

## Human Fertilisation and Embryology Authority Scientific and Clinical Advances Group

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| <b>Committee:</b>                       | Scientific and Clinical Advances Group                                                                                                                                                                                                        |
| <b>Meeting Date:</b>                    | 24 <sup>th</sup> November 2005                                                                                                                                                                                                                |
| <b>Agenda Item:</b>                     | 8                                                                                                                                                                                                                                             |
| <b>Paper Number:</b>                    | SCAG (11/05)04                                                                                                                                                                                                                                |
| <b>Paper Title:</b>                     | <b>Germinal vesicle transfer (GVT)</b>                                                                                                                                                                                                        |
| <b>Author:</b>                          | Katy Berry Annex A Justin St John                                                                                                                                                                                                             |
| <b>For Information or Decision?</b>     | Information and decision                                                                                                                                                                                                                      |
| <b>Resource Implications:</b>           | Information                                                                                                                                                                                                                                   |
| <b>Recommendation to the Committee:</b> | <ul style="list-style-type: none"> <li>• Note the data and views presented in the paper from Justin St John</li> <li>• Come to a view about the use of GVT in treatment of i) infertility in older women ii) mitochondrial disease</li> </ul> |

### 1. Background

1.1 Germinal vesicle transfer (GVT) was identified through the horizon scanning process as an issue that may have an impact on assisted reproduction in the near future.

1.2 At the last meeting Justin St John presented information to SCAG about the potential uses and safety of GVT. It was agreed that Justin would write a summary of his presentation and views for consideration by SCAG. The summary is attached in Annex A.

### 2. Introduction

2.1 In germinal vesicle transfer, the nucleus, at the germinal vesicle stage is removed from the patient's oocyte and transferred into an enucleated donor egg. The germinal vesicle stage is the point in oocyte maturation, before the oocyte has one set of chromosomes, when two copies of each of the 23 pairs of chromosomes are present (4N). The oocyte is then matured *in vitro* and transferred back into the patient. This means that successful use of this technology relies on the successful development of *in vitro* maturation.

2.2 Following germinal vesicle transfer and fertilisation the embryo will contain genetic material from three individuals: chromosomal DNA (and possibly some mtDNA) from the patient, mtDNA from the host oocyte and chromosomal DNA from the sperm.

2.3 This technique may benefit women of advanced reproductive age and women with mitochondrial diseases. The reduced pregnancy rates in women of advanced reproductive age is thought to be due to aneuploidy (chromosome abnormalities) caused by an uneven division of the genetic material in the meiosis stage of oocyte maturation. In oocyte maturation the division of the chromosomes is dependent on components of the ooplasm (e.g. mitochondria), which may become dysfunctional in older women. GVT may also potentially allow women with inherited mitochondrial diseases (there are more than 500 diseases) to have healthy children.

### **3. GVT paper**

3.1 The paper (Annex A) summarises studies of GVT carried out in both humans and animals and discusses the use of GVT in treatment of older women and to avoid passing on mitochondrial diseases.

3.2 Justin St John concludes that although useful for developmental biologists studying molecular biology, GVT is not well enough understood to be used in treatment.

### **4. Conclusions**

4.1 Members are asked to:

- Comment on the data and views presented in the paper (Annex A)
- Comment on the use of GVT in treatment of
  - i) infertility in older women
  - ii) mitochondrial disease

### **Germinal Vesicle Transfer and Mitochondrial DNA Transmission.**

Justin C. St. John, The Mitochondrial and Reproductive Genetics Group, The Medical School, The University of Birmingham, B15 2TT, UK. [j.stjohn.1@bham.ac.uk](mailto:j.stjohn.1@bham.ac.uk)

Germinal Vesicle Transfer (GVT) and Pronuclear Transfer (PNT) are two techniques that require the transfer of either a GV from an unfertilised oocyte or pronuclei from a fertilised oocyte into an enucleated recipient oocyte. In many cases, this is likely to be a transfer from an aged oocyte into a younger oocyte, preferably from the same stage of development. Following reconstruction, the GV is allowed to develop to Metaphase II through in vitro maturation (IVM) and is then fertilised through either IVF or ICSI. The PN following PNT will already consist of sperm and oocyte chromosomes and will subsequently need to be activated. The resultant zygotes are then allowed to develop in culture before transfer to patients.

These procedures have been proposed as potential treatments for those women whose oocytes fail to fertilise or arrest during development or are associated with aneuploidy. Infertility in older women is associated with an increase in oocyte aneuploidy, which could be attributed to abnormal chromosomal segregation during meiosis (Battaglia et al., 1996; Volarcik et al., 1998). Indeed, studies using human oocytes have shown that GVT from aged oocytes introduced into the enucleated ooplasm of young oocytes (Zhang et al., 1999) or sibling oocytes (Takeuchi et al., 2001) can overcome oocyte aneuploidy, with the majority of reconstructions having normal karyotypes. However, whilst these findings are encouraging, only a small number of oocytes were used in these studies (see Table).

The use of senescence accelerated mice has also been used to study oocyte aneuploidy, as they demonstrate age related misalignment of chromosomes in their oocytes. One study showed that there was a significantly greater level (57.1%) of chromosomal abnormalities in reconstructions when the GV was transferred from aged mice to the enucleated ooplasm of young mice (Cui et al. 2005). However, GVT from young oocytes into the enucleated ooplasm of aged oocytes resulted in only 16.7% of reconstructions possessing chromosomal misalignment compared with 16.3%

for young GVs transferred into young ooplasm. In this instance, the authors argued that the GV, as opposed to the ooplasm, determined the correct distribution of the spindle and the chromosomes in such reconstructions.

A series of other studies have reported varying outcomes (see Table). Interestingly though, autologous (the same oocyte source) and heterologous (surrogate oocyte source) GVTs appear not to give rise to significantly different developmental outcomes (Liu et al., 1999; Li et al., 2001a). In cattle though, oocyte diameter at the GV stage appears to be an indicator of success, where a diameter of approximately 110µm appears necessary for successful maturation following transfer (Bao et al., 2003). However, only 11 to 15% of GVTs of these managed to reach blastocyst. Of the 27 morulae and blastocysts transferred, 3 resulted in live births, though they died shortly after.

Other reports have suggested that the ooplasm may contain the necessary factors required to rescue damaged oocytes. Induction of mitochondrial damage in mouse oocytes can prevent oocyte maturation, spindle body formation and chromosomal segregation (Takeuchi et al., 2005). This damage was overcome by GVT of the karyoplast derived from a damaged oocyte into the ooplasm of a healthy enucleated oocyte. Cytogenetic analysis showed that 20/21 of these reconstructions had a normal number of chromosomes. Furthermore, performing ICSI on GVT reconstructions and transferring the resulting 2 cell embryos led to the production of live offspring. Consequently, it is still unclear whether the cytoplasm or the GV contains the crucial factors which enable correct chromosomal segregation or whether it is a combination of both.

GVT has also been widely proposed as a method of preventing the transmission of mitochondria associated diseases from mother to offspring (Cummins et al., 1998, Trounson et al., 2001). Through its various biochemical pathways, the mitochondrion generates ATP, which is necessary for cellular homeostasis and function (Moyes et al., 1998). The inner membrane of the mitochondrion houses the mitochondrial genome (mtDNA). In the human, this extranuclear

genome is approximately 16.6kb in size (Anderson et al., 1981). MtDNA encodes 13 of the subunits of the electron transfer chain (ETC) complexes, associated with the process of oxidative phosphorylation (OXPHOS), along with 22 tRNAs and 2 rRNAs that are necessary for mRNA expression (Anderson et al., 1981). Expression of these mitochondrial genes is vital for cellular function, especially as the ETC is the cell's major generator of ATP (Moyes et al., 1998).

Normally, mtDNA is inherited through the oocyte (Birky, 2001; 1995) and tends to be homoplasmically transmitted, i.e. the presence of only one mtDNA genotype (Monnat et al., 1985). Occasionally, two or more mtDNA genotypes can be present, often due to either a pathological or non-pathological rearrangement, resulting in a state of heteroplasmy, as in the oocytes of a woman harbouring a mtDNA disease ranging from 0 to 95% (Blok et al., 1997). Those pathological mutations or deletions can result in severe cellular impairment and can be lethal (Wallace, 1992). However, the clinical phenotypes resulting from mtDNA mutations are dependent on the proportion of mutated mtDNAs to wild type. It has been reported that, in the case of Leber's Hereditary Optic Neuropathy (LHON), >60% mutant mtDNA load is required before the disease phenotype presents (Chinnery et al., 2001). In some mitochondrial diseases, including a case of Myoclonic Epilepsy with Ragged Red Fibres (MERRF), over 85% mutant mtDNAs need to be present before the affects are observed (Boulet et al., 1992).

A major concern is that the transferred GV is still surrounded by a population of tightly packed mitochondria which will also be introduced into the donor ooplasm. These mitochondria remain close to centre of the immature reconstruction and disperse throughout the cytoplasm as maturation ensues (Fulka, 2004). The initial close proximity of the mitochondria to the nucleus may allow for preferential amplification of mtDNA and its uniform dispersal throughout the cytoplasm will ensure that it will be found in most tissues. Indeed, exogenous mtDNA accompanying the murine zygotic karyoplast is found in higher concentrations at blastocyst than mtDNA from transferred cytoplasts (Meirelles & Smith, 1998). This has been further highlighted by studies where zygotic karyoplast transfer into zygotes resulted in varying amounts of mtDNA

being transmitted to offspring (0 - 69% (Meirelles & Smith, 1997), suggesting that those molecules introduced can be transmitted at random frequency. This may also account for the large amount of donor mtDNA detected in offspring derived from cytoplasmic transfer (Brenner et al., 2000), especially as this was delivered into the oocyte at the same time as the sperm. Consequently, the position of 'foreign' mitochondria in the early oocyte and embryo may play a role in determining which mtDNA genomes are selected for amplification. Thus, it would be difficult to predict to what extent a mutated or deleted mtDNA molecule would be selected for. However, a recent report suggests that the varying levels of mtDNA deletion in mouse zygotes could be reduced to levels lower than those expected for the phenotypic onset of mtDNA disease when the zygotic nucleus is transferred into an enucleated healthy oocyte (Sato et al., 2005). The subsequent offspring also remained under the level for pathological onset even with the accumulative increase of the deletion with time. However, as a point of caution, these authors state that, in the human, this form of nuclear transplantation could not be applied to those mtDNA diseases where the pathogenic mutation had significant replicative advantage over wild type mtDNA. This is most likely as in humans the prenatal period is 13 times longer than in the mouse and this represents the window in which mtDNA mutant replication is most proliferative (Sato et al., 2005).

Not all mtDNA mutations are however neutral which therefore make the levels of their transmission difficult to assess (see Wallace, 1992). To this extent, somatic cell fusions have also demonstrated that the nuclear background of a differentiated cell could preferentially select for either wild type or mutated mtDNA molecules (Dunbar et al. 1995). This would suggest that if mutated or deleted mtDNA molecules persist in a particular cell type or tissue that is a high ATP user then preferential amplification of those molecules could be of considerable disadvantage to the offspring.

The issue of heteroplasmy is not just confined to mtDNA rearrangements. The increasing use of nuclear transfer, cytoplasmic transfer and GVT can create offspring with two or more populations

of mtDNA from differing strains or species (see St. John et al., 2004 for extensive review) with differing sequences. Consequently, sequence differences due to mtDNA from a 'foreign' source may give rise to proteins with slightly altered amino acid sequence. This has been demonstrated in both pigs (St. John *et al.*, 2005) and cattle (Steinborn et al., 2002) and may, in the long term, result in inadequate interaction between the separate subunits of the electron transport chain. In its extreme, such cytoplasmic conflict can have serious effects on ATP production (McKenzie & Trounce, 2000).

Currently, no appropriate strategies have been developed to eliminate mutated mtDNA from oocytes. Although the use of toxins on (Fulka et al., 2004) or the centrifugation of (Van Blerkom et al., 1998) the mitochondria surrounding the GV have been suggested, the use of drugs or techniques that would induce cytoplasmic disorganisation could be harmful to oocyte function. It would therefore appear that GVT is neither sufficiently refined nor are the subsequent anomalies sufficiently well-understood to introduce its use therapeutically in order to treat infertility or mitochondrial associated disease. However, the technique does provide developmental biologists with an important tool for deciphering the molecular mechanisms involved in oocyte aneuploidy and the involvement of functional and dysfunctional mitochondria in such outcomes.

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