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Original Article

Evaluation of a high implantation potential (HIP) embryo grading system designed to reduce multiple pregnancy

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ABSTRACT

Aims: Although we have progressed a long way in performing in vitro fertilization, one hurdle that still remains is the assessment of embryos. The aim of the current paper was to evaluate an embryo selection protocol based on the presence of high implantation potential (HIP) embryos on Day 3 embryo transfer outcomes.

Methods: The analysis of cycles from 6573 IVF cycles in a private fertility clinic was done. Patients included IVF couples having day 3 embryos transferred. Rates of clinical pregnancy, twinning, and high order multiples (HOM) were investigated.

Results: When one or more HIP embryos were present in the cohort, there were no significant differences in clinical pregnancy rates when the decision was made to reduce the scheduled number of embryos to transfer by 1, that is, when comparing 2 embryos transferred with 2 reduced to 1, 3 with 3 reduced to 2, or 4 with 4 reduced to 3. Twinning rates were the same when at least 2 embryos were transferred and high order multiple rates were not different when at least 3 embryos were transferred. The presence of HIP embryos increases the chance of pregnancy when 1–2 embryos are transferred. With 3–4 embryos, the presence or absence of a HIP embryo becomes less of a factor in establishing pregnancy.

Conclusions: Selecting HIP embryos can reduce the number of embryos transferred without adversely affecting pregnancy rates. Their presence is a strong predictor of pregnancy in patients ≤37 years old.

1. Introduction

The drive to reduce the risk of multiple pregnancy as a consequence of In Vitro Fertilization means that IVF programs must place fewer embryos into each patient than in the past without compromising the chance of achieving a pregnancy. In order to accomplish this goal, IVF centers have developed grading systems that contain semi-quantitative descriptors of the morphology of the early zygote, embryo, or blastocyst. Zygote grading systems evaluate pronuclear size and position, nucleoli number and distribution, and cytoplasmic appearance.[1–3] Several different criteria including the uniformity of blastomeres, percentage of fragmentation, rate of cleavage, and

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blastomere multinucleation are used to grade early stage cleaved embryos.[3–6] Later stage blastocyst grading systems evaluate expansion, zona thinning, and quality of the trophectoderm and inner cell mass.[7,8] Some systems look at each stage separately and some have combined different stages and incorporate them into a "graduated" or "cumulative" embryo score.[9–12]

The ultimate aim of any grading system is to identify the zygote, embryo, or blastocyst that is most likely to implant and become a baby. The morphologic assessment is taken into account when deciding how many embryos to replace with the ultimate goal being a single embryo transfer (SET). Given the well-known morbidity associated with multiple pregnancy,[13–15] many programs are shifting toward elective single embryo transfer (eSET). Although several countries have enacted legislation to allow the transfer of only one or two embryos,[16,17] the USA currently has no laws to limit the number of embryos transferred but does have recommended limits issued by professional societies to encourage eSET. The American Society of Reproductive Medicine suggests that eSET is appropriate for women under age 35 with a good prognosis and a "top quality embryo" available. However, despite the recommendation the rates of eSET in the USA remain below 7%.[18] The question remains for all IVF physicians and embryologists: what criteria do we use to help us pick the best embryo for transfer?

Several studies have attempted to determine what characteristics constitute a "top quality" embryo.[19,20] Our program began a series of studies in the early 2000s to identify embryos with a high implantation potential (HIP).[21,22] These preliminary studies led us to conclude that a combination of day 2 and day 3 cell number (4–6 cells on day 2 and ≥7 cells on day 3) along with a low percentage of fragmentation (<20%) and the absence of multinucleation characterized HIP embryos. The studies also showed that 1–2 HIP embryos per embryo transfer were sufficient to maintain a satisfactory pregnancy rate without increasing the rate of high order multiple pregnancies. Later studies performed at our facility confirmed that HIP embryo grading correlates with increased blastocyst development rates, euploid chromosomal status and increased pregnancy potential whether transfers were performed on day 3 or day 5.[23–25]

Based on our own early studies, we developed a selection protocol for embryo transfer customized for each patient. The protocol is based on age and number of failed cycles, with one option being to place one less embryo than what was ordered prior to cycle start if there is at least one HIP embryo available on the day of transfer. The objective of the present study is to evaluate our embryo selection protocol in a large series of patients and to determine if using the automatic option to transfer one less embryo (as requested by the treating physician) has been successful in maintaining clinical pregnancy rates while decreasing the risk of multiple pregnancy. We also sought to determine the effect of HIP embryo availability and quantity on embryo transfer outcome collectively and stratified by age.

2. Materials and methods

Records of treatment cycles between January 1st, 2008 and December 31st, 2010 at Boston IVF were reviewed. For the first part of the study, 2641 cycles in which at least one HIP day 3 embryo was transferred were evaluated. Embryo transfer protocols were customized for each patient based on age and number of failed cycles, with one option being to reduce the number of embryos transferred by one if there was at least one HIP. The physician of record determined which cases would have the "reduce" approach. These cycles were compared with cycles in which the number of embryos was not reduced even though there was a HIP embryo available. For the second part of the analysis, 4932 cycles from the same time period in which ≤4 embryos were transferred were assessed according to the availability and quantity of HIP embryos.

Patients underwent ovarian stimulation with either human urinary or recombinant FSH or human menopausal gonadotropins. Ovulation was inhibited with either an antagonist or agonist according to physician preference. Oocyte maturation was induced with hCG (human urinary or human recombinant) and oocytes were retrieved 36 h later by transvaginal ultrasound-guided aspiration.

The medium used for gamete culture was Human Tubal Fluid Fertilization and Cleavage Medium (HTF; Sage In Vitro Fertilization, Inc.) supplemented with 10% Plasmanate® (Bayer Corporation) or Serum Protein Substitute (Sage In Vitro Fertilization, Inc.) in microdrops under oil. Motile sperm were isolated using a discontinuous PureCeption™ (Sage In Vitro Fertilization, Inc.) gradient consisting of an upper and lower layer. Gradient and centrifugation parameters were individualized for each semen sample. All oocytes scheduled for conventional fertilization were coincubated with 50,000 sperm in 30 μl drops 4–6 h after retrieval. Those oocytes destined for ICSI were stripped of their cumulus layer and injected with a single sperm following sperm preparation.

At 17–20 h post-insemination, the oocytes were assessed for fertilization. Normal fertilization was defined as the presence of two pronuclei and two polar bodies. A recheck was performed 4–6 h later to identify any oocytes with delayed or abnormal fertilization. Embryos were assessed on days 2 and 3 after oocyte retrieval by analyzing cell number and fragmentation. Embryos were considered HIP if they contained at least 4–6 cells on day 2 and ≥7 cells on day 3 along with a low percentage of fragmentation (<20%) and the absence of multinucleation. The actual rates of clinical pregnancy, twinning, and high order multiples (HOM) were recorded for cycles in which HIP embryos were or were not generated. The rates in each group were compared using Chi Square analysis. p-Values <0.05 were considered statistically significant.

3. Results

A summary of results of implantation data when at least one HIP embryo was transferred can be found in Table 1. The transfer of one fewer embryo than what was ordered prior to cycle start does not change the clinical pregnancy rate when at least one HIP embryo is present. The transfer of one fewer embryo does dramatically reduce the risk of twins when one embryo is placed rather than two. Higher order multiple pregnancies are also eliminated when only one or two embryos are transferred. Twin pregnancy rates were all statistically the same when at least two embryos were