Literature Review

In Vitro Fertilization (IVF) means fertilizing an ovum with a spermatozoon outside the body in a culture dish under controlled culture conditions. It is being a principle option to treat most of the infertility causes, is a field of contentious research. Efforts have been made to improve each and every step of in vitro culture so as to increase overall success of IVF treatment. Technical aspect of in-vitro fertilization has been changed significantly in last 35 years resulting in overall improvement in results. However success of the treatment is affected by both intrinsic as well as extrinsic factors. It is not conceivable to enhance intrinsic potential of fertility artificially hence the emphasis has been set on extrinsic factors such as drugs used for stimulation protocol, culture conditions, as well of selection of gametes and embryos from cohort to enhance the chance of conception.

Development of embryo starts from gametogenesis – formation of male and female gametes. Sperms are produced in millions on daily basis by the series of complex processes called spermatogenesis. However very few sperms in semen sample exhibit normal morphology as described by Laboratory Manual published by World Health Organization (WHO) for examination of human semen. Morphogenetic process during sperm formation and maturation can result in anomalies which can be observed microscopically [Auger J et. al. 2001]. Sperm morphology provides vital information on the quality of sperm sample [Menkveld R et. al. 2001, Franken DR et. al. 1999, Auger J et. al. 2001]. Morphology has been an important parameter in deciding which form of assisted reproductive treatment will be effective in treating a particular patient (Agarwal A et. al. 2009).

Oocyte is a female gamete which provides the genetic material as well as the nutritive environment for developing embryo. However for IVF treatment, ovaries are stimulated with exogenous hormones to allow maturation of many oocytes. This is called as controlled ovarian hyper stimulation (COHS) which bypasses the complicated selection procedure that usually occurs during single oocyte development and maturation. Oocyte quality is influenced by COHS as well as many factors such as, nuclear genome, mitochondrial genome, microenvironment provided by the ovary and pre-ovulatory follicles [Balaban B et. al. 2006, De Cassia et. al.2010]. Mature oocytes with perfectly spherical shape and a single intact first polar body, clear, moderately granular, homogeneous and translucent cytoplasm without any inclusion bodies and
small peri-vitelline space, a clear and regular zona-pellucida have been reported to be ideal [Alikani et. al. 1995, Ebner et. al. 2000, 2006, Xia et. al. 1997]. Oocytes collected after COHS display varying stages of maturations and viability. Only Oocyte exhibiting first polar body is considered as mature and is suitable for ICSI. However, nuclear maturity alone is, not enough for successful fertilization. Oocyte cytoplasmic maturation should be completed along with the nuclear maturation. Especially in the oocytes retrieved following COHS, the cytoplasmic maturation lags behind the nuclear maturation [Rienzi et. al. 2005, De Santis et. al. 2005, Montag M et. al. 2006]. Asynchrony in maturation of cytoplasm and nuclear processes exhibits deviations in intra-cytoplasmic as well as extra-cytoplasmic morphology of oocyte [Hassan et. al. 1998, Eichenlaub et. al. 1995, Kahraman et. al. 2000]. Although the influence of these deviations on ICSI outcome is not clearly understood, the attempts have been made to correlate these dysmorphisms with oocyte quality and subsequent embryonic development. According to some studies slight deviation in oocyte shape does not affect developmental competence [De Sutter et. al. 1996, Balabn et. al. 1998, 2008, Rienzi et. al. 2005]. However, Embryos derived from oocyte with abnormally large size (30% larger in diameter than normal oocyte) is associated with digync-triploidy [Rosenbuschet et. al. 2002, Planchot M. 2003, Balakier et. al. 2002, Ebner T et. al. 2006]. Such oocytes are called as giant and are recommended to be excluded from the ART program. Extra cytoplasmic abnormalities like thickness of zona pellucida (ZP) or dark ZP shows no effect on the fertilization rate, pronuclear development, embryo quality or implantation rate [De Sutter et. al. 1996, Balabn et. al. 1998, 2008, Rienzi et. al. 2012]. Only broken or empty zona pellucida is not suitable for IVF treatment [Loutradis et.al.1999]. Association of increased Peri-Viteline Space (PVS) with embryo quality and its development has also been documented [De Sutter et. al. 1996, Xia P et. al. 1997, Sathanathan H et.al. 1997, Balabn et. al. 1998, Chamayou et. al. 2006]. According to some studies morphology of first polar body is a predictive marker of oocyte quality. Fragmented first polar body reflects post ovulatory age of the oocyte indicating in-vitro ageing of the oocyte. Degenerative first polar body is associated with asynchrony between nuclear and cytoplasmic maturation which may lower the developmental potential of the oocyte [Ebner et. al. 2000, Ciotti et. al. 2004, De Santis et. al. 2005, Balaban et. al. 2008]. However most of the studies indicate no correlation between polar body morphology and embryo

Influence of zygote morphology on subsequent embryo development has been observed. Study of zygote morphology includes careful analysis of number, size and symmetry of pronuclei, appearance of cytoplasm as well as size, number and distribution of the Nuclear Precursor Bodies (NPBs) between two nuclei. Pioneer efforts have been taken by Scott et. al. in 1998 and Tesarik and Greco in 1999 to find out effect of zygote morphology on ICSI outcome. Scott et. al. graded the zygotes into four types viz. Z1, Z2, Z3, and Z4 whereas Tesarik et. al. classified zygote from pattern ‘0’ to pattern ‘5’. Both systems are based on correlating the pattern of NPBs in fertilized oocytes with predictive ability of embryo development and the grades or patterns are assigned to the zygote in the descending order of deviations of their pronuclear morphology. Zygote should have two equal sized pronuclei. Deviation in PN size and number i.e. presence of single PN indicates fusion of two pronuclei or parthenogenic activation. Presence of 3 PN is mostly caused by dispermy or digynic triploidy [Munne S et. al. 1998, ALPHA Scientist in reproductive medicine, 2011]. Normal human zygote should have five to seven nucleoli. Increased number of nucleoli indicates fragmentation and in vitro oocyte ageing [Schwartz et al. 2003]. The number and arrangement of NPBs in both nuclei have been correlated with implantation and development [Scott et. al. 2007, Ludwig et. al. 2000, Scott L et. al. 2002, Zollner U et. al. 2003, Borini et. al. 2005]. Significant improvement in Pregnancy Rate (PR) in
cycles where at least one embryo derived from optimum zygote morphology was transferred has been reported [Van De Ban et. al. 2001].

The zygote eventually divides mitotically to form two celled embryo. Within a cohort onset of first cleavage occurs at variable time points. The embryos showing onset of first mitotic division resulting into two celled embryo at 25 to 27 hour after Intra Cytoplasmic Sperm Injection (ICSI) is called as Early Cleaved Embryo (EC). The concept of Early Cleavage (EC) and its effect on embryo quality was published for the first time by Edward and his colleagues in 1998. It is documented that embryos which show EC have more chance to implant than Non-Early Cleaved (NEC) embryos. This correlation of early cleavage with embryo quality at subsequent in vitro developmental stages has been supported by several studies (Shoukir et. al. 1997, Sakkas D et. al. 1998, Bos-Mikich et.al. 2001, Lundin K et. al. 2001, Fenwick J et. al. 2002, Salumets A et.al 2003, Van Montfoort et. al. 2004, Giorgetti C et. al. 2007).

Positive correlation has been observed between good PN morphology and early onset of first mitotic division (EC) [Balaban B et. al. 2001, Christopher Chen et. al. 2005]. Uneven distribution of cellular and genetic material results in unequal sized blastomeres which is detrimental to embryo viability and negatively correlates with Implantation Rate (IR) and pregnancy Rate (PR). The significantly higher percentage of early cleaved embryos show normal cleavage pattern and even sized blastomeres as compared to non-early cleaved embryos [Meng-Ju et. al. 2012, d. hlinka et.al. 2012, Maria Cruz et. al. 2012, Mina Alikani et. al. 2012]. Correlation of unequal division has been shown with higher incidences of aneuploidy and multinucleation [Harderson et. al. 2001, Magli et.al. 2001].

Morphological anomalies in first few cleavages and poor quality of embryonic development are strongly associated with error in duration of cell cycle [Thorir Harderson et. al. 2001, Laëtitia Hesters et. al. 2007, P Terriou et. al. 2007, Irene Rubio et. al. 2012, Mina Alikani et. al. 2012]. Improvement in the rate of implantation has been documented in early cleaved mono-nucleated embryos [Isaac Kligman et. al. 1998]. It has been also shown that EC embryos frequently produce mono-nucleated blastomeres than NEC embryos [Brezinová J et. al. 2004, Laëtitia Hesters et. al 2007, M.J.Pelinck et.al.1998]. Literature search also indicates that there is reduction in the rate of spontaneous abortions of the implantation of EC embryos than NEC embryos. This results in improvement in implantation and pregnancy rate as well as rate of birth [Lundin K et. al. 2001, Meng-Ju Lee et. al. 2012, hlinka et. al. 2012]. --- The ultimate goal of IVF unit.
Very few embryos reach the 2 cell stage at 25h post ICSI and few patients benefit from such an early assessment and selection for transfer (Bos-Mikich et. al. 2001). It is not known, however, at which time point of completion of the first cell cycle, embryo viability starts to drop. 50% of embryos show cleavage at 29hr Post Injection (PI) and has equal potential as those of embryos showing cleavage up to 27h PI. This extended period maximizes the number of potential embryos available for transfer. Onset of EC is a better independent marker of implantation potential than zygote morphology, but the best outcome can be achieved with system including both the criteria [Balaban B et. al. 2001, Christopher Chen et. al. 2005].

Number of scoring systems based on microscopic observations of developing embryos during in vitro culture have been proposed [Giorgetti et. al.1995, Veeck et. al.1999, Fisch et. al. 2001, de Placio et. al. 2002, Baczkowski et. al. 2007]. These systems include evaluation of number and symmetry of blastomeres, degree of fragmentation and nuclear status. The development stage of embryo at particular time after ICSI is exhibited by number of blastomeres. Significantly higher Pregnancy Rate (PR) has been documented with transfer of embryo exhibiting 2 cells on day 1 (at 26-28 h after ICSI), 4 cells on day 2 (at 46-48 h after ICSI) and 8 cells on day 3 (at 66-68h after ICSI) [Lundin et. al. 2001, Fenwik et. al. 2002, Sccott et. al. 2007, Van Rayen et. al. 1999, Racowsky et. al. 2011, Meng-Ju Lee et. al. 2012, d. hlinka et. al. 2012, Irene Rubio et. al. 2012, María Cruz et. al. 2012 ]. Embryos exhibiting less or more number of cells at distinct observation time point show chromosomal error and early pregnancy loss [Munn et. al. 2006, Magli et. al. 2007, Finn et. al. 2010].

Fragmentation is a small portion of cytoplasm without DNA. It is frequently observed under in vitro embryo culture. This is an anucleated structure enclosed by a cell membrane originated from blastomeres. Degree of fragmentation plays important role in embryo potential, and is expressed as the percentage of the total cytoplasmic volume. Increase in fragmentation is associated with reduced blastocyst formation rate, increased chromosomal abnormalities and negatively correlates with implantation and pregnancy rates [Hardy et. al. 2003, Racowsky et. al. 2000, Magli et. al. 2007].

Cytoplasmic anomalies like granularity and presence of vacuoles are often observed in IVF embryos. Predictive value of these cytoplasmic features on embryos potential is not clearly understood. However the cleavage stage embryo should have pale and clear or finely granular

Nuclear status of the blastomeres is one of the important features for prediction of embryo potential in IVF treatment. Presence or absence of nuclei and their number plays important role in cleavage stage embryo evaluation. Each blastomere having single nucleus is an index of chromosomal normality of the embryo [Isaac Kligman et. al. 1998, M.J.Pelinck et. al 1998, Jackson et. al. 1998 Katharine V. et. al 1998, Van Royen et. al. 2003, Ciray N et al. 2006, P Terriou et. al. 2007, M Takayuki et. a. 2014]. IVF embryos frequently exhibit multinucleated blastomers which is usually associated with chromosomal abnormalities, impaired cleavage, developmental arrest, poor implantation rate and increased risk of spontaneous abortion. Such embryos are suggested to be excluded from transfer [P Terriou et.al. 2007, Jackson et. al. 1998, VanRoyer et. al. 2003]. Multi nucleated embryos frequently exhibit late onset of first mitotic division and uneven cleavage.

Several methods have been proposed to ensure the correct selection of potential embryo [Giorgetti et. al.1995, Desai et. al. 2000, Fisch et. al. 2003, 2007, de Placio et. al. 2002, Baczkowski et. al. 2007]. Most of these systems are based on the evaluation of blastomeric number along with symmetry and the degree of fragmentations. The existing system of embryo selection based on these observations differentiates embryos into various grades like Grade I, Grade II, Grade III and Grade IV (Alpha Scientist in Reproductive Medicine and ESHRE Special interest Group of Embryology, 2011).

In some studies embryo morphology is qualified in numerical score, while in some other studies logical regression analysis is applied [Giorgetti et. al. 1995, Desai. N et. al. 2000, Fisch J et. al.2003, Sjoblom P et. al. 2006]. Graduated embryo scoring system proposed by Fisch et. al. includes parameters such as pronuclear morphology, early cleavage and day 3 morphology for scoring the embryo. Neuber et. al. proposed a multistep assessment system that includes morphological parameters up to day 5 and have shown positive correlation of EC and subsequent good quality with blastocyst development. These studies are based on the consideration of limited parameters of the morphological characteristics of embryos and its development stages. With most of these systems more than three embryos of variable qualities are to be transferred making it difficult to identify the quality of implanted embryos. With this scope in mind we thought of framing a system which will provide a selection score to the embryo in form of
numeral indicating its implantation potential. This system is step taken forward to minimize the possibility of subjective selection and multiple transfers of IVF embryos. The system is novel, simple and highly reliable.