reminded that we have to put it in context is perfectly fair, and I think we will have to come back to it because you articulated some of the issues we are going to have to deal with in about half an hour. But in that context, it is okay. I just don't think we have to defend why we are talking about mitochondria right now. I think that is what we are supposed to be doing.

DR. SCHON: This is not really in the realm of safety but I would just like to bring it to the floor. The transfer of ooplasm means the transfer of mitochondria right now, unless the protocol is changed. So, I would like to spend just a couple of minutes talking about the evolutionary implications of this, not safety, not

viability.

It comes to the heart of why nature invented maternal inheritance in the first place. So, why is that? In fact, nobody really knows but the most reasonable answer is the same reason why nature invented sex, and it comes down to something Muller's ratchet which in economics would be called Gresham's law--all things being equal, things go from bad to worse. I think that would be the best way to describe Muller's ratchet.

So, if you had clonal expansions of DNAs that were going to their progeny, eventually they would call up mutations and wipe out that organism in evolutionary time. So, sex was invented to erase that--well, that is a little bald statement there. That is part of the reason I think sex was invented, to help accommodate, to deal with those kinds of mutations.

Now, when you have an organelle that is present not at one or two copies per cell but at thousands, it is very difficult to deal with that kind of a problem of Muller's ratchet where, if a mutation arises, it just naturally will spread through the population, as you saw so dramatically. So, what appears to have happened is that maternal

inheritance came around so that when mutations arose you shut them down. In fact, when we look at pedigrees with real diseases, first of all, the pedigrees are short, meaning they go from great-grandmother to proband and might go one more generation and then, like a light going out, that pedigree is extinguished carrying that mutation. That is what is really going on.

That mutation only passes through the maternal line and goes nowhere else. So, all mitochondrial mutations that we study are really only a few hundred years old, if you will, or less in time. They come on and they go out.

So, what does this have to do with ooplasmic transfer? So, now we are taking oocytes, ooplasm containing mitochondrial haplotype A and sticking it into a recipient cell with mitochondrial haplotype B. This is lateral genetic transfer. All right? We haven't eliminated Muller's ratchet but we haven't made things that much better either because now you are putting in a new genotype from this pedigree into a new pedigree. If you do this with one person, two people, ten people, a hundred people it is probably irrelevant. But if you start doing this with tens

of thousands of people--I don't expect this ever to happen at that scale but it is something just to think about--y are now transferring mitochondrial genotypes horizontally through the population that otherwise would never have been transferred because they all pass vertically. That is the only point I am trying to make. I can't quantitate the impact of this, it is just a fact.

DR. MURRAY: This will be a question for Dr. Van Blerkom. Thanks to both speakers. Fascinating, I have learned a lot from both presentations. I am going to focus on one thing which we may actually be able to put aside, but one of the striking things in your presentation was the information about the dynamic patterning and remodeling of the location of mitochondria in the egg. You showed us some slides of how that might affect calcium ion transport, and the like. Is there any reason to think that the injection of another 10,000, a bolus of cytoplasm with 10,000 mitochondria in some particular site in the egg would be either readily integrated and made to dance the same way as the native ones, or might there be some disruption of, say, fine structure of transport structures, the architecture within the

cell that might make it more difficult? One, is this important enough to worry about? Two, are there ways to sort of answer that question?

DR. VAN BLERKOM: I don't think I have an answer for that, except to say that the work we have done with regard to mitochondrial transfer indicates that you can't predict how they we dance. In some eggs they will remain where you place them as the cells divide; in others there is a more pronounced distribution. So, that is the level of predictability, which is a problem.

As far as interrupting, I don't get the sense that the amount of cytoplasm that is put in and the number of mitochondria that are transferred is actually significant in terms of disrupting any of the normal cell functions or even contributing to them, for that matter.

DR. MURRAY: You don't think it makes a difference?

DR. VAN BLERKOM: I don't think it makes a difference.

DR. MULLIGAN: Is there anything that aggregates the mitochondria or keeps them in any constrained fashion that, upon transfer--this is kind of a similar question to what Tom was asking,

that is, some cytoskeletal structure that you transfer like a precipitative mitochondria?

DR. VAN BLERKOM: I think Jacques actually alluded to this when he spoke about differences in the cytoplasmic texture, and we have to think in terms of the human and our experience, those of us who have experience in working with human eggs, is that even with the standard ICSI procedure eggs differ substantially in how they receive sperm, how the cytoplasm is withdrawn, the viscosity of the cytoplasm, and you can actually see this as you do it. I have seen this many times. I think the situation that Jacques has described, where you have different cytoplasmic textures and you can actually see in his cytoplasmic transfer studies the cytoplasm that is injected in some eggs but not in others, I think indicates why in some cases when you put in a bolus of mitochondria or a bolus or cytoplasm they remain fixed in position and in other cases they are more diffuse. I think you cannot predict that. I don't think you want to relax the cytoplasm by treating it with drugs so that you have some sort of uniform distribution or some controllable distribution.

DR. MULLIGAN: Can you alter the viscosity

or whatever you want to call it--

DR. VAN BLERKOM: In a sense you can relax the cytoplasm. It usually requires treatment with some relaxant drugs that will relax cytoarchitectural components. I don't think you want to do that in clinical IVF. The problem in the cytoplasm injection is that you have already injected the cytoplasm and now you discover that, in fact, the recipient egg has, let's say, a particular viscosity where the cytoplasm remains intact in one position. Maybe those type of studies will be useful to determine whether or not the mitochondria remain fixed or not as a prelude to a clinical trial. But they are differences that are egg specific. They are hard to predict and what I tried to emphasize is that just by looking at an egg you really can't tell.

DR. SALOMON: Dr. Hursh and then Dr. Sausville. Then what I would like to do is move on to Dr. Knowles, only because I am just trying to have some time at the end.

DR. MALTER: Very brief?

DR. SALOMON: Yes, sure.

DR. MALTER: I am Henry Malter, from St. Barnabas. Jonathan, the experience you showed,

what exactly did you do? Was that where you were isolating essentially mitochondria in part of the cytoplasm and taking it from there?

DR. VAN BLERKOM: The experiments I showed were not cytoplasmic injections. These were procedure where we have actually compartmentalized the mitochondria and then took mitochondria in relatively small drops, smaller than you would actually use in a cytoplasmic transfer, and actually deposited it into the egg. So, those were enriched mitochondrial fractions.

DR. MALTER: I just wanted to remind of some images that actually Jacques showed because we have done this as well. In fact, we have done it with spare human material and it is essentially duplicating exactly what is done during the clinical cytoplasmic transfer material, loading an egg with labeled mitochondria and injecting them. Those were not extensive experiments but we never saw that just sitting in one place. Basically, you showed right after injection you can see this bolus, this red image in part of the cytoplasm and then, as development proceeded, they just essentially seemed to disperse and it was just variable. You would see it in some blastomeres.

DR. VAN BLERKOM: So, these were fertilized eggs after injection?

DR. MALTER: Yes.

DR. HURSH: This question is for Dr. Shoubridge. You don't feel that heteroplasmy itself is a problem, but if there was a situation where the mitochondria became asymmetrically distributed so you had one, say, organ that was primarily donor mitochondria could you foresee any problems with that mitochondria with a disconnect with the nucleus in any way? Would that be a safety consideration that we need to be considering?

DR. SHOUBRIDGE: Our data would suggest that it is not a big problem, but I don't think you can rule it out because, I mean, what happens biologically is that every time you have a child, of course, the father's nuclear DNA is introduced. So, now that nuclear DNA is introduced to mitochondrial DNA that it has never seen and the mother's genome has seen that mitochondrial DNA. So, it is a natural process for new nuclear genes to be introduced into mitochondrial DNA genes to dance with them and they have never danced with them before, to follow the dancing analogy. But in

the case of our mice, of course, that is exactly what we have, we have complete fixation of a donor genotype in the liver. In that case it doesn't seem to produce any particular phenotype that we can recognize but we haven't done any liver function tests. The mice seem to be pretty normal, but I don't think you can rule it out.

DR. SAUSVILLE: So, this question's last comment sort of follows along on that. First of all, I want to thank both of the speakers this afternoon because I think they have put, at least for me, a lot of the biological issues somewhat in greater perspective.

But, I guess, addressing one of the other major concerns that goes into the IND and, again, this is somewhat to what Dr. Hursh's question alludes to, is the issue of safety. I seem to be hearing that if one looks to safety either from the implications for the recipient, the organism who receives it, the mouse experiments don't suggest that there is a tremendously great effect for having radically different mitochondrial genomes and, moreover, do suggest that if there were to be a bad different you would have to have an enormous amount of penetration in one participant organ.

Then, the comment that you made subsequently is that if one looks at safety from the standpoint of evolutionary safety, at one level you could construe that as an argument that the mechanism is designed to keep itself safe because it is going to extinguish itself within a very few generations and you would have to posit that if this were a threat to our collective genomes you would have to have a succession of almost continued maintenance through some sort of artificial system.

So, I guess quite apart from the issue of whether mitochondria really do anything for you or whether, indeed, the cytoplasm does anything for you, my initial reaction to this is that it is hard to make the case that the procedure appears unsafe, at least from the standpoint of mitochondrial related matters.

DR. SALOMON: Yes, I think I was earlier saying the same thing in another way, that it seems like with the threshold issue there is a lot of safety.

DR. SHOUBRIDGE: I guess the only thing I would add there is that the slight caution is that because we know there are mechanisms that increase the proportion of bad guys in cells from patients

who have disease, if you unwittingly put in something from an individual that is below the threshold you could select for it in a tissue-specific way. I think that may be a very small risk but I don't think it is zero.

DR. MULLIGAN: I think that one issue about mechanism that is important is that if you really did think that mitochondria weren't important, by not having mitochondria in your ooplasm you could, obviously, reduce whatever risk you otherwise would be concerned about. So, it is a relevant issue just because you have wiped out that risk completely if you didn't have any.

DR. SAUSVILLE: But then that becomes impossible to investigate in the conventionally clinically oriented situation since what we have heard is that while, in an ideal sense, you would parse out precisely which part of this works, I inferred from the discussion earlier that that is going to be very difficult from a practical point of view to ever do in a meaningful sense clinically.

DR. MULLIGAN: There might be people who don't feel that that is an important part of the method and would choose to go down the regulatory

pathway that wouldn't make use of mitochondria.

DR. SAUSVILLE: I don't think there is anything that would prevent that from a regulatory standpoint, but the issue is whether or not the user community would actually go down that path. I think that is uncertain to me from what I have heard.

DR. SALOMON: We certainly haven't gotten to where we need to be by the end of the day, but I think we have made some progress along that line. Ms. Knowles is going to talk to us about ethical issues and then, just to give you the lay of the land, we are going to do the public comment section, take a break and come back and really get into the key questions, and that is when we will have to have it all in perspective, mitochondrial safety, ooplasm, other components of the ooplasm and its impact on this group of scientists and physicians.

Ethical Issues in Human Ooplasm Transfer Experimentation

MS. KNOWLES: Thank you for inviting me to be a part of this. I have been charged with elucidating the ethical issues in human ooplasm transfer experimentation. So, we are going to step

back a little bit from all the mitochondrial data we have been talking about, and stepping back from the animal models, and we are looking now at the issue that we started with today, looking at the experimentation of ooplasm transfer, that I am going to call OT just for shorthand, in humans, and looking at some of the ethical issues.

In terms of context, I just want to highlight that all medical experimentation takes place in the context of some risk and some uncertainty. The question, therefore, is what is the threshold of risk and uncertainty that is acceptable? One way that we can better understand the risk and uncertainty of OT experimentation is by looking at what I am calling the knowns and unknowns.

So, in terms of elucidating safety and efficacy concerns, we are going to say to ourselves what threshold of risk and uncertainty exists in this context and so what are the knowns and unknowns. I am going to look at the implications this has not only for whether it is ethical to proceed with this technique in women and to create children, but also the implications for informed consent.

Considerable amount of thought, discussion and work has been devoted to the question of the ethics and science of both therapies and experiments that result in inheritable genetic modifications, and I adopt that term from the AAAS report of 2000. In the time that is allotted to me, I can't do justice to that work but what I can do is nod to some of the work and some of the issues that are on the table when we are talking about inheritable genetic modifications. Similarly, I don't actually have time to address the depth of the issue of what I call the invisible woman, the other woman who is involved in all of these procedures, the egg provider.

So, it is extremely important to realize that the implications of proceeding with OT both in experiments and as a clinical technique have larger ripple effects which implicate the safety of the women who undergo the egg provision, the egg donation as it is called, to enable this technique to go forward. So, whereas ooplasm transfer is primarily concerned with transplanting genetic material that is believed, although we don't know certainly at all, to not have an impact on phenotypic development of the embryo, there is a

likelihood then that the market for oocytes will be increased and will pull on women who have not typically been pulled on for provision of eggs based on their phenotypic characteristics which aren't going to be, we assume, as important in this market. So, that has some larger social ripples and ramifications that we should be thinking about as well.

That leads me to my last area of concern that I am actually not going to touch on. Given FDA's mandate, I am not going to address the social and legal ramifications of this technique but I think it is necessary to underline the importance these issues have, the uncertainty that exists where genetic parenthood is tripartite and the ethical imperative now to have a broad and multidisciplinary review of the ethical and scientific issues. So, somebody needs to be free to deliberate about these larger ethical issues as well, and I think it is my responsibility to just outline that.

Turning then to safety and efficacy, we are asking ourselves what are the unknowns. There are clearly more unknowns than I have on this list so I am just going to highlight what I think some

of the most important unknowns are. The first is it is not known, and we have heard this many times so a lot of what I am going to say is going to be sort of summarizing--what is not known are the defects that ooplasm transfer is trying to correct.

It is not known what is doing the work in OT. Although we have concentrated on mitochondria recently, we have to remember that we don't actually know what is doing the work. We don't know whether OT techniques have an adverse effect on transferred material. We don't know that. We don't know whether OT helps actualize abnormal embryos that would not otherwise be actualized. And, we don't know the effects on embryos, infants and toddlers--humans--with heteroplasmy. Would don't know what its effects are.

So, let's delve a little bit into that. Our scientific understanding of why an embryo does not develop is still incomplete. We heard that a number of different ways today. We know there are a number of different factors that may be implicated including maternal age and including ATP deficiencies. So, let's look at what some of the other factors may be.

This is a partial quotation from The New

England Journal of Medicine, March 7, 2002, many factors can lead to poor embryonic development, including chromosomal abnormalities, genetic defects, and cellular abnormalities. Impaired embryonic development may also be consequence of other problems within the embryo or in its immediate environment.

In the Huang experiment, in fertility and sterility, October, 1999 it was stated, the reasons for previous implantation, and this is in describing the failure of the nine patients in that study, the reasons for previous implantation failure in these nine patients are not clear because their oocytes appeared morphologically normal and the embryo transferred were of fair quality.

This is complicated by a great variation in the women in each of the studies, incomplete histories of the techniques each woman underwent prior to OT, the number of attempts, the techniques tried after OT and inclusion and exclusion criteria for the women in each group. This is complicated by what Dr. Lanzendorf and her colleagues refer to as the subjective grading of embryos in vitro performed by various embryologists, which renders a

comparison between patients' previous IVF cycles and treatment cycles unavailable. So, we know that that information in terms of comparison is not available to us in many circumstances.

Continuing with the unknowns, what is doing the work? We don't know this. Since we don't know what is doing the work and whether in all cases it is the same beneficial factor, which we can't assume it necessarily is and we don't even know if the same beneficial factors are transferred, it is not actually possible to know whether OT is clinically indicated in a particular case.

I have shorthanded the citations because I have so many words on these slides, but I have the citations if you would like them. The mechanisms involved are still enigmatic. It remains unclear as to which cellular components are transferred in the donor ooplasm. Exact mechanisms and factors that help to rescue the function of the defective oocytes remain unknown. It is not yet clear how ooplasm transfer works. Specialized proteins or messenger RNAs may direct subsequent cell cycle events. it is also possible that donor mitochondria is providing the benefit.

So, can transfer techniques have an adverse effect on material that is transferred, transferred material? Here, of course, we are concerned with the risks that are implicit in this technique. Interestingly, it seems that all the research of the clinicians in the protocols that we were provided express some concern with the source of ooplasm or cytoplasm used either in their own experiment outcome used by other in the other experiments. These concerns include the effects of cryopreservation of the material transferred since that has been studied and shows that cryopreservation can have negative impact on oocytes and embryos. That, obviously, has to be considered.

So, let's look at what they said, because it is still not known what is being transferred to recipient oocytes, it cannot be determined if cryopreservation may have an averse effect on these factors.

This is the 3PN protocol and they are commenting on the use of metaphase II oocytes, one concern we have is the risk of transferring donor chromosomes, and we heard about this earlier, from metaphase II oocytes of donors into the recipient's

oocytes.

We feel validation is still required to provide absolute proof that donor nuclear DNA has not been accidentally transferred. That is referring to the 3PN protocol.

What are the effects on embryos? Well, the bottom line is we don't actually know. Let's take a look at what they said. Even though the use of cytoplasmic transfer has been employed in several IVF clinics--this is from the abstract, by the way, of this report--and pregnancies have resulted, it is not known definitively whether the physiology of the early embryo is affected.

There may be an improved developmental potential of hybrid cytoplasm in chromosomally normals as well as abnormal embryos. So, here we know the following risk exists with respect to the effect that OT may have on embryos and that abnormal embryos may be actualized as well as normal embryos getting the boost that we talked about.

We do know at this point that ooplasmic transfer can alter the normal inheritance of mitochondrial DNA resulting in sustained heteroplasmy representing both donor and recipient

mitochondrial DNA. That is also a quotation.

What are the effects on the embryos, infants and toddlers with heteroplasmy? And, we are talking about humans. Well, because little is understood about the maintenance of mitochondrial heteroplasmy and its nuclear regulation during human development, the effects of potentially mixing of two mitochondrial populations are still being debated. In other words, we don't know.

We do know that mitochondrial heteroplasmy may result in embryos, approximately 50 percent from my reading that particular study, of non-viable embryos used in Barritt's study exhibited this trait. We also know that two children now exhibit mitochondrial heteroplasmy, but we don't know what this means and it is unclear whether all the children created from ooplasm transfer have been tested for mitochondrial heteroplasmy. It sounds like, from the first speaker's presentation, that we know that, in fact, not all the children that have been created this way have been tested.

So, let's look at what we do know. Well, we know that the incidence of chromosomal anomalies is higher in this population than the rate of major

congenital abnormalities observed in the natural population. This is a quotation from page 430 of Barritt et al. in the European Society of Human Reproduction and Embryology journal.

We know that one 18-month old boy, as Dr. Cohen was mentioning this morning, has been diagnosed with PDD. And, we know that the mitochondrial DNA inheritance is changed in some children resulting in an inheritable genetic modification.

Let's talk about inheritable genetic modification. I want to say first of all that there has been kind of an interesting discussion going on in the literature about whether this is, in fact, a case of germline genetic modification. I think that is, in fact, interesting in and of itself, the fact that there is a lot of energy being spent to make sure that we are not labeling this a germline genetic modification. That should be telling us something. I have seen some very interesting arguments about why it is not a case of germline genetic modification, including one that I have mentioned to several people before, that it can't be considered a germline genetic modification because is doesn't pass through males. Well, I am

not actually going to discuss that particular argument but the energy that is being expended should be telling us something about whether, in fact, it is an inheritable genetic modification.

Well, why are we concerned about IGMs? Here I have to be really very concise. This term IGM, inheritable genetic modification, as I mentioned, I am taking from the AAAS, the American Association for the Advancement of Science, their 2000 report which brought together a group of eminent scientists including gene therapists, ethicists and policy analysts, and they say the following, they say essentially due to the transmission of inheritable genetic modifications, there would need to be compelling scientific evidence that these procedures are safe and effective, compelling scientific evidence. For those techniques that have foreign material, their stability across generations would need to be determined based initially on molecular and animal studies before proceeding with germline interventions in humans. It is not yet possible to meet these standards, nor is it possible to predict when we will be able to do so. One footnote I should add that was correctly mentioned earlier, we

don't know whether, because the blood only of these children has been tested, the germ cells have also inherited this mitochondrial heteroplasmy. But we haven't tested for that yet because we can't at this point. So, it is important to recognize that that, in fact, is true but it doesn't mean that this is not inheritable genetic modification. That is important.

They also go on and say the possibility of genetic problems occurring as a result of the unintended germline side effects seems at least as great or greater than those that might arise from intentional inheritable genetic modifications which at this time we don't permit in many, many countries. Why? Because knowing you were creating an IGM assumes that you would have safeguards and rigorous monitoring in place and we know that in this case that is actually not true because they allegedly didn't think that they were going to be transmitting genetic modification.

So, those are the AAAS conclusions. Clearly, we have a duty to future generations--there is a lot of theoretical work on this, but we can intuit that we do have a duty to future generations to be thinking about what we

are, in fact, passing on to them, to be doing it carefully if we are going to do it.

I would note that there is almost never consensus in the international community, but there is pretty close to a consensus in the international community that we should not be doing research that results in inheritable genetic modifications. I just want to highlight, in terms of the international work, that this would not be permitted in most countries, this kind of protocol, and in the U.K., which is arguably the most liberal with respect to embryo research, they are going to allow some stem cell protocols that we are not in this country, they prohibit germline modification, and the House of Lords stem cell report noted that--they didn't discuss OT in the context of fertility treatments at all, but discussed what we were discussing, the use of a similar procedure with respect to screening out mitochondrial disease and they said that very little research has been carried out on this procedure and it would need extensive testing in animal models and in human eggs before it could be used therapeutically in humans. Remember that they are talking about a therapy in a disease, not fertility, in that

context.

What does heteroplasmy of this type, the type that we have been discussing in humans in the two children that we have been talking about, what does it mean? Well, the bottom line is we don't know. We do know that there are diseases associated with mitochondrial heteroplasmy. We know that. Yet, there is no reason to consider this mitochondrial DNA heteroplasmy from this OT protocol as harmful because it is known to occur naturally in normal individuals.

Well, to be fair, we don't know if this type of heteroplasmy resulting from these experiments results in mitochondrial disease because it doesn't occur naturally. So, we haven't been able yet to determine that it is benign. We simply know that this other type of heteroplasmy can occur in normal individuals and it can occur and be associated with disease states as well. So, we cannot say that it is benign because we don't know. We don't have the information at hand to know. We haven't done the experiments yet to know or the follow-up to know.

We do know that one child has PDD but we don't know whether that child actually is

heteroplasmic or not. I would like to know that if we have that information available. I don't think we know that.

What else? Well, since mitochondrial diseases are associated with heteroplasmy that can be early or late onset, we cannot know whether this heteroplasmy is benign until these children grow up. That is a basic conclusion from logic.

Limitations of clinical data, well we heard very candidly from our speakers, and it is much appreciated, some limitations of the clinical data. It is very helpful. Small sample sizes; incomplete information on the women in the experiment for a number of very legitimate reasons. We don't know necessarily whether previous procedures are the reasons for their failure. Incomplete testing of the children who have been born; and the lack of long-term follow-up.

This is particularly troubling. There is clearly a need for long-term monitoring of the children that are born with a heteroplasmic condition and those that aren't born with a heteroplasmic condition. In addition, there is likely going to need to be extensive follow-up of these children until they have children to

determine whether, in fact, we have an inheritable genetic modification and what happens to it through the generations.

This follow-up can, and will likely be very intrusive because, as we were hearing on the mouse models, the mitochondrial segregation is tissue specific and differs. So, if you are going to do proper follow-up you would need to take tissue biopsies from different tissues to understand how the mitochondria has been differentially segregated. This, of course, could be extremely intrusive. Whether one could ethically consent to this kind of long-term monitoring and invasive follow-up for a child that is not yet conceived has to be added to the ethical picture when we are looking at this.

So, what do the knowns and unknowns tell us? Well, this has pretty profound implications for informed consent. How you get meaningful informed consent in this environment is a real question and a real challenge, not only because of all the information that we don't know but also because of the specific environment which we are dealing with. We are dealing with the environment of reproductive medicine which has a reputation for

having a tremendous overlap between clinical innovation and human experimentation. This environment has to be factored into the whole question of the meaningfulness of informed consent.

Added on to that is the fact that patients that come into fertility clinics are desperate, truly desperate for real reasons to get pregnant. We heard very candidly that they will pressure concentrations, researchers, to provide techniques for them even when they are not necessarily indicated. We have clinicians who are very thoughtful people but who have developed their practice as clinician researchers where much of their practice is the practice of experimentation because they can. This is an interesting area where they can actually do a lot of clinical innovation and human experimentation.

So, what does that mean? It means that perhaps this is not the best environment for basic research to be conducted when down the road the risks could be much more than society or even the individuals are actually willing to bear despite what they say in this context. There is near consensus in the literature, in the briefing package, the protocols that we have been

discussing, that this is not ready for widespread clinical applications. Pretty much all the protocols we read or people who have spoken to us earlier today indicate in their work that they do not believe it is appropriate to conduct this experiment in a widespread fashion in fertility clinics in this country. They are very candid about that.

So, should there be more animal testing? Yes. At the very least, one of the things I was struck by was when Dr. Shoubridge was talking is that at the very least we could be doing the tests on his animals, tissue-specific tests to find out whether they are, in fact, normal. He says they appear normal, very candidly, but he doesn't know. They haven't tested for that. So, we could be doing that work.

Given the level of uncertainty of the risk, I think the answer is quite clearly yes. All the studies that we look at rely on animal studies. So little is known about the function of mitochondria, about heteroplasmy, about the bottleneck, about mitochondrial diseases that animal experimentation of various kinds, mice, primates, can surely help elucidate these

underlying uncertainties.

Finally, must there be further human embryo experimentation before embryos are implanted and children are born? Yes. There must be more human embryo experimentation before implantation. This is a lovely quote from The New England Journal of Medicine, the use of novel reproductive techniques must be based on more than their mere availability. There has to be clear clinical indication for using such techniques, evidence of their efficacy and consideration of the risks to the mother and society.

This is difficult. We make decisions about bringing techniques to human trials by looking at the risks and uncertainties, the potential harm to the patients, offspring and other individuals involved. But we have to also factor in the nature of the condition that is the focus of these experiments in examining the risk to the patients. Here we are talking about how quickly we move forward. How imperative is it that this results in human experimentation in the clinics tomorrow? So, this is a factor in our deliberations.

In this case, although infertility can be

a very serious condition with serious and real emotional impacts and personal side effects, this is not always the case with infertility. More importantly, we are talking about the ability to have a genetically related child. Let's make it even more of a finer point here. The inability to have a genetically related child is not a life-threatening or fatal condition.

So, my point is simply that when we discuss how quickly we move forward, the necessity of making this happen quickly in fertility clinics, we have to keep this in mind as well. Finally and very importantly, we have a duty to the children that we help to be born to do our utmost to see that they are born free from disease or impairment, and we are not there yet.

The combination of these factors quite clearly, in my mind, mandates that further trials not be conducted on human embryos that will be implanted in women with the hope of creating more children at this time. If the FDA decides otherwise, there are, in fact, all kinds of factors that should be introduced, that I don't have time to go through--informed consent procedures, rigorous screening, etc. that we can discuss at

another time. That is the end of my remarks.

DR. SALOMON: Thank you for a really superb presentation and actually an excellent transition. What I would like to do now, before the break, is to invite three people who are on the official docket for public comment. We have allotted seven minutes each for these people. Then we will take a break and then come back and face the set of questions, many of which we have set groundwork for and some of which we will have to try and put in a proper context.

The first person I would call for the public hearing is Dr. Jamie Grifo, representing the American Society for Reproductive Technology. Welcome, Dr. Grifo.

Open Public Hearing

DR. GRIFO: Thank you. I appreciate the opportunity to speak. My name is Jamie Grifo. I am a clinician researcher. I am a reproductive endocrinologist. I am the division director at MIU, University School of Medicine for Reproductive Endocrinology. I oversee our laboratory; I oversee our research. I run the fellowship and I am a practicing clinician.

In my spare time I am the president of

SARD. SARD is an organization of the American Society of Reproductive Medicine. It has been in existence since 1988. We are composed of physicians, scientists, researchers, embryologists, nurses, mental health providers and patient advocates. We set the standard for the practice of our medicine.

You have never heard a story about this organization because we are not sensational and there is no journalist that will tell our story. We have effectively set the standard for our field; we have self-regulated and no one knows this story. We are the only group of physicians in the world who collect data, validate data, publish data in collaboration with the CDC about clinic specific and national birth rates. We have strict membership guidelines. We have strict criteria for lab and medical directors of programs. We validate data by random site visits. We have ethical guidelines and practice guidelines that are required to be followed in order to maintain membership. We have teeth. We have eliminated 30 people from our membership for failure to adhere to our guidelines.

More recently, we now require performance

standards and if they are not met we offer remedial services to these clinics to assure quality of care. We have also issued a statement saying that we do not think reproductive cloning should be done at the current time until it is proven to be safe and effective.

So, we have set the standard for our field. We do regulate our field, and we have done a very good job. Unfortunately, the media prefers to talk about people who are not our members and who are not doing things that people say they are doing.

We are very pleased that the FDA has taken an active role in regulating the medicines and the devices that we use to assure safety for our patients. Our goal is that our patients have healthy outcomes.

I do not believe, and we do not believe that ooplasmic transfer is a food or a drug. It is a research protocol. Research protocols traditionally have been regulated by a very fine situation that has withstood the test of time. It is called informed consent and institutional review board. That method has worked. Human research has been done ethically. Results have been good.

Safety has been assured.

One must realize that you can never assure safety in any new technique. The safest thing that we can do is stop all research in our field. Unfortunately, the series of letters sent out from FDA has just done that in our field. That has assured that our work will be done in other countries by people who perhaps do not have the skills or the support to do what we, Americans, can do. We have been the best in our field. Unfortunately, we have had that privilege taken away from us.

Through informed consent and IRB we have introduced in our specialty, in very rapid sequence, techniques that did not exist. We have made the practice of IVF better. We have helped more patients. Techniques such as ICSI, assisted hatching, embryo biopsy in co-culture have been in existence and have helped many patients. Embryo biopsy was done initially in England. It took me four years to get institutional review board approval to do embryo biopsy. In collaboration with Jacques, we had the first baby in the United States. We were the second group in the world. There have been hundreds of thousands of babies

born free of genetic disease by this technique. If we attempted to institute this practice into our field today in this environment, we would not be able to do that.

I applaud the FDA in wanting to assure safety, but human research will always have inherent risks. You cannot get rid of risk. With informed consent patients are educated about what those risks may be and they make a decision whether or not to undergo those risks.

The FDA must add value to the practice of research in this field. I hope that there is a better mechanism, other than stopping us from doing our research, that can exist. Thank you for the opportunity to speak.

DR. SALOMON: Thank you. The next speaker is Dr. Sean Tipton, also from the American Society for Reproductive Medicine. Does anyone know, is Dr. Tipton here? Mr. Tipton, sorry. Maybe I could invite the third speaker since there wasn't any particular order or priority here, Pamela Madson, from the American Infertility Association.

MS. MADSEN: It is an honor to be with all of you here today. It is an encouraging and auspicious start that so many members of the

medical and scientific research and government communities have come together.

For the millions of us who are locked together in the wrenching battles against infertility, this meeting embodies the hope of achieving increasingly effective and safe treatments as quickly as possible because we have no time to waste.

The population of the infertile is growing, with one in six couples actively experiencing problems. Let's be clear, we are raw. Recent headlines made public what most of us already know, that our collective ignorance about fertility is extracting an enormous toll. That women who delayed childbearing, either by choice or force of circumstance, feel duped out of their shot at genetic motherhood. That their partners, who also long for the children that are uniquely theirs, are just as saddened and infuriated by the loss. That the individual and societal costs of infertility are intolerable. Let me respond to you, no, it is not life-threatening; it is life-stopping.

What do we do about it? Certainly we raise public awareness about infertility, its

prevalence, its causes and prevention. We make a concerted effort to educate everyone about the human reproductive life cycle. But we must also rededicate ourselves to refining the infertility treatments we have and to discovering new ones. Like any other ruthless disease, infertility ravages not just the immediate sufferers but their families and friends, employers, peers and employees. With age, a genetic inheritance, a physiological fluke or a medical condition is to blame, all those affected by infertility have one thing in common, an urgent need for reliable paths to biological parenthood.

As patients, we understand, to a large extent, that the fees we pay for services propel developments in reproductive technology. It is worth noting, however, that we are here when our government does not provide any funding for research. Yes, we need more embryo research. No, it is not funded by our government. We are cognizant of the risks we voluntarily take as the subjects of clinical experimentation that are required to move the research expeditiously. We know that we are treading on uncharted territory.

To date, ooplasm transfer research offers

the greatest potential to help women with oocyte problems. It is potential. We need research, we need it to move forward. It is the avenue that seems to be leading to many different technologies that may deal with the multiple forms of egg-related infertility. We want to do everything we can to facilitate this work because right now, as far as we know, there is nothing else.

Of course, we are concerned that researchers adhere to the highest standards possible. It is not only our health at stake, but the health of future generations as well. We have always relied on the twin mechanisms of IRBs and informed patient consent, and it is our understanding that the system has worked reasonably well.

As willing participants in experimental procedures, patients have the right to honest and forthright information before giving consent. That includes anticipated outcomes and possible pitfalls; what is known and best guesses about what isn't. We wonder why IRBs can't be overhauled to include a broader array of interests--patient advocates and possibly government representatives among them. We wonder why we don't have uniform

IRB standards. This is likely to be far less intrusive and economically onerous than the creation of an entirely new system.

If, however, the government is committed in its current plans, we do urge restraint. We would like to know that government federal guidelines will not be so cumbersome and expensive that they inhibit researchers from pursuing promising leads. We want to know that the costs of regulation which are passed down to consumers will be reasonable and contained. Remember, most of the infertile around this country are paying out of pocket. We don't have coverage.

Otherwise, we jeopardize the access to treatment for all but a very wealthy few. As it is, the financial burden of largely uninsured reproductive technology puts an enormous strain on the infertile. We are asking that we build on the cooperation and open communication that we have witnessed here today, and we would urge, if we are going to work together, that whatever body it is, whether it is through overhauling of systems that are in place or a new body, that it be composed of regulators, researchers, reproductive clinicians and patient advocates to ensure that politics do

not interfere with the community's need for scientific breakthroughs. We are depending on a true collaborative process. The infertile cannot afford, and do not deserve any less. Thank you.

DR. SALOMON: Very nicely spoken. As I said, we are going to take basically a ten-minute break. It is 4:15 right now. We will start again at 4:25 regardless of anyone who isn't here, just so you take me seriously this time. I want to make sure we have enough time. Thanks.

[Brief recess]

Questions to the Committee

DR. SALOMON: To initiate the final phase of this afternoon and where things have to come together, all the different pieces that we have explored all day, is in dealing with a series of specific FDA questions. These will be briefly reviewed by Dr. Moos.

DR. MOOS: I am just going to try and tie together a few things that we have heard today by way of introducing our list of questions. I am not going to subject you to a detailed reiteration of this list; it is in the briefing package.

The first thing I want to say is directed to the folks whom we consider really the most

important people in the room, who are the patient interest advocates. I think that if you have a look at the kinds of questions we have been asking and discussing, implicit in the entire format of the meeting and the discussion is that we have no intention of stopping any kind of research. Our intention is to balance carefully the avoidable risks and the benefits in a way that we optimize the balance between the two.

To do that, we need to make use of the best scientific and medical evidence and analysis. I think the presenters have done an excellent job of laying out much of the critical information that we will need to make use of to synthesize how we go ahead with this.

Many of our judgments will depend on some kind of treatment of numerical data. We have seen a great many mentions of how small the numbers are and what the statistics are like. And, one of the things which, over in the FDA corner, we found very striking is just this fact. We heard some very useful information suggesting that experiments might be quite feasible and relatively straightforward to design that would satisfy us that heteroplasmy per se represents a manageable

risk.

But there is a fly in the ointment, particularly with respect to the incidence of Turner syndrome that has been reported in some of the data. We know that it is very common. The best information that we can get out of the literature suggests that the incidence of Turner syndrome in the general population is perhaps 1/100 conceptions, not live births but conceptions. If someone wants to weigh in with a better number, we are all ears. In contrast, the series that has been reported has an incidence of 23 percent, more than 20-fold higher. If you factor in the biochemical pregnancies, which were very likely aneuploid, the figure becomes higher.

We acknowledge that the confidence interval around 3/13 is very, very large, but this is something that can't be ignored. There are a couple of scenarios. We can reduce this with respect to the efficacy question either to a situation in which ooplasm transfer has no beneficial effect on fertility, in which case the additional risks of instrumentation, of superovulation and so forth are not reasonable, or that it does give a boost, in which case the

potential to bring marginal embryos that perhaps should not come to term to a point where something bad might happen actually exists. So, this is an issue that we have not heard sufficient discussion on and that I would like for the committee to keep in mind as we tackle the question.

If we can address the salient safety issues, I just want to say one or two words about product characterization. There has been I think a very interesting discussion about what it is that is doing something. I would like to point out that the better we characterize the material that is being transferred, the better we will be able to manage those risks from a number of standpoints. There will be questions that we will need to consider both to initiate experiments in what we call Phase I or safety studies, and there will be questions that we will need to confront at the time of the licensure which will, indeed, require much more detailed information about what is in the product that is making it work and definitive proof that the product, in fact, is working.

With that brief introduction to the questions, I will yield the floor to our chairman, with thanks for his able service, and to all the

members of the committee and panelists for the discussion today. Thank you.

DR. SALOMON: Thank you very much. So, there are two pages of questions, but some of them are more important than others and I will do my best to prioritize them.

As stated here, to me, there are a couple of principal goals. The first is to determine whether there are data available right now that support the safety or support the rationale for ooplasm transfer that is sufficient to justify any perceived risk involved in the clinical trial. We need to deal with that.

We also need to determine a separate issue, what additional data are needed prior to initiation of a broader use of this technology or clinical trials if the first discussion should come to the conclusion that clinical trials shouldn't go forward.

So, I think there are a couple of different options that the committee can now consider. You can consider that, no, there is not enough data; no clinical trials. But you can't just say that. You have to say what exactly has to be done. We have to come to some grips with the

concept of where is the bar going to be set for this. We can also say, no, there is sufficient data; go forward with clinical trials but, in parallel, we need additional data. You know, you need to be show us evidence that the field is working on these additional data but we can also then go forward and talk about what is a good clinical trial. So, I think that is a major issue. We can't leave without really trying to come to grips with it.

A second major issue to me is regardless of the answer to either of those, even though they have such important immediate implications, another issue here is to begin at least a dialogue with the community regarding what you will need to characterize this product. I mean, that is going to be something that you can't change. Whether we are talking about islet transplantation, therapeutic gene transfer in any number of cells, stem cells of any sort, you have to have a sense of product. We are not talking about, "hey, trust me with this wonderfully ethical group of scientists," it has to be, "trust me, we are going to do this in 40 centers, in 50 states and charge money for it." I mean, that is okay. That is fine; that is the

American way. But in the process of doing that, the direction that the FDA has to have from us is how you are going to make sure that in 50 states and 50 places or 100 places, or whatever, there is a sense of objective measurements for the quality of the product, what we call lot release criteria. Those things are much more difficult to do in a biologic. I know that. We all know that. But they are not impossible.

So, with that background let's start kind of with the first concept. I am getting off the strict question order a little bit but I am going to do that on purpose. So, the first question here is we have heard the clinical presentations and I have to start with a discussion of is there enough data, preclinical or clinical, right now to do a human clinical trial? Let's assume that that is a really good clinical trial that is going to answer a question, just are we comfortable doing a clinical trial or should we say, no, we are not comfortable; it should be put on hold and then we have to set a bar?

MS. WOLFSON: Well, as one of the few non-scientists here, first of all, I would like to say I really thought that Lori posed very

interesting questions and I don't think you can answer your question without kind of addressing all of those questions.

From what I have heard and what I have read up to this point, I do not think we have enough clinical data to allow human studies in any form. I think that there are so many things that have to be answered that haven't been answered. When Lori spoke about informed consent, I thought to myself, well, it is one thing for a couple to give informed consent for any dangers that they might encounter, but how can they give informed consent for future generations? I would even wonder if they could really give informed consent for their own possible child if there is a risk that, for instance, there is a 23 percent chance that that child would have Turner syndrome?

I think these questions have to be addressed. I don't think we got enough information here today to say that there is enough clinical data out there at all.

DR. SALOMON: Okay, that is clear. What are other thoughts here?

DR. NAVIAUX: An alternative to that would be a limited number of expert centers, one, two--a

small number that would be guided by the recommendations of this body in obtaining some of the human data that is necessary in the process of offering the technique. I will leave it at that for now.

MS. WOLFSON: Just a point of clarification, do you mean human data as in pregnancies, or are you talking about experimentation with human embryos?

DR. NAVIAUX: I think there are practical difficulties. We definitely need the embryo research but we have kind of left the human reproductive technology people out on a limb without any support because there is no mechanism for funding human gamete research. So, yes, I we need that data but, you know, in the U.S. there may not be a mechanism, and someone can correct me perhaps.

DR. SALOMON: Dr. Sausville?

DR. SAUSVILLE: I think there returns a little bit to the importance of the animal experiments that were discussed previously. Recognizing that the lack of support for human gamete related research and subsequent production of zygotes is an issue that is ultimately one that

this committee does not have the purview to, shall we say, change, I do think that the scientific rationale that might emerge from a considerably larger body of research that can be funded on animal-related matters would increase my enthusiasm for the possibility, and possibly the fact, that there is something actually happening here. We heard that we don't know what components of this process convey a salubrious outcome. Maybe the whole combination of things is necessary, but then that gets to the product issue that was raised. I mean, do you define this product as having a lot of ATP? Do you define it as having a certain minimum level of ATP or calcium, or whatever you favorite component is?

So, to me, while I actually want the field to move ahead and potentially give what benefit it can within the context of its limitations, I just feel that in comparison to many other therapies that have come to this committee before, some of which are very specialized, in each case the proponents were able to make the scientific case preclinically for the ones that went forward; that there was a basis for actually regarding this as an ultimately successful outcome and I don't actually

see that here.

DR. SALOMON: Lori?

MS. KNOWLES: I just want to make the point that it is true, and we are obviously not going to discuss it at any length, that there is a lack of publicly funded human embryo research, but there is private money for human embryo research and the fact that there isn't public money for it doesn't, to me, say that then you skip that stage and do the experimentation in humans, live humans.

So, if you actually want to be able to offer this technique and make money from it, you have to do the experimentation that shows that it is safe. It is just part of the equation, the way that I see it.

DR. SALOMON: I just want to point out that here is where it gets kind of complicated because we have to be very careful. One is talking about efficacy and one is talking about safety. I am not saying that we don't have to discuss both but we need to be careful. Ed is talking about efficacy and I was talking about efficacy, and now you kind of throw in safety, that is okay but we need to be sure that we stay intellectually clear that the domains of safety and efficacy are

different.

MS. KNOWLES: Right, and I actually agree. My feeling is exactly what you were saying, that there are all kinds of information that we can get from animal models--it sounds like, about the efficacy.

DR. SAUSVILLE: And I would go so far as to say that both safety and efficacy are uncertain to me.

DR. VAN BLERKOM: As far as efficacy, we are dealing with long-standing infertile couples, women whether have been through lots of treatments unsuccessfully. What animal model do you propose that will be relevant? I mean, as far as a mouse, put in cytoplasm and get mice. Would you use a primate model. I don't know if there are any long-standing infertile Macaques. Maybe there are. So, I am not sure about the relevancy specifically of animal models.

I think the basic question is, is this effective? If you look at all the publications on cytoplasm transfer, they all say we don't know that this is effective. We don't know what is causing, if anything, a boost in efficacy. So, I think in reality what it is going to come down to is that

the only system that is really suitable for a test of efficacy is going to be the human. I just don't see an animal system providing the types of information that you would like to see.

DR. SAUSVILLE: I would respectfully suggest that while I can understand the ultimate human relevance of both the use of the procedure and the judgment of its value, what we are talking about here is the setting up of some boundary conditions which would begin to be able to be applied to that which is used in this critical human experiment. I mean, the very presentation that I believe came from you showed that there is a great deal of variability in terms of where you stick the needle, the different types of eggs--I mean, this becomes very problematic, therefore, for deciding how we would set up the human experiments, at least to me it does.

DR. VAN BLERKOM: That is the whole point. I think the human experiment is unique, unique in the sense that I think there are confounding issues that happen in human eggs that you are not going to find in other species.

DR. SIEGEL: May I interrupt? We really need to focus this in a context that will be more

useful to us if we are trying to deal with the questions. The question you asked is whether there is enough data to do clinical research but then you are focusing on the efficacy side. We worded our question somewhat differently, and for a reason, and that has to do with what our regulatory authorities are. I would like to have this discussion within the context of what our regulatory authorities are.

So, your question bears some significant similarity to question number three, which I would like to take just a moment to read and explain the context of why it is worded that way. Are these data, referring to the clinical and preclinical data currently availability, sufficient to determine that ooplasm transfer does not present an unreasonable and significant risk to offspring and mother, and to support further clinical investigations?

The determination we need to make specifically is whether there is an unreasonable and significant risk. That is largely a safety determination, but what risks are reasonably and what risks are not reasonable is clearly linked to the issues of what disease is being treated, what