Introduction

World’s first IVF baby is a result of natural cycle IVF. There was no hyper-stimulation used, naturally developed single follicle was retrieved and fertilization was achieved by conventional IVF procedure. Briefly, sperm suspension and oocyte cumulus complexes were cultured together in the test tube containing culture medium at 37\(^\circ\). Sophisticated culture conditions were not developed at that time. Over last 35 years advent in pharmaceuticals made availability of more pure and powerful stimulation drugs. Increased knowledge of pathophysiology made the stimulation protocols more effective with minimum discomfort to the patient. Development in technology like invention of ICSI technique, made it possible to manipulate human gametes in Vitro to achieve fertilization. Improvement in culture conditions like development of sequential culture media, applications of clean air, heated surface and triple gas incubators has improved quality of embryos grown in the laboratory. This results in the production of multiple good quality embryos with equivalent cell number and morphology by individual patient. Although IVF procedures have been greatly improved over the years, implantation rate of an individual embryo is very. The efficacy of IVF treatment in terms of live birth rate is still only 30-40\%. To overcome low implantation rate is common practice to transfer multiple embryos in the intention that at least one will implant and result in to pregnancy. This may result in undesirable multiple gestations.

Multiple gestations can be avoided only by reducing the number of embryos transferred to one. In order to enable the reduction in number of embryos to be transferred without significantly lowering the pregnancy rate, it is important to select most potential embryo for transfer in IVF programs. Thus the discrimination of potential embryo from a cohort is the demanding task for an embryologist. Most of the embryo grading systems are based on the evaluation of blastomeric number along with symmetry and the degree of fragmentations.

In present study systematic screening of all morphological parameters and their deviations from ideal characteristics at every stage of the developing embryo under in vitro culture conditions has been carried out. The differences in the characteristics of implanted embryos and those failed to implant at each stage of their development were examined. With this we made an attempt to increase our knowledge to identify most
potential embryo within cohort. Present study includes cases with total implantation or total failure. This study is a retrospective analysis of data accumulated at Niramay IVF Center, Chinchwad, Pune during June 2010 to March 2016. Images captured during embryo assessment were observed and analyzed for different morphological parameters at each in vitro development stage. A numerical score is assigned to the selected embryo considering all microscopic observations at each stage of its in vitro development.

Materials and Methods

Materials:
Images of embryos captured at different in vitro developmental stages were analyzed in the previous chapters and assigned scores as described before. These were studied to calculate the cumulative Selection Score (CSSc) of the embryo.

Methods

Method for Assigning Cumulative Selection Score to the Embryo.
Selection Score is assigned to the Embryo by making use of following individual scores-

i. Selection Score of the Gametes (SScG) – Sperms and oocytes are assigned numerical scores depending upon their morphological observations as described in Chapter 4. Selection Score of the Gamete (SScG) is obtained by multiplying the score of gamete with \(10^0\) for successfully completing the gametogenesis.

ii. Selection score for zygote (SScZ) – Zygotes are assigned numerical scores depending upon their morphological observations as described in Chapter 5. Selection Score of the Zygote (SScZ) is obtained by multiplying the score of zygote with \(10^1\) for successful zygote formation i.e completion of first stage of embryo development.

iii. Early cleavage – Cohort was observed for embryos exhibiting onset of first mitotic division between 27-29 h PI called as early cleaved embryo (EC) and are merited with positive sign (+).

iv. Selection Score of embryo at 2cell stage (SScE2) -
2 cell stage Embryos (E2) are assigned numerical scores depending upon their morphological observations as described in Chapter 7. Selection Score of the E2 (SScE2)
is obtained by multiply the score of E2 with $10^2$ for successful transition from zygote to 2 cell stage embryo i.e completion of second stage of embryo development.

v. **Selection Score of embryo at 4cell stage (SScE4) -**

4 cell stage Embryos (E4) are assigned numerical scores depending upon their morphological observations as described in Chapter 7. Selection Score of the E4 (SScE4) is obtained by multiply the score of E2 with $10^3$ for successful transition from zygote to 2 cell stage and from 2 cell stage to 4 cell stage embryo i.e completion of third stage of embryo development.

vi. **Calculation of cumulative Selection Score of the embryo (CSScE):**

Cumulative Selection score of embryo (CSScE) is obtained by arithmetic sum of individual selection scores of all in vitro developmental stages.

Thus

$$
\text{CSScE} = \text{SScG} + \text{SScZ} + \text{SScE2} + \text{SScE4}
$$

**Results**

1) Images of embryos at different in vitro developmental stages analyzed and scored in the previous chapters 4, 5, 6 and 7 were studied to calculate the cumulative Selection Score (CSSc) of the embryo. Table 8.1 displays scheme employed for calculation of CSSc for representative embryos by novel numerical scoring system framed in this study.
2. Score for 119 embryos was calculated employing the numerical scoring system framed in this study. Embryos were grouped into four groups according to the range of their cumulative scores. Percentage of embryos in the respective group and corresponding Implantation Rate (IR) was calculated (Table 8.2). Figure 8.1 is the graphical representation of these results. Graphical expression of the data is in accordance with the trend line. The distribution of EC and NEC among different score groups was calculated. Their IR rate was calculated (Table 8.3 and 8.4 respectively) and graphically represented in Fig.8.2 and 8.3
3. Minimum score at which implantation has occurred is 5559.
4. Implantation rate has been increased with the increasing cumulative selection score.
5. The percentage of embryos obtaining higher score is very low; however their implantation rate is significantly high as compared to embryos with lower score.
6. Significantly higher the percentage of EC embryos are observed in higher score group.
Table 8.2 Distribution and Implantation Rate of embryos in different score groups

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Group</th>
<th>Score of embryos</th>
<th>No. of Embryos</th>
<th>No of implanted Embryos</th>
<th>Percentage of Embryo(%)</th>
<th>Implantation Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Below 5000</td>
<td>10</td>
<td>0</td>
<td>8.4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>5000-7000</td>
<td>54</td>
<td>5</td>
<td>45.37</td>
<td>11.02</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>7000-9000</td>
<td>44</td>
<td>17</td>
<td>36.97</td>
<td>45.98</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>9000 Above</td>
<td>11</td>
<td>5</td>
<td>9.2</td>
<td>54.09</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>119</td>
<td>27</td>
<td>22.68</td>
<td></td>
</tr>
</tbody>
</table>

Images of 119 captured during IVF treatment at Niramaya center during June 2010 - March 2016 were retrospectively analyzed and scored according to present frame.

Fig 8.1 Distribution and Implantation Rate of embryos in different score groups
**Table 8.3 Distribution of EC and their IR among different score groups**

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Embryo Score</th>
<th>Group</th>
<th>Total Embryos</th>
<th>No. EC Embryos</th>
<th>%EC</th>
<th>EC IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Below 5000</td>
<td>I</td>
<td>10</td>
<td>1</td>
<td>2.17</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5000-7000</td>
<td>II</td>
<td>54</td>
<td>11</td>
<td>23.91</td>
<td>27.27</td>
</tr>
<tr>
<td>3</td>
<td>7000-9000</td>
<td>III</td>
<td>44</td>
<td>25</td>
<td>54.35</td>
<td>52.00</td>
</tr>
<tr>
<td>4</td>
<td>9000 Above</td>
<td>IV</td>
<td>11</td>
<td>9</td>
<td>19.57</td>
<td>44.44</td>
</tr>
</tbody>
</table>

Embryos obtained during IVF treatment at Niramaya center during June 2010 to March 2016 were retrospectively analyzed for EC and NEC embryos and their IR was calculated.

**Fig 8.4 Distribution of EC and their IR among different score groups**
Table 8.3 Distribution of NEC embryos and their IR among different score groups

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>Embryo Score</th>
<th>Group</th>
<th>Total Embryos</th>
<th>NEC</th>
<th>% NEC</th>
<th>NEC IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Below 5000</td>
<td>I</td>
<td>10</td>
<td>9</td>
<td>12.33</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5000-7000</td>
<td>II</td>
<td>54</td>
<td>43</td>
<td>58.90</td>
<td>4.65</td>
</tr>
<tr>
<td>3</td>
<td>7000-9000</td>
<td>III</td>
<td>44</td>
<td>19</td>
<td>26.03</td>
<td>15.79</td>
</tr>
<tr>
<td>4</td>
<td>9000 Above</td>
<td>IV</td>
<td>11</td>
<td>2</td>
<td>2.74</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Embryos obtained during IVF treatment at Niramaya center during June 2010 to March 2016 were retrospectively analyzed for EC and NEC embryos and their IR was calculated.

Fig 8.3. Distribution of NEC embryos and their IR among different score groups
Discussion
In the global scenario of the impact of genetic makeup along with nutrition, environmental pollution, stress, life style has been shown to affect fertility resulting in increasing the complexity of causes and treatment of infertility. At the same time due to late marriages and demand of carrier orientated life style there is increase in the age of motherhood. This poses problems for conception. Being an important part of the human life, inability to bear child represents major life problem. It imparts various psychological and psychosomatic effects among the suffering couples.

With this scenario there is increasing need of assisted conception. Invention of fertility drugs in 1950 began the medication for infertility. The therapy gets developed rapidly with introduction of ART. In Vitro Fertilization programs making use of transfer of in vitro cultured embryos developed from oocytes obtained due to hormonal stimulation is a break through invention of 21st century. It has provided a treatment option for couples who would be otherwise unable to conceive through normal means because of various issues. IVF with ICSI enabled to bypass problems with women’s fallopian tubes, male’s sperm motility and many more.

Although a large amount of study has been carried out on various aspects of IVF treatments for improving its success rate, it is an accepted fact that the success rate is not very impressive i.e. it is within 25-30%. Efforts have been made in studying the quality of gametes, zygotes and embryos up to its stage of transfer. The existing system of In Vitro Fertilization makes use of embryos selected on the basis of their morphology. Gametes, zygotes and Embryos are assessed for their morphological characteristics by observing under the phase contrast microscope. Selected gametes are used for ICSI, the zygotes are cultured under ideal culture conditions and monitored for their morphological assessment at the different check points as per ESHERE guidelines.

In the present scoring system we made daily light microscopic observations of all morphological parameters of all sequential stages of embryo development starting from gametes till embryo transfer under in vitro condition. Results of screening of microscopic observations were correlated for assessment and assigning cumulative selection score to the embryo. Assessment of morphology at each stage with respect to all microscopic parameters is expressed in the form of a numerical score. Completion of previous stage
and transition to next developmental stage is merited by exponential stage specific multiplication factor (St MF) like \(10^0, 10^1, 10^2, 10^3\) for gamete, zygote, E2, E4 respectively. The Cumulative Selection Score (CSSc) is obtained by arithmetic sum of scores at all stages from gamete till embryo transfer. Cumulative Selection Score endorses the embryo with its potential for implantation. For example, cumulative selection score between 5000-7000 of Group II embryos endorses them with 11% implantation potential Table 6.1.

Thus scoring of each stage takes cognizance of every parameter of that stage noted microscopically and multiplication factor takes account of timely development. While cumulative selection score is the reflection of the score of each of these stages. Thus the potential of the embryo for implantation is evaluated on the basis of its numerical score.

This Novel Numerical Scoring System is quick, efficient and inexpensive. It is very precise and can accommodate almost all characters of in vitro embryos. Multistage and multifactorial analysis enhanced the ability of current system in selecting the potential embryo for transfer. Microscopy, the least invasive assessment method which imparts minimum stress on embryo along with numerical scoring has made this system more objective. Application of this system should allow the more correct selection of potential embryo for transfer.

The novel numerical system for selection of embryo for IVF treatment is comparable with the existing grading systems. Embryo which is assigned graded I in existing system is comparable with embryo which is assigned a score of 9. Embryo with grade 2 is comparable with embryo which is given scored 7 and so on. However, in the existing system of assigning grade to the embryo, quality of gamete from which it is derived is not reflected. In the scoring system developed by us, since the score is given to every stage and is accounted while assigning score to the embryo, it becomes easy to know the quality of gametes from which it is developed. According to us, since the gamete quality plays important role in development of potential embryo present system merits the gamete quality appropriately. The system allows the discrimination of embryos which are apparently similar at particular time point. The results on the screening of EC and NEC embryos and their scoring according to this scheme displayed in the table 8.2 and Fig. 8.1, 8.2 and 8.3 are highly impressive and in accordance with literature trends.
scheme although makes use of the merits of the existing grading systems is more precise, simple and accommodative. Every possible morphological features of each stage of development has been assigned an independent score of either 1 or 2, making the scheme most versatile for scoring all variations in all stages of development of the most dynamic system of embryo. Nonetheless, since every system has its own advantages and limitations routine application of this system is necessary to appreciate its use in improving not only the IR and PR but also the rates of take home baby -- the ultimate aim of ART!

All efforts in this study have been made as a step towards fulfilling the urge of motherhood of my patients.