Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential

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With increased use of comprehensive chromosome screening (CCS), the question remains as to why some practices do not experience the same high levels of clinical success after implementation of the approach. Indeed, the debate surrounding the efficacy and usefulness of blastocyst biopsy and CCS continues. Importantly, several variables impact the success of an assisted reproductive technology cycle. Transfer of a euploid embryo is but one factor in an intricate system that requires numerous steps to occur successfully. Certainly, the culture environment and the manipulations of the embryo during its time in the laboratory can impact its reproductive potential. Environmental stressors ranging from culture media to culture conditions and even culture platform can impact biochemical, metabolic, and epigenetic patterns that can affect the developing cell independent of chromosome number. Furthermore, accompanying procedures, such as biopsy and vitrification, are complex and, when performed improperly, can negatively impact embryo quality. These are areas that likely still carry room for improvement within the IVF laboratory. (Fertil Steril® 2016;105:571–87. ©2016 by American Society for Reproductive Medicine.)

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Since the early days of embryo culture in vitro, various modifications to the culture system have been explored in attempts to optimize embryo development and increase the number of good-quality embryos available for transfer. This has resulted in most labs being able to grow numerous high-quality embryos, often to the blastocyst stage, and has helped lead to improved overall pregnancy rates. In turn, embryo selection methods have become important as IVF centers strive to further increase efficacy, promote single ETs, and reduce time to pregnancy and live birth.

Toward this end, comprehensive chromosome screening (CCS), or preimplantation genetic screening (PGS) examining embryos for all 23 pairs of chromosomes, has received an immense amount of attention. However, this approach is controversial, and the ability to improve outcomes is questioned by some investigators.

Of note, different laboratories seem to experience differing levels of success when applying CCS. This may suggest that laboratory conditions could be impacting efficacy. Despite the significant impact of gamete quality on subsequent embryo development, it is often assumed that suboptimal embryo culture conditions are largely responsible for poor embryo development in vitro. While embryo culture conditions may not be able to overcome the inherent limitations imposed by the sperm or egg, the quest to improve the culture environment continues. Similarly, optimizing other procedures within the IVF laboratory to minimize the

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stress imposed on the embryo is an ongoing endeavor. The IVF laboratory must not only grow competent embryos but must ensure this competency is maintained after various manipulations, such as biopsy and cryopreservation.

CULTURE MEDIA

Culture media have vastly improved since the first attempt at IVF, when simple somatic cell media were used with serum supplementation. Laboratories often made their own media, which introduced variables that could impact efficacy. Various modifications were made, often using animal embryos as model systems, and eventually media were formulated specifically for human embryos (1, 2). The introduction of commercially produced media vastly improved consistency, resulted in the ability to culture embryos to the blastocyst stage, and undoubtedly aided in the success of IVF.

While numerous studies have explored the impact on embryo quality of altering the energy substrate composition, supplementing macromolecules, adding growth factors, and modifying other media constituents and culture conditions, with technological improvements and new approaches to assess embryo metabolism, morphokinetics, and other means of viability assessment, there remains the possibility that media formulations may still be further refined and improved to benefit embryo development and improve resulting clinical outcomes.

Media Comparisons

Various studies have attempted to compare the efficacy of various embryo culture media with the goal of determining whether one is superior to another for the growth of human embryos (3–12). Unfortunately, many of these studies are underpowered, do not use proper experimental design, and fail to control for critical variables that affect culture media performance (13). Thus, drawing concrete conclusions about the superiority of any individual commercially available culture medium is difficult. Furthermore, no study has compared all available media in a controlled fashion, making it impossible to declare an "optimal" recipe. A recent systematic review on randomized control trials (RCTs) concluded that a conventional meta-analysis comparing embryo culture media was not possible (5). Only four trials reported on live birth (9,14–16), and one reported a significant difference. Nine trials reported ongoing and/or clinical pregnancy rates, of which only four showed a significant difference (16). Pooling the data did not reveal a superior culture medium. A follow-up review found similar results. Thus, it is unknown what culture medium leads to the best success rates in IVF/intracytoplasmic sperm injection (ICSI) (5, 17).

Currently, human preimplantation embryo culture media are broadly grouped into single-step or sequential media, with the former lending itself to interrupted or uninterrupted culture. Several reviews are available describing the evolution of these media systems and the potential benefits of either approach (18–23). Unfortunately, few well-designed, prospective RCTs exist that compare single-step media to sequential media, and it is impossible to say which approach is superior (Tables 1 and 2). Most culture media within these two broad categories are similar in terms of composition. They contain glucose, pyruvate, and lactate at varying concentrations to permit embryo development past traditional developmental blocks. The majority of culture media also contain some assortment of amino acids. Because commercial embryo culture companies do not publish concentrations of media components, it is difficult to discern why one culture media might be superior to another, although methods exist to approximate media composition (Table 3) (27).

Recently, several single-step media have been introduced to be compatible with uninterrupted culture and use with time-lapse technology. Whether any observed improvements in outcomes are due to the media composition itself, to less handling and stress to the cells for routine observation, or to the newer incubator design or gas concentrations is unknown.

That being said, composition of culture media is still important in trying to improve current culture conditions. The balance of organic and inorganic salts is crucial (28). The composition and ratios of energy substrates is also critical. One area that likely deserves extra attention in optimizing media performance is the composition of amino acids. Amino acids support numerous cellular processes, including acting as metabolites, osmolytes, antioxidants, and buffers, and likely help alleviate stress in the embryo culture system (29). Even a brief period of culture with no amino acids can impair mouse embryo development (30). Thus, all embryo culture media should contain some assortment of amino acids. While animal studies have given some insight into the positive and negative effects of individual and combinations of amino acids (31–33), detailed assessments of individual amino acids and all possible combinations and ratios have not been conducted. Additionally, while controversial, a concern with the inclusion of amino acids in media is the buildup of ammonium and its potential negative impact on embryo and fetal development (34). This potential problem may be alleviated through media change in a sequential system. However, use of proper assortment and concentrations of amino acids and culture conditions is also a feasible approach. Modern embryo culture media should include the dipeptide form of glutamine to reduce ammonium production (35–38). Whether the use of other dipeptide amino acids could further improve the culture environment is unknown (39), although a clearer understanding of embryo metabolism and use of dipeptides is likely warranted (39, 40).

Macromolecule supplementation is another area that may lend itself to media improvement. Primary protein supplements used in clinical embryo culture media include human serum albumin (HSA), as well as complex protein supplements containing HSA and a combination of alpha and beta globulins (41, 42). Macromolecules can act as a surfactant and as a nitrogen source, stabilize membranes and modulate the physical microenvironment, act as a carrier molecule for other compounds, or even bind trace elements