THE USE OF MITOCHONDRIAL TRANSFER TO IMPROVE ART OUTCOME

Björn HEINDRYCKX, Cristina EGUIZABAL, Susana CHUVA DE SOUSA LOPES, Mieke GEENS, Rita VASSENA

for the ESHRE SIG Stem Cells

A recent report by Fakih *et al.* (2015) describes the use of mitochondria, harvested from autologous egg precursor cells, to improve pregnancy outcome after ICSI in a proprietary treatment known as "AUGMENT^{SM}". These findings were published in the open access Journal of Fertilization: In Vitro, IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology in August 2015. The report has generated much interest and the technology has been described by the company marketing it as the "latest major innovation" in ART.

As a Special Interest Group of ESHRE, we certainly welcome innovative stem cell based approaches to infertility. However, as scientists and physicians we have a responsibility to evaluate newly reported findings of such magnitude and with such long-term potential impact with caution. From a safety perspective, we would recommend certain quality controls for further studies to substantiate and unify the significance of the outcomes.

We have several concerns with the published report of Fakih *et al*:

1. In their introduction the authors state: *"Studies have demonstrated that mitochondria isolated from egg precursor cells are of high quality and can therefore serve as an autologous source of mitochondria for women."* This comment is supported by a citation from 1998 (Van Blerkom *et al.*, 1998), long before the existence of egg precursor cells was proposed.

2. As rationale for their study the authors state: "*The support and use of the AUGMENT treatment is based upon case reports of clinical success using human donor egg cytoplasm injection as well as multiple published animal studies that have demonstrated that the addition of mitochondria during IVF treatment is safe, improves the quality of the embryos, and increases the success of IVF."* This explanation is supported by reference to a report by Cohen *et al.* (1998), a study of ooplasmic transfer between mature human oocytes. With only seven cases performed, this study did not demonstrate to any acceptable extent the safety of mitochondrial transfer, an improvement in embryo quality, or any increase in success rate after IVF. Evidently, the mitochondria used in the study of Cohen *et al.* (1998) did not originate from egg precursor cells. Importantly, the study of Cohen et al. (1998) makes none of the claims now proposed by Fakih *et al.* (2015).

3. Unfortunately, Fakih *et al.* (2015) do not describe an adequate control group for the first series of 93 patients included in the study, which makes any conclusion drawn from the present data unreliable. For the second group of 25 patients, the authors report that the oocytes were allocated to either the Augment group, for mitochondrial injection, or to the ICSI-only group. This allocation was not randomised, with only 38% allocated to the ICSI-only

group and 62% to the Augment group. Such an imbalanced allocation should favour the Augment group in embryo transfer and pregnancy rates, even though the authors claim otherwise. Thus, comparing the pregnancy rates of women having the Augment treatment with those with similar infertility problems who didn't use the technique would substantiate significantly the claims made by Fakih *et al.*

4. An additional source of bias was that the embryologists performing embryo scoring and selection for transfer were not blinded for the treatment groups.

Independently of these major methodological shortcomings, we should also be cautious about the general experimental and clinical applications of these techniques in ART. What are the proven benefits of this technology? Have safety issues been addressed sufficiently in advance? One can argue, as the authors do, that many newly developed techniques (such as IVF itself or ICSI) were "most often adopted into clinical practice without any demonstrated benefit in randomized controlled clinical trials". However, the fact that some techniques have been adopted without appropriate evidence does not constitute a justification for being less cautious with new techniques. Moreover, mitochondrial transfer is especially invasive, requiring laparoscopic surgery before the ART treatment, and we believe that proof-of-concept studies should be performed thoroughly before clinical translation is considered.

Further studies on mitochondrial transfer should address the following safety issues:

1. Quality assessment of the reagents, the isolated mitochondria and the manipulated oocytes. How will purification occur? How will quality be assessed? What is the best source of cells for the mitochondria? What number of mitochondria should be injected per oocyte?
2. The critical threshold of the number of mtDNA copies needed to support embryonic developmental potential. For example, it has been shown that excessively high mtDNA copy numbers can have detrimental effects in mice (Ylikallio *et al.,* 2010). There seems scant evidence of a minimal threshold number of mtDNA copies for the efficient support of embryonic development. Studies have found wide variation in the number of mtDNA copies in oocytes and embryos, but without any obvious correlation between infertility disorders and mitochondrial insufficiency. In contrast, however, recent quantifications at the blastocyst level seem to suggest an adverse correlation between mtDNA copy number and blastocyst quality and implantation rate in the human (Fragouli *et al.*, 2015).
3. Risks of mtDNA heteroplasmy. Although the study of Fakih *et al.* (2015) used autologous mitochondria, the consequence of different mitochondrial genetic backgrounds in cells or embryos remains unclear. The accumulation of mtDNA occurring over time must also be taken into account, which may amplify the consequences. Some evidence in mice points to abnormalities in pulmonary function, metabolism and in altered behaviour and cognition due to mtDNA heteroplasmy (Acton et al., 2007; Sharpley et al., 2012).

4. Nuclear-mitochondrial incompatibility. For example, cytoplast fusion experiments in cows suggest that certain mtDNA haplotypes are more compatible with a given nuclear genome than others (Bowles et al., 2008). This is, however, not a point of attention for the Augment study, as in this case autologous mitochondria were used, as stated before.
5. Epigenetic changes caused by extra micromanipulation or resulting from nuclear-mitochondrial incompatibility or mtDNA heteroplasmy. Such consequences might manifest only during later life.

It is also critical that the potential benefits of these techniques are first demonstrated in animal models, and so far this is lacking for mitochondrial transfer from egg precursor cells. Certainly, cytoplasmic transfer has been applied in the past to overcome repeated implantation failure in IVF, and this led to the birth of more than 20 babies. But because of the small sample size of these different studies, we should not claim that such cytoplasmic transfer was of benefit to the treated patients, or that the abnormalities in some of the children born were the result of the technique itself. A few animal studies have investigated the beneficial effect of the transfer of mitochondria harvested from sources other than egg precursor cells. However, an effect was only verified in early events - such as fertilisation in the pig (El Shourbagy *et al*., 2006), or during early pre-implantation development in bovine and mice models (Yi *et al.*, 2007; Hua *et al.*, 2007); these animal models do not reflect adequately infertility issues.

In summary, the SIG Stem Cells recommends caution when interpreting the results reported by Fakih *et al.* (2015), and urges all stakeholders to perform extensive safety studies and prove the beneficial effect of mitochondria transfer on infertility before considering the possible clinical benefit of this, so far, unproven technology.

References

Acton BM, Lai I, Shang X, Jurisicova A, Casper RF. Neutral mitochondrial heteroplasmy alters physiological function in mice. Biol Reprod 2007; 77: 569-576.

Bowles EJ, Tecirlioglu RT, French AJ, Holland MK, St John JC. Mitochondrial DNA transmission and transcription after somatic cell fusion to one or more cytoplasts. Stem Cells 2008; 26**:** 775-782.

Cohen J, Scott R, Alikani M, Schimmel T, Munné S, et al. Ooplasmic transfer in mature human oocytes. Mol Hum Reprod 1998; 4: 269-280.

El Shourbagy SH, Spikings EC, Freitas M, St John JC. Mitochondria directly influence fertilisation outcome in the pig. Reproduction 2006; 131: 233-245.

Fakih MH, Shmoury MEl, Szeptycki J, et al. The AUGMENT^{SM} Treatment: Physician Reported Outcomes of the Initial Global Patient Experience. JFIV Reprod Med Genet 2015; 3: 154. doi:10.4172/2375-4508.1000154

Fragouli E, Spath K, Alfarawati S, et al. Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. PLoS Genet 2015; 11(6): e1005241.

Hua S, Zhang Y, Li XC, et al. Effects of granulosa cell mitochondria transfer on the early development of bovine embryos in vitro. Cloning Stem Cells 2007; 9: 237-246.

Sharpley, M.S. et al. Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. Cell 2012; 151: 333-343.

Van Blerkom J, Sinclair J, Davis P. Mitochondrial transfer between oocytes: Potential applications of mitochondrial donation and the issue of heteroplasmy. Hum Reprod 1998; 13: 2857-2868.

Yi YC, Chen MJ, Ho JY, Guu HF, Ho ES. Mitochondria transfer can enhance the murine embryo development. J Assist Reprod Genet 2007; 24: 445-449.

Ylikallio E, Tyynismaa H, Tsutsui H, et al. High mitochondrial DNA copy number has detrimental effects in mice. Hum Mol Genet 2010; 19: 2695-2705.