Original Article

Comparison of physiological-ICSI (PICSI) with ICSI in cases of moderate to severe oligoasthenoteratozoospermia (OAT) – a randomized control study

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Abstract

Objective: To compare the efficiency of physiological intracytoplasmic sperm injection (PICSI) over intracytoplasmic sperm injection (ICSI) in Oligoasthenoteratozoospermic patients. **Design:** Randomized control study. **Setting:** KJIVF and laparoscopy center Delhi. **Methods:** From September 2019 to May 2020, 45 patients of male factor felled under our criteria of OAT were divided into two groups of PICSI and ICSI. Sperm selection was performed under high magnification in both the groups and results were compared. **Outcome measure:** Primary outcome: Fertilization rate, cleavage rate, blastulation, and utility rate; Secondary outcome: Clinical pregnancy rates and miscarriage rates. **Results:** 20 PICSI and 25 ICSI treatments were performed and observed that the rate of blastulation and embryo utility shows significant difference in PICSI group ($P = 0.013$). The pregnancy percentage was also better in PICSI but implantation, fertilization, cleavage; clinical pregnancy rates were clinically comparable in both the groups but not statistically significant. **Conclusion:** The outcomes of our study are independent of male factor and it only depends on intervention whether it was PICSI or ICSI. The blastulation rate and embryo utility shows statistically significant difference between PICSI and ICSI groups. Hence, we are slowly progressing toward the superiority of PICSI over ICSI but enough evidences are still not available.

Keywords: Blastulation, fertilization, intracytoplasmic sperm injection, physiological intracytoplasmic sperm injection and morphology

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INTRODUCTION

The 10% to 15% of total population is affected by infertility and the male factor is solely responsible in 40%.[1,3]. Intracytoplasmic sperm injection (ICSI) was introduced to increase the pregnancy in severe male infertility.[2,5,13] ICSI children born is around 10 per 1000 with live birth rates of 24% and remained unchanged in decades.[14,16,22,23] Toxicity of PVP affects rate of fertilization and sperm head decondensation.[4,26,27] The oocyte is surrounded by HA; a high-molecular weight glycosaminoglycan and basis is a specific receptor that binds to HA is present on the head of mature sperms.[5,17,20] There is no known adverse impact on zygote development.[20] This study is conducted to determine the efficacy of physiological

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intracytoplasmic sperm injection (PICSI) over ICSI in OAT.

MATERIALS AND METHODS

The randomization of 25 ICSI cycle with 20 PICSI cycle was conducted with the help of 'Random number generation' App, assigning 25 & 20 subjects to each treatment group.

Design of study: Randomized control study

Place of study: KJIVF and Laparoscopy centre Delhi

Period of proposed study: 9 months from September 2019 to May 2020.

Sample size: All the subjects with male factor infertility who took consultation with us were the possible participants of our study. A total of 45 subjects during the 9 months of study fulfilled our criteria. All the 45 subjects consented and participated in the study. The participants were randomly divided into experimental [A group] and controlled group [B group].

Inclusion criteria: Patients with male factor infertility (moderate to severe OAT) were selected.

Count: The criteria for OAT sperm concentration 1×10^6/mL to 10×10^6/mL, motility ≥5% and <40%, and strict morphology < 4%.

Exclusion criteria: Patient with other compounding factor like decreases ovarian reserve, metabolic and endocrinal problem and testicular sperm.

Outcomes **of study ⇒ analyzed:** Primary outcomes: Fertilization rate, cleavage rate, blastulation, and utility rate

Secondary outcomes: clinical pregnancy rates and miscarriage rates.

The embryologist performing the ICSI must be experienced, to avoid any variability between different operators. All patients were aware of the procedure and have signed the written consent.

This study was approved by the ethical committee of IFS.

Ovarian stimulation, oocyte retrieval and selection

GNRH analogs in combination with a graded menotropin controlled ovarian stimulation in women were carried out. When the size of two or more follicles reached 18 mm, a dose of 10,000 IU of human chorionic gonadotropin (HCG) was given and transvaginal ultrasound-guided oocyte retrieval was preformed 36 hours after HCG trigger.

The oocytes were incubated for 1 to 2 hours. At 37°C in 6% CO_2 and 5% O_2, and 89% N_2 in Heracell incubator before complete removal of cumulus mass and corona cells in 80 IU hyaluronidase (Sage) by gentle mechanical aspiration with denuding pipettes.

Sperm preparation

The semen collection was done in sperm wash medium to avoid damage caused by the seminal fluid factors in cases of poor semen parameters to get good viable sperms,[7] before the process of denudation in a well-labelled sterile container.

After proper liquification of sample the spermatozoa were treated by density gradient (80% and 40% Sage density gradient and HEPES flushing medium) method according to World Health Organization (WHO) guidelines.[28]

Before each procedure an embryologist checks the randomization with the help of app "Random number Generation" to assign the patient for PICSI or ICSI group.

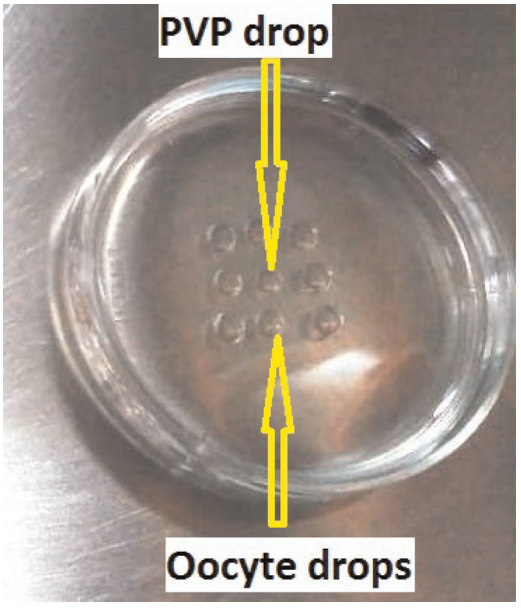

Figure 1: Preparation of intracytoplasmic sperm injection Dish: a 5 µl drop of polyvinylpyrrolidone for priming the injrction pipette. 5 µl HEPES medium drops for keeping the oocytes during ICSI. The number of HEPES drops depends on the number of oocytes to be injected. Everything is covered under oil

Conventional intracytoplasmic sperm injection

After denudation the eggs were checked for M1, polar body and M2 stage and were cultured in HEPES medium for 1 to 2 hours. The ICSI dish was prepared as shown in Figure 1. The ICSI procedure was carried out on heated stage of an inverted Olympus microscope equipped with Narishige micromanipulator (Narishige, Tokyo, Japan) under 200x to 400x magnifications. The sperms with normal morphology were selected and injected into the mature oocytes.

PICSI procedure

For PICSI conventional plastic culture dishes prepared with three microdots of powdered HA. By adding 5 mL droplet (in a long-tail shape) of fresh culture medium (flushing medium HEPES) to each of the three microdots the powered HA was rehydrated. Near each 5 mL culture medium droplet (tail-shaped) a 2 mL droplet with suspension of treated spermatozoa was placed and connected to the droplet using tip of the pipette [Figure 2]. The PICSI dish was incubated at 37°C under oil; within 5 minutes the mature sperms binds by their head to the surface of HA microdots and were spinning around their head. Spermatozoa spinning faster were preferred and with ICSI injecting pipette HA-bound sperm was picked up and injected one by one into an oocyte. Both PICSI and ICSI were performed at x400 magnification; the spermatozoa were selected for their morphology according to WHO2010 guidelines.

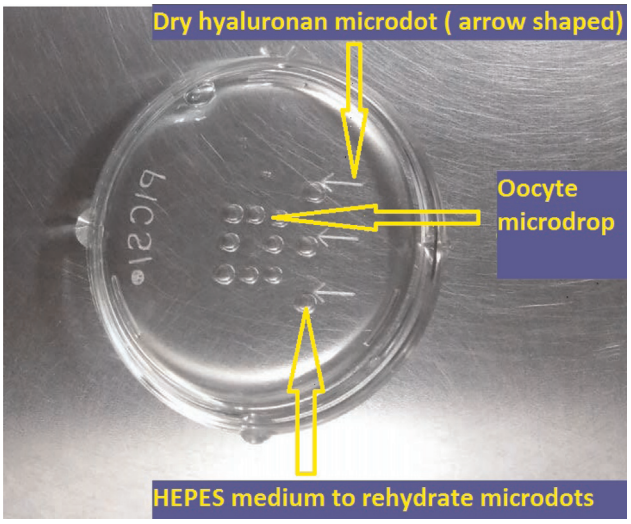

Figure 2: Preparation of physiological intracytoplasmic sperm injection dish: one 5 µl drop of polyvinylpyrrolidone for priming the injection pipette in the centre of oocytes micro drop. Three tail shaped 5 µl drops to rehydrate dry hyaluronan microdots; 5 µl HEPES drops for keeping the oocytes during ICSI. The number of HEPES drops depends on the number of oocytes to be injected. Everything is covered under oil

Embryo culture and transfer, main outcome measures

The injected oocytes were cultured in cleavage medium (Medi cult – origio) at 37°C in 6% CO_2, 5% O_2, and 89% N_2 in planer benchtop incubators (origio). The inverted microscope with grading 1 to 5, that is (best to worst) based on the criteria mentioned in "Gardner" was used for checking fertilization and cleavage of embryos. After 3 or 5 days since oocyte retrieval ET was carried out if good quality embryos were less than or equal to three. On Day 3 not more than three embryos per patient were transferred and on Day 5 two embryos were transferred and the remaining good quality embryos were vitrified.

β-HCG test was done after 17 days in Day 3 transfer and after 15 days in Day 5 transfer. The clinical pregnancy was decided by ultrasound examination a week after positive β-HCG. The primary and secondary outcomes were analyzed.

Statistical analysis

For statistical analysis, data after collection was entered in Microsoft excel and analysis was done in statistical software SPSS v 20.0 (IBM). Data thus analyzed was presented in the form of tables and diagrams. Categorical data was presented as frequency/percentage and compared using Chi square and Fisher exact tests while as continuous data was presented as mean ± SD and compared using independent T test. Risk was calculated as odds ratio with 95% confidence interval. For any observed difference *P* value of < 0.05 was considered as statistically significant.

RESULTS AND OBSERVATIONS

The result of study is analyzed between 20 PICSI and 25 ICSI treatments.

In general characteristics [Table 1 and Figure 3] of patients the mean age and standard deviation are almost similar between both the groups (30.10 ± 4.983) and is not statistically significant ($P = 0.584$). The experimental group has slightly more prevalence of addiction (smoking, alcohol, and tobacco) 20% than control group 12.0% but overall the P value is not significant (P=0.682). The moderate male factor prevalence is more in ICSI group 60.0% (45.0% in PICSI group) and severe male factor is lightly more in PICSI 55.0% (40.0% in ICSI), although *P* value is not significant ($P = 0.337$). Stimulation wise two groups are similar ($P = 0.105$).

Table 1: Comparison of effects of general characteristics in patients of both the groups. ('N' is the number of procedures).

		Procedure		Total	Odds ratio	*P* value
		PICSI*N* = 20	ICSI*N* = 25	*N* = 45		
Mean Age ± SD (Years)		30.10 ± 4.909	30.92 ± 4.983			0.584
Addiction (smoking, alcohol and tobacco)	Yes	4	3	7	1.833(0.359-9.353)	0.682
		20.0%	12.0%	15.6%		
	No	16	22	38		
		80.0%	88.0%	84.4%		
Diagnosis	Moderate male factor	9	15	24	0.545(0.166-1.793)	0.377
		45.0%	60.0%	53.3%		
	Severe male factor	11	10	21		
		55.0%	40.0%	46.7%		
Stimulation	Agonist	1	6	7	0.278(0.044-1.754)	0.105
		5.0%	24.0%	15.6%		
	Antagonist	19	18	37		
		95.0%	72.0%	82.2%		
	Egg donation	0	1	1		
		0.0%	4.0%	2.2%		

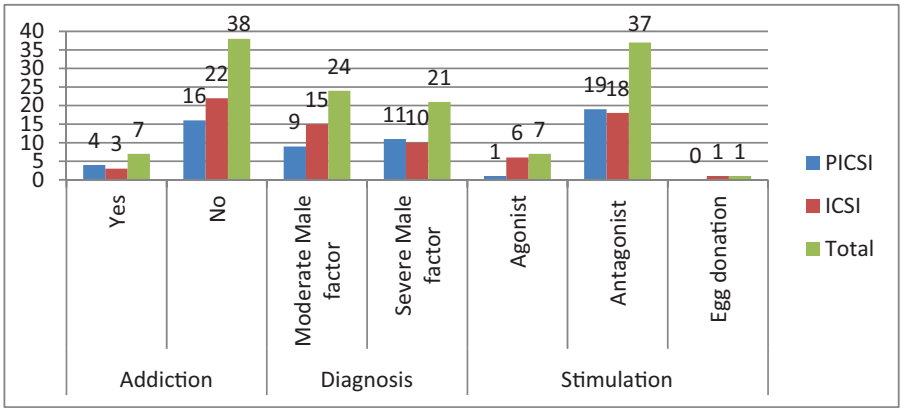

Figure 3: Comparison between general characteristics of PICSI and ICSI groups

Table 2: Comparison between numbers of variable in both the groups.

	Procedure	*N*	Mean	SD	*P* value
No. of follicle	PICSI	16	12.06	4.640	0.056
	ICSI	25	9.48	3.721	
Sperm count (million/ml)	PICSI	14	11.43	16.066	0.185
	ICSI	21	6.57	3.265	
Motility (%)	PICSI	10	16.40	16.480	0.511
	ICSI	16	19.56	7.642	
No. of Egg	PICSI	20	10.15	4.727	0.436
	ICSI	25	9.20	3.367	

The *P* value is not significant in any of the studied outcomes [Table 2]: no. of follicles ($P = 0.056$), sperm count ($P = 0.185$), motility ($P = 0.511$), no. of egg ($P = 0.436$).

Table 3 shows no statistical significance difference in any of the outcomes: no. of eggs injected ($P = 0.693$), fertilization ($P = 0.745$), cleavage ($P = 0.602$), and embryo transfer ($P = 0.625$). The mean blastocyst (PICSI = 3.50 and ICSI = 1.87) and mean embryo freezing rate (PICSI = 4.89 and ICSI = 2.35) is better in PICSI group and shows statistically significant difference ($P = 0.013$) between the two group.

Table 3: Depicts the primary and secondary outcomes of this study ('N' number of procedures including self and egg donation).

	Procedure	N	Mean	SD	*P* value
Injected	PICSI	19	8.68	4.110	0.693
	ICSI	25	8.24	3.308	
Fertilized	PICSI	20	6.20	3.238	0.745
	ICSI	25	6.52	3.268	
Cleaved	PICSI	20	5.90	3.110	0.602
	ICSI	25	6.40	3.227	
Blastocyst	PICSI	20	3.50	1.638	0.013
	ICSI	23	1.87	2.341	
Embryo transfer	PICSI	20	9.550	1.535	0.625
	ICSI	24	9.804	1.853	
Embryo freeze	PICSI	9	4.89	1.965	0.013
	ICSI	20	2.35	2.519	

The clinical pregnancy [Table 4; Figure 4] is also not statistically significant ($P = 1$) between both the groups but the percentage of pregnancy is better in PICSI group 40.0% with only 36.0% in ICSI group.

Outcomes based on moderate and severe male factor in ICSI and PICSI group [Tables 5–7 Figures 5 and 6] shows no significant difference between both the groups. The pregnancy outcome based on male factor in ICSI group [Table 6] is almost similar but it is slightly more in severe male factor (40.0%) than moderate male factor (33.3%) although it is statistically not significant. Similarly, the percentage of moderate male factor pregnancy outcome [Table 8] is more (44.4%) than severe male factor (36.4%) in PICSI group, but this is also not significant ($P = 1.000$).

DISCUSSION

In ICSI, the chances of aneuploidy and chromosome aberration is high as sperms are chosen based on their morphology and this increases the chances of fertilization of oocyte with damaged DNA which may lead to pregnancy loss.[11,19] The common risks associated with ICSI are damage to embryos, multiple pregnancy, and complications during pregnancy and child birth; whereas defects in ICSI occur in far less than 1% of ICSI babies.[2,24]

Previously, Strehler et al. (1998) in a study of effects of PVP on spermatozoa found that the acrosome, plasma membrane and mitochondria are the most affected components of sperm. It is also observed that after the process egg penetration PVP hinder nuclear sperm

Table 4: Result of clinical pregnancy in both groups.

		Procedure		Total	Odds ratio	*P* value
		PICSI *N* = 20	ICSI *N* = 25	*N* = 45		
Pregnancy	Yes	8	9	17	1.185(0.353-3.980)	1
		40.0%	36.0%	37.8%		
	No	12	16	28		
		60.0%	64.0%	62.2%		

Table 5: Outcome based on male factor in ICSI

	Diagnosis	*N*	Mean	SD	*P* value
Injected	Moderate male factor	15	7.27	3.173	0.070
	Severe male factor	10	9.70	3.093	
Fertilized	Moderate male factor	15	5.80	3.468	0.183
	Severe male factor	10	7.60	2.757	
Cleaved	Moderate male factor	15	5.80	3.468	0.264
	Severe male factor	10	7.30	2.751	
Blastocyst	Moderate male factor	13	1.31	1.843	0.196
	Severe male factor	10	2.60	2.797	
Embryo transfer	Moderate male factor	15	10.020	1.9065	0.474
	Severe male factor	9	9.444	1.8105	
Embryo freeze	Moderate male factor	11	1.91	2.119	0.401
	Severe male factor	9	2.89	2.977	

Table 6: Pregnancy outcome based on male factor in ICSI

		Diagnosis		Total	Odds ratio	*P* value
		Moderate male factor	Severe male factor			
Pregnancy	Yes	5	4	9	0.750	1
		33.3%	40.0%	36.0%	(0.143-3.941)	
	No	10	6	16		
		66.7%	60.0%	64.0%		
Total		15	10	25		
		100.0%	100.0%	100.0%		

Table 7: Outcome based on male factor in PICSI

	Diagnosis	*N*	Mean	SD	*P* value
Injected	Moderate male factor	9	9.67	4.637	0.337
	Severe male factor	10	7.80	3.584	
Fertilized	Moderate male factor	9	7.22	2.949	0.210
	Severe male factor	11	5.36	3.355	
Cleaved	Moderate male factor	9	7.00	3.041	0.158
	Severe male factor	11	5.00	3.000	
Blastocyst	Moderate male factor	9	4.00	1.803	0.226
	Severe male factor	11	3.09	1.446	
Embryo transferred	Moderate male factor	9	10.111	1.9650	0.135
	Severe male factor	11	9.091	.8312	
Embryo freeze	Moderate male factor	6	5.17	1.602	0.584
	Severe male factor	3	4.33	2.887	

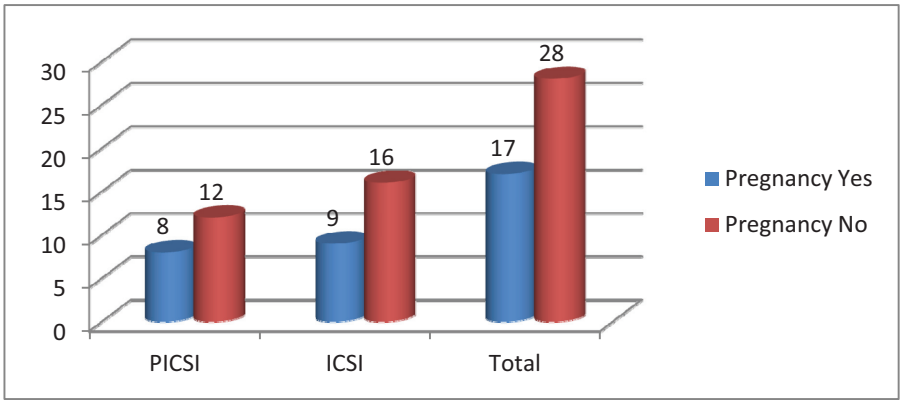

Figure 4: Comparison of pregnancy outcomes between both the groups

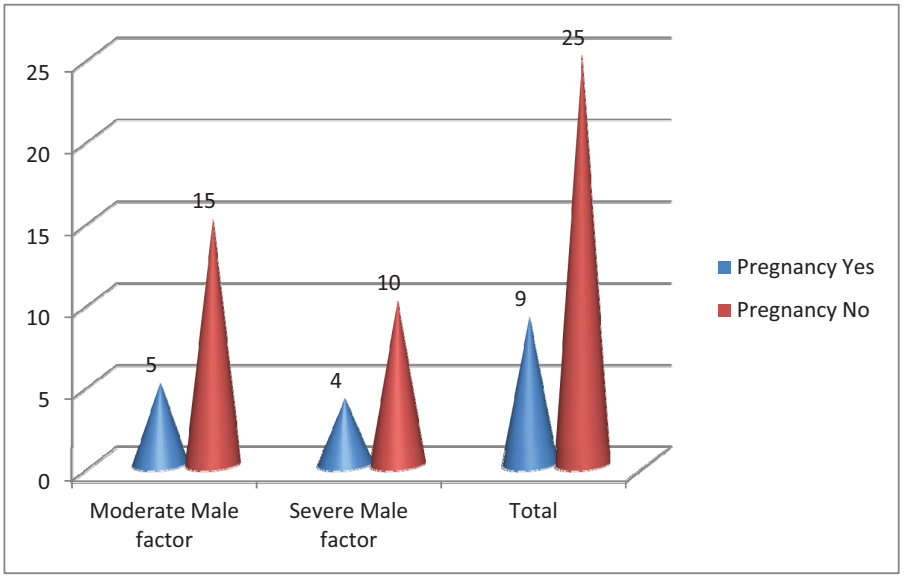

Figure 5: Comparison of pregnancy with respect to severe and moderate male factors in control group

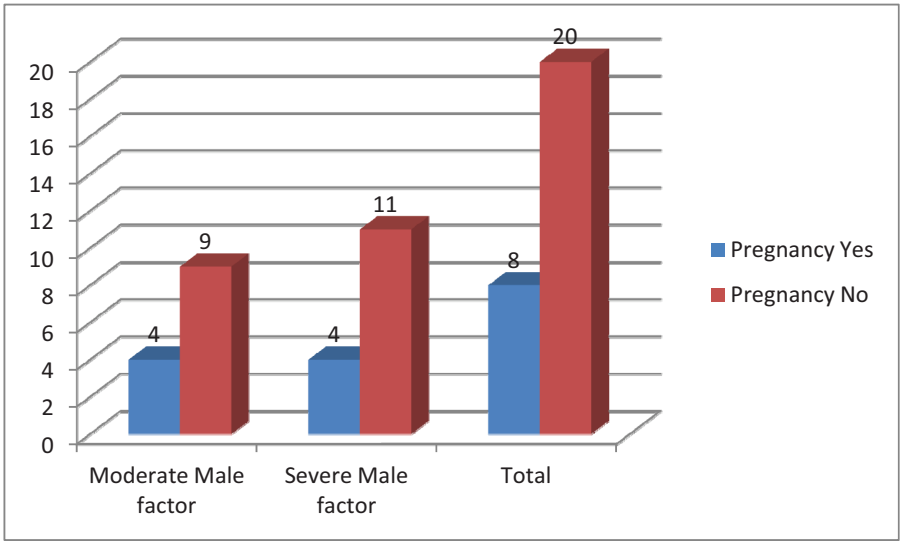

Figure 6: Comparison of pregnancy with respect to severe and moderate male factors

Table 8: Pregnancy outcome based on male factor in PICSI

		Diagnosis		Total	Odds ratio	*P* value
		Moderate male factor	Severe male factor			
Pregnancy	**Yes**	4	4	8	1.400(0.232–8.464)	1
		44.4%	36.4%	40.0%		
	No	5	7	12		
		55.6%	63.6%	60.0%		
Total		9	11	20		
		100.0%	100.0%	100.0%		

decondensation. In this study the nucleus is most damaged component both in context to shape and structure of chromatin.[25] Many studies have shown correlation between higher DNA fragmentation rates in teratozoospermic men than normozoospermic men. The head defects like tapered head, increased head length with slightly change in width, decreased acrosome coverage, all result in chromosomal aneuploidy and poor chromatin packaging.[6,24] The shape of sperm does not define absence or presence of chromosomal abnormalities; thus it is invalid criteria for selection of mature sperm devoid of chromosomal degradation.[12] The HA-bound spermatozoa

shows reduced chromosomal aneuploidies and DNA fragmentation and results in good embryo quality.[26] The use of HA do not cause any toxicity to the sperm and to the oocyte, as it is a part of female reproductive tract and cumulus complex. HA is carried into oocyte during in-vivo-conception by spermatozoa therefore HA-sperm selection is safer than ICSI selection.[9,21]

The spermatozoal hyaluronidase is primarily located at the regions of plasma and inner acrosomal membranes; however PH-20: a bifunctional protein present on the sperm plasma membrane has a hyaluronidase activity and role in secondary sperm-zona binding is produced during the exocytosis of acrosome.[8,15] The hyaluronidase activity is depicted by the N-terminal domain of PH-20, which enables the penetration of cumulus cell surrounding the oocyte.[18,29]

Some studies have shown HA-sperm selection improves blastocyst rate by PICSI fertilization,[4] pregnancy rate,[10,30] decreased fragmentation rate in embryos,[30] miscarriage rate[30] when compared with conventional ICSI. Some authors also found no statistical differences in fertilization, pregnancy, implantation rates, and in blastocyst rates.[5,16]

In our study the general characteristics [Table 1 and Figure 3] of patients like age, addiction, diagnosis of moderate and severe male factor and stimulation, that is, agonist and antagonist between two groups PICSI and ICSI is compared and analyzed and found no age difference between two groups, that is, the mean age and standard deviation are almost similar in both the groups (30.10 ± 4.983) and is not statistically significant ($P = 0.584$). So there is no effect of age in the outcomes of result between two groups and age is not a concern may be due to small sample size. The PICSI group has slightly more prevalence of addiction (20%) than ICSI group (12.0%) but the *P* value is not significant ($P = 0.682$); so the two groups are also similar in addiction wise. The moderate male factor prevalence is more in ICSI group 60.0% (45.0% in PICSI group) but severe male factor is lightly more in PICSI 55.0% (40.0% in ICSI) although *P* value is not significant ($P = 0.337$) and stimulation wise also two groups are similar ($P = 0.105$). Thus, overall there is no effect of age, addiction, diagnosis, and stimulation protocol on the outcome of our study.

In Table 2, the number of variables which are almost similar in both the groups and the *P* value is not significant in any of the outcomes, that is, No. of follicle ($P = 0.056$), sperm count (million/mL) ($P = 0.185$), motility (%; $P = 0.511$), no. of egg ($P = 0.436$) between the two groups. Thus, two groups are comparable to each other and this is in favor of our study.

When primary and secondary outcomes are compared [Table 3] found that there is no statistical significance difference in any of the outcomes: no. of eggs injected ($P = 0.693$), fertilization ($P = 0.745$), cleavage ($P = 0.602$), embryo transferred ($P = 0.625$); but the mean blastocyst (PICSI = 3.50 and ICSI = 1.87) and mean embryo freezing rate (PICSI = 4.89 and ICSI = 2.35) are better in PICSI group with statistically significant difference ($P = 0.013$).

The result of clinical pregnancy [Table 4 and Figure 4] also shows no statistically significant difference ($P = 1.000$) between two groups but the pregnancy percentage is better in PICSI group 40.0% with only 36.0% in ICSI group. The odd ratio 1.185 shows that pregnancy rate 1.185 times more in PICSI compared to ICSI group

The outcomes based on moderate and severe male factor in ICSI and PICSI groups [Tables 5–7] shows no significant difference between two groups; this means outcomes hardly depends on whether the male factor is moderate or severe and thus the outcome in independent of male factor and is only depends on intervention whether it was PICSI or ICSI.

The pregnancy outcome based on male factor in ICSI group [Table 6 and Figure 5] is almost similar but it is slightly more in severe male factor (40.0%) than moderate male factor (33.3%); although it is statistically not significant between two groups of male factor.

Similarly, the percentage of moderate male factor pregnancy outcome [Table 8 and Figure 6] is more (44.4%) than severe male factor (36.4%) in PICSI group, but it is also not significant ($P = 1$) between the two groups of male factor.

CONCLUSION

The conclusion of our study is that PICSI gives good outcomes in blastulation rate and embryo utility (embryo freeze) are statistically significant in PICSI versus ICSI. Pregnancy percentage was better in PICSI group; although it was not statistically significant. The outcomes of this study are independent of male factor and only depend on intervention whether it was PICSI or ICSI.

Hence, we are slowly progressing toward the superiority of PICSI over ICSI but enough evidences are still not available.

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Conflicts of interest

There are no conflicts of interest.

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