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Recent Advances in Assisted Reproductive Technology

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Abstract In 1978 the world witnessed the birth of the first "test tube baby." Since this time there have been explosive advances in assisted reproductive technologies (ART). Current optimizations surrounding the delivery of in vitro fertilization (IVF) including the utilization of minimal stimulation protocols and gonadotropin releasing hormone (GnRH) agonist cycle triggers are being increasingly utilized to maximize patient safety. Modifications, such as those seen in the embryology laboratory, continue to improve pregnancy rates. Concurrent with these advancements in IVF have been the emergence of related technologies, such as embryonic genetic testing and oocyte preservation, which potentially have broad applications to both fertile and infertile couples. As these relevant applications of ART become increasingly utilized, it is incumbent upon society to ensure that these resources are made available in a morally responsible and equitable manner.

Keywords In vitro Fertilization · IVF · Preimplantation genetic screening · PGS · Preimplantation genetic diagnosis · PGD · Preimplantation genetic testing · Metabolomics · Proteomics · In vitro maturation · Review · Embryo biopsy · Fluorescence in situ hybridization · FISH · Embryo cryopreservation · Oocyte cryopreservation · Egg bank · Oocyte bank · Fertility preservation · Oncology · Time lapse · Videography · Fertility · Infertility · Infertile

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Introduction

In July of 1978, the world witnessed the birth of Louise Brown, the first successful in vitro fertilization (IVF) pregnancy achieved by Drs. Robert Edwards and Patrick Steptoe [1, 2•]. Since that time, advances surrounding assisted reproductive technologies (ART) have experienced explosive development and growth. Indeed, what was in 1978 perceived by many as a controversial medical curiosity has radically changed the prognosis of couples suffering with infertility and is responsible for an increasing portion of the world's births [2•]. Many of the advances in ART have both increased success rates and offered a broad range of options to couples undergoing treatment.

Technological advances being developed and perfected currently hold the potential to change the field of ART in still more dramatic and exciting ways well into the future. Some of these technologies, such as those focusing on the genetic evaluation of developing embryos or oocyte cryopreservation, impact the medical care of individuals both with and without a diagnosis of infertility [3••]. In this

review we will discuss some of the cutting edge innovations in the field of ART and the implications of these technologies in the future.

In Vitro Fertilization Advancements

IVF has literally transformed the field of infertility since its inception in 1978 [2•]. Initially, the techniques required to perform IVF were unrefined with relatively poor pregnancy rates [2•]. Major milestones in perfecting IVF, however, were developed quickly, including the use of controlled ovarian hyperstimulation, luteal phase support, and improved culture media [2•, 4, 5]. Other significant advances soon followed with the development of intracytoplasmic sperm injection (ICSI), assisted hatching, and description of optimized ET techniques [6–8]. These advances both improved the success rates associated with ART and increased the number of individuals that were candidates to pursue infertility treatments [2•]. However, some trends in the use of ART during this period were less than positive. Two of the chief concerns that emerged during the development of ART were the persistent risk of patients experiencing ovarian hyperstimulation syndrome (OHSS) and/or a marked increase in the risk of multiple gestation pregnancies [3••].

The concept of controlled ovarian hyperstimulation (COH) in the context of ART was pioneered by Howard and Georgeanna Jones in the early 1980's [9•]. This initial COH protocol utilized 150 IU of human menopausal gonadotropin (hMG) which yielded an average of 3.7 oocytes retrieved per IVF cycles [10]. Later trials in the same center using higher doses of gonadotropins resulted in increased numbers of retrieved oocytes but did not improve pregnancy rates [11]. In the late 1980's and early 1990's many centers adopted increasingly aggressive COH dosing protocols which were associated with increases in various complications, the most notable being OHSS [9•].

Not only did these aggressive COH dosing protocols result in increased risk, they also presented a significant financial burden on patients [9•]. Recently, there has been an increased emphasis on exploring "minimal stimulation" COH protocols [9•]. In several trials, utilization of such protocols that utilize lower doses of injectable gonadotropins results in comparable pregnancy rates with decreased medical complications and cost compared with more standard COH protocols [9•, 12]. Another recent strategy for minimizing the risk of OHSS in COH cycles is the use of gonadotropin releasing hormone (GnRH) agonist, instead of human chorionic gonadotropin (hCG), to trigger ovulation during an IVF cycle [13]. Taken together, minimal stimulation COH protocols and the appropriate utilization of GnRH agonist to trigger ovulation have the potential to mitigate the incidence of IVF associated OHSS, significantly improving the safety of women undergoing ART.

ART, as currently practiced, is also associated with an increased rate of multiple gestation pregnancies [3••]. In IVF cycles, this is principally a result of the routine transfer of multiple embryos into the uterus [14]. Multiple gestation pregnancies are clearly associated with increased health risks, both to mother and child, as well as increased medical costs [14]. For these reasons, there is a concerted effort by many to limit the number of embryos transferred in IVF cycles. Many countries outside the United States have mandated that only certain numbers of embryos may be legally transferred [3••, 14]. The American Society for Reproductive Medicine (ASRM) has made formal recommendations regarding this issue (Pract Committee 2009 [15]). Many advocate the use of single embryo transfer (SET) in IVF cycles, essentially eliminating the risk of IVF associated multiple gestations. Several clinics using SET report no decrease in the pregnancy success rate while almost eliminating multiple gestation pregnancies [16•]. The trend toward decreasing the number of transferred embryos represents an improvement in ART that will significantly improve the cost and safety associated with IVF in the coming years.

Laboratory Advancements

Probably the single most significant factor in the dramatic improvement in IVF pregnancy rates over the past 10-15 years has been the technological modifications in the embryology laboratory [2•]. Of these modifications, perfecting the embryo culture media has likely been the most significant [2•]. Indeed, pregnancy rates increased dramatically since the introduction of the culture media formula titled "Human Tubal Fluid" first introduced in 1985 [17]. Since that time, there have been essentially constant improvements in the composition of embryo culture media that have continuously improved IVF outcomes [2•]. Various other modifications are constantly being evaluated to optimize embryology laboratories. One such modification that is the subject of much research today is the optimal oxygen (O2) concentration in embryo incubators [18]. Other areas of ongoing research are evaluating the optimal pH of embryology media [19, 20]. In addition to media, various other techniques have also been introduced that have optimized outcomes. For example, assisted hatching and ICSI have been of significant benefit [2•, 7].

Laboratory Advancements: In Vitro Maturation (IVM)

The ability to mature oocytes in a laboratory environment, in vitro maturation (IVM), is another technology that is

being currently perfected by many centers. IVM, as currently described, is the practice of retrieving immature human oocytes which then complete the transition from prophase I to metaphase II, including extrusion of the 1st polar body, in vitro [21–23]. Over the past decade or so, there have been many modifications in IVM protocols including priming with hCG, follicle stimulating hormone (FSH), and/or luteinizing hormone (LH) and specific changes in IMV oocyte culture media [24–28]. Additionally, the retrieval procedures to obtain oocytes for IVM are also continuously being perfected [29]. These modifications have resulted in several relatively small trials that reported IVM pregnancy rates approaching, though still lower than, IVF cycles [30–34]. IVM does not appear to introduce more risks, such as imprinting defects, as compared to IVF cycles [35, 36].

The chief benefit of IVM is often cited as a technology that could eliminate the risk of OHSS in PCOS patients [21, 22, 32]. However, the recent introduction of GnRH antagonist cycles utilizing a GnRH agonist trigger and the increased use of mild gonadotropin stimulation protocols offer the ability to reduce or eliminate OHSS in otherwise conventional IVF cycles [9•, 12, 13]. Other investigators have put forth IVM as a possible treatment for women who have had suboptimal responses to traditional IVF cycles [31, 34]. The benefit of IVM in today's current clinical environment is unclear and will need to be further explored.

Laboratory Advancements: Cryopreservation

Perhaps the most significant advancement in embryologic laboratory technology in recent years has been in the field of cryopreservation. The concept of gamete cryopreservation is not new. In 1942, cryopreservation and subsequent revival of a mammalian sperm cell was first described [37]. Applying cryopreservation to IVF embryos was achieved relatively early in 1983 when the first pregnancy resulting from a cryopreserved embryo was reported [38]. The process used to cryopreserve embryos has evolved since this time. For example, prior to cryopreservation today, embryos are exposed to low concentrations of glycerol and propanediol with sucrose supplementation to decrease the content of intracellular water [39]. These compounds result in decreasing the intracellular ice crystal formation that may be associated with cryopreservation [39]. The new technique of vitrification requires decreased concentrations of these compounds and therefore, in theory, may minimize theoretical concerns surrounding possible embryonic toxicity [39]. Increased efficiency in performing embryo cryopreservation has increased ultimate pregnancy rates per stimulation cycle and has provided increased flexibility within IVF cycles that has proved critical for many functions including some forms of preimplantation genetic testing [2•]. There is constant improvement in the protocols surrounding the processes required for embryo cryopreservation and reanimation of the embryo following the thaw [39].

A more recent advance in cryopreservation technology is the ability to cryopreserve unfertilized oocytes. Oocyte cryopreservation is a significant advancement in ART and has the potential to have a monumental impact on the field of infertility as well as society in general. The first pregnancy resulting from a cryopreserved oocyte was in 1986 [40]. However, following this significant achievement, trials evaluating the pregnancy rates of cycles utilizing oocyte cryopreservation consistently yielded unacceptably low pregnancy rates [41]. These poor pregnancy rates were likely due to cellular damage during the cryopreservation process [42•]. Early oocyte cryopreservation techniques relied on controlled-rate or slow-cooling [41, 43•]. In 1999, the first birth following oocyte cryopreservation using vitrification was reported [44]. Since that time, oocyte cryopreservation protocols using vitrification, including the introduction of the equipment innovations such as the cryotop, have been continuously optimized [41, 42•, 43•]. These techniques resulted in oocyte survival rates following cryopreservation approaching 90 % by 2005 [43•]. Currently, multiple clinics report pregnancy rates in cycles utilizing oocyte cryopreservation that approach those seen in fresh IVF cycles [45, 46]. However, the practice of oocyte cryopreservation is still considered an experimental technology according to the American Society for Reproductive Medicine (ASRM)[47].

The demonstration that oocyte cryopreservation is now a viable alternative to embryo cryopreservation has broad implications. The application of this technology that could impact the largest proportion of society is for women who wish to preserve their fertility. Women who pursue professional careers are increasingly delaying childbearing compared to historical norms[48]. While this trend has added to the richness and productivity of the world's economy, it has also increased the rate of age related female subfertility and infertility [49]. While ART has improved the prospects of achieving fertility for sub/infertile women in their late 30's and early 40's, there still exists a significant rate of failure to conceive for this population [49].

The introduction of the birth control pill in the latter half of the 20th century empowered women to have unprecedented control to determine their desired involvement in the workforce [50]. In a similar manner, the ability to preserve a woman's fertility potential at a young age through oocyte cryopreservation has monumental social implications. The "biological clock" that women face as they approach their late 30s and early 40s certainly influences career decisions and places disproportionate pressure on women, as compared to men, to begin childbearing at relatively young ages

[49]. As oocyte cryopreservation technology continues to improve and become less costly, its widespread use for elective fertility preservation by large segments of the population seems likely. With this new opportunity, however, society must be mindful of economic barriers that would likely lead to inequitable access to this technology that could further empower the options of the wealth at the expense of the poor.

Oocyte cryopreservation has additional applications that are currently being utilized. Oocyte cryopreservation is increasingly being offered as a method of fertility preservation for oncology patients prior to receiving chemo or radiation therapy [51•]. The use of oocyte cryopreservation for the purpose of fertility preservation is supported by professional societies [47]. Cryopreservation of unstimulated ovarian cortical tissue for the purpose of fertility preservation for oncology patients has also been performed, although with limited success [51•]. At the present time, embryo and/or oocyte cryopreservation in the context of conventional IVF is the recommended method of female oncologic fertility preservation.

Another application for oocyte cryopreservation is the recent creation of donor oocyte "egg banks" for use in donor oocyte cycles. Traditionally, donor oocyte cycles demanded that the cycles of the donor and recipient be coordinated [42•]. Egg banks eliminate the need for this time consuming and costly process while offering increased choices to couples undergoing a donor oocyte IVF cycle [42•]. As donor oocyte cycles currently comprise approximately 10 % of all IVF cycles, egg banks, from a systems point of view, represent a significant advance in quality and efficiency [42•, 52•].

Technologies Evaluating Embryos

A significant challenge in ART has been and continues to be determining which embryos in a given IVF cycle are optimal for uterine transfer. Traditionally, embryo morphology has been the most utilized method of determining embryo quality. There are numerous grading systems that have been developed to grade embryo morphology [53, 54]. However, embryo morphology alone has been shown to be a suboptimal indicator of determining which embryos have normal chromosomal status (euploidy) or optimal implantation potential [53, 54]. For this reason, a variety of different modalities have been developed to evaluate embryo quality both directly and indirectly.

Technologies Evaluating Embryos: Preimplantation Genetic Diagnosis (PGD)

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) involve obtaining one or more cells from the developing embryo and evaluating the genetic composition of this cell(s) for either a specific genetic defect known to exist in the parents (PGD) or to screen for the presence of embryo aneuploidy (PGS) [55•, 56]. The results of this information then guide the decision as to which embryos are appropriate for embryo transfer [55•]. In 1990, Handyside et al. reported the first established pregnancies using this procedure in two couples known to be at risk for transmitting adrenoleukodystrophy and X-linked mental retardation [57]. Since then, strides in molecular biology and IVF techniques have enabled the perfecting of PGD. PGD has successfully detected the presence of numerous genetic based disorders, such as sickle cell anemia and retinoblastoma [58, 59]. Advancements in genetic medicine are increasingly linking medical conditions to specific genetic markers [60]. The expansion of genetic medicine in the future will certainly broaden the applications of medically indicated PGD.

Another application for PGD is Human Leukocyte Antigen (HLA) typing [61]. This technology is generally employed by parents who have a child affected by a particular disorder that could benefit from some sort of human tissue transplant. For example, a child with leukemia who requires a bone marrow transplant. In these cases, PGD has been employed as a modality to ensure that the next child that the couple conceives will be HLA compatible with their existing child with the given illness. This practice is relatively uncommon but has generated considerable debate regarding the ethics of HLA typing PGD [3••].

In couples with recurrent pregnancy loss (RPL) and a documented balanced reciprocal/Robertsonian translocation or chromosomal inversion in one or both parents, preimpantation genetic diagnosis (PGD) coupled with IVF has been shown to have some benefit in improving pregnancy and live birth rates [62–64]. Traditionally, florescence in situ hybridization (FISH) has been used to identify the presence of translocations in PGD translocation cases. FISH is able to identify both balanced and unbalanced chromosomal translocations. In recent years, microarrays are being increasingly utilized. Microarrays are able to evaluate all 23 pairs of chromosomes and thus evaluate the chromosomes involved in the structural aberration as well other chromosomes for aneuploidy or other chromosomal imbalances [63, 65].

Technologies Evaluating Embryos: Preimplantation Genetic Screening (PGS)

Chromosomal aneuploidy is believed to be the single greatest causal factor in pregnancy failure [66]. PGS is the practice of evaluating cells from a developing embryo for the purposes of identifying aneuploidy. PGS was introduced as a technology that could greatly improve pregnancy

efficiency in IVF patients at risk for miscarriage such as couples suffering from recurrent pregnancy loss or patients with advanced maternal age [67].

There are many different technologies that are used to determine the ploidy status of embryonic cells for PGS. PGS for aneuploidy was first performed with FISH evaluation for approximately 5 chromosomes using a cell taken from the embryo at the cleavage stage. FISH technology then progressed to the routine evaluation of 9-14 chromosomes [55•]. This PGS methodology resulted in suboptimal results and was sharply criticized [68]. There are several reasons why PGS using FISH at the cleavage stage did not produce optimal results. Firstly, FISH only evaluates a portion of possible aneuploidies (9-14 out of 23 pairs of chromosomes [56]. Therefore, FISH is unable to detect many chromosomal aneuploidies. Furthermore, high rates of aneuploidy/euploid mosaicism are known to exist in cleavage stage embryos [69]. Therefore, results obtained from a cleavage stage embryo may not represent the chromosomal status of the remainder of cells comprising the embryo.

In recent years, these two limitations have been addressed by the introduction of technologies that evaluate all 23 chromosome pairs and the ability to perform trophectoderm biopsy at day 5 of development (Fig. 1). The most common methods of performing 23 chromosome PGS employ microarray technology, utilizing either a single nucleotide polymorphism (SNP) or comparative genomic hybridization (CGH) platform [56]. Other forms of 23 chromosome PGS evaluation include CGH on metaphase chromosomes and real time polymerase chain reaction (PCR) [70, 71]. PGS evaluating 23 chromosomes and day 5 biopsy has resulted in excellent pregnancy rates in specific patient populations such as couples suffering from recurrent pregnancy loss (RPL) [72–74].

The recent technological advances in preimplantation genetic testing suggest that there will be a wider implementation of PGD/PGS in the future. However, PGD and PGS require close collaboration between obstetricians, fertility specialists, IVF laboratory staff, and geneticists. Technical limitations and the known phenomenon of mosaicism in the embryonic complex can confuse diagnostic results obtained through PGS/PGD. This poses a risk for which couples need to be appropriately counseled.

Technologies Evaluating Embryos: Secretomics and Metabolomics

Secretomics and metabolomics offer another strategy of determining which embryos are optimal to consider for uterine transfer [75, 76]. These technologies attempt to determine information about embryos from byproducts that can be measured in the media culture fluid surrounding the developing embryo [75]. One important advantage of these approaches is that they provide a noninvasive manner to evaluate embryos versus invasive techniques that require embryo biopsy.

Secretomics is the evaluation of specific protein profiles found in the media culture fluid surrounding the developing embryo [75]. The concept of secretomics was pioneered in animal models but has recently been applied to human embryos [77]. While a variety of methods have been used in the past, mass spectrometry using surface-enhanced laser desorption/ionization coupled to time-of-flight analysis is the most commonly utilized modality currently to perform embryo secretomics [75, 78, 79]. Specific protein profiles evaluated using this process has been shown by some investigators to be predictive of implantation and pregnancy potential [78–80]. Some of these protocols focus on determining protein patterns that reflect cellular growth or apoptosis [80–82]. Other protocols are designed to detect specific protein markers, such as ubiquitin, that are thought to be critical for embryo implantation [78, 79, 83]. Recently, more invasive applications of this technology have been described that evaluate the fluid present in a blastocyst embryo [84]. However, this application of secretomics is experimental and not yet widely utilized.

Metabolomics is the evaluation of the metabolic byproducts present in embryo culture media [75]. These metabolic byproducts contain complex metabolite patterns that may prove detailed information regarding the metabolic status of the embryo [75, 76]. The primary method of performing metabolomics is through the use of various spectroscopic

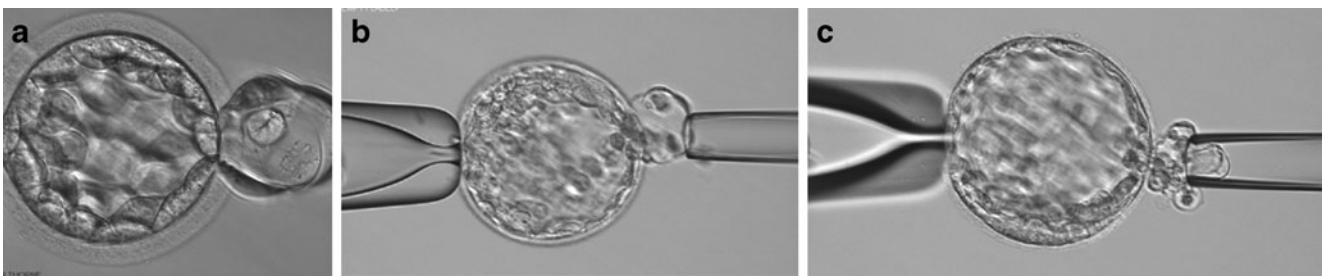

Fig. 1 These photographs show an embryo at the blastocyst stage. **a** shows the herniation of TE cells after the application of a laser to breach the zona pellucida. **b** and **c** show the process of obtaining a sheet of TE cells that will be analyzed for PGS

techniques which are capable of identifying and comparing specific metabolites [75].

Metabolomics first developed by attempting to correlate embryo potential to concentrations of specific metabolites. For example, early reports suggested that nitric oxide metabolites may correlate with blastulation rates in developing embryos [85]. Currently, however, the field is much more complex and evaluates relative concentrations of numerous metabolites simultaneously [86•]. The specific metabolites evaluated in these models are complex and include carbohydrates, amino acids, carboxylic acids, fatty acids, and nucleotides [86•]. Complicating matters further, there is a wide variation of "normal" for these compounds and their relative concentrations change throughout embryonic development [86•]. Despite these challenges, however, significant advancements utilizing metabolomics are currently being reported. Metabolomic platforms have been shown in some studies to predict embryonic implantation potential [87].

Technologies Evaluating Embryos: Time Lapse Imaging

Another recent development in noninvasive evaluation of developing embryos is the use of time lapse imaging and videography. Early embryogenesis is a dynamic process that until recently has not been documented with great specificity [88]. Recent advances in incubators equipped with built in time lapse and video equipment now allow the real time evaluation of the dynamic changes that occur during early embryo development [88]. By evaluating specific growth patterns, some centers have reported the ability to predict embryo developmental potential by evaluating cellular division patterns obtained through these time lapse camera equipped incubators [89•]. This emerging field offers yet another technology that, as it is perfected, may be a powerful tool to select embryos best suited for uterine transfer in IVF cycles.

Conclusions

The scope of recent advances in the field of assisted reproductive technologies is staggering. ART has had a tremendous impact on medicine since its introduction in 1978. The technologies that are coming of age now and are visible on the horizon have the potential to expand the utilization of ART to broad portions of society with and without an infertility diagnosis. For infertile couples, these advances promise to further improve the effectiveness, convenience, and availability of infertility treatment while driving down costs with economies of scale and other efficiencies. For individuals without a diagnosis of infertility, innovations such as oocyte cryopreservation and preimplantation genetic testing offer applications that may be applied socially on a grand scale. As these relevant applications of ART become increasingly utilized, it is incumbent upon society to ensure that these resources are made available in a morally responsible and equitable manner. This moral responsibility may prove to be one of the largest challenges surrounding ART moving forward.

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