Abstract

In Vitro Fertilization (IVF) is a technique designed to increase probability of conception in couples for whom other fertility therapies have been unsuccessful or are not possible. This treatment is commonly called as test tube baby where eggs are fertilized by sperms in the laboratory - outside the body.

After the birth of Louise Brown, the first IVF baby in 1998, development in biotechnology enables manipulation of human gametes for in-vitro-fertilization (IVF) treatments. Developments in IVF protocols have enabled the availability of more number of good quality embryos for transfer. This resulted in considerable increase in success rate of assisted conception. However the total success in terms, of implantation rate of embryos and total pregnancy rate is not satisfactory. It is widely accepted that, after in vitro fertilization pregnancy rate increases with number of embryos transferred. To overcome the low implantation potential, it is practice to transfer more numbers of embryos in effort that at least one get implant and results in the pregnancy. This may lead in multiple pregnancies. High order multiple pregnancies i.e. three or more gestations is an undesirable outcome of Assisted Reproductive Technology (ART). Pregnancy with multiple gestations is associated with adverse physiological consequences. Although embryo reduction can be performed to reduce fetal number, the procedure may result in the loss of total pregnancy. In reality multi fetal pregnancy reduction is not an acceptable option for many women.

Ideally, the ultimate aim of ART during IVF is to achieve a single conception. Single embryo transfer has been encouraged to avoid multiple gestations. To achieve the highest success in IVF and to keep the proportion of multiple pregnancies at minimum level is the challenging task. In order to be able to reduce number of embryo to be transferred without significantly lowering the pregnancy rate, it is essential to increase our knowledge of choosing the potential embryo for transfer from a cohort.

Most widely used criteria for selecting the best embryo for transfer have been based on cell number and morphology. Biochemical methods evaluating follicular fluid composition or metabolic activity of embryo are also available to assess embryo quality. However, these methods are very complex, expensive, time consuming and impractical in
most of the busy ART laboratory. Therefore, the assessment of morphology has been, and will remain the primary choice for selection. Morphological evaluation by microscopy is quick, easy and inexpensive with a predictive aptitude.

All morphological methods rely on single static observation of embryo at the time of embryo transfer. However implantation potential of an embryo is influenced by gamete quality, fertilization events, growth rate and culture conditions. Embryos has to undergo time bound, complex development processes. The development consists of union of gametes to form single cell zygote after fertilization of oocyte, commencement of mitotic divisions, maternal to embryonic genome activation, initiation of protein synthesis and cell differentiation. The embryo after fertilizations is on stringent set of clocks, which directs division and initiation of key events from gene activation through compaction and blastulation with energetic process. Each stage is dependent on successful completion of previous stage.

Pioneer efforts have been taken in correlating embryo morphology at particular development stage with pregnancy outcome. Literature survey shows the impact of embryo quality at individual developmental stage on pregnancy rate. Each stage has the predictive value at that particular check point. However embryo has to pass through different developmental stages. Successful completion of the previous stage makes the foundation for next stage of the complex development process. This limits the selection of potential embryo by single static observation at the time of transfer. It is difficult to define potential embryo that will implant and give rise to healthy baby. For optimal evaluation assessment of embryo at each of the development checkpoint is essential. Frequent observations of embryo for long period of time can analyze the growth rate and development potential. However removing culture dish containing embryos for microscopy from the incubator impart shift in Ph., temperature as well as O₂ and CO₂ concentration. It certainly induces a stress on embryo.

The assessment procedure should be rapid, accurate and non-subjective with minimal effect on developmental competence of embryos. Application of efficient grading system has advantages in terms of i) selection of embryo with potential of implantation ii) reduction in number of embryos to be transfer to avoid multiple gestation iii) selection of capable embryo for cryo preservation to elude unnecessary expenses iv) limiting
discrepancy between observers so that the selection will become unbiased and non-subjective.

The objective of this study is to develop a novel method for selection of IVF embryos for potential implantation taking cognizance of all stages of in-vitro development. We attempted to express embryo quality in numerical values so as to make unbiased and inexpensive embryo selection with quick and easy method.

**Methods**

Existing selection methods are studied to find correlation of morphological features of oocytes, sperms, zygotes and embryos at each stage with success of implantation during in vitro culture. The retrospective study was performed on embryos produced from the patients underwent their IVF treatment at Niramaya IVF center. Routinely used stimulation protocol, oocyte retrieval technique, in vitro fertilization and culture procedures were followed. Since the sperm are available in millions, sperm having absolutely normal morphology were selected form semen sample for injections. Cycles with morphologically abnormal spermatozoa or surgically removed spermatozoa are excluded from this study. The study included transfer of single embryo or transfer of two or three embryos of identical morphology.

**Embryo grading Scheme**

The embryos were observed at definite time to assess each development stage starting from gametes, zygote, time required for onset of first mitotic division after injection of spermatozoa and morphology of embryo at subsequent cleavage stages till transfer. Generally we performed embryo transfers on day 2 at which embryos are at 4 cell stages and rarely on day 3 at 8 cell stage of embryo. Microscopic images at 400X magnification were captured with camera attached to inverted microscope (Olympus IX 70) at different check point. Thus, in each transfer the morphology and cleavage stage of all embryos at different in vitro developmental stages were recorded.
The embryos are differentiated according to their morphological characteristics at different development stages. Numerical score is given in descending order with increasing dysmorphisms.

All in vitro development stages, till embryo transfer are allotted with a numerical score according the quality. Each development stage has an additional aptitude to distinguish competent embryo over the previous stage. Therefore, completion of previous stage and transition in to next stage is merited by applying an exponential stage specific multiplication factor. Embryos having overall good morphology and normal growth rate at each stage has more chance to implant. An embryo with leading growth rate has more potential compare to embryo lagging in growth irrespective of morphology. Hence the increasing multiplication factors for each succeeding stage accurately assess the embryo quality. Cumulative score is calculated at the time of embryo transfer by addition of individual stage score.

Pregnancy was confirmed by evaluating serum beta hCG (human Chorionic Gonadotropin) concentration after 12 days of embryo transfer. If pregnancy occurred, ultrasound evaluation was performed to ensure the presence of an intrauterine gestational sac and observation of fetal cardiac activity. In this study we have included the cases in which either all transferred embryos are implanted or all are not implanted. Minimum score among the implanted embryos is found out. Implantation rate is calculate which is define as fraction of transferred embryos resulted in an implanted embryos or gestational sac. Embryos with overall score more than seventy thousand as per present numerical system exhibit good implantation potential. Reduction in Implantation rate is observed as embryo score declines below seventy thousand and significant increase in IR is observed among embryos having embryo score above ninety thousand.

During in-vitro development each stage has consistently been shown to have some predictive value. The present study enables to exploit selection potential of each stage for final embryo selection. Aptitude of present grading system is get augmented by multifactorial morphological assessment at different stages. This grading system facilitates easy, inexpensive and unbiased embryo selection which is applicable even for small IVF set up.