Fixing oocytes? A bovine model provides new hope

Henry Malter
Genesis Fertility and Reproductive Medicine, Brooklyn NY 11228, USA
E-mail address: hemalter@gmail.com.

Henry Malter, PhD

Abstract In a previous issue of Reproductive BioMedicine Online, Chiaratti and co-workers presented a bovine model for ooplasmic transfer, which demonstrated a positive effect on early development. Developmental deficits resulting from artificial treatment of recipient eggs with a toxic compound were ameliorated by the addition of small volumes of healthy donor cytoplasm. This model provides an important advance in the understanding of ooplasmic effects in early development and addresses issues about the prior human trials in this area.

KEYWORDS: bovine, cytoplasmic transfer, mitochondria, oocytes

This article was published in Reproductive BioMedicine Online, Vol 22, 2011, Malter, p 229-231, 'Fixing oocytes? A bovine model provides new hope'. Copyright Elsevier. It is reprinted here with permission.

Ooplasm is literally the stuff of life and provides the maternally derived molecular requirements and driving force of early development. A complete and accurate assessment of the ooplasm remains to be accomplished and this Sisyphean task would seem to be constantly expanding. New components and even new species of components such as small RNAs and peptides are identified every year. There can be no doubt that general or specific deficits in the ooplasm disturb the processes of maturation, fertilization and embryonic development leading to infertility. Considerable effort is being exerted to describe early human developmental determinants towards a strategy of embryo selection. However, direct intervention to 'correct' potential deficits in specific oocyte components presents a much more difficult prospect.

The lack of a complete ooplasmic component catalogue has failed to hinder experiments involving the functional assessment and manipulation of whole ooplasm. Research using such manipulation clearly shows that ooplasmic components can be transferred from one egg to another with often profound effects on development. For example, the excellent early study by Muggleton-Harris and co-workers (1982) demonstrated that small quantities of ooplasm transferred from F1 hybrid oocytes could circumvent the characteristic '2-cell' block in a standard lab strain. Perhaps the most extensive body of work concerns the transfer of ooplasm between different strains and in some cases even different species harbouring distinctive mitochondria (Ferreira et al., 2010; Smith and Alcivar, 1993; Takeda et al., 2000). Such studies have helped to clarify the behavior of mitochondria during early development and the interaction between the mitochondria and other ooplasmic constituents. While in some very specific scenarios (the DKK and DBA/2 mouse strains and similar inbred aberrations) development can be compromised due to genetic incompatibilities and dysfunction, taken as a whole this body of work seems to indicate that the simple transfer of ooplasm and the creation of oocytes and early embryos with mixed-cytoplasmic and mitochondrial make-up is readily compatible with normal term development and unremarkable life histories in the resulting offspring (Babinet et al., 1990; Latham and Solter, 1991; Smith et al., 2000).

Based on such prior research, several human assisted reproduction clinics pursued the use of ooplasmic transfer as a means to circumvent deficits in early human development. In the most extensive trial, the initial results were quite promising with an excellent implantation rate and many babies born to couples with intractable infertility and 100% prior failure with treatment (Barrit et al., 2001; Cohen et al., 1997). However, due to the associated transfer of genetic material (mitochondrial DNA) between individuals, the US Food and Drug Administration reviewed and eventually suggested the technique be restricted pending the completion of a successful Investigational New Drug protocol. The FDA considered transferred cytoplasm to be a biological 'product' of which safety and efficacy had to be determined. Unfortunately, conducting such a complex and costly protocol was far beyond the resources of the small private clinic involved and so instead the technique was simply discontinued. At the time, much misinformation and negative hysteria surrounded this situation and the application of the technique in human patients, which was considered premature by some.

Chiaratti et al. (2011), pioneers in ooplasm/mitochondrial

transfer research present the results of a challenging and complex study in the bovine demonstrating the 'rescue' of

damaged oocytes by the transfer of donor cytoplasm, leading to term development. In some ways, this latest work from the laboratories of Lawrence Smith and Flavio Meirelles is a continuation of their extensive prior research – creating embryos/offspring with mixed cytoplasmic/mitochondrial make-up using diverse donor and recipient sources. However, in this case, the recipient ooplasm was artificially compromised by exposure to the cytotoxic DNA stain ethidium bromide (EtBr). Oocytes exposed to high doses of EtBr failed to mature and arrested while lower doses resulted in developmental deficits. This negative effect could be overcome by the addition of modest volumes of healthy donor ooplasm. Therefore the study constitutes a successful animal model for ooplasmic transfer as a therapeutic protocol. The authors attempted to determine if the EtBr effect (and its subsequent rescue) was via mitochondrial function. However, the level of heteroplasmy, mitochondrial DNA and ATP content and membrane potential did not differ between treated and control embryos. Obviously many other cellular components (proteins, nucleic acids, etc.) are present in donor cytoplasm and could be the source of any observed effects and simple dilution of the toxin is also a likely contributor. However, a key point is that, regardless of the specific nature of the positive effect, this is yet another study showing no negative effect or consequences from ooplasmic transfer. The bovine is a reasonable and perhaps underutilized model for basic human reproductive aspects and has some advantages over the mouse (Ferreira et al., 2010; Menezo and Herubel, 2002).

Over the past 10 years since the human clinical ooplasmic transfer work (in which I played only a small peripheral role in follow-up studies), I have been amazed at the level of misunderstanding of not only the technique and trial itself but the underlying science as well. As a developmental biologist, I felt that the human ooplasmic transfer trial presented one of the most interesting embryological results of the last decade. It provided strong evidence for a dramatic amelioration of cryptic and otherwise intractable oocyte-related developmental dysfunction via the modest addition of whole

donor ooplasm. Something in the small mass of donor ooplasm clearly seemed to have a positive effect on early development with further downstream consequences. These

fascinating results were unfortunately lost in a rush to condemn the work and speculate on the potentially negative aspects.

The concept that heterologous ooplasm or mitochondrial heteroplasmy per se is somehow inherently deleterious is unfounded and in fact is contradicted by a large body of basic science research including the current excellent study. However, multiple critical reviews and assessments of ooplasmic transfer have and continue to suggest dire consequences based on unique observations in inbred mice and other aberrant genetic scenarios or simply drawn from thin air (Cummins, 2001; Liang et al., 2009; Winston and Hardy, 2002). A recent publication showing the expected 'negative' consequences in ooplasmic transfer experiments using the well-characterized DBA/2 mouse strain suggested in somewhat inflammatory fashion a critical lesson for potential

human manipulations (Liang et al., 2009). Of course this work simply reveals an expected outcome based on the identical developmental 'mismatch' this strain exhibits in crossbreeding with other mouse strains. Mechanistically this phenomenon is an important piece of the ooplasmic puzzle and it certainly may be 'bad news' for DBA/2 mice. However, the connection between this outcome – clearly resulting from unique genetic abnormalities fixed in the inbred genome involved – and any possible reproductive incompatibility in outbred humans is highly speculative. Others have proposed that mitochondrial heteroplasmy is itself a rare and dangerous condition and even alarmingly speculated that the mitochondria in heteroplasmic ooplasmic transfer offspring will simply 'stop working' at some point (Cummins, 2001; KH Campbell, personal communication). This is, however, without any real basis and is contradicted by the vast majority of current science in this area. Hypervariable region (noncoding) heteroplasmy of the type/level observed in some ooplasmic transfer individuals is in fact common in the normal human population with no discernible effect (Tully et al., 2000). As discussed, a large body of research in multiple mammalian species confirms the great flexibility of mitochondrial/cytoplasmic interaction. While some congenic mice strains bred to complete cross-strain divergence in nuclear/mitochondrial backgrounds have displayed functional deficits, multiple experimental heteroplasmic animals created by cytoplasmic manipulation do not and exhibit life histories showing no evidence of dysfunction, much less catastrophic failure (Nagao et al., 1998; Smith et al., 2000). It is a shame that competent scientists feel free to extrapolate from unique scenarios, ignore majority results and engage in irresponsible, unfounded speculation with ominous reference to the future health of patients who deserve better respect.

A clear understanding of the molecular determinants of gamete-based developmental deficits in the human is an important goal but treatment options for such deficits remain

elusive. Those of us with a mandate to help infertile patients do not have the luxury of simply ignoring these deficits and their correction. The introduction of specific molecular reagents into human gametes would seem to be precluded at least for the moment. The use of whole

gametes from healthy fertile donors has become a 'work-around' to allow patients with intractable gamete-related issues to conceive children with at least a partial genetic and physical connection. This would have been the only treatment option available to the individuals who consented to the ooplasmic transfer trial. Of course most patients seeking assistance with reproduction want their own genetic offspring and ooplasmic transfer offered a solution in this regard.

Seeking a complete determination of the safety and efficacy of such protocols will certainly require furthering our understanding of the ooplasm. Excellent basic experiments like the informative DBA/2 strain work discussed above continue to demonstrate that the interaction between the parental genomes and ooplasm can have profound downstream effects. However, there are no truly satisfactory model systems for human infertility and so ideally such research takes place in the species of interest. The human ooplasmic transfer trial represented a tantalizing glimpse at the kind of key research that is needed. Gametes derived from human embryonic stem cells will hopefully provide a source of appropriate and acceptable research material for the future and also could form the basis for unique new clinical scenarios. Studies like the current bovine publication, in a divergent but appropriate model, provide another path for advancement and I extend my hearty congratulations and encouragement to the authors for their efforts.

Ooplasm is clearly powerful stuff. The potential for negative consequences cannot be taken lightly in its manipulation. However, evidence of basic safety and the potential

for positive manipulative outcomes, such as demonstrated in the current study, can also not be denied. Those wishing to grapple with the development of interventional strategies in this area need to exercise both caution and courage. Patients seeking solutions to their health challenges from human biomedicine, who exhibit these qualities as well, deserve nothing less.

References

Babinet, C., Richoux, V., Guenet, J.-L., Renard, J.-P., 1990. The DKK inbred strain as a model for the study of interactions between the parental genomes and egg cytoplasm in mouse preimplantation development. Development (Suppl.), 81–87.

Barrit, J.A., Brenner, C.A., Malter, H.E., Cohen, J., 2001. Mitochondria in human offspring derived from ooplasmic transplantation. Hum. Reprod. 16, 513–516.

Chiaratti, M.R., Ferreira, C.R., Perecin, F., et al., 2011. Ooplast-mediated developmental rescue of bovine oocytes exposed to ethidium bromide. Reprod. BioMed. Online 22, 172–183.

Cohen, J., Scott, R., Schimmel, T., Levron, J., Willadsen, S., 1997. Birth of infant after transfer of anucleate donor cytoplasm into recipient eggs. Lancet 350, 186–187.

Cummins, J.M., 2001. Mitochondria: potential roles in embryogenesis and nucleocytoplasmic transfer. Hum. Reprod. Update 7, 217–228.

Ferreira, C.R., Burgstaller, J.P., Perecin, F., et al., 2010. Pronounced segregation of donor mitochondria introduced by bovine ooplasmic transfer to the female germline. Biol. Reprod. 82, 563–571.

Latham, K.E., Solter, D., 1991. Effect of egg composition on the developmental capacity of androgenetic mouse embryos. Development 113, 561–568.

Liang, C.-G., Han, Z., Cheng, Y., Zhong, Z., Latham, K.E., 2009. Effects of ooplasm transfer on paternal genome function in mice. Hum. Reprod. 24, 2718–2728.

Menezo, Y.J., Herubel, F., 2002. Mouse and bovine models for human IVF. Reprod. Biomed. Online 4, 170–175. Muggleton-Harris, A., Wittingham, D.G., Wilson, L., 1982. Cytoplasmic control of preimplantation development in vitro in the mouse. Nature 299, 460–462.

Nagao, Y., Totsuka, Y., Atomi, Y., et al., 1998. Decreased physical performance of congenic mice with mismatch between the nuclear and the mitochondrial genome. Genes Genet. Syst. 73, 21–27.

Smith, L.C., Alcivar, A.A., 1993. Cytoplasmic inheritance and its effects on development and performance. J. Reprod. Fertil. 48 (Suppl.), 31–43.

Smith, L.C., Bordignon, V., Garcia, J.M., Meirelles, F.V., 2000. Mitochondrial genotype segregation and effects during mammalian development: applications to biotechnology. Theriogenology 53, 35–46.

Takeda, K., Takahashi, S., Onishi, A., Hanada, J., Imai, H., 2000. Replicative advantage and tissue-specific segregation of RR mitochondrial DNA between C57BL/6 and RR heteroplasmic mice. Genetics 155, 777–783.

Tully, L.A., Parsons, T.J., Steighner, R.J., Holland, M.M., Marino, M.A., Prenger, V.L., 2000. A sensitive denaturing gradient–gel electrophoresis assay reveals a high frequency of heteroplasmy in hypervariable region 1 of the human mtDNA control region. Am. J. Hum. Genet. 67, 432–443.

Winston, R.M.L., Hardy, K., 2002. Are we ignoring potential dangers of in vitro fertilization and related treatments? Nat. Cell Biol. 4 (Suppl.), 14–18.