In Vitro Fertilization Research is Translational Research

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Abstract

In vitro fertilization (IVF) is the perfect example of translational research. Changes in IVF and the IVF laboratory have been transmitted to clinical care, showing dramatic improvements in health outcomes, including notable increases in the cumulative pregnancy rate. Current research in the laboratory focusing on culture media, embryo selection criteria, and implementation of genetic testing and manipulation promises to translate to further improvements in our ability to assist human reproduction. The field of IVF and ART remains a large source for clinical and scientific discovery and development, and will require the proper interested and invested personnel, occupational structuring, and funding for continued success.

Keywords

IVF, translational research, evidence-based medicine, ART, in vitro fertilization, assisted reproductive technologies

Translational research is “turning discoveries made in academic labs into innovative therapies,”¹ or perhaps a more catchy definition is “bench to bedside.” The thesis here is that changes in the laboratory have been transmitted to clinical care, showing dramatic improvements in health outcomes. In vitro fertilization (IVF) is the perfect example of translational research, with an increase in pregnancy rate from 5% to 50% since 1978 and the rate of multiple gestations in a dramatic decline secondary to the development of technology to assess embryos. Recent hot topics of investigation have been the microbiome of embryo culture media and the embryo as a source of material for total genome sequencing. A problem however is that the IVF laboratory is not viewed as a basic science laboratory, but this is contentious.

A familiar term is evidence-based medicine but hopefully this could be replaced by coining science-based medicine. The clinical part of assisted reproductive technologies (ARTs) has seen a strong development toward evidence-based clinical practice. New drugs and new treatment protocols are tested in properly powered prospective controlled trials before being introduced to general clinical practice.² A strategy moving forward has been the initiation of collaborative research efforts through multicenter trials to assist with funding for large clinical trials for validation of equipment, consumables, and procedures in the ART laboratory. As future ART methods and strategies are being developed, there is emphasis on the prospective trials before medical community acceptance for routine clinical practice.

Terms that are being utilized today are precision medicine and personalized medicine. Precision medicine is utilizing big data applications for medical research and personalized medicine is identifying defects in a biochemical pathway that can be

targeted to cure or impact a disease process. A perfect example of this is the development of the drug Gleevec, where the tyrosine kinase target enzyme is blocked. This blockage turns chronic myelogenous leukemia into a chronic and nonfatal disease. An issue here is that most manipulations to a gene or metabolic pathway could potentially have profound changes in all the cells of the body, which could create increased morbidity or a new source for mortality, that is, the disease created is worse than the disease attempted to be cured. Gleevec is designed to block only the tyrosine kinase in the white blood cells but additionally affects the thyroid gland; therefore, patients have to take supplemental thyroid, a seemingly small price to pay. Much scientific excellence and clinical progress has been paved in obstetrics, gynecology, and the reproductive sciences, but a recent decline in the population of physician scientists, whose work has made this possible, leads us to question sustainability.

The problem with translational medicine is that the medical system has been broken down into 2 separate occupational pathways, 1 being basic scientists and the other being clinicians. In the past, there were a fair number of physician scientists who would work in both arenas. This cadre of individuals is unfortunately diminishing in number each year. There are a number of reasons for this, including inadequate salary,

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insufficient funding for the complexity of research, and a great deal of dedicated time required in the basic science laboratory. An area that is rapidly expanding with a scientific bent is clinical research or clinical trial research. This has taken on a new significant meaning and this is additionally a great place for physicians with research inclination to exercise their passion. Much of the translational research being done in some way leads back to the National Institutes of Health (NIH), with its extramural branch providing funding for universities and programs throughout the country. Another large funding source for clinical discovery has been through that of biomedical and pharmaceutical industry.

The IVF demonstrates, in a number of ways, bench to bedside research. A prime example of this is studies of the effect of the culture media formulation on embryo outcome. Referencing the fetal origins hypothesis, it has been proposed that many adult diseases originate in utero secondary to adaptations made by the fetus to its encountered environment. Research has been directed to study the IVF culture medium and its ability to influence fetal growth rates.³ These types of studies draw attention to a growing need for studies investigating fetal growth patterns after ART and the long-term health outcomes of IVF children.

Another way in which embryo culture media has been studied is potential for epigenetic environmental changes with impact on the developing embryo. Research has shown evidence that children born after ART have an increased risk of cardiometabolic abnormalities as well as subtle genome-wide changes in deoxyribonucleic acid methylation. Beckwith-Wiedeman and Angelman Syndrome are the 2 examples of imprinting, where a methylation inactivates 1 of the parental alleles resulting in disease. This biochemical example of a disease pathway has prompted clinicians to look at changes in the embryo culture media environment for measurable effects in reproductive outcome. Temperature, atmosphere, contaminants, and pollutants are the embryo culture media parameters that have been undergoing scientific evaluation and experimentation. Additional investigation is ongoing to clarify the relationship between ART, genome-wide alterations in imprinted genes, and their possible relevance to subtle metabolic consequences reported in ART offspring.

The search for the perfect embryo, the one that can undergo single embryo transfer with genetic screening for success, is the Holy Grail of IVF. This process has progressed from utilizing fluorescence in situ hybridization (FISH), to single-nucleotide polymorphism (SNP) evaluation, and now to whole gene sequencing of embryos prior to transfer. There are 2 ways that genetic material is scrutinized for the evaluation of the embryo. One is preimplantation genetic screening (PGS) and the other is preimplantation genetic diagnosis (PGD). The latter is where specific gene defects are known and primers are made to identify these mutations in order to detect affected embryos. In addition to comprehensive chromosomal screening (CCS), examples of diseases for which specific primers have been designed for the purpose of de-selection of target gene

Figure 1. Noninvasive analysis of metabolites and proteins that can be associated with embryo quality.

mutations in offspring include cystic fibrosis, Marfan syndrome, Parkinson disease as well as hundreds more.

As genetic screening tests continue to improve, microdeletions and defects are being found and even implicated in male factor infertility when concerning the Y chromosome. The PGD and intracytoplasmic sperm injection have been used to successfully overcome, in some instances, these found male factors. With our ability to identify more genetic variations of unknown significance, it will be a challenge going forward to interpret these findings and assess hypothetical risk to parents when these variants are identified with PGS. It is important further to realize the potential for *de novo* deletions and mutations for which an identified phenotype is not yet known and whether PGS should be recommended to all future parents undergoing ART.

With interest in optimization of the embryo selection process and improved pregnancy rates with single embryo transfers, work has been done to determine what parameter is most predictive of a successful implantation. Looking at trophoctoderm (TE), inner cell mass, and blastocyst expansion to predict implantation and live birth, it was determined that TE grade is the most significant predictor among the standard blastocyst morphologic assessments.⁴ More recently, work with time-lapse monitoring systems has shown improvement in the evaluation of embryo morphology and morphokinetics. Research continues to further improve our ability to maximize outcomes of IVF and provide optimal counseling for expectant outcomes to patients.

The investigation of embryo physiology using “omics” technologies has shown an application of noninvasive metabolomic and proteomic platforms to understand embryo viability and selection criteria. The more information that can be gathered about the embryo prior to transfer will allow selection of the highest quality embryo and thereby, potentially, the ideal pregnancy success outcomes. Embryos have metabolic plasticity, which allows for adaptation to the nutrients and resources allotted in the cultured *in vitro* environment. This fact revealed

Figure 2. Two mitochondrial replacement techniques: (A) maternal spindle transfer and (B) pronuclear transfer.

a window for study and optimization of the culture and assessment of embryo development (see Figure 1). The metabolomics technology is able to measure uptake and production of multiple substrates by analyzing the medium after *in vitro* culture, a process that is noninvasive for the embryo.⁵ Certainly, this technology will allow for improved understanding of embryo metabolism and holds promise to improve implantation and pregnancy rates.

In addition, the biochemical environment of culture media, the microbiome of the female reproductive tract has been recently looked at with regard to the effect of composition on implantation and pregnancy rates. Further studies are needed to

investigate hydrogen peroxide-producing lactobacillus species and their dominant role in the vaginal microflora. Additional work looking at the impact of antibiotic prophylaxis at the time of embryo transfer or colonization of the reproductive tract with healthy lactobacilli to improve ART are areas of interest, although the exact interaction between genes and environment is yet to be known.

New work with mitochondrial transfer illustrates another way that basic science impacts clinical disease, genetics, and reproductive outcome. Simply stated, nuclear genetic material from a woman with mitochondrial disease is transferred to a donor cytoplasm and subsequently fertilized with the partner's

sperm.⁶ Figure 2 depicts 2 mitochondrial replacement techniques, maternal spindle transfer and pronuclear transfer. This development eliminates the disease potential in the resulting embryo, substituting unaffected donor mitochondria, with the minor downside of a change in the ancestry profile of the offspring. Clustered regularly interspaced short palindromic repeats (CRISPR) is another nascent application for editing the human genome; however, this requires altering the nuclear profile and therefore presents profound germ line changes.

The development of IVF has also afforded those with disease burden not only the opportunity to de-select for this outcome in offspring but also the options for fertility preservation in the face of reprotoxic with potentially curative treatments. The concept of oocyte or sperm cryopreservation has the potential to allow for procreative choices even after the potential for irreversible damage to gametes by radiation prior to bone marrow transplant, for example. Improvements in vitrification and thawing have made this process a realistic venture. Future embryos created could theoretically also be subjected to CCS and PGD to save a child from withstanding what his or her parents endured in disease.

The NIH currently has a clinical trial to study oocyte freezing for the purpose of fertility preservation in advance of planned gonadotoxic therapy for chronic disease states. The study is specifically enrolling females who will be undergoing gonadotoxic therapy, hematopoietic stem cell transplantation (HSCT), and females with sickle cell disease (SCD). The purpose is to study the effects of the therapy on infertility and the utility of cryopreservation for future reproductive options in this population of female patients. Treatment with chemotherapeutic drugs, HSCT, and pelvic radiotherapy has the potential to markedly increase the risk of iatrogenic ovarian failure leading to infertility in women. The SCD as a specific example is the most common hemoglobinopathy in the United States. The HSCT is the only treatment currently available for SCD that results in a complete cure. Multiple studies have unfortunately demonstrated that infertility and premature ovarian insufficiency are quite common following HSCT.⁷ It has been observed that largely preserved ovarian function exists prior to transplantation but profound gonadotoxicity occurs following transplant. There is a clinical need for additional, effective fertility preservation methods for these at-risk populations and continued study in this arena.

Clinical improvements have been notable, with increases in the cumulative pregnancy rates after IVF. Current research in the laboratory, focusing on culture media, embryo selection criteria, and implementation of genetic testing, promises to translate to further improvements in our ability to assist human reproduction. Our ability to provide fertility preservation as a realistic venture additionally allows for reproduction in a population where this opportunity is lost. Our success can only soar as we continue to question, evaluate, and improve our benchside processes for valuable clinical outcomes.

As society is introduced to new technology and options for genetic engineering of embryos, some applications have been well tolerated—including de-selection for gene mutations and creation of embryos to eliminate disease; however, trait engineering is not considered acceptable. As research progresses in this exciting translational field, some questions that need to be answered include the following:

Does ART affect the health of resultant children as they progress into adulthood?

Does ART lead to chronic pathologic conditions down the line?

What is the impact on fertility status for individuals who are a product of ART?

Can ART be implicated separately from infertility as the issue causing potential chronic disease and other complications seen in the offspring of new reproductive technologies?

The field of IVF and ART remains a large source for clinical and scientific discovery and development and will require a proper interested and invested personnel, occupational structuring, and funding for continued success.

Review Questions

- Which is the best example of translational research?
 - Discovering the enzyme associated with the development of protein X.
 - Discovering the gene that suppresses an oncogene.
 - Making a significant change to a prescribed cough medicine.
 - Discovery of an immunologic substance that retards the growth of melanoma.
- The new trend in innovation is:
 - Evidence-based medicine
 - Science-based medicine
 - Precision medicine
 - Patient-based medicine
- The largest percentage of money allocated for research is located in:
 - Federal funding
 - Industrial funding
 - University funding
 - Philanthropic sources
- In the past 5 years, the number of physician scientists has:
 - Plateaued
 - Increased
 - Declined
- Culture media in IVF has been associated with:
 - DNA changes
 - RNA changes
 - Epigenetic changes
 - Protein changes

Answers: 1. D; 2. C; 3. B; 4. C; 5. C.

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