Biomechanics and developmental potential of oocytes and embryos

Jonathan Kort, M.D. and Barry Behr, Ph.D.

Department of Reproductive Endocrinology and Infertility, Stanford University, Stanford, California

The high incidence of multiple embryo transfers is evidence of the need for better methods of embryo selection. Additionally, methods to determine the reproductive competence of unfertilized oocytes are critically needed to inform the growing population of patients undergoing fertility preservation. The ideal method of oocyte and embryo selection would be noninvasive, inexpensive, and able to be incorporated into embryology workflow with minimal disruption. Methods to assess the biomechanical properties of cells offer many of these traits, and there is a growing body of evidence in multiple cell types demonstrating the biomechanical properties of cells are reflective of a cell's intrinsic health. The associations with these properties are not mere coincidence, as many of the biomechanical properties are critical to cellular function. The biomechanical properties of oocytes and embryos undergo a dynamic, characteristic transformation from oocyte maturation through blastocyst formation, lending itself to biomechanical assessment. Many of the assessments made by embryologists, from ease of microinjection during intracytoplasmic sperm injection to degree of blastocyst expansion, are direct proxies for cellular biomechanics. Newer, objective and quantitative methods of biomechanical assessment are being applied to oocyte and embryo selection, with early use supporting their application in assisted reproduction. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)

Key Words: Oocyte, embryo selection, biomechanics, noninvasive

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NEED FOR NONINVASIVE OOCYTE AND EMBRYO SELECTION

In vitro fertilization (IVF) providers and patients are often challenged when determining which embryo to transfer because there is limited, and sometimes conflicting information, to guide their decision. Many embryos, despite being morphologically and chromosomally normal, fail to implant. To compensate, clinicians transfer multiple embryos in 67%–89% of cases (1), leading to an unacceptably high rate of multiple births and their consequent risk of prematurity and other complications (2).

The slow adoption of elective single-embryo transfer (eSET) is evidence for the need for better embryo selection methods to augment currently employed methods such as morphological assessment, blastocyst culture, preimplantation genetic screening (PGS), and morphokinetics. While each method has inherent strengths and limitations, even the most reliable methods are predictive of a successful pregnancy after transfer only 60%–70% of the time (3) and all require fertilization to occur—precluding providing any information about the reproductive competence of oocytes to the ever increasing fertility preservation patient population. Blastocyst morphology assessment is routinely done and is noninvasive and free. However, the inter-observer consistency between morphology assessments is poor, as is its correlation with embryo ploidy (3), although when used in conjunction with PGS it may have additional value (4). The development of PGS for embryo selection has garnered excitement as a major advance as it identifies and deselects aneuploid embryos thought to be responsible for the majority of failed IVF cycles, particularly those associated with increasing maternal age (5). Randomized-controlled trials have demonstrated an increase in success rates after employing this method that justify its use to facilitate eSET (6, 7), and while the American Society for Reproductive Medicine has even changed its transfer guidelines for cases employing PGS (8), additional investigation is still needed to test its efficacy.

While these are significant advantages, PGS is invasive and expensive, requiring a highly-trained embryologist to safely biopsy the trophectoderm, significantly increasing the workload for IVF clinics that perform a high volume of PGS cycles. In contrast to blastomere biopsy of cleavage-stage embryos which has proven harmful to treatment outcomes (9), initial data

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Reprint requests: Jonathan Kort, M.D., Department of Reproductive Endocrinology and Infertility, Stanford University, 1195 West Fremont Avenue, Sunnyvale, California 94087 (E-mail: JKort@stanford.edu).

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regarding trophectoderm biopsy appears reassuring, but longer followup is clearly needed.

The ideal embryo selection method would be strongly predictive for viability, applicable to both oocytes and embryos, inexpensive, and require little disruption to the embryology lab workflow. Embryo and oocyte biomechanic assessment methodologies offer many of these traits, and investigations in this arena may provide novel embryo selection techniques.

USE OF BIOMECHANICAL PROPERTIES AS A MARKER FOR CELLULAR HEALTH

Researchers are increasingly finding the biomechanical properties of cells reflect a cell's intrinsic health or ability to function. Often cellular function is dictated by its structure and biophysical properties (10). The significance of relative viscoelastic properties of cells has been demonstrated in a variety of cell types: ovarian cancer cells have been shown to be softer than non-malignant epithelial cells, and softer cancer cells are more likely to metastasize than stiffer lower grade malignant cells (11). In this case, transcriptomics have revealed actin cytoskeleton remodeling is responsible for this correlation. However, in red blood cells, which undergo a 10-fold increase in shear modulus—a quantification of rigidity or deformity when a shear stress is applied (12)—after infection with malaria, the observed biomechanical change is primarily due to chemical properties resulting from aberrant protein-protein-cytoskeleton interactions (13). These biomechanical changes are not mere inconsequential incidental findings—it is the change in cell stiffness and elasticity after plasmodium infection that causes sequestration of infected cells in major organs precipitating sickle crises, and in cancer cells, greater elasticity among higher grade cancers likely facilitates the squeezing through membranous pores required to metastasize (14).

BIOMECHANICAL PROPERTIES CRITICAL FOR OOCYTE AND EMBRYO FUNCTION

Given the critical changes in oocyte and early embryo viscoelasticity, it is logical that alterations in the biomechanical properties of oocytes or embryos may offer insight into their viability or health. Observed changes to embryos' viscoelasticity may be necessary for embryo development and implantation, and many of the current methods for oocyte/embryo assessment, such as blastocyst expansion or ease of penetrating the oolemma during ICSI, are direct proxies for biomechanical characteristics of oocytes and embryos.

During oocyte maturation and fertilization, the zona pellucida—a network of sulfated glycoproteins configured to form fibrils—undergoes characteristic changes (15) as the oocyte transitions through different stages of meiosis concluding with the development into a zygote. The zona pellucida undergoes softening during maturation (16, 17) which perhaps is a mechanism to facilitate sperm penetration. In contrast, during fertilization the zona hardens as a result of the cortical reaction in which the cortical granules release their contents into the perivitelline space (16). This process, in which hardness originally implied resistance to proteolytic digestion, is an enzyme-mediated mechanism to prevent polyspermy (18) and is thought to be derived by changes in the covalently and non-covalently cross-linkage of fibril polymers that result in true stiffening (16). Independent of maturation, differences in zona pellucida structure and thickness have been associated with the reproductive competence of corresponding oocytes (19–22), with a slightly thinner zona or undistinguishable inner layer corresponding with significantly lower rates of conception or blastocyst development (19). These differences have been correlated with the corresponding patients' prognosis as well as the type of ovarian stimulation (19, 23). Tangentially it is the concept of an abnormally rigid or thickened zona that forms the basis for performing assisted hatching on poor prognosis patients (23, 24). The correspondence of variations in zona pellucida structure, thickness and rigidity to oocyte competence supports the notion that biomechanical properties of oocytes or embryos may be an effective method of oocyte or embryo selection.

The transition in the physical properties of the zona pellucida also parallels changes to the oocyte cytoplasm viscosity during folliculogenesis and maturation (25). Between the onset of follicle recruitment and ovulation, the cytoplasm changes from an aqueous to a more viscous substance. In ovarian hyperstimulation cycles, this cytoplasmic maturation process often becomes desynchronized from nuclear maturation. The size and persistence of the injection funnel after ICSI has proven to be a marker of cytoplasm viscosity, with larger more persistent funnels indicating greater cytoplasmic viscosity (26). With a more aqueous cytoplasm, there is a greater tendency to restore the original shape after injection. Additionally, variation in the viscosity of metaphase II (MII) oocytes has been observed, creating the potential for a viscosity measurement to be a differentiator of mature oocyte quality (25), and other studies have found that higher viscosity, or injection funnel persistence, is a poor prognostic indicator of subsequent preimplantation development (26). Viscoelastic differences in oocyte cytoplasm and cell membrane may explain why variations in oolemma breakage have been observed to correlate with clinical outcomes, with membranes breaking immediately or failing to break even with aspiration portending a significantly lower fertilization and higher degeneration rates after ICSI (27).

Blastocyst expansion has been a key morphological indicator of embryo viability (28), however this subjective grading is essentially a reflection of the underlying biomechanical properties of multiple subunits of the developing embryo. In cycles without assisted hatching, the elasticity and rigidity of the zona pellucida, in conjunction with compaction, cavitation and development of the blastocoel determines the relative expansion and the timing of the critical hatching process needed for the blastocyst to interact with the endometrium. The elasticity of the zona pellucida, as discussed previously, varies between oocytes and embryo and changes throughout maturation, fertilization and preimplantation development. Using a micropipette aspiration technique, Maître et al. demonstrated that compaction results from pulsatile contractions resulting from the actomyosin cortex, which result from and alter the cell-cell adhesions as the embryo