



Effectiveness of using “Physiological ICSI” in patients with severe male factor infertility: A randomized controlled trial

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ABSTRACT

A randomised controlled trial was conducted to investigate whether the patients with severe male factor infertility will benefit by “Physiological ICSI”. Written informed consent was obtained from each subject once they agreed for the study. Randomization was done with sealed envelopes [Conventional ICSI (ICSI) and “Physiological ICSI” (PICS)] on the day of oocyte retrieval after examining the semen sample of the husband. The pregnancy rate among the two different groups did not vary. There was only one biochemical pregnancy which belonged to the ICSI group. So the clinical pregnancy rate was higher than in the PICS group, the difference of which is not statistically significant. Even though the cumulative pregnancy rate was 58 % in PICS group and 33 % in ICSI group, the difference was not statistically significant.

KEYWORDS : male factor infertility, physiological ICSI, conventional ICSI

Introduction

The technological advance of intracytoplasmic sperm injection (ICSI) enabled a single sperm to be injected into an oocyte. In this approach, the natural process of sperm–oocyte self-selection is superseded by a random choice of an embryologist and the selection is based on sperm morphology and motility. However, this selection procedure is not discriminatory with respect to the identification of spermatozoa with normal haploid chromosome, intact chromatin or DNA. In addition, in severe male infertility cases, sperm used for ICSI may have a detrimental effect and lead to production of potentially defective embryos, low pregnancy rate and high abortion rate. Therefore, an effective procedure for selection of normal sperm based on membrane maturity for ICSI is greatly needed.

In the natural human fertilization process, hyaluronic acid seems to play a pivotal role in physiological sperm selection. In the fertilization process human oocytes are naturally surrounded by HA, which is then involved in the mechanism of sperm selection. In fact, mature spermatozoa able to bind to and digest HA have the best chance of reaching the oocyte and fertilizing it.

However, there are conflicting data regarding subsequent improvement of fertilization rate and cleavage rate after ICSI using HA-bound spermatozoa. The present study was aimed to determine the role of HA for selection of mature and chromosomally competent spermatozoa to optimize ICSI outcome.

Objective

To investigate whether the patients with severe male factor infertility will benefit by “Physiological ICSI”.

Materials and Methods

Study design – Randomized control trial

Study period - 1 ½ years (April 2012 to August 2013)

Setup- Department of Reproductive Medicine Unit, Christian Medical College and Hospital, Vellore,

Project proposal was approved by Institutional review Board, Christian Medical College, Vellore.

Method of allocation concealment

With sealed opaque envelopes

Inclusion Criteria

1. Severe male factor infertility (concentration 1- 10 million/ ml , motility- \leq 30 %, morphology \leq 4 % normal sperms)
2. Female partner \leq 37 years
3. Female partner with more than three dominant follicles by ultrasonography on the day of hCG.

Exclusion Criteria:

1. Females with significant pelvic pathology like fibroid, moderate to severe endometriosis, hydrosalpinx.
2. Female partner with baseline follicle stimulating hormone (FSH) level $>$ 12 IU/l and/ or Anti mullerian Hormone (AMH) level $<$ 2.2ng/ ml
3. Surgically retrieved sperm.

Methods

Written informed consent was obtained from each subject once they agreed for the study. Randomization was done with sealed envelopes [Conventional ICSI (ICSI) and “Physiological ICSI” (PICS)] on the day of oocyte retrieval after examining the semen sample of the husband.

Outcome measures:

The primary outcome measure tabulated was the Fertilisation rate - was calculated by the ratio of fertilized oocytes to the total number of survived injected metaphase II (MII) oocytes multiplied by 100 in both groups.

Secondary outcome measures were

1. Embryo quality rate which is defined as the number of grade 1, grade 2 and grade 3 embryos in day 2 and day 3 out of the total number of embryos developed. Grading of embryos was as accepted by the Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology in Istanbul consensus workshop on embryo Assessment, 2011.

For Day-3 embryos

- Grade 1 – $<$ 10% Fragmentation, Stage-specific cell number, No multinucleation
 Grade 2 – 10–25% Fragmentation, Stage-specific cell number for majority of cells, No evidence of multinucleation
 Grade 3- Fragmentation $>$ 25%, Cell number not stage-specific,

Evidence of multinucleation

2. Clinical pregnancy rate as the number of clinical pregnancies expressed per 100 embryo transfer cycles

Results

Total number of patients (ICSI cycles) - 21
 PICSI group – 11, ICSI group- 10

Table 1. Patient characteristics between PICSI and ICSI groups

		PICSI	ICSI	p- value
Age Median (IQR)	Male	35 (32- 38)	38.5 (36- 46)	0.004
	Female	27 (24- 30)	33 (31.5-34.2)	0.006
Infertility (n)	Primary	11	9	0.96
	Secondary	0	1	
BMI Median (IQR)		23 (21- 25)	27 (21.5- 29.2)	0.2
Type of protocol (n)	Long	2	0	0.23
	Short	0	1	
	Antagonist	9	9	

Table 2: Fresh spermatozoa Motility and morphology among PICSI & ICSI groups

	Fresh Motility Median (IQR)	p value	Morphology Median (IQR)	P value
PICSI	25(21-36)	0.597	3(2-5)	0.715
ICSI	22.5(19.75-32.75)		3(3-4)	

Sperm hyaluronan binding median among was 45(29-50) PICSI & 54 (28.75-99.9) ICSI groups. Blastulation rate on D5 among PICSI was 50(0-100.00) & 0(0-30) in ICSI groups. Blastulation rate on D6 among PICSI was 36.36(0-50.00) & 0(0-20.00) in ICSI groups. Implantation Rate among PICSI was 18% (22) & 27.7% (22) in ICSI groups. Pregnancy rate was 4/9 (44%) among PICSI & ICSI groups. Clinical Pregnancy rate among PICSI was 4/9 (44%) & 3/9 (33%) in ICSI groups. Cumulative Pregnancy Rate among PICSI was 7/12 (58%) & 3/9 (33%) in ICSI groups. The median hyaluronan binding in PICSI and ICSI are 45 and 54 % respectively. The total number of MI oocytes obtained groups and the oocyte morphology between the two groups were comparable. The time taken for the procedure differs significantly with median of 35 and 17minutes in PICSI and ICSI groups respectively (p value- 0.015). The fertilization rate, embryo development rate, embryo quality rate among the study and control group were not different significantly. The Blastulation rate on day 5 and day 6 were higher for PICSI group was higher, but the difference is not statistically significant. There was no difference found between the implantation rate between the PICSI and ICSI groups. The pregnancy rate among the two different groups did not vary. There was only one biochemical pregnancy which belonged to the ICSI group. **So the clinical pregnancy rate was higher than in the PICSI group, the difference of which is not statistically significant.** Even though the cumulative pregnancy rate was 58 % in PICSI group and 33 % in ICSI group, the difference was not statistically significant. There was only one patient with pregnancy loss which belonged to the PICSI group.

Discussion

In the present study, the preliminary observation was made for an interim analysis with limitation over interpretation due to inadequate sample size that may have influenced base line characteristics like age of the male and female between PICSI and ICSI group to have difference significantly.

We observed a significant difference in ICSI procedure duration, which was almost double the time longer in the PICSI group compared to the study group, even though it does not influence the clinical outcome. It can be hypothesized that the PICSI duration might be longer when injecting a higher number of oocytes and this could be considered as a potential PICSI drawback. Parmegiani et al had a similar observation when he compared the two ready to use systems- PICSI dish and Sperm slow and reported that the mean ICSI

procedure duration was almost 3 minutes longer in the PICSI group than in the Sperm Slow group, when injecting a mean number of five oocytes. He hypothesized that PICSI duration was longer than with Sperm Slow because we used a two-step procedure: [1] collection of HA-sperm, and [2] subsequent sperm immobilization in Sperm Slow(31).

A multicentric study demonstrated that where the proportion of HB binding sperm is ≤ 65%, sperm selection by hyaluronan binding resulted in a statistically significant decrease in miscarriage rate and promising although not significant improvements in implantation rate and clinical pregnancy rate. The study also noted that improvements were more pronounced if subjects were stratified by the washed HB score rather than fresh HB score(32). Our study has not included a diagnostic HB scoring mainly due to the cost constraint as well as the fact that we have included only couples with severe male factor. But Hyaluronic acid binding was assessed during or after the ICSI procedure from the microdot and the proportion of sperm Hyaluronic acid binding in PICSI and ICSI groups were 45 % and 54% respectively which was not significantly different.

During this initial interim analysis, it was noted that it would take at least an additional 2 years to obtain the sample size and power originally planned. The insignificant difference is likely to be due to the low n

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