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SYMPOSIUM

The Past, Present, and Future of Embryo Selection in *In Vitro* Fertilization

Frontiers in Reproduction Conference

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Assisted reproductive technology (ART†) has been recognized for its success in treating infertility, a condition that affects 15 percent of couples in the United States. The most popular option is *in vitro* fertilization (IVF), which relies on embryo culture, selection, and transfer for implantation, with the ultimate aim of pregnancy. Previous embryo selection methods relied on morphological factors to select for greatest viability. At Yale's Frontiers in Reproduction Conference on April 29, 2011, at the New Haven Lawn Club, Dr. Denny Sakkas of Yale's Department of Obstetrics, Gynecology, and Reproductive Sciences presented a paradigm shift: using morphological factors along with metabolic, protein, and genetic markers in culture media to enhance embryo selection and IVF success rates.

Infertility affects more than 6.1 million couples in the United States [1,2]. Infertility refers to either the inability to conceive after a year of unprotected intercourse or the inability to carry a pregnancy to term. The countless contributing factors are contingent on the fertility of both the male and female and include genetics, lifestyle, environmental toxins, tubal blockage, low semen quantity and quality, and age. Although drugs exist for the management and treatment of reproductive disorders such as oligospermia in males and endometriosis in females, they prove to be marginally helpful at best [3].

As a result, many couples turn to assisted reproductive technology (ART) and, in particular, *in vitro* fertilization (IVF), a

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†Abbreviations: ART, assisted reproductive technology; IVF, *in vitro* fertilization; sHLA-G, soluble human leukocyte antigen-G; CCs, cumulus cells; COX2, cyclooxygenase 2; STAR, steroidogenic acute regulatory protein; PTX3, pentraxin 3; FDA, Food and Drug Administration.

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technique that has existed for 30 years and assists in the birth of more than 38,000 babies worldwide each year [4]. The future of fertility and IVF were underscored at Yale's Frontiers in Reproduction Conference on April 29, 2011, at the New Haven Lawn Club. Expanding upon the current methods of embryo culture, selection, and transfer, Dr. Denny Sakkas of Yale's Department of Obstetrics, Gynecology, and Reproductive Sciences presented on "The IVF Laboratory of the Future" and groundbreaking developments furthering the field of reproductive fertility.

In 1978, Robert Edwards and Patrick Steptoe published "Birth After the Reimplantation of a Human Embryo" in *The Lancet* [5]. This was the first case of a successful birth through IVF, and 30 years later, 3 million babies have been born through the same technique. The method is a multi-step process consisting of ovarian hyperstimulation, oocyte retrieval, fertilization, embryo culture, embryo selection, and embryo transfer. The lack of current treatments to increase the quality of the sperm or egg is compensated for by using increased numbers: Multiple follicles and eggs in the woman are induced to mature and ovulate per menstrual cycle [6]. One criticism of IVF is the increased rates of multiple pregnancies, which heightens risks of premature delivery and low birth weights and endangers both the mother and children. Restrictions in some countries on the embryo number transferred have reduced multiple pregnancy dangers [7]. At the conference, Dr. Sakkas discussed current and prospective methods in improving embryo selection to optimize pregnancy and minimize the risk of multiple births.

Currently, embryo selection is based on embryo morphology and the rate of embryo development in culture. Positive selection criteria include the number of blastomeres, the absence of multinucleation, early cleavage to the two-cell stage, and a low percentage of cell fragments in embryos [8]. Further factors found to increase pregnancy and implantation rates include the bastocoelic cavity expansion state and the cohesiveness and number of the inner cell mass and trophectodermal cells [9]. A sequential embryo assessment model along with a computer algorithm is currently used to take these factors into account and has been able to select for embryo development into blastocysts in 86 percent of cases [10].

Despite the stringent and vigilant morphological criteria, per transfer of 2.3 embryos, only 52.3 percent result in live birth. Of these ART pregnancies, more than 30 percent are multiple-infant births [11]. The increased incidence of preterm delivery in these multiple pregnancies has drastic consequences on public health, as preterm infants require longer stays in the neonatal intensive care unit and are more vulnerable to respiratory, gastrointestinal, central nervous, and immune system complications. These infants can also sustain longer-term problems, including cerebral palsy, mental retardation, and learning difficulties. Thus, it becomes imperative to develop embryo grading and evaluation systems to select for the greatest viability. Current developments beyond morphological criteria have looked into metabolic parameters of embryos in culture media. These metabolic markers include decreased pyruvate [12] and increased glucose uptake [13] by the embryos, as well as elevated asparagine and decreased glycine and leucine levels [14] in the culture media.

Dr. Sakkas reported that in 2008, the Department of Obstetrics, Gynecology, and Reproductive Sciences at Yale, in conjunction with the Department of Chemistry at McGill University, investigated the metabolomic profiling of embryo culture media through proton nuclear magnetic resonance (1H NMR). They discovered that the metabolomics profile correlated with embryo reproductive potential. From the proton NMR spectrum, alanine, pyruvate, and glucose levels were reduced in the culture media of embryos that resulted in pregnancy. Glutamate levels were found to be higher compared to embryos that failed to implant, possibly due to its generation from α-ketoglutarate and ammonium, thereby lowering the potentially toxic ammonium to

developing embryos. A sensitivity — the ability to identify true implantations/pregnancies — of 88.2 percent and a specificity — the ability to correctly predict no implantations/pregnancies — of 88.2 percent was achieved through 1H NMR [15].

Further reproductive potential can be facilitated by the examination of protein markers in the embryo culture media. In one study by Noci et al., soluble human leukocyte antigen-G (sHLA-G) was isolated and considered as a possible protein marker of embryo reproductive potential. The presence of sHLA-G shows no correlation with embryo morphology, and the lack of sHLA-G in culture media has a negative predictive value [16]. In another study in which sHLA-G-positive embryos were transferred, implantation and pregnancy rates were 44 percent and 75 percent, respectively, compared to 14 percent and 23 percent of transferred sHLA-G-negative embryos [17]. A protein biomarker that has been found to be upregulated and increased during embryo maturation into the blastocyst stage is a Day 5 secretome — a set of proteins secreted from the cell — resembling ubiquitin. Ubiquitin has been implicated in the turnover of key signaling molecules during implantation [18].

Genomic markers are at the research forefront of improving embryo selection and IVF success. The cumulus cells (CCs) that surround the oocyte from fertilization until implantation have been analyzed and gene-profiled to gauge embryo potential: the likelihood of an embryo to implant and lead to a successful pregnancy. Several genes expressed in CCs have been correlated with predicting pregnancy, including cyclooxygenase 2 (COX2) [19,20], steroidogenic acute regulatory protein (STAR), and pentraxin 3 (PTX3) [21]. Two upregulated biomarkers have been identified in the CCs of successful pregnancies, BCL2L11 and PCK1, which are involved in apoptosis of abnormal cells and gluconeogenesis [22]. Implications of these findings can lead to future IVF techniques of CC collection post oocyte retrieval, followed by gene profiling of embryos to recognize which need fresh placement and which are most viable.

Pioneering developments in the field of ART have expanded the embryo selection process beyond measures of morphology. Although current methods in selection have offered some success, recent noninvasive assessment of embryo potential will allow for more proficient selection of the most viable embryos. From Dr. Sakkas' discussion, the selection process that was once a "beauty contest," simply evaluating embryo appearance, will soon include metabolic, protein, and genomic markers as assessment criteria.

Machines employing metabolomics culture assessment have been available since the start of 2011 in Europe and India through the Massachusetts-based firm Molecular Biometrics. The company is aiming for its "ViaMetrics-E" system to acquire Food and Drug Administration (FDA) approval and begin United States sales by the fourth quarter of 2011. This metabolomics machine is expected to improve IVF success rates, reduce costs, and diminish dangers associated with multiple pregnancies and preterm deliveries. These combined components provide an improved understanding of embryo viability, allowing for the identification of embryos that are most likely to result in a pregnancy. Ultimately, the amalgamation of all factors will provide greater success to the field of IVF toward achieving the goal of one healthy baby per pregnancy.

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