Cumulus cell gene expression as a potential biomarker for oocyte quality

The sole reason to transfer more than one embryo to an in vitro fertilization (IVF) patient rests on our continued inability to identify the single most developmentally competent embryo in any cohort. If we could identify that one embryo in every cycle, elective single-embryo transfer could be universally performed, multiple pregnancies would be virtually eliminated (except for the rare instance of monozygotic twinning), and the probability of pregnancy for each patient would be maximized. It is not surprising, therefore, that much effort continues to focus on identifying an embryo evaluation paradigm that meets this goal.

Although a plethora of technologies have been assessed above and beyond standard morphological assessment, time-lapse imaging (TLI) and preimplantation genetic testing for aneuploidy (PGT-A) are currently the most favored. Nevertheless, there is at best low-quality evidence that TLI improves selection over the standard morphology evaluation (1). Although PGT-A improves the time to pregnancy and reduces miscarriage rates, it requires a trophectoderm biopsy, which makes it an invasive test. Also, we are struggling with the interpretation of PGT-A results relating to mosaicism (2). Efforts continue to focus, therefore, on development of noninvasive evaluation technologies.

One of the less explored potential noninvasive approaches involves analysis of the somatic cells surrounding the oocyte, the cumulus cells. The intimate relationship between these cell types via heterologous gap junctions allows bidirectional communication, which is essential for regulation of oocyte growth and acquisition of developmental competence through completion of nuclear and cytoplasmic maturation. In this issue of *Fertility and Sterility*, Green et al. (3) report on the comparison of transcriptomic profiles of cumulus cells from oocytes that led to euploid embryos, which either did or did not result in sustained implantation after double-embryo transfer. After controlling for multiple hypothesis testing, no gene was differentially expressed between "implanter" versus "nonimplanter" embryos. The authors concluded that the cumulus cell transcriptome was not predictive of live birth within a cohort of sibling embryos.

The authors of this study are to be commended for their study design which, by using the sibling paired approach, mitigated patient heterogeneities and potential associated confounding in the analyses. Indeed, the study rigorously showed that, in the investigated samples, there was no gene whose expression level in cumulus cells was significantly associated with the outcome. However, this does not rule out the possibility that gene expression in cumulus cells is predictive of outcome. There are at least two reasons why both these apparently contradictory statements could be simultaneously correct. [1] Genes are organized into networks, so it is possible that changes across a network may be significant, even if changes in any individual gene in that network are not. [2] Correction for multiple testing, which is essential to avoid false-positive results, also makes it more difficult to identify true positives. This is a well-known problem in the analysis of biological "omics" data that has no easy solution and that closely relates to the general challenge of determining and interpreting statistical significance (4).

These issues cannot be overcome by hypothesis-free statistical tests, even with improved data and analysis methods. There are simply too many formal possibilities, so no amount of data will ever be sufficient to test them all. Rather, it will be necessary to formulate and test specific, mechanistically motivated hypotheses generated by considering the underlying biology. In this regard it is noteworthy that a recent investigation of genes in the phosphoinositol 1,3 kinase/protein kinase B (P13K/Akt) pathway, which is well known to play a central role in cross-talk between the oocyte and surrounding cumulus cells, has suggested that 11 genes in this pathway were significantly down-regulated for oocytes resulting in a pregnancy compared with those that did not (5).

Aside from these considerations regarding the authors' approach to hypothesis testing and data analyses, other challenges are worth mentioning. We note that this study involved only 17 patients; the possibility exists that any differential gene expression between implanters and nonimplanters may have been identified in a larger population. We also note that the patients ranged in age from 18 to 42 years; despite the commendable paired design of this study, is it not possible that age-dependent variations in gene expression obscured any expression differences? Moreover, these patients were likely to have been of poorer prognosis because they underwent double-embryo transfer. One wonders, therefore, whether similar results would have been obtained in a moderate to good prognosis patient group. Finally, we note that only day-6 embryos were included in the analyses. Presumably these embryos had developed more slowly and so did not meet the criteria for biopsy on day 5. Would similar results have been obtained with embryos after the normal development timeline? And conversely, because de facto the design included only euploid embryos suitable for trophectoderm biopsy, did restriction of the study sample to superior embryos capable of good quality blastocyst formation potentially mask the possibility of detecting differential gene expression in a more heterogeneous group?

In conclusion, the authors must again be congratulated for tackling the challenges associated with developing a noninvasive test with high sensitivity and specificity for identifying biomarkers for embryo competency. Again, they should be commended for their strong study design. However, as we have noted, we feel that their results do not close the exploration of relationships between cumulus cell gene expression and oocyte quality. Rather, we encourage future investigations focused on testing specific mechanistically motivated hypotheses based in the underlying biology. Such an approach may reveal relevant cumulus cell "omics," perhaps in conjunction with TLI, PGT-A, and standard morphology evaluation, all incorporated into an algorithm for embryo evaluation.

Regarding standard morphology, despite its use for embryo evaluation for nearly 40 years, it continues to be the most common method for embryo selection. Even when other technologies are employed, it continues to be the tie-breaker when all else is equal. It goes without saying that any clinical test requires a development phase, which, if promising results are obtained, provides the foundation for a validation phase with appropriately powered randomized, controlled trials for assessment of efficacy before being applied in the clinical realm. We are working in exciting times, with ever-advancing technologies emerging on the horizon, including exploration of the utility of imaging approaches other than TLI, such as fluorescence light imaging microscopy, as potential tools for oocyte and embryo evaluation.

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https://doi.org/10.1016/j.fertnstert.2017.12.013

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