The clinical need for a method of identification of embryos destined to become a blastocyst in assisted reproductive technology cycles

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Abstract

Purpose To provide a rationale for continuation of efforts to improve the outcome of Assisted Reproductive Technology outcomes, thereby increasing the likelihood of the live birth of healthy neonates.

Methods Description of rationale and a framework leading to improvement in Assisted Reproductive Technology outcomes.

Results The opportunity for improvement in the success rate for Assisted Reproductive Technology outcome is predicated on selection of the highest quality embryo(s) for transfer. However, such approaches must be balanced by a limit to the number of embryos transferred so as to reduce the risk for multiple births and particularly higher order multiple gestations. Blastocyst transfer offers one such successful approach, but is confounded by suggestions

of an increased risk of both pregnancy complications and epigenetic disorders.

Conclusion There is a need for development of approaches which, individually or in combination, may assist in the early detection of embryos destined to develop into blastocysts.

Keywords Blastocyst · Blastocyst prediction · Assisted reproductive technologies · Embryo selection · IVF · Pregnancy outcome

Capsule A clinical need exist for establishment of approaches which can be utilized to assist in identification of embryos destined to develop into blastocysts.

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Infertility—a major U.S. health disorder

Infertility is estimated to afflict approximately one in six of all couples trying to conceive in the United States; however this number may be an underestimation due to the secrecy many couples display because of feelings of shame and guilt, as well as the feeling of futility many perceive because of their inability to pay for infertility services [13, 43, 50]. Even if this lower number is correct, infertility would still rank as one of the most common chronic health disorders in reproductive-age individuals in the United States [46]. Though substantial advances have been made in the diagnosis and treatment of infertility, there still remain far too many couples who are unsuccessful in their attempts to have a healthy baby through standard fertility assessments and treatments. These couples often use fertility-enhancing drugs including gonadotropins, which in combination with intrauterine insemination, have pregnancy rates of approximately 10–15% per cycle in women under the age of 40. However, use of fertility-enhancing drugs is associated with increased risks for both Ovarian Hyperstimulation Syndrome (a rare, but potentially, life threatening disorder)

and multiple gestation pregnancies, which occur in an estimated one quarter of women who are successful in conceiving.

The role of infertility therapy including ART

The use of Assisted Reproductive Technologies (ART) such as in vitro fertilization and embryo transfer (IVF-ET) results in a significantly greater percentage of couples with success in their efforts to build a family. While originally IVF-ET was used primarily by couples with tubal disease as the etiology of their infertility, it is now utilized by couples with virtually all etiologies of infertility including unexplained infertility following failure with simpler modalities of treatment. Despite increases in the success of IVF-ET, which reached 41% per cycle in the most recent national success reports from the U.S. Centers for Disease Control report for women <35 years old, the majority of couples do not conceive ([7] CDC ART Report). The per cycle prognosis is even worse for couples in older age groups of 35–37, 38–40, 41–42, 43–44, and >44 years old, where only 31%, 22%, 12%, 5%, and 1% respectively, were reported to have successful outcomes ([7] CDC ART Report). Of importance, the age-specific incidence of success is primarily attributable to the *quality of the oocytes* of women in these age groups (and hence the embryos that develop from them), as opposed to other factors such as the endometrium where the embryos implant, or the quality of the male partner's semen.

Risks associated with ART

One opportunity for couples who fail to conceive in an IVF-ET cycle is a repeat attempt. In most states, fertility treatments are not covered by insurance carriers. IVF is associated with a significant economic cost (~\$12,000–\$15,000 per cycle), as well as emotional stress on each partner in the couple, strain on the couple's relationship (with a high percentage of couples undergoing infertility treatment divorcing), and time lost from work or other activities. In addition, there is the rare but potential risk in subsequent procedures of complications including injuries to major vessels and the bowel. Coexisting with these safety issues are the efficacy considerations which represent the two major challenges of IVF treatment today: improving the success rate and increasing the incidence of singleton gestations (e.g. decreasing the rates of multiple gestations).

The present day approach to minimize the risk of failure in IVF-ET cycles is to increase the number of embryos to be transferred in a single cycle, thereby increasing the likelihood that at least one will implant [3]. However, such an approach also increases the potential for multiple gestation

pregnancies including higher order multiple gestations. The incidence of twin gestation reported in the 2008 National CDC Summary in women under 35 years of age was 33.8%, and the triplet pregnancy rate was 3.2% despite an average of 2.2 embryos transferred (www.cdc.gov/art). Viewed differently, of the 63.5% of IVF cycles in women 37 years of age and younger, 33% are multiple pregnancy live births, and thus 50% of the infants born in the maternal age groups are twins and triplets. The associated complications for both mother and baby [4, 10, 19, 29, 31, 32] include preterm delivery [14, 26, 35] which increases exponentially with the number of fetuses. The rate for delivery before completion of 32 weeks gestation is only 1.6% in singleton pregnancies, but it increases to 11.8% in twin gestations, 36.7% with triplets, and 64.5% with quadruplets according to the National Vital Statistics report. Furthermore, extreme prematurity, defined as delivery before 28 weeks gestation, has been reported to be as high as 14% for triplet and quadruplet pregnancies [8, 12, 24]. Higher order multiple gestation pregnancies are also associated with higher hospital maternity and nursery costs. In a report by Callahan et al. [5], hospital charges for a singleton pregnancy were \$9,845, but rapidly increased to \$37,947 for twin gestations (for a per baby cost of \$18,974), and \$109,765 for triplet gestations (for a per baby charge of \$36,588). These increased costs are only a beginning, as it has been estimated that the cost to the healthcare system for even twins and triplets can reach 100–200 times the hospital costs for a baby from a singleton gestation [1, 5].

Selective fetal reduction developed as a medical intervention to reduce the risk of preterm delivery of high order fetal pregnancy, the majority of which occur as a consequence of infertility treatment. While the physical risks of such procedures and the likelihood for loss of non-reduced fetuses are low in experienced hands, performance of such procedures may not be congruent with the ethical and moral principles of many couples. For those patients who do rely on fetal reduction to reduce their risk of preterm delivery and its complications, they are likely to suffer long term sorrow and feelings of guilt at the anniversary of the procedures, or at the birthdays of the surviving siblings.

The medical community has become increasingly aware of the medical risks and societal burden of high order multiple pregnancy. Recently the patients themselves have been demonstrating a strong desire for as natural a pregnancy as possible, which is a singleton pregnancy. A recent article in the New York Time Magazine titled “Unnatural Selection” reported that 38% of the 101 selective fetal terminations performed in 2010 at a New York Medical center were selective reductions of twin pregnancies to a singleton pregnancy (NYT Magazine, Aug 14, 2011) [39].

Collectively, these concerns of the negative outcomes of multiple pregnancies have reached such a magnitude that

guidelines have been established to limit the number of embryos transferred in IVF-ET cycles in the United States. Similar laws limit the number of embryos to be transferred in other countries. Yet despite adherence to these guidelines, the incidence of multiple pregnancies remains high with an average embryo transfer number of 2.3 in all age groups [7].

Limitations of current ART methodology

The Reproductive Endocrinology and Infertility discipline, and the couples they provide care for, would clearly benefit from a method which would improve the success rate of IVF-ET cycles by enabling selection of *a single embryo* for transfer. Such an approach currently is severely hampered by limited ability to differentiate embryo quality among a cohort of embryos from an individual cycle. While individuals with diminished ovarian reserve may have few oocytes and a limited number of good embryos on current morphologic assessments, for most women participating in an IVF-ET cycle, a majority of their embryos appear to be of a reasonable quality deemed appropriate for transfer, and many women will have more good quality embryos than would be appropriate to transfer.

Importantly however, morphological criteria currently in use frequently cannot distinguish embryos likely to succeed in establishing a pregnancy. In fact, of all of the component stages of an IVF-ET cycle (e.g. ovarian stimulation, oocyte retrieval, oocyte fertilization, zygote cleavage, embryo transfer, and implantation), the stage most commonly unsuccessful is embryo implantation. A non-invasive method to select the embryo most likely to develop to the blastocyst stage could therefore offer a significant improvement in overall outcome and safety [51].

Blastocyst transfer—opportunity to improve ART outcome

Thus, to minimize the risk for multiples, to avoid the need for selective reduction, and to improve the success rate of IVF-ET cycles, what is needed is an improvement in our ability to successfully select which embryo to transfer. A series of articles have now appeared that suggest one way in which to accomplish improved embryo selection is to culture embryos beyond the cleavage stages at which transfer is most common (Day 3), to the blastocyst stage (day 5 after oocyte retrieval), thereby transferring embryos which have successfully progressed to this stage [2, 15, 17, 34, 36, 40, 42]. In fact, with blastocyst transfer, the implantation rate has been reported to be as high as 50–60%, which is nearly two fold higher than that reported with transfer of a day 3

embryo (at which time they are usually at the eight cell stage) [16, 37]. However, until recently, there have not been recognized, reproducible factors which can predict in vitro blastocyst development [11, 18]. Unfortunately, blastocyst culture is not without risks; 45% of healthy appearing eight cell embryos placed in extended culture with the intention of performing a blastocyst transfer end up with arrest in growth and development, or become atretic, with this number increasing to 69% of lower quality embryos [41]. In some cycles this can result in there being no remaining embryos to transfer [20, 47], which occurs as often as one in every five cycles [11] in one report. Furthermore, predicting day 5 transfer based on day 3 morphology was successful for only 51% of embryos [41]. Thus while current morphological assessment is helpful in choosing an embryo(s) to transfer, there remains significant opportunity for improvements in the ability to predict which embryo(s) is destined to develop to a blastocyst, with its enhanced likelihood of establishing a successful pregnancy.

Additionally, there has been possible concern that embryos cultured to the blastocyst stage increases the risk for epigenetic disorders such as Beckwith-Wiedemann Syndrome [23, 25, 30, 33]. With an association between embryo culture and genetic imprinting, there could be some concern with regards to the more subtle long term effects of extended culture. There is an increased risk of monozygotic twinning which is associated with a higher risk of preterm delivery, congenital defects, and miscarriage [45]. There is also an observed increased incidence of pregnancy complications including preterm delivery, low APGAR scores, respiratory distress, and low birth weight in all IVF pregnancies [22]. Recently a small increased risk of low birth weight babies following blastocyst transfer was observed, with the suggestion that it was due to the extended culture [21]. The concern of low birth weight extends beyond the perinatal period. Prematurity has been found to be associated with advanced pubertal growth [49], and low birth weight has been correlated with insulin insensitivity and the development of Type II diabetes in adults [38, 44]. Finally, a recent Swedish study assessing long term mortality following preterm birth has reported that in infants with late (34–36 week gestation) preterm birth, there is an increase in early childhood and young adulthood death related to congenital anomalies, endocrine, respiratory, and cardiovascular disorders [9]. Additionally, there is concern that the early nutritional environment for the embryo may also have influences well into adulthood, with effects on cardiovascular health [6, 27, 48]. These factors may mediate adult vascular disease. In part because of these concerns, while some ART centers in the United States routinely practice blastocyst transfer to limit the number of embryos transferred, the majority of IVF programs are continuing to primarily employ day 3 embryo transfers ([7] CDC ART Report).

Unmet need to improve clinical outcomes by identification of embryos destined to develop into blastocysts

Given the increase in pregnancy rates associated with selection of embryos that reached the blastocyst stage, but in light of risks of culturing embryos to the blastocyst stage, there is a great clinical need for the ability to successfully select embryos at an earlier stage of development which are destined to have a high propensity for blastocyst development and subsequent implantation. Additionally, among couples with multiple blastocysts available to transfer, there is a clinical need to be able to successfully identify which would have the greatest propensity for implantation and subsequent delivery of a healthy baby.

Clinical value of blastocyst prediction

There would be significant value, to both infertile couples and care providers, in the early detection of embryos most likely to implant. For patients, this would improve their likelihood of a successful IVF attempt, thereby reducing stress and anxiety and improving their quality of life, while reducing the expense and time commitment required for multiple cycles of IVF-ET. For the ART programs that currently conduct blastocyst transfer, this will allow reduction of the labor intensive time the embryos have to be cared for in the IVF laboratory, and eliminate those cycles in which there are no embryos to transfer because of arrest or atresia of all embryos during prolonged culture. Additionally, for ART programs that currently perform day 3 embryo transfers, identification of embryos destined to become blastocysts will result in improvement of pregnancy rates, without the risks associated with prolonged culture. Finally, it is expected that the transfer of embryos earlier may negate detrimental epigenetic errors that may be associated with extended culture. Thus, identification of a highly sensitive and highly specific approach to predict blastocyst development on day 2 or 3 after oocyte retrieval would represent a clinically significant breakthrough, and provide an opportunity to dramatically improve ART success rates.

Furthermore, there are significant benefits that would accrue from the ability to identify embryos destined to develop into blastocysts, and subsequently implant. First, with improved predictability, the pressure to transfer multiple embryos would be considerably diminished, ultimately leading to a *single embryo transfer*. It has been observed that in a patient's acceptance of a single embryo transfer, the predictability of a pregnancy was the most important factor influencing a patient's decision [28]. This would potentially be of particular value to the 63.5% of U.S. IVF cycles conducted in women 37 years of age and

younger [7] where currently only 7.2% (<35 years of age) and 4.0% (35–37 years of age) of cycles are single embryo transfers. Elective single embryo transfer would become increasingly acceptable as success rates of IVF improve. The risk for multiple gestations and particularly higher order multiples would thus be decreased. This alone would thereby reduce the economic and societal costs associated with hospitalization of babies delivered prematurely, the life-long societal cost for care of preterm newborns with significant major morbidities, and the stress on the couple and their family from caring for multiples. Second, the ability to predict which embryo would develop into a blastocyst will result in fewer implantation failures and unproductive IVF cycles. Further, the potential benefit of early identification of repetitive reproductive failure would be identification of couples who may not benefit from conventional IVF, and who would be better candidates for oocyte and embryo donation, or adoption. Additionally, less vigorous stimulation will reduce the cost of fertility drugs and intensive monitoring of the ovarian response. These reductions in cost will have the beneficial effect of expanding the number of infertile couples who will be able to financially afford ART therapy.

Thus for both short term benefits to individual couples afflicted with infertility, as well as long term societal benefits, there is tremendous potential clinical value in the development of approaches, including preimplantation genetic diagnosis, metabolomics, and time lapse embryo imaging, which may assist in the ability to accurately predict on embryo culture days 2 and/or 3 which embryos are destined to implant.

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