A History of Developments to Improve *in vitro* Fertilization

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The central goal of infertility treatment has not changed over time—to help build healthy families.

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Abstract

Methods of *in vitro* fertilization (IVF) have advanced dramatically since the first IVF baby was born in 1978. Originally yielding single-digit success rates, IVF is now successful in nearly 50% of cases in which the woman is younger than 35 years. Here, we describe the improvements in laboratory techniques and advances in our abilities to manipulate reproductive physiology that have facilitated this improvement. Additionally, we describe efforts to ensure safety standards in this competitive field.

A Brief History of *in vitro* Fertilization

Human reproduction research has always been fraught with both scientific and ethical challenges that initially hindered development of treatments for infertility. However, in the 1960s and 1970s, our understanding of the events in human oocyte fertilization grew to the point that *in vitro* fertilization (IVF) of human oocytes became possible. Ultimately, this knowledge led to the widely acclaimed first live birth of a "test tube baby," Louise Brown, in England in 1978.[1] In this sentinel IVF birth, the mother had a natural menstrual cycle, physicians laparoscopically retrieved a single pre-ovulatory oocyte from her ovary, fertilized it *in vitro*, and then transferred the resulting eight-cell embryo into her uterus.

Three years later, the first IVF baby in the U.S., and the 15th worldwide, was born. In this case, rather than rely on the one oocyte that would be produced naturally, the mother was injected for several days with human menopausal gonadotropin to induce several follicles in the ovary to produce oocytes. After this process, termed controlled ovarian stimulation (COS), physicians laparoscopically retrieved the pre-ovulatory oocytes, fertilized them *in vitro*, and then transferred day 3 or day 5 embryos into the mother's uterus. In 1985, the first IVF baby in Missouri was born to a couple who underwent IVF at Washington University, and delivered at what is now Barnes-Jewish Hospital[2]. Since that time, the practice of IVF has continued to evolve at an astounding pace.

Today, IVF accounts for millions of births worldwide and 1-3% of all births every year in the U.S. and Europe.[3] The increasing demand for fertility treatment drives research and development of technologies to optimize IVF regimens and success. In the vast majority of IVF cases, infertile couples undergo treatment to conceive a genetically-related child. However, couples are also undergoing IVF so that their embryos can be genetically tested to decrease transmission of single-gene mutations associated with morbidity.[4,5] Additionally, use of donor sperm and oocytes is becoming increasingly common, and women who are unable to carry a pregnancy are now able to use gestational carriers.

Below, we highlight several of the major milestones that have made IVF an extremely effective tool to care for these patients.

Controlled Ovarian Stimulation

The initial studies of IVF conducted in women undergoing natural menstrual cycles yielded on average 0.7 oocytes per retrieval and a 6% per cycle pregnancy rate.[6] In the 1980s, researchers at the Jones Institute in Norfolk, Virginia, began injecting women with gonadotropins to stimulate multiple ovarian follicles to produce oocytes. These oocytes were then fertilized *in vitro,* and the healthiest appearing embryos were implanted in the woman's uterus. This advent of controlled ovarian stimulation (COS) improved average oocyte yields to 2.1–2.6 and average pregnancy rates to 23.5% per cycle in 1982 and 30% in 1983.[4,6] Initially, human chorionic gonadotropin (hCG) was used to trigger ovulation because it is physiologically homologous to luteinizing hormone, which increases rapidly in a natural cycle to trigger ovulation. In early IVF procedures, an important concern was premature ovulation, which would make retrieving oocytes impossible despite careful and labor-intensive COS. However, two innovations to IVF practice, including the use of gonadotropin releasing hormone (GnRH) agonists in the 1980s and of GnRH antagonists in 2001, made it possible to prevent premature ovulation and reliably control oocyte retrieval. Several different medication regimens exist, but all follow the same concept: injectable medications upregulate endogenous hormones to recruit multiple ovarian follicles to yield multiple oocytes at retrieval.[7]

Ovarian Hyperstimulation Syndrome

Two problems occurred as a result of injections of supraphysiologic doses of gonadotropins. First, to improve the chances that a woman would become pregnant with one fetus that would survive to term, physicians began fertilizing multiple oocytes and implanting multiple embryos. This practice sometimes results in women carrying twins and even higher order multiples of fetuses, putting the fetuses at risk of low birth weight and preterm birth. Second, the most common and severe iatrogenic complication of ovarian stimulation is ovarian hyperstimulation syndrome (OHSS). OHSS occurs when the ovaries are excessively stimulated and then either triggered with injected hCG to stimulate ovulation or by the endogenous increase in hCG that occurs when a woman gets pregnant. OHSS is characterized by hemoconcentration from leaky vessels and third spacing of fluid that leads to ascites and electrolyte abnormalities. Symptoms range from mild abdominal distention to renal failure and death as a result of thromboembolic phenomena or end-organ damage. Despite extensive research, the exact pathogenesis of this syndrome remains unclear but is noted to have increasing incidence with an increasing number of developing follicles and elevated levels of estradiol, which is made by the ovarian follicles. To address this concern, in 1979, physicians began monitoring COS by serially measuring the serum estradiol levels and transvaginally assessing ovarian follicles to better monitor for risk factors. Identifying patients at risk allowed physicians to take preventative measures such as adjusting medications appropriately and more frequently monitoring symptoms.[8-10]

The current limitation to COS is that it requires time and labor-intensive monitoring. Additionally, gonadotropin is rapidly degraded in the body, so women have to undergo daily injections for 10 days. However, scientists such as Washington University professor Irving Boime are developing long-acting forms of gonadotropins that may one day reduce the number of required injections and the amount of monitoring.[11]

Embryo Culture

Since the early days of *in vitro* embryo culture, efforts have been directed toward improving the culture system to optimize embryo development and increase the number of high-quality embryos available for transfer. Initially, embryo culture media was fashioned from media intended for culture of somatic cells and supplemented with serum.[12,13] Numerous researchers have optimized media for embryo metabolism and development by supplementing it with various macromolecules, altering the energy substrate composition and amino acid balance, and adding growth factors. For many years, laboratories made their own culture media, but now it is commercially produced, resulting in improved consistency and quality control between different laboratories and practices.[14] Much attention will undoubtedly continue to be directed toward refining culture media to further optimize embryo development and clinical outcomes.

Improvements in embryo culture over the years have allowed us to extend *in vitro* culture of embryos to the blastocyst stage, permitting detailed morphologic assessment of embryos and better selection of embryos for transfer. This has been key to our ability to maximize pregnancy rates in IVF while minimizing the number of embryos transferred and thus minimizing the risk of multiple gestations. Extended culture has also allowed us to perform preimplantation genetic testing of embryos, a process that is best applied when the embryos are far enough developed in culture to sustain removal of several cells for genetic testing.

Improved embryo culture, in combination with improved COS, allow us to generate more embryos than are initially transferred. Today, approximately 50% of IVF cycles performed via COS in our center result in the creation of excess embryos of good quality that can be frozen for the patient's future use. Thus, the woman can often

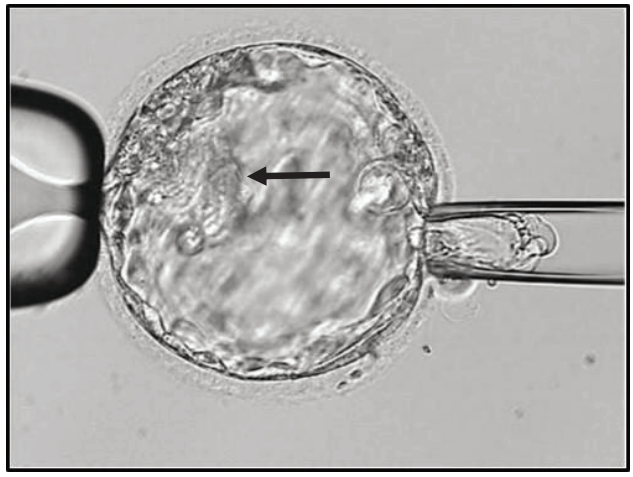
avoid further COS injections and invasive oocyte retrieval. This process is now efficient enough that women facing gonadotoxic treatments such as chemotherapy can preserve future fertility by undergoing COS and having their oocytes retrieved and frozen.

Preimplantation Genetic Testing

Before 1990, options to prevent transmission of genetic defects were limited to invasive techniques such as chorionic villus sampling and amniocentesis, after which termination could be offered if the fetus was found to be affected. Throughout the 1990s, as surplus embryos became available, techniques were developed to utilize the time between days three and five after oocyte fertilization as an opportunity to identify which embryos were affected by chromosomal imbalance or a specific gene disorder before transfer to the uterus.[15] The initial technique was to screen cleavage-stage embryos by fluorescence in situ hybridization, but that was later found to lower birth rates and cause more harm than good.[16,17] Now, we biopsy cells from the trophectoderm of blastocyst-stage embryos, (see Figure 1), and perform one of two types of preimplantation genetic testing. The first, preimplantation genetic diagnosis (PGD) applies when one or both genetic parents carry a mutation, such as those linked to Huntington's disease or cystic fibrosis, and testing is performed to ensure the single-gene trait has not been passed to the embryo. PGD is commonly done by polymerase chain reaction as this method is more accurate than fluorescence in situ hybridization and allows us to obtain sufficient genetic material for evaluation from only a few cells, thus decreasing harm. Although this process requires vitrification of the embryo to allow sufficient time for analysis, recent studies suggest that cycles using frozen and fresh embryos are nearly equally successful, making this a feasible option for couples.[18,19] Importantly, PGD does not appear to increase the risk of obstetric complications, including fetal malformation related to the biopsy procedure.[20]

The other type of testing is preimplantation genetic screening (PGS), which is used to look for embryonic aneuploidy. Although not routinely recommended as a standard of care in IVF as it has not been shown to improve outcomes in low-risk patients, PGS can be beneficial in a select patient population. PGS offers prognostic value for patients who are deemed high risk for embryo aneuploidy (abnormal number of chromosomes), including those of advanced maternal age (≥35 years old) and those diagnosed with recurrent pregnancy loss. Patients should receive genetic counseling before electing PGS or PGD to ensure they fully understand the risks and limitations of these techniques. Genetics will certainly continue to play a large role in shaping the future of practice in reproductive medicine, and although innumerable advances have been made, there is still work to be done to discern how PGS and PGD are best applied in IVF.

Figure 1: A pipette (left) holding a blastocyst near the inner cell mass (arrow) while a needle (right) biopsies trophectoderm cells.



Reducing the Risk of Multiple Gestations Associated with IVF

In the early years of IVF, several embryos were implanted with the hope that at least one would survive, often leading to multiple births. For example, in 2004, 36.6% of women younger than 35 years of age undergoing IVF had a live birth after being implanted with, on average, 2.5 embryos per cycle. As a result, 32.7% of the women delivered twins and 4.9% delivered triplets. Improvements in embryo culture and cryopreservation techniques, in addition to guidelines regarding the number of embryos to be transferred, (see Table 1), have reduced the quantity but increased the quality of embryos transferred, thus reducing the risk of multiples. Thus, in 2014, 48.7% of women younger than 35 undergoing

Table 1
Recommended limits on the numbers of embryos to transfer

Age (years)	< 35	35-37	38-40	41-42
Prognosis				
Cleavage Stage Embryos				
- Favorable	1	1	≤ 3	≤ 4
- All others	≤ 2	≤ 3	≤ 4	≤ 5
Blastocysts				
- Euploid	1	1	1	1
- Favorable	1	1	≤ 2	≤ 3
- All others	≤ 2	≤ 2	≤ 3	≤ 3

Adapted from ASRM Committee Opinion: Limits on number of embryos to transfer. Fertil Steril 2017.

IVF had a live birth—11.8% of those births were twin deliveries, and 0.4% were triplet deliveries. This reduction in multiples is largely the result of minimizing the number of embryos transferred to just one.

IVF Outcomes Reporting

Efforts to track IVF activity and outcomes started in 1985 and were initially voluntary. However, since Congress passed the Fertility Clinic Success Rate and Certification Act in 1992, clinics have been required to report IVF outcomes data to the Centers for Disease Control (CDC) to provide transparency and protect patients from false claims of IVF success.[21,22] Public reporting of outcomes has become increasingly viewed as a promising strategy to improve health care outcomes.[23] IVF success rates for all reputable clinics are now available on the web from both the CDC and the Society for Assisted Reproductive Technology (SART), an affiliate of the American Society for Reproductive Medicine.[22,24] SART is an excellent resource for both patients and physicians that provides ample information including detailed guides of various ART protocols and procedures, as well as success rates of individual technologies at practices across the country. Their first annual publication was in 1988 and has been increasingly used to help guide continued improvement and evaluation of ART programs. SART reporting differs from that of the CDC in that it includes cycle start information, whereas CDC data only offers outcome statistics for completed cycles.[22,24] More than 90% of clinics are SART members, and the SART registry reports data on more than 95% of ART treatment cycles in the U.S. Between the two reporting systems, a large amount of information is available allowing for detailed analysis of data for transparency and continued opportunities for improvement of patient outcomes. SART also makes it easy for patients to understand the quality of the IVF lab they are entrusting their care to—one of the greatest predictors in their chances of achieving a live birth through IVF.

Conclusion

The field of reproductive endocrinology and infertility has progressed at an astounding pace over the past three decades as we have developed new techniques, medications, testing, and strategies to treat infertile couples. Now, many previously sterile couples are able to conceive, carry, and deliver healthy children of their own. Despite the major advances described here, much attention will remain devoted to assessing the long-term outcomes of the children born as a result of IVF; the oldest child of IVF is merely 38 years old. The central goal of infertility treatment has not changed over time—to help build healthy families.

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Disclosure

None reported. **MM**