

**Human Fertilisation and Embryology Authority
The Scientific and Clinical Advances Group**

Committee:	Scientific and Clinical Advances Group
Meeting Date:	21 st February 2008
Agenda Item:	6
Paper Number:	SCAG(01/08)02
Paper Title:	Developments in treating mitochondrial disease
Author:	Helen Richens
For Information or Decision?	Decision
Resource Implications:	Accounted for in the business plan (horizon scanning)
Recommendation to the Committee:	<p>Members are asked to:</p> <ul style="list-style-type: none"> • note the developments outlined regarding germinal vesicle transfer, pronuclei transfer and microcytoplasm cryopreservation (study at Annex A) • consider whether germinal vesicle or pronuclei transfer would be safe to use in treatment • consider whether it is likely that centres will want to use microcytoplasm cryopreservation for research or treatment

1 Introduction

- 1.1 Mitochondrial diseases are caused by alterations in the small amount of DNA contained within mitochondria. They can cause brain, neural, muscle, cardiac, endocrine, renal or bone marrow disease. There are few treatments for mitochondrial disease and for many patients the disease progresses and can be fatal. Mitochondrial DNA (mtDNA) is inherited exclusively from the mother through the mitochondria present in her eggs.
- 1.2 This paper will look at developments in the following areas of research into mitochondrial disease:
- germinal vesicle transfer
 - pronuclei transfer (research project at Newcastle Fertility Centre for Life)
 - microcytoplasm cryopreservation

2 Germinal vesicle transfer

Background

- 2.1 Germinal vesicle transfer (GVT) was first identified by members of the Horizon Scanning Panel in 2005 and SCAG asked to be regularly updated with developments. Evidence on GVT was presented to SCAG in June 2005, November 2005 and September 2006.

- 2.2 GVT involves transplanting the nucleus of an immature oocyte (the germinal vesicle) from a patient into an enucleated recipient oocyte from a donor. Following GVT and fertilisation the embryos will contain genetic material from three individuals: nuclear DNA and possibly some mitochondrial DNA (mtDNA) from the patient, mtDNA from the recipient oocyte donor, and nuclear DNA from the sperm.
- 2.3 The resulting embryo will therefore have mitochondria either partially or fully derived from the recipient oocyte donor. The technique could allow women to avoid passing on mitochondrial disease. In theory it could also benefit women of advanced reproductive age whose cytoplasm and zona pellucida may have declined in quality.
- 2.4 However following Royal Assent of the Human Fertilisation and Embryology Bill (as it currently stands), this technique will only be possible if regulations are passed to allow it to treat mitochondrial disease.
- 2.5 At the September 2006 meeting SCAG decided that there was not currently enough evidence on the safety and efficacy of the technique for it to be used clinically. SCAG was concerned that the technique may not guarantee elimination of all unhealthy mitochondria. They thought that the risk of heteroplasmy (having more than one genetic population of mitochondria within a cell) needs to be taken into account.

Developments in germinal vesicle transfer

- 2.6 A recent paper by Kobayashi & Sato (2008) looked at the behaviour of mitochondria transferred with the donor GV nucleus and mitochondria in the enucleated recipient oocyte in mice. They labelled the mitochondria from both sources then fused the GV nucleus and recipient oocyte together. They used microscopy to look at the behaviour of the mitochondria during *in vitro* maturation and preimplantation development. The group found that the two types of mitochondria behaved in a similar way to mitochondria in a normal oocyte during meiosis. The mitochondrial heteroplasmy of the oocytes did not influence their *in vitro* maturation and preimplantation development.
- 2.7 The executive carried out an extensive literature review as part of the scientific consultation for the Authority paper on human animal hybrids in September 2007. Part of this literature review focused on the effect of nuclear transfer on mitochondrial DNA replication (Bowles et al 2007, Lloyd et al 2006).
- 2.8 These studies found that nuclear transfer can result in both donor cell and recipient oocyte mtDNA persisting through to blastocyst and being transmitted to offspring (heteroplasmy). It was not known what factors affect donor mtDNA transmission. Nucleo-mitochondrial interactions were found to be different in nuclear transfer (NT)-derived embryos than embryos generated by IVF. MtDNA transcription and replication factors, which are encoded by the nucleus, persist in NT-derived embryos, but not in IVF ones. The nucleo-mitochondrial interaction following nuclear transfer is therefore out of sequence and this may have safety implications for embryos derived following GVT.
- 2.9 Justin St John will provide us with an update on any other developments in GVT shortly. This will be either presented to members at the February meeting or circulated at a later date.

3 Pronuclei transfer

Background

- 3.1 Researchers at the Newcastle Fertility Centre at Life were granted a licence in 2005 to study possible methods of preventing the transmission of mitochondrial DNA disorders (Project: R0153).
- 3.2 Pronuclei transfer involves transferring the pronuclei (the haploid nuclei of an oocyte or sperm during fertilisation) from a zygote containing unhealthy mitochondria into an enucleated zygote containing healthy mitochondria.
- 3.3 Previous experiments in mice suggested that it was possible to prevent the transmission of mitochondrial disease by pronuclei transfer between zygotes. Pronuclei were transferred from zygotes with abnormal mitochondria into ones with healthy mitochondria. The zygotes developed into blastocysts with >20% donor DNA. Resulting offspring were normal and had no or low levels of donor DNA.
- 3.4 The group wanted to test the feasibility of pronuclei transfer in human zygotes.

Developments in pronuclei transfer

- 3.5 The group at Newcastle Fertility Centre at Life recently announced that they have created human embryos that reached blastocyst stage, following pronuclei transfer. The group used zygotes with an abnormal number of pronuclei that would have otherwise been discarded from IVF treatment. The resulting blastocysts contained mtDNA from donor recipient zygote as well as DNA from the transferred female pronucleus and male pronucleus.
- 3.6 The work has not yet been published but the HFEA is due to receive a progress report from the group on the 12th February.

4 Microcytoplasm cryopreservation

Background

- 4.1 The issue of microcytoplasm cryopreservation was identified as a high priority issue in the 2007 Horizon Scanning process. SCAG decided that microcytoplasm cryopreservation should be considered in conjunction with the updates on GVT.
- 4.2 Microcytoplasm cryopreservation involves removing a segment of the oocyte (microcytoplasm) from the parent oocyte and freezing the segment and oocyte. These are then thawed and reconstructed into a complete oocyte.
- 4.3 This technique could have an impact on assisted reproduction if it provides a more effective way to cryopreserve oocytes than conventional methods.
- 4.4 Freezing oocyte segments would also allow ooplasm (cytoplasm) to be stored and potentially transferred to improve the developmental potential of oocytes or embryos in assisted reproduction treatment. This is unlikely to be allowed under the Human Fertilisation and Embryology Bill unless it was for the treatment of mitochondrial disease.
- 4.5 SCAG has previously considered cytoplasm transfer in conjunction with GVT in June 2005. Members looked at a US study that found there were some chromosomal and developmental problems in fetuses and children conceived

in this way. The research was suspended in the USA in 2001. SCAG members were of the opinion at this meeting that cytoplasm transfer had 'been and gone' as a technique and GVT was likely to progress more rapidly.

Information on microcytoplasm cryopreservation

- 4.6 A group in the USA Goud et al (2007) has developed the technique of microcytoplasm cryopreservation in mouse oocytes (Annex A). Oocytes were micromanipulated to remove ooplasm fragments (microcytoplasts). The microcytoplasts were then injected into the perivitelline space and cryopreserved along with parent and sibling control oocytes using a conventional slow freezing technique. The microcytoplasts were thawed and used to reconstruct an oocyte by electrofusion, either within or without a zona pellucida.
- 4.7 Survival of the total fragments (parent segments and microcytoplasts) was significantly higher (75.5%) than the survival of control whole oocytes (64.2%) after thawing. However the survival of parent oocytes segments only (66.9%) was not significantly higher than the control oocytes.
- 4.8 Electrofusion of frozen-thawed microcytoplasm segments with parent or recipient oocytes was more successful when carried out within the zona pellucida (91.4%), compared to those fused without zona pellucida (56.2%). The group also assessed spindle morphology in reconstructed fresh oocytes.
- 4.9 The group thought that the technique could potentially improve the low survival rate of conventional oocyte cryopreservation because it increases the surface area to volume ratio of the oocyte.
- 4.10 When SCAG briefly considered the issue at the November Horizon meeting members were worried that the technique would disrupt the internal components of the oocyte. Though the study showed that the spindle was intact following reconstruction of the segment and parent oocyte, the group did not investigate if the oocyte would develop any further.
- 4.11 As far as we are aware the technique is at an early stage and has only been carried out in mouse oocytes. The technique has not yet been shown to be effective at producing oocytes that are able to be fertilised and develop. We are unaware of any other groups attempting this technique with mouse or human oocytes.
- 4.12 Clinics are unlikely to want to use microcytoplasm cryopreservation in clinical treatment, but may want to use this or a similar technique for research.
- 4.13 There are safety implications of using this technique as a means for ooplasm transfer. SCAG already considered some of the safety implications of cytoplasm transfer in the June 2005 SCAG meeting. A recent study by Acton et al (2007) found significant physiological differences between heteroplasmic and control mice. Problems in heteroplasmic mice included pulmonary hypertension, increased body mass and fat mass and abnormal electrolyte levels. The group suggested that as ooplasm transfer appears to be significantly associated with the mitochondrial heteroplasmy, children conceived through ooplasm transfer would need to be closely monitored for health problems.

5 Legislation

- 5.1 Pronuclei transfer is currently allowed with a licence from the HFEA for research purposes.
- 5.2 The Licence Committee originally rejected the research application from Newcastle Fertility Centre at Life to carry out pronuclei transfer. They agreed the research would be prohibited under paragraph 3(4) of Schedule 2 of the Human Fertilisation and Embryology Act 1990:
- “A licence under this paragraph cannot authorise altering the genetic structure of any cell while it forms part of an embryo, except in such circumstances (if any) as may be specified in or determined in pursuance of regulations”*
- 5.3 However an Appeal Committee reversed this decision and licensed the project. The Appeal Committee accepted that pronuclei transfer changed the genetic *constitution* or *composition* of the cell (or embryo as defined by the Act) but agreed that it did not alter the genetic *structure*. They defined altering the genetic *structure* as altering the expression of nuclear genes that result in heritable characteristics.
- 5.4 The same circumstances could apply to licensing the creation of an embryo following GVT.
- 5.5 Under the Human Fertilisation and Embryology Bill a permitted egg or embryo is one whose nuclear or mitochondrial DNA has not been altered. This means that if the Bill was passed, centres that wanted to carry out GVT, pronuclei transfer or cytoplasm transfer would not be able to use the resulting eggs or embryos in treatment because the mitochondrial DNA would have been altered. However regulations may allow it for the treatment of serious mitochondrial disease:
- “Regulations may provide that –
an egg can be a permitted egg, or
an embryo can be a permitted embryo,
even though the egg or embryo has had applied to it in prescribed
circumstances a prescribed process designed to prevent the transmission of
serious mitochondrial disease.”*
- 5.6 Therefore if regulations allow, GVT, pronuclei transfer or cytoplasm transfer could be used in treatment of mitochondrial disease, subject to licence. However there are no regulation making powers that would allow these techniques to be used for other purposes, for example to treat infertility in older women.
- 5.7 An amendment was tabled by Lord Walton of Detchant in the House of Lords Report Stage allowing the HFEA to license treatment for mitochondrial disease without the need for additional regulations from Parliament. The amendment was not moved but will be debated further at a later date.

6 Conclusion

- 6.1 Members are asked to:
- note the developments outlined regarding GVT, pronuclei transfer and microcytoplast cryopreservation

- consider whether germinal vesicle or pronuclei transfer would be safe to use in treatment
- consider whether it is likely that centres will want to use microcytoplasm cryopreservation for research or treatment

7 References

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- Goud A P et al. (2007) Reconstruction of ooplasm recipient oocytes with frozen-thawed donor microcytoplasts and influence on the microtubular spindle *Fertility and Sterility*. 87: 923-933
- Acton B M et al (2007) Neutral mitochondrial heteroplasmy alters physiological function in mice. *Biol Reprod*. 77(3): 569-576