EDITORIAL

IMPROVING EGGS: MORE QUESTIONS THAN ANSWERS

Assisted reproduction practitioners face an increasingly difficult challenge in attempting to facilitate the birth of a healthy baby for any and all infertile couples. The “easy” problems have been mostly solved leaving a variety of complex and poorly understood fertility deficits. One current struggle involves addressing cases of poor development and implantation failure associated with dysfunctional gametes. Compromised oocytes can simply be replaced by donor substitutes but at the price of losing the patient’s genetic contribution. A variety of controversial strategies have been proposed to get around this problem including the creation of functional “oocytes” from the patient’s somatic cells (1) and oocyte to donor ooplast nuclear transfer (2). Perhaps the simplest idea has been to “improve” the patient’s own eggs by an infusion of theoretically healthy ooplasm from a donor egg (3). As originally proposed, this technique seemed like a well-founded concept. Furthermore, the initial results from applying the technique in a defined group of patients seemed to be positive. Implantation was improved with a 43% clinical pregnancy rate (and several births) in 27 couples with a combined history of almost 100 failed assisted reproduction cycles (4,5). Despite the lack of a controlled trial, this result was at least intriguing.

Several variations on ooplasmic infusion have been suggested including the transfer of polyspermic zygote cytoplasm (6) or cytoplasm from cryopreserved oocytes (7), and even the injection of supposedly purified mitochondria from the patient’s cumulus cells (8).

In this issue, Opsahl and co-workers present the results of a study in which the oocytes of older patients and those with high basal FSH were injected with cytoplasm from cryopreserved donor oocytes. However, the results of this protocol were poor. This negative outcome may help to define two important aspects of the ooplasmic infusion concept. The first concerns what patients might be benefited by such protocols.

The second concerns the source of any positive developmental effectors that might be introduced.

The question of patient selection for ooplasmic infusion treatment is particularly complex due to our lack of basic knowledge about the biological deficits involved with poor embryonic development and implantation failure. Furthermore, some of these issues, such as the direct relationship between aneuploidy and maternal age, may not be addressable through ooplasmic transfer (9,10). The source of the aneuploidy age effect remains obscure. However, it is clear that the majority of nondisjunction events apparently arise in the meiotic divisions leading to the generation of the mature MII oocyte (11). While it is not impossible that immediate ooplasmic conditions in the mature egg may be involved with the generation of subsequent errors, it seems unlikely that minor “augmentation” of the potentially compromised ooplasm at this stage could correct or prevent this nondisjunction. Recently, it was discovered in mouse (12) as well as in human embryos (Munne, personal com.) that mitotic nondisjunction in cleavage stages can lead to a specific form of mosaicism that appears to be maternal-age related. It is possible that such later forms of nondisjunction can be addressed by ooplasmic transfer. Patients for the original ooplasmic transplantation investigation were selected only based on the incidence of oocyte-related deficits in embryonic development and implantation (4). This selection avoided those of advanced maternal age and decreased ovarian reserve. Patient age was slightly higher in the first ooplasmic transplantation patients and, in fact, one aneuploidy-related loss as well as an aneuploid-related selective reduction was observed following the technique’s application (13). The older patients (aged 40–49) in the current study apparently did not benefit from ooplasmic transfer. The five cycles that did exhibit elevated hCG all failed. Among these, a single clinical pregnancy was lost at 8 weeks and identified as aneuploid. A previous case report on the transfer of cryopreserved ooplasm reported that three patients of advanced maternal age did not exhibit improved embryonic development or implantation following the technique, while a single 35-year old patient apparently did and delivered healthy twins (7). While the nature of any beneficial effect from ooplasmic infusion remains to be elucidated, it seems that a robust correction of oocyte aneuploidy is not part of this effect. Therefore, the technique may not be indicated with older patients and, at the minimum, aneuploidy assessment should be a part of such treatment.

The second issue raised by the current report concerns the source of the ooplasm used for infusion. This would seem to be a critical factor if the intent of the procedure is to transfer some positive effectors from donor to recipient. In the current study, donor oocytes were used following cryopreservation. This was done to avoid the necessity of arranging synchronous donor and recipient cycles. However, the post-thaw survival of these oocytes was very poor and no evidence is presented as to the true viability and quality of the few surviving oocytes used as the source of donor cytoplasm. It seems likely that the application of such a traumatic cryopreservation protocol might result in a degradation of key ooplasmic factors or alter the oocyte structure in such a way that the extracted ooplasm was compromised. One of the key questions in oocyte cryopreservation has been whether the lack of success is the result of detrimental changes in the nucleus or the ooplasm or both. Although distinguishing between these options is complicated when assessing cryopreservation data of single cells, some experimental evidence in the mouse suggests that ooplasmic damage can be serious (14). The previous successful trial using cryopreserved donor oocytes reported a much greater post-thaw survival rate (7). Based on these results, the validity of using cryopreserved donor material remains an open question. However, it seems certain that all donor ooplasm is not created equal and ooplasmic transplantation protocols need to be based on the most healthy and appropriate donor material possible.

Combining all of the currently published clinical trials of ooplasmic infusion protocols still provides us with only a meager data set confounded by the kinds of differences in patient selection and technical specifics discussed here. Obviously, a great deal more research will be necessary before a clear picture emerges concerning the basis and optimal strategies for oocyte "improvement" via ooplasmic infusion protocols. Unfortunately, the controversial nature of such human gamete manipulation has resulted in a premature de-facto ban on the clinical trials necessary to address these issues—at least in the United States. The Food and Drug Administration has issued a statement declaring that ooplasmic transfer and related protocols constitute treatment options that are subject to formal review and approval (15). Affected clinics are currently evaluating this situation but it remains to be determined if such review and approval will even be feasible. It is ironic and unfortunate that this regulatory intrusion follows many years of a questionable government policy that has failed to

support (and in some cases actively discouraged) the very type of research necessary to address these valid and critical human health issues. In any case, gamete-related problems are not going to solve themselves and will constitute an ongoing treatment challenge for our field. Hopefully, the many questions that remain can eventually be addressed through appropriate research and clinical trials, and we can proceed toward the goal of successfully treating infertile patients, men and women, according to their needs and desires.

REFERENCES

1. Nagy PZ, Bourq de Mello MR, Tesarik J, Visentin JA, Abdelmassih R: Fertilizable bovine oocytes reconstructed using somatic cell nuclei and metaphase-II oocytes. *Hum Reprod* 2001;16 (Suppl 1), abstr # O-009
2. Takeuchi T, Gong J, Veek LL, Rosenwaks Z, Palermo GD: Preliminary findings in germinal vesicle transplantation of immature human oocytes. *Hum Reprod* 2001;16:730-736
3. Cohen J, Scott R, Schimmel T, Levron J, Willadsen S: Birth of infant after transfer of anucleate donor cytoplasm into recipient eggs. *Lancet* 1997;350:186-187
4. Cohen J, Scott R, Alikani M, Schimmel T, Munné S, Levron J, Wu L, Brenner C, Warner C, Willadsen S: Ooplasmic transfer in mature human oocytes. *Mol Hum Reprod* 1998;4:269-280
5. Barritt JA, Tomkin G, Sable DB, Cohen J: Effects of cytoplasmic transfer on embryo quality are post-genomic. *Fertil Steril* 2001;76 (Suppl 3S), abstr #O-14
6. Huang C, Cheng T, Chang H, Chang C, Chen C, Liu J, Lee M: Birth after the injection of sperm and the cytoplasm of trippronucleate zygotes into metaphase II oocytes in patients with repeated implantation failure after assisted fertilization procedures. *Fertil Steril* 1999;72:702-706
7. Lanzendorf SE, Mayer JF, Toner J, Oehninger S, Saffan DS, Muasher S: Pregnancy following transfer of ooplasm from cryopreserved-thawed donor oocytes into recipient oocytes. *Fertil Steril* 1999;71:575-577
8. Tzeng C, Hsieh S, Chang S, Tsai N, Cheng Y, Wei Y: Pregnancy derived from mitochondrial transfer (MIT) into oocyte from patient's own cumulus cells (cGCs). *Fertil Steril* 2001;76 (Suppl 3S), abstr # O-180
9. Munné S, Alikani M, Tomkin G, Grifo J, Cohen J: Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995;64:382-391
10. Dailey T, Dale B, Cohen J, Munné S: Association between non-disjunction and maternal age in meiosis-II human oocytes. *Am J Hum Genet* 1996;59:176-184
11. Hassold T, Chiu D: Maternal age-specific rates of numerical chromosome abnormalities with specific reference to trisomy. *Hum Genet* 1985;70:11-17
12. Bean CJ, Hunt PA, Millie EA, Hassold TJ: Analysis of a malsegregating Y chromosome: Evidence that the earliest cleavage divisions of the mammalian embryo are non-disjunction prone. *Hum Mol Genet* 2001;10:963-972
13. Barritt JA, Brenner C, Willadsen S, Cohen J: Spontaneous and artificial changes in human ooplasmic mitochondria. *Hum Reprod* 2000;15:207-217

14. Levron J, Willadsen SM, Shimmel T, Cohen J: Cryopreservation of activated mouse oocytes and zygote reconstitution after thaw. *Hum Reprod* 1998;4 (Suppl):109–116
15. Zoon KC: Letter to sponsors/researchers—human cells used in therapy involving the transfer of genetic material by means other than the union of gamete nuclei 2001; Available at <http://www.fda.gov/cber/ltr/cytotrans070601.htm>

Dr Henry E. Malter, PhD

Senior Scientist

Gamete and Embryo Research Laboratory

The Institute for Reproductive Medicine and Science

St. Barnabas Medical Center

101 Old Short Hills Road, Suite 501

West Orange, NJ 07052