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Research Article

ROLE OF EARLY CLEAVAGE IN PREDICTING SUCCESS OF INTRA CYTOPLASMIC SPERM INJECTION IN ASSISTED REPRODUCTIVE TECHNOLOGIES

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ABSTRACT

Aim and Objective: The present study is aimed to carry out the impact of early cleavage over late cleavage in assessing the pregnancy outcome using of Intra Cytoplasmic Sperm Injection (ICSI) in assisted reproductive technologies. **Materials and Methods** A total of 154 patients enrolled for Intra Cytoplasmic Sperm Injection (ICSI) fulfilling the selection criteria were recruited for the study at a tertiary care assisted reproductive centre. ICSI was performed 3–5 h after oocyte aspiration with the prepared sperm. All embryos were checked for early cleavage at 27 hours post intra cytoplasmic sperm injection. They were divided into two groups. Group I- Embryos which cleaved before 27 hours after Intra Cytoplasmic Sperm Injection (ICSI). Group II- Embryos which cleaved after 27 hours. The pregnancy rates were compared between the two groups. **Results:** All the 154 patients were analysed. There was no difference in the mean age, duration of ovarian stimulation, number of oocytes retrieved, fertilization, cleavage rates and embryo quality between the two groups. Early cleavage was observed in 98 patients (63.64 %). Late cleavage was observed in 56 patients (36.36%). The clinical pregnancy was confirmed in 59 patients (60.20%) in Group I and 20 patients (35.71%) in Group II which was statistically significant P <0.001. **Conclusion**: Early cleavage is a strong predictor of embryo quality and can predict ICSI outcome.

Keywords: Clinical pregnancy, Early cleavage, Embryo quality, Intracytoplasmic sperm injection, Ovarian stimulation.

INTRODUCTION

Assisted reproductive technology (ART) is a general term referring to methods used to achieve pregnancy by artificial or partially artificial means. All treatments or procedures that include the in vitro handling of both human oocytes and sperms or of embryos for the purpose of establishing a pregnancy. It is a reproductive technology used primarily in infertility treatments. Different methods of embryo transfer have been followed in this treatment are fresh embryo transfer and frozen embryo transfer.

The success of assisted reproductive technologies (ART) depends primarily on the quality of the embryos transferred and endometrial receptivity. Routinely the selection of embryos for transfer is based on embryo morphology and developmental stage. Sometimes, implantation may not occur after transferring good quality embryos to a receptive endometrium.[1] Other methods of selection of embryos include pronuclear morphology, oocyte and pronuclear polarity, blastomere symmetry and blastocyst culture.[2] Pronuclear zygote morphology

may vary during the dynamic process of syngamy.[3] According to previous studies, selection of embryos on the basis of cell number and quality at the time of transfer is of more significant benefit.[4] Other morphological features such as variation in zona thickness and the presence of multinucleated blastomeres have also been affect the implantation and pregnancy.[5] Some authors scored blastocyst on the basis of inner cell mass and trophectodermal cells and selecting high quality blastocyst, which leads to higher pregnancy and implantation rates.[6] Several biochemical methods have been used to assess the human embryo quality, such as O_2 consumption, pyruvate uptake, glucose uptake, lactate production and secretion of platelet-activating factor production or amino acid turnover.[7] These procedures are all more complex and time-consuming and it is very difficult to follow in routine practice. There is still a need for an easy, simple, and more efficient method of viable embryo selection. A recent study showed that assessment of the time of cleavage to the two cell stage was a reliable parameter for the selection of embryos with the highest capability of implantation and successful pregnancy after transfer.[8] The aim of the present study was done to evaluate the impact of early cleavage over late cleavage in assessing pregnancy outcome using Intra Cytoplasmic sperm injection.

MATERIALS AND METHODS

It was a prospective observational study conducted in the Department of Reproductive Medicine, at a tertiary care centre from Oct 2010-May 2012. A total of 154 patients who underwent Intra Cytoplasmic Sperm Injection (ICSI) were included in the study in the age group of 21-45 years. **Inclusion criteria:** All patients enrolled for ICSI during this study period were included in the study. The patient has only early cleavage embryos and the patient having only late cleavage embryos for transfer were included in the study. **Exclusion criteria:** Patient having both early and late cleavage embryos for transfer was excluded from the study. Informed consent was taken before the enrollment of each participant and the Institutional ethical committee approval was obtained (IEC/10/JULY/83/29).

Two stimulation protocols were used in this study; The A gonadotropin-releasing hormone (GnRH) agonist protocol- A gonodotropin releasing hormone agonist is an analogue that activates the receptors resulting in increased secretion of Follicle stimulating hormone (FSH), Luteinizing hormone (LH). The GnRH antagonist protocol -A gonadotropin-releasing hormone antagonist is an analogue that blocks the GnRH receptor resulting in an immediate drop in gonadotropin (FSH, LH). In the GnRH agonist protocol, pituitary down regulation was done with GnRH agonists. Once the patient was down regulated completely (had menses, E2 <30 pg/ml) gonadotropin injections (recombinant follicle stimulating hormone/human menopausal gonadotropin) were given until the day of hCG administration. The doses were adjusted according to the patient's ovarian response. In the GnRH antagonist protocol, without down regulation gonadotropin injections were administrated daily from the second day of the menstrual cycle. The doses were adjusted according to the patient's individual ovarian response. Once the dominant follicle reached 14 mm in mean diameter, GnRH antagonist was administered subcutaneously at a dose of 0.25 mg daily until the day of hCG administration. In both groups, ovulation was induced by the administration of either recombinant h CG or urinary h CG when at least two follicles reached 18 mm in diameter, and oocyte retrieval was performed 34–36 hours later. Oocytes were retrieved transvaginally under ultrasound- guidance. Motile sperms were isolated by a swim-up or gradient centrifugation. Ejaculated, testicular biopsy; cryopreserved ejaculated and cryopreserved testicular biopsy semen specimens were all included in the study. Intra Cytoplasmic Sperm Injection (ICSI) was performed 3–5 h after oocyte aspiration with the prepared sperm. Normal fertilization was confirmed by the presence of two pronuclei and two polar bodies 16–20 h (day1) after Intra Cytoplasmic Sperm Injection (ICSI). Normally fertilized oocytes (Zygotes) were spherical and had two polar bodies and two PNs. PNs had approximately the same size, centrally positioned in the cytoplasm with two distinctly clear, visible membranes. The presence of nucleolar precursor bodies, their number and size aligned at the PN junction were assessed. On the same day, early cleavage examination was performed on the zygotes within 27 hours after Intra Cytoplasmic Sperm Injection
(ICSI). Embryos displaying two cells at inspection were designated as 'early cleavage'. The embryos that

had not yet cleaved to the 2-cell stage after 27 hours were designated as 'late cleavage'. Two or three embryos were transferred on Day2 depending on the patient's age and embryo quality. The embryos that were not transferred were cryopreserved. The luteal phase was supported by vaginal supplementation of progesterone or intramuscular injection of progesterone.

Pregnancy was determined by a serum β human Chorionic Gonodotropin (β h CG) test 14 days post transfer. The clinical pregnancy was confirmed by the presence of an intrauterine gestational sac with fetal cardiac activity by ultrasound examination at 4 weeks after embryo transfer. Patients were divided into two groups. Group I- Embryos which cleaved to two cells before 27 hours after injection. Group II- Embryos which cleaved to two cells after 27 hours. The pregnancy rates were compared between the two groups.

Statistical analysis: The collected data were analysed with SPSS 16.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis, means and standard deviation were used. For the numerical data nonparametric Mann–Whitney *U* test was used to find the significance. To find the significance in categorical data Chi - Square test was used. In all the statistical tools, the probability value of $p<0.05$ was considered as significant level.

RESULTS

A total of 154 patients were analyzed. The baseline characteristics were shown in (Table1). About 65% of the patients were in the age group of 26-35 years. Early cleavage was observed in 98 patients (63.64 %) and late cleavage was observed in 56 patients (36.36%) (Table 1). In our study 71.78% of MII oocytes retrieved in the early cleavage and 28.22% in the late cleavage group ($P<0.0001$) (Fig 1). The results showed that the good quality embryos were significantly higher in the early cleavage group than in the late cleavage group (78.30% vs. 21.70%) ($P<0.0001$) (Fig 2). The transfer of early cleavage embryos resulted in a significantly higher pregnancy rate than those with late cleavage embryos. (66.33% vs. 39.29%) ($p<0.001$) (Fig 3) The clinical pregnancy was confirmed in 60.20% in the early cleavage group and 35.71% in the late cleavage group which was statistically significant $p <0.001$. (Fig 4)

Table 1: Baseline Characteristics

Parameters	Early Cleavage (Group I)	Late Cleavage (Group II)
Mean Age (years)	31 ± 4	32 ± 5
Mean Duration of Infertility (years)	7 ± 4	8 ± 5
No of oocytes retrieved	15 ± 8	11 ± 8
No of MII Oocytes retrieved	12 ± 7	8 ± 7
No of Grade I Embryos	7 ± 5	4 ± 4
No of patients	98 (63.64 %)	56 (36.36%)

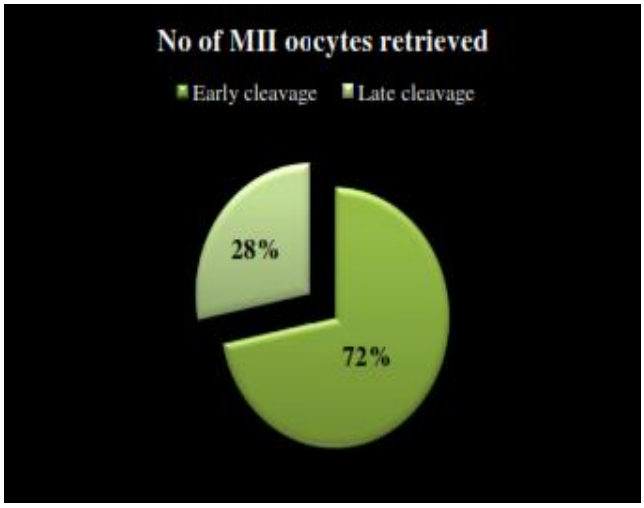

Fig 1: Comparison of early cleavage and late cleavage with No. of MII oocytes retieved

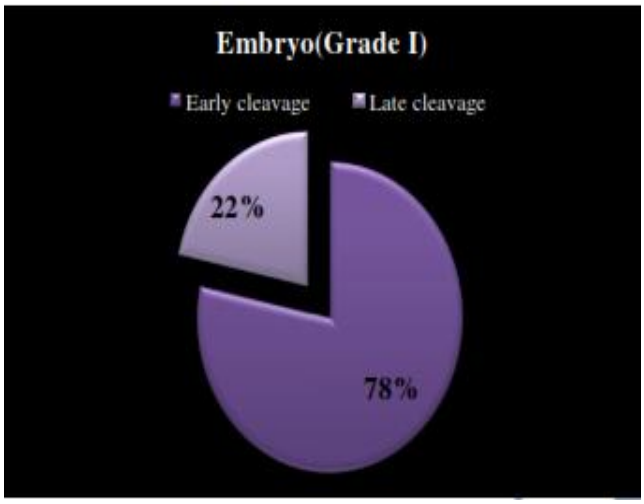

Fig 2: Comparison of early cleavage and late cleavage with good quality embryo

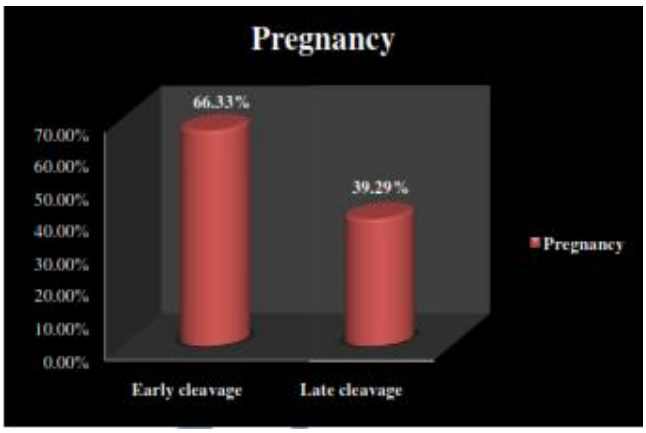

Fig 3: Pregnancy rate in early cleavage and late cleavage group.

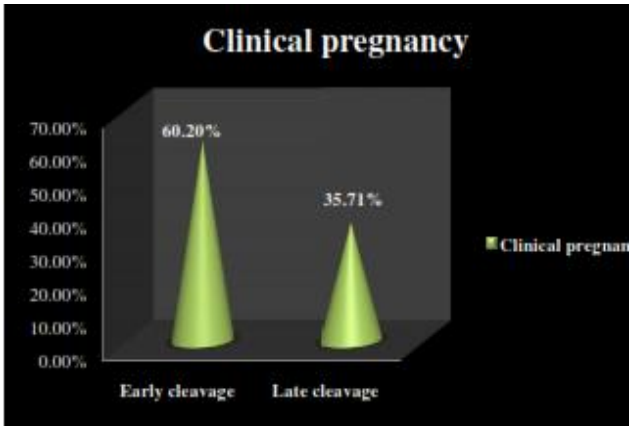

Fig 4: The clinical pregnancy rate in early cleavage and late cleavage group.

DISCUSSION

In the present study , the effect of the early cleavage of transferred embryos were evaluated aiming to increase the pregnancy rate and prevent multiple pregnancies.In the previous studies, transfer of more embryos has been the approach to increase pregnancy rates. However, this also increases the multiple pregnancy with increased medical risks, cost to the patient and society[1]. Some authors they found that the selection of embryos at the time of transfer based on cell number and quality was more benefit.[4- 6] Good quality embryos must exhibit appropriate kinetics and synchrony of cell division[10]. In normal-developing embryos, cell division occurs in every 18–20 h. If we observed a group of four cell embryos at the time of transfer, it was not possible to distinguish which has just cleaved to the four cells or which has been at the four cells for several hours. Hence, selection of the more advanced embryo was difficult to assess.[4- 6] Cleavage stage embryos range from the 2-cell to the compacted morula composed of 8–16 cells.[10]

The types of cleavage on day 3 embryos were classified according to blastomere number as rapid cleavage (>9 cells), normal cleavage (7-8 cells), or slow cleavage (<6 cells). On the basis of quality of embryos on day 3 were classified as good embryos (<20 % fragmentation and an even blastomere) or poor embryos (>20% fragmentation and an uneven blastomere).[15] Embryos which are dividing either too slow or too fast may have metabolic and/or chromosomal defects.[10] Recent time-lapse studies found that not only the timing of cleavage, but also the time between each cell division is also important. In cleavage stage embryos if all blastomeres divide in exact synchrony, only 2-, 4- or 8-cell embryos would be observed. However, we frequently observed 3-, 5-, 6-, 7- or 9-cell embryos, which is an indication of asynchronous development of embryos.[10] Some authors found that implantation increased fourfold in embryos with low glycolytic activity.[1] Selection of embryos by pronuclear assessment has some drawbacks. Accurate pronuclear assessment needs considerable manipulation of zygotes outside the incubator[8]. According to some studies the blastocyst transfer has been successfully used as a means of embryo selection. It is not in routine use because of lack of experience in prolonged embryo culture, as well as anxieties about those patients whose embryos arrest before blastocyst formation[8]. Although several factors influence the result of an assisted reproductive technology (e.g. stimulation response, endometrial receptivity, oocyte maturity, culture conditions), embryo quality is also one of the most important factors[9]. More recently they showed the assessment of the time of cleavage to the two cell stage was a reliable parameter for the selection of viable embryos with the highest capability of implantation and successful pregnancy after transfer[8]. The early cleaving embryos give rise to better embryo quality due to intrinsic, unknown factor within the oocyte. This unknown factor improves the viability of embryos.[1,4,7] One of the possible important mechanisms of delaying cleavage may be delayed fertilization. Oocyte immaturity is the most important factor responsible for delayed fertilization. Since only metaphase II oocytes were injected in Intra Cytoplasmic Sperm Injection (ICSI) procedure, the possibility of oocyte immaturity was eliminated in the present study. Although there may a difference in fertilization time between In vitro fertilization (IVF)

and Intra Cytoplasmic Sperm Injection (ICSI), there seems to be no correlation between the time of fertilization and cleavage[1]. Semen parameters may also affect fertilization and cleavage time.[1] Different morphological abnormalities of the oocytes caused by the reduced blood supply of the follicle during stimulation resulting in oxygen deficiency leads to reduced viability.[3] In our present study, we observed that a significantly higher number of early cleaving embryos became good quality embryos (Fig 2) and indicating an indirect way of selecting the best quality viable embryos. Several other studies have also strongly supported this approach and showed the value of early cleavage as a marker of embryo viability.[5,7,11] In the recent study they found significantly more embryos in the best category showing signs of early cleavage (51.1%) compared with the Non-early cleavage (38.7%) .[13] A number of reports have been published and found that the transfer of an early cleavage embryo resulted in a significantly higher pregnancy rate.[5, 11, 12] The results from our study were similar to these reported studies (Fig 3). We did not transfer mixed early cleavage embryos and late cleavage embryos together in order to evaluate the outcome of early cleavage clearly. One of the recent studies supported this approach[15]. Many articles in the literature deal with the importance of early cleavage to improve embryo selection before transfer and help to reduce multiple pregnancies[13]. In the previous study, they found the clinical pregnancy rate was significantly higher in the early cleavage group than in late cleavage group[14]. In the present study, we investigated that a significantly higher pregnancy rate and the clinical pregnancy rates when early cleaved embryos were transferred compared with late cleavage embryos. (66.33 versus 39.29% and 60.20 versus 35.71% respectively). (Fig 3 and Fig 4).Our data strongly support the previously published studies dealing with early cleavage.[5, 11, 14] So from our study the assessment of early cleavage seems to be a simple, easy, non invasive, effective and valuable method of assessing the embryo viability with higher clinical pregnancy rate.

CONCLUSION

In conclusion the assessment of early cleavage is a strong predictor of embryo quality and can predict ICSI outcome. Therefore, early cleavage criteria can be included for selecting embryos with a higher potential of implantation and successful pregnancy while avoiding multiple pregnancies.

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Conflict of interest: None

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