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A SIMPLE CRITERION FOR IMPROVING PREGNANCY RATE IN INTRACYTOPLASMIC SPERM INJECTION BY ASSESSING THE EARLYCLEAVAGE

Clinical Research

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ABSTRACT

Aim and Objective: The aim of this study was to establish a simple criterion for improving the pregnancy rate by assessing the early cleavage in Intra Cytoplasmic Sperm Injection (ICSI). **Materials and Methods:** A total of 294 patients enrolled for ICSI fulfilling the selection criteria were recruited for the study at a tertiary care assisted reproductive centre. ICSI was performed 2-3 h after oocyte aspiration with the prepared sperm. All embryos were checked for early cleavage at 27 hours post ICSI. They were divided into two groups. Group I embryos which cleaved before 27 hours after ICSI. Group II- embryos which cleaved after 27 hours. The pregnancy rates were compared between the two groups. **Results:** All the 294 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 165 patients (56.12 %). Late cleavage was observed in 129 patients (43.88%). The pregnancy was confirmed in 129 patients out of 165 (78.20 %) in Group I and 50 patients out of 129 (38.80%) in Group II which was statistically significant P <0.005. **Conclusion:** Simple selection criteria using the early cleavage embryo may improve the pregnancy rate in ICSI.

KEYWORDS

Early cleavage, embryo quality, intracytoplasmic sperm injection, ovarian stimulation, pregnancy rate.

INTRODUCTION

The success of assisted reproductive technologies (ART) depends mainly on the quality of the embryos and endometrial receptivity.[1] The successful delivery of a healthy baby and reduce the multiple pregnancies are the main goals of embryo selection in assisted reproductive technologies (ART) [2] Despite great improvements of assisted reproduction, IVF live birth rates remains relatively low. Delayed childbearing and the well-known negative effect of age on reproduction is one explanation. Another explanation is that, we still cannot know how to select the embryo with the highest implantation potential for transfer.[3] Identification of highest implantation potential of an embryo is of primary importance in assisted reproduction.[4] Evaluation of morphology is presently considered the single most important predictive measure for assessing embryo viability .[5] For selecting the viable embryos many scoring and grading criteria have been developed.[6] To improve embryo selection various advanced technologies (metabolomics, proteomics, timelapse algorithm-based selection (TLM), preimplantation genetic testing for aneuploidy (PGT-A)) have been practiced as tools.[3,7] These procedures are all more complex and time-consuming and it is very difficult to follow in routine practice. In addition, most of the randomized trials do not support their use. Transfer of more embryos has been the approach to increase pregnancy rates. This also increases the multiple pregnancy with increased medical risks, cost to the patient and society. In 2009, over 400,000 fresh *In Vitro* Fertilization (IVF) and ICSI cycles were performed in Europe. One out of every five treated, resulted in a live birth. To achieve this birth rate, in 75% of the cycles more than one embryo were transferred. Twenty percent of the deliveries were multiple pregnancies.[8, 9] In 2012, close to 100,000 fresh IVF/ICSI cycles were performed in the US. The average number of embryos transferred was between 1.9 and 2.9 (dependingon the age of the patient). The implantation rate was 37.5% in the less than 35 yrs age group. In this same age group, a 40.7% live birth rate was achieved but almost 30% of the deliveries were twin deliveries.[10, 9] There are additional maternal and neonatal risks in multiple pregenancies.[11,9] In order to prevent these unfavourable results brought about by ART, it is necessary to restrict the number of embryos for transfer. However, concerns have been raised that restricting the number of embryos for transfer might jeopardize the success rate of IVF treatment. So in order to reduce multiple pregnancies and achieve a maximal rate of implantation, selection of most viable embryo for transfer has become a high priority in assisted reproduction. One of the main problems in ART today is selection of best embryos for transfer. Therefore, it is important to increase our knowledge on embryo selection. It has been shown that early-cleaved (EC) zygotes develops in to better quality embryos and gives a higher pregnancy rate compared with those cleaved later in IVF and ICSI cycles (Shoukir *et al*., 1997; Sakkas *et al*., 1998; Bos-Mikich *et al*., 2001; Lundin *et al*., 2001).[12] With this in background, the study was conducted. The aim of this study was to establish a simple criterion for improving the pregnancy rate by assessing the early cleavage in ICSI.

MATERIALS AND METHODS

It was a prospective observational study conducted in the Department of Reproductive Medicine & Surgery, at a tertiary care centre from Oct 2010-Dec 2013. A total of 294 patients who underwent 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 had only early cleavage embryos and the patient had only late cleavage embryos for transfer were included in the study.

Exclusion criteria: Patient had both early and late cleavage embryos for transfer was excluded from the study. Informed consent was taken before the enrolment of each participant and the Institutional ethical committee of Sri Ramachandra Institute of Higher Education and Research (SRIHER) approval was obtained (IEC/10/JULY/83/29).

Ovarian stimulation and ICSI procedure

Ovulation induction was performed using a long Gonadotrophin-Releasing Hormone (GnRH) analogue suppression protocol or a GnRH antagonist protocol using human menopausal gonadotrophins or recombinant Follicle-Stimulating Hormone (FSH).

Ovulation and final maturation of the oocyte were induced with human chorionic gonadotrophin (hCG) when the leading follicle reached 18 mm in average diameter. Oocytes were aspirated 34-36 hours after hCG administration. Oocyte retrieval was performed by transvaginal ultrasound guided puncture. Removal of the surrounding cumulus cells was accomplished by a combined enzymatic and mechanical treatment carried out under a stereoscopic dissecting microscope. Oocytes were denudated from cumulus oophorus by exposure to 80 IU/ml hyaluronidase enzyme in HEPES-buffered medium followed by mechanical removal of the corona radiate with the use of plastic pipette stripper tips. 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. For the ICSI procedure, 1-2 µL washed spermatozoa was placed in 10% polyvinylpyr-rolidone (PVP; sage) .After denudation, a single sperm is injected into the cytoplasm of the oocyte.

Assessment of fertilization, embryo cleavage and embryo Transfer
Fertilization assessments were performed 17±1 hour post injection. Normally, fertilized oocytes should be spherical and have two polar bodies and two PNs. PNs should be juxtaposed, approximately the same size, centrally positioned in the cytoplasm with two distinctly clear, visible membranes. On the same day, early cleavage examination was performed on the zygotes at 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'. Embryo quality was evaluated under an inverted microscope. Two or three embryos were transferred on Day 2 or day 3 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 294 patients were analyzed. The baseline characteristics were shown in (table1). Early cleavage was observed in 165 patients (56.12%) and late cleavage was observed in 129 patients (43.88%).

Table 1: Comparison Of Baseline Characteristics

Parameters		Group I N=165 (%)	Group II N=129 (%)	p value
Age	Age distribution(Yrs) (Mean±SD)	30.7±4.8	31.9±5.2	#0.116
	Age <35 Years-N=234	142(60.68)	92(39.32)	*0.002
	Age >35 Years –N=60	23(38.33)	37(61.67)	
Duration of infertility(Yrs) (Mean±SD)		7.0±3.9	7.7±4.7	#0.222
Type of infertility	Primary infertility-N=257	144(56.03)	113(43.97)	#0.934
	Secondary infertility-N=37	21 (56.76)	16 (43.24)	

*p<0.05 is significant #p>0.05 is not significant

This table shows the base line characteristics and the type of cleavage among the study subjects. There was no significant difference in age (p=0.116), duration of infertility (p=0.222), type of infertility (p=0.934) in both the groups. The study population was divided in to two groups based on age as ≤35 yrs and >35yrs. We observed statistically significant difference in the age distribution among these two groups, (p<0.002) with early cleavage rate of 60.68% in patients ≤35 yrs and 38.33% in patients >35yrs (table1).

Table 2: Comparison Of Outcome Of Controlled Ovarian Stimulation

Parameters	Group I N=165	Group II N=129	p value
Oocytes injected(Mean±SD)	13.14±6.	7.84±5.5	*0.005
Oocytes fertilized(Mean±SD)	10.72±5.5	5.78±4.3	*0.005
Fertilization rate (%)	80.66%	72.33%c	*0.001
Embryo grading Grade I(Mean±SD)	8.19±4.9	3.18±3.01	*0.005
Grade II(Mean±SD)	2.52±3.2	2.19±2.4	#0.641
Grade III(Mean±SD)	0.09±.6	0.50±1.3	*0.005

*p<0.05 is significant #p>0.05 is not significant

The number of MII oocytes, good quality oocytes, oocytes fertilised and good quality embryos were significantly more with early cleavage group followed by late cleavage group (p<0.05) (table 2).

Table 3: Comparison Of Good Quality Embryos Between The Groups

Parameters	Group I N=165 (%)	Group II N=129 (%)	p value
Good quality embryo(1762)	1352(76.73%)	410(23.27%)	*0.0001

*p<0.05 is significant #p>0.05 is not significant

So good quality oocytes significantly influence the cleavage of embryos with more early cleavage embryos. 76.73% of good quality embryos in early cleavage group and 23.27% in late cleavage group which was statistically significant (P<0.0001) (table3).

The hypothesis that transfer of embryos formed from EC zygotes may yield a higher pregnancy rate than transfer of embryos from LC zygotes was tested. In our study the transfer of early cleavage embryos resulted in a significantly higher pregnancy rate than those with late cleavage embryos. (78.20%) vs. (38.80%) (p<0.005) (fig1).

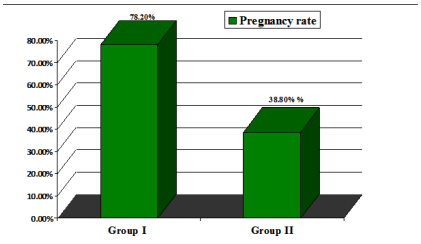

Figure 1: Comparison Of Outcome Of Treatment

DISCUSSION

Despite many parameters may influence the outcome of ICSI, embryo selection is one of the most important factors that affect the outcome. Many parameters have been used to make this decision, including pronuclear morphology, blastomere morphology, blastocyst grading and choosing most viable embryos to increase implantation, pregnancy, and live birth rates is of great significance. The use of early cleaved embryos to select the best embryos was first reported by Edwards et al.[6] In our study the mean number of oocytes injected in EC group were 13.14±6. and the mean number of oocytes fertilized were 10.72±5.5. But the mean number of oocytes injected in LC group were 7.84±5.5. and the mean number of oocytes fertilized were 5.78±4.3. But in Haydar Nadir Çiray et al study [4] in which the mean oocytes injected were 11.8±6.9 and mean fertilized oocytes were 8.6±5.7. In our study the fertilization was achieved in 80% of the oocytes injected. But in the Jing fu et al study the fertilization rate was slightly lower (67.7%)[13] Our results showed significantly higher fertilization rates, seems to reflect a good quality oocyte. In our study, we found early cleavage in 56.12% in ICSI. Similar findings were studied by Eddesy et al., with 46.20% [14] Haydar Nadir Çiray et al, with 42.5% early cleavage in ICSI [4] But in Isiklar et al study they found slightly lower percentage of early cleavage in ICSI (25.6%) [15] ICSI procedure is ideal for this type of observation because it requires removal of cumulus–corona cells prior insemination. Nuclear maturity, cytoplasmic features and the time of sperm entry are thus precisely known.[16] However, most of the studies examined transfers, in which two or more embryos were transferred, of them at least one embryo had shown early cleavage. This makes it difficult to conclude to which embryo the pregnancy can be attributed.[14] In our study two or three embryos were transferred either all EC or LC embryos, which makes it possible to determine the relationship between early cleavage and pregnancy arising from one specific embryo category. In our study majority (69.37 %) were grade I embryos. Of this EC group yielded about 76.73% and LC group yielded 23.27% of good quality embryos which was statistically significant (p<0.0001). This also correlates

with the study conducted by Jing Fu et al where they found that 52.5% had excellent quality embryos in EC group and 28.9% in the LC group ($p<0.01$).[13] It can be the reason that the early cleaving embryos stem from oocytes in which cytoplasmic and nuclear maturation are better synchronized [17]. In the study by Fancsovits et al, where they reported that the number of top quality embryos, regarding the number of cells and the morphology score, was significantly higher in the EC group (35.9%) than in the LC group of embryos (19.7%) [18] It has been demonstrated that EC embryos have better morphology.[19, 14,20,12,21] 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.[15, 22, 23] In the study by Nagy *et al. (*1994), cleavage to the two-cell stage was initially observed 20 h after ICSI, whereby 10 out of the 93 normally fertilized oocytes (11%) had cleaved to the two-cell stage. But in our study, fertilized oocytes were examined at 27 h after ICSI and at this stage (56.12%) had cleaved to the two-cell stage.[22] 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 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 ICSI there seems to be no correlation between the time of fertilization and cleavage. In the previous study also found that the early cleavage of embryos to the two-cell stage is not entirely influenced by the timing of fertilization, and is therefore more likely due to intrinsic factors within the oocyte or embryo that promote embryo cleavage after fertilization. Semen parameters may also affect fertilization and cleavage time.[15, 22] In our present study, we observed that a significantly higher number of early cleaving embryos became good quality embryos (table 3) 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.[24,23,17] We did not transfer mixed early cleavage embryos and late cleavage embryos together in order to evaluate the outcome of early cleavage clearly. Our study revealed that transfer of EC led to significantly higher pregnancy rates as compared to LC, 78.2% versus 38.8%; ($p<0.05$). This was similar to studies by Tesarik et al., Salumets et al., Fancsovits et al., Hammoud et al., Brezinova et al.[25,24,18,26,27] The possibility that EC embryos might be a highly significant biological indicator of embryo growth potential, may predict IVF outcome was first proposed by., Sakkas et al and Shoukir et al.[22,23]

CONCLUSION

In conclusion, the assessment of early cleavage seems to be simple, easy, non invasive, effective and valuable criteria of assessing the embryo viability and using the early cleavage embryo may improve the pregnancy rate in ICSI.

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Conflict of interest: None

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