



ORIGINAL

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HYPO-OSMATIC SWELLING TEST

DR. SAQIB SIDDIQ

Associate Professor of Obs. Gynae.
Postgraduate Medical Institute, Lahore.

Dr. Rashid Latif Khan.

14 Abubakar Block New Garden Town
Lahore

DR. MOHAMMAD TAYYAB

DR. MAHRUKH KHAN

ABSTRACT

Hypo-Osmotic Swelling (HOS) test is considered as a reliable method to find out live sperms. **OBJECTIVE:** To evaluate the effect of HOS test for selection of alive sperms. **DESIGN:** Prospective randomized study. **PERIOD:** June 2000-March 2002. **SETTING:** Infertility Advisory Centre Lahore. **METHODS AND RESULTS:** Twenty four ICSI cycles were included in the study. In all the cases the sperms were non motile they were divided in two groups. In group A (12 cases) sperms were used which were tested by HOS and labeled as alive sperms. In group B (12 cases) in which sperms were randomly selected (without HOS Test) and used in ICSI Fertilization, embryo quality and clinical pregnancy rate were compared in two groups. **CONCLUSIONS:** HOS test is an effective and safe method to be used for selecting alive sperms in cases of necrostermia when they are used in Intracytoplasmic sperm injection (ICSI) to achieve a pregnancy.

KEY WORDS: Non motile sperms, HOS Test, ICSI

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) is a major break through in the field of male infertility, first successful experiment was done by Plaemmro et al¹. Sperms are collected either from epididymis or from testes in cases of Azoospermia. In these circumstances, the sperms are very few in number and sluggish so ICSI is more suitable option for them². The source of the immotile sperms affects the outcome of the treatment³⁻⁶.

Lack of sperm motility is one of the factors contributing to male infertility and affects the outcome of ICSI⁷. If immotile sperms are seen during ICSI procedure the scientist would like to pick up the sperm for injection which is more likely to result in positive fertilization & implantation. The HOS Test can

differentiate between alive & dead sperms⁸. The correlation between HOS Test and its outcome in terms of fertilization and implantation is variable in different reports that means some reports have shown no correlation whereas others showed good correlation⁹⁻¹².

The aim of this study was to evaluate the HOS Test selected sperms versus non selected sperms when used during ICSI in cases of complete lack of sperm motility. Whether the sperms are obtained from ejaculate or surgically retrieved.

MATERIAL & METHODS

A total of twenty four cycles between June 2000 to March 2002 were included in the study. The study was carried out at The Infertility Advisory Services,

Lahore, which is the pioneer and centre of excellence for ART.

Cases showing non motility of sperms were included. They were randomly divided in two groups. In group A the live sperms were selected by HOS test. In which HOS medium by the name of HYPO™ was used for Hypo-Osmatic sperm swelling test, manufactured by VITROLIFE made in Gutenberg.

Sperms are placed in HOS medium & under ICSI microscope (IX70 Olympus Japan) sperms are observed for curling of the tail, these changes indicate that the sperm is alive.

Then these sperm are washed in polyvinyl-prolidone and these sperms are called HOS test positive then ICSI was performed as described by Palemo¹. In Group B the sperms are not selected by HOS test and these non selected sperms are injected into the oocytes.

Ejaculated Sperms

Semen was allowed to liquify for 30 minutes. Sperm count & motility was assessed. Separation or washing was performed (medium PURE SPERM VITROLIFE Gutenberg (40% and 80%) to find out whether motile sperm was present, in the concentrated liquot.

Surgically Retrieved Sperms

Sperms are obtained either from epididymis or testes in cases of Azoospermia as described by Jaroudi¹³. Epididymal sample is obtained by using a 24 gauge butterfly needle. If no sperm was obtained then testicular biopsy was performed under local anaesthesia using 1% xylocaine in the form of infiltration. (5mL).

The biopsy tissue was vortexed for 5 minutes. Then samples were spun immediately at 1800G for 5 minutes. The supernatant was removed and the pallet was resuspended in 50 to 100 ML of media. Sperms were checked under light microscope then a sperm search for ICSI was performed. If the specimen contains blood, a red blood cell lysing buffer was used as described by Verheyen et al¹⁴.

Ovarian Stimulation

Down regulation was carried out by long protocol using subcutaneous injection of decapeptyl 0.1mg daily from 21st day of the cycle and FSH (Puregon, Organon, Holland) was added after two weeks and follicular tracking was done by transvaginal ultrasound. The HCG 10000 i.u (Pregnyl Organon) was injected when at least 3 follicles reaches ≥ 18 mm.

Ovum Pick Up

OPU was carried out by transvaginal ultrasound guidance after 35 hours of HCG injection. The follicular aspirate was poured into 60mm Falcon dish and cumulus oocyte complexes were transferred into another dish with ASP medium of Vitrolife. Denudation of oocytes were carried out after 4 hours and ICSI was performed.

Fertilization was confirmed by the presence of two pronuclei. The number of blastomeres and their evenness were recorded at 42 & 66 hours after ICSI. Embryos were graded as good, fair or poor with the use of criteria described already¹⁵.

RESULTS

Twelve cases were placed in group A among 24 cycles, in which selected sperms by HOS test were used (alive sperms) & Twelve cases in group B in which non selected sperms were used. In Group A, (Table 1) out of 12 cases four patients became clinically pregnant (33%).

In four cases (33%) sperms were obtained surgically where as in remaining 67% ejaculated sperms were used. All four patients who became pregnant were young. Their ages were ≤ 35 years. In all these cases collective fertilization rate was 75%.

In Group B, Table-II where non selected sperms were used in ICSI, three (25%) clinical pregnancies were achieved. All these pregnant women were young i.e age ≤ 35 years. In these twelve cases collective fertilization rate was 69%.

Table-I. Group A. Result of individual patients in HOS Test selected sperms group.

Age of Wife	Sperm	No of Eggs	Fertilization	ET	Pregnancy
36	Ejaculate	8	5	3	–
35	Ejaculate	6	3	3	
28	Testicular	11	9	3	–
30	Testicular	9	7	3	–
38	Ejaculate	4	2	2	
25	Ejaculate	13	11	3	
33	Testicular	9	6	3	–
31	Ejaculate	9	7	3	–
26	Testicular	14	10	3	–
36	Ejaculate	7	2	2	–
27	Ejaculate	10	8	3	
32	Ejaculate	8	6	3	

Table-II. Group B. Result of individual patients in Non selected sperms group (No HOS Test).

Age of Wife	Sperm	No of Eggs	Fertilization	ET	Pregnancy
28	Ejaculate	12	8	3	
36	Ejaculate	7	5	3	–
32	Testicular	9	7	3	–
30	Testicular	8	5	3	
27	Ejaculate	11	8	3	
35	Ejaculate	5	2	2	
35	Ejaculate	6	4	3	
33	Testicular	9	7	3	
34	Ejaculate	6	4	3	
27	Ejaculate	10	7	3	
28	Ejaculate	11	9	3	
37	Ejaculate	5	2	2	

Table-III. Effect of the source of sperms in the outcome of selected & Non selected sperms by HOS Test.

Testicular		Ejaculated	
HOS Test	No of HOS Test	HOS Test	No HOS Test

Table-III. Effect of the source of sperms in the outcome of selected & Non selected sperms by HOS Test.

	Testicular		Ejaculated	
	No of cases	4	3	8
Fertilization	74%	73%	68%	60%
Pregnancies	3	0	1	3

DISCUSSION

Motile sperms finding during ICSI is not always possible in a small group of patients. So it is important to find live sperms. The viability of nonmotile spermatozoa can be detected by a dye exclusion test using eosin Y¹⁶. This method has been very reliable for diagnostic purposes, but the toxicity of this substance in clinical use is unknown. HOST was developed to evaluate the vitality of sperm without any toxicity concerns, because physiologic substances are used. It has been used clinically in selecting viable nonmotile sperm for ICSI. The injection of such sperm has resulted in fair fertilization, good cleavage rates, and some pregnancies⁵.

The injection of HOS test positive spermatozoa in our study resulted in 35% pregnancy rate. Although these rates were not statistically significant in the Non-HOS test group (probably due to the small number of participants). The pregnancy rates do tend to be significantly different when increased number of patients are included.

There was a overall tendency for higher pregnancy rates in the HOS positive group. This may be due to the reason that selected sperms have a higher chance of picking viable or normal spermatozoa for injection. Zeyneloglu et al¹⁷ reported that the HOS test positive nonmotile spermatozoa have similar aneuploidy rate compared to the motile ones. On the other hand in non selected sperms there is chance of injecting nonviable or aged spermatozoa. Any patients coming for an ICSI cycle with immotile spermatozoa can be offered HOS test as a first line of treatment because so many ongoing pregnancies have been reported in the literature.⁵.

CONCLUSIONS

It is safe to use a hypo-osmotic solution to select viable nonmotile sperm from all kinds of sperm

retrievals to use in ICSI pregnancies. HOS test groups tend to have higher rates of penance and implantation, although the number is not statistically significant. Especially ejaculated and epididymal spermatozoa need assessment of the effectiveness of HOST positive sperm over random injection of immotile sperm. There is a need to do a multicenter trial or a metaanalysis of published reports from different centers once a sufficient number of studies are available.

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**A lady is women who makes a man
behave like a gentleman.**

Reussell Lynes