ORIGINAL ARTICLE

COMPARATIVE STUDY OF INTRACYTOPLASMIC SPERM INJECTION OUTCOME FROM SPERMS RETRIEVED BY DIFFERENT PROCEDURES

Shazia Ali, Nasim Ashraf*, Anjum Ara Siddique*, Samina Jalali, Sajjad Shami****

Islamic International Medical College Rawalpindi, *Islamabad Clinic Serving Infertile Couples (ICSI), Islamabad, **Quaid-e-Azam University Islamabad, Pakistan

Background: Different sperm retrieval procedures can help retrieve sperms that can be used for Intracytoplasmic Sperm Injection (ICSI) in azoospermic men. The objective was to compare the Intracytoplasmic sperm injection outcome, through different sperm retrieval procedures in azoospermic men. **Methods:** A Retrospective Study was carried out at Islamabad Clinic Serving Infertile Couples from September 1999 to March 2005. The study includes 105 female subjects and 105 male azoospermic subjects. In 105 male subjects 50 subjects had Non-Obstructive Azoospermia (NOA) and 55 subjects had Obstructive azoospermia (OA). These subjects underwent surgical sperm retrieval procedures like Percutaneous Epididymal Sperm Aspiration (PESA) or Fine Needle Aspiration Biopsy. Intracytoplasmic sperm injection (ICSI) was performed from both and outcome was compared. All the values were expressed as Mean±SE. Limit of significance was set at (p<0.05). Mean values were compared using unpaired Students t-test. **Results:** Subjects who underwent ICSI procedure with motile sperms retrieved through Biopsy had significantly (p<0.05) raised cleavage rate and live birth rate. **Conclusion:** No matter what ever the procedure of sperm retrieval used, the sperm motility has great significance. Significantly raised live birth rate was detected in subjects where sperms retrieved through biopsy were used for ICSI procedure.

Keywords: Intracytoplasmic Sperm Injection (ICSI), Percutaneous epididymal sperm aspirate (PESA), testicular biopsy, azoospermia, infertility

INTRODUCTION

A male factor is solely responsible in about 20 percent of infertile couples and is contributory in another 30–40%.[1] Azoospermia is defined as complete absence of sperms from the ejaculate. It is present in about one percent of all men and 10–15% of infertile men.[2] It can be of obstructive or non-obstructive origin.

Common causes of obstructive azoospermia (OA) include previous vasectomy, congenital absence of vas deferens and post infective epididymitis and rare causes such as Young's syndrome, testicular trauma and retrograde ejaculation. In this group of patients sperm retrieval is successful in almost 100% of cases.

The second group of azoospermic men are those with non-obstructive azoospermia (NOA), characterized by impaired spermatogenesis, ranging from varying degrees of maturation arrest to Sertoli cell-only syndrome. Clinically, they often have testes of decreased volume and raised Follicular Stimulating Hormone (FSH) levels. The common causes of (NOA) include Klinefelters syndrome, iatrogenic (e.g. radiotherapy), torsion, mumps, orchitis and Cryptorchidism. Sperm retrieval in this group is effective in approximately 50% of cases.[3–5]

A limited evaluation of both partners is important before reaching a final decision on the management of the couple with infertility.[6] An evaluation should be done before one year if: i) Male infertility risk factors such as history of bilateral cryptorchidism are known to be present, ii) Female infertility risk factors including advanced female age (over 35-years) are suspected, iii) If the couple questions the male partners fertility potential. The initial screening evaluation of the male partner of an infertile couple should include a reproductive history and two semen analyses. If possible the two semen analyses should be separated by a time period of one month. If a man has history of previous fertility this does not exclude the possibility that he can not acquire a new secondary male infertility factor. Men with secondary infertility should be evaluated in the same way as men who have never initiated a pregnancy.[7]

Semen Analysis is the cornerstone of the laboratory evaluation of the infertile male and it helps to define the severity of the male factor causing infertility. Standard instructions have been published by the World Health Organization (WHO). Common methods of sperm retrieval are Microsurgical Epididymal Sperm Aspiration (MESA), Percutaneous Epididymal Sperm Aspiration (PESA), Testicular Sperm Extraction (TESE) and Fine Needle Aspiration Biopsy. It has recently been demonstrated that 50% of the total group of (NOA) men will have a minute amount of ongoing spermatogenesis within their testicular parenchyma which can be used for Intracytoplasmic sperm injection (ICSI) procedure. The evident ability of ICSI to achieve high fertilization and pregnancy rates has extended its application to azoospermic patients.[8]

PATIENTS AND METHODS

The study includes 105 female subjects and 105 male azoospermic patients, 50 with Non-Obstructive Azoospermia (NOA) and 55 with Obstructive Azoospermia (OA). Duration of study was from September 1999 to March 2005. Detailed history was taken and couples were explained the basic physiology and pathology of infertility. Semen analysis of the male subjects was performed according to WHO recommendations. Diagnosis of azoospermia was made when no spermatozoa were detected in the seminal fluid.[9,10] Hormonal profile of the female was performed on the third day of the menstrual cycle.

After obtaining informed consent patient was anaesthetised with Propofol 1% injection, 1.5–2.5 mg/Kg body weight. Right epididymis was repeatedly penetrated with needle attached to a disposable syringe containing 2 ml tissue culture medium. The process was then repeated on the left epididymis. Specimen was examined under the microscope for presence of motile and non-motile sperms. When no motile sperm were found in PESA in specimen from both left and right side then Testicular Biopsy was done. Aspirate from both testes were taken by using 18g needle attached to 20 ml syringe and was examined under the microscope for the presence of motile and non-motile sperms.

All female subjects were desensitised by giving subcutaneous (s/c) Gonadotropin Releasing Hormone Agonist (depot preparation Decapeptyl 3.75 mg) from the mid-luteal phase of proceeding spontaneous menstrual cycle or after cyclic replacement of oestrogen and progestins in amenorrhic female subjects for 21 days. Desensitisation was considered complete, when after 4 weeks of treatment there was absence of greater than 10 mm follicles in both ovaries on vaginal ultrasound. Follicular stimulation of the female subjects was carried out by using subcutaneous administration of recombinant FSH 50-IU preparation the dose of which was calculated by keeping the age and basal serum FSH level of the female subject under consideration. On alternate days the ovarian follicular response was monitored by transvaginal ultrasound and dose of medication was adjusted accordingly. Human Chorionic Gonadotrophin, 10,000 IU was given intramuscularly when the leading follicles on ultrasound were >20 mm in size. Oocyte retrieval was done 35.5 hours after Human Chorionic Gonadotropin injection using the vaginal ultrasound technique under general anaesthesia. Mature ova were identified by presence of corona radiata and thick cumulus mass around them. All collected eggs were transferred to the incubator at 37 ºC, 5% CO_2 for about 1–2 hours prior to microinjection by ICSI. Presence of two pronuclei and polar bodies under the microscope, 18 hours after microinjection of eggs confirmed fertilisation. Cleavage of embryos was confirmed after another 24 hours of *in vitro* culture. All embryos were graded before embryo replacement. Embryo transfer was carried out at 2–4 cell stage on Day-2, Day-3, or at blastocyst stage on Day-5 of egg collection under ultrasound guidance. Progesterone vaginal pessaries were given from the day of egg retrieval till the day of pregnancy test, i.e., 2 weeks after embryo transfer. This luteal support was continued for another 12 weeks if pregnancy was achieved.

All values were expressed as Mean±SE. Limit of significance was set at $p<0.05$. Mean values were compared by using unpaired Student's *t*-test. The whole statistical analysis was done using the statistical Graph Pad Prism software, verizon 4.03 (Graph Pad Prism Inc. USA).

RESULTS

In a total of 105 male subjects, 55 underwent PESA, in which 47 (85%) had motile sperms and 8 (14.5%) had non-motile sperms. Rest of the 50 male subjects underwent testicular aspiration biopsy, in which 39 (78%) had motile sperms and 11 (22%) had non-motile sperms. Female subjects who underwent ICSI-cycle with motile or non-motile sperms retrieved through PESA or Biopsy had serum FSH levels of 5–7 mIU/ml (Table-1).

Table-1: Clinical characteristics and Day-3 FSH levels of females undergoing ICSI cycles

Characteristics	PESA		BIOPSY	
	Motile sperm	Immotile sperm	Motile sperm	Immotile sperm
Age of female at presentation (yrs)	30.55±0.82	31.25±2.10	31.97±0.76	26.73±1.84
Day-3 (FSH) levels of female mIU/ml	6.362±0.30	5.225±0.37	6.774±0.33	5.927±0.70
No. of ampoules of Gonoadotropin	6.645±0.32	6.625±0.67	7.154±0.36	6.455±0.705

All values are expressed as Mean±SE

Statistically, significant ($p<0.05$) increased number of oocyte were retrieved from female subjects undergoing ICSI-cycles where motile sperms were retrieved through PESA (Table-2).

Table-2: Ovarian response of the Female Subjects Undergoing ICSI-Cycles

Characteristics	PESA		BIOPSY	
	Motile sperm	Immotile sperm	Motile sperm	Immotile sperm
No. of oocyte retrieved	16.94±1.35 a*	8.875±1.63	14.46±1.12	13.91±2.19
Oocyte maturity Rate%	81.03	88.7	78.7	93.4 c*
No. of metaphase II	13.72±1.05 b*	7.875±1.60	11.38±0.93	13.0±2.16

All values are expressed as Mean± SE, *significant ($p<0.05$)
a) motile sperm from Pesa vs immotile sperm from Pesa
b) motile sperm from Pesa vs immotile sperm from Pesa
c) motile sperm from biopsy vs immotile sperm from biopsy

Similarly, number of 2-Pronuclei and fertilization rate were significantly raised (p<0.05) in ICSI–cycles where motile sperms were retrieved through PESA. But, significantly raised (p<0.05) cleavage rate were observed in ICSI-cycles where motile sperms were retrieved through biopsy (Table-3).

Table-3: Pronuclear development, fertilization and cleavage rate in subjects of PESA and biopsy in ICSI cycles

Characteristics	PESA		BIOPSY	
	Motile sperm	Immotile sperm	Motile sperm	Immotile sperm
No. of 2-Pronuclei	9.255±0.81d*	5.625±1.36	6.462±0.59	6.727±1.15
Fertilization rate %	67.4 e*	51.4	56.7	51.4
No. of cleaved embryos	7.191±0.68	4 .125±1.27	5.59±0.52	5.273±0.858
Cleavage rate%	77.7	73.3	86.5 fg*	78. 3

All values are expressed as Mean±SE, *Significant (p<0.05)
d) motile sperm from Pesa vs motile sperm from biopsy,
e) motile sperm from Pesa vs immotile sperm from Pesa
f) motile sperm from Pesa vs motile sperm from biopsy
g) motile sperm from biopsy vs immotile sperm from biopsy

Pregnancy rate were raised in couples undergoing ICSI cycles with motile sperms retrieved through biopsy. Also live birth rate were significantly raised (p<0.05) in ICSI–cycles where motile sperms retrieved through biopsy were used (Table-4).

Table-4: Pregnancy, abortion and live birth rate in PESA and biopsy subjects undergoing ICSI-cycles

Characteristics	PESA		BIOPSY	
	Motile sperm	Immotile sperm	Motile sperm	Immotile sperm
No. of embryo transferred	56.00±3.54	7.0±7.0	44.50±23.79	13.50±5.72
Percent of Total pregnancies	36.1	12.5	48.7	-
Pregnancy rate per cycle	36.1	12.5	48.7	-
Abortion rate	64. 7	-	52.6	-
Live birth rate per embryo transfer	3.57	3.75	7.861 h*	-

All values are expressed as Mean±SE, *Significant (p<0.05).
h) motile sperm from biopsy vs motile sperm from Pesa.

DISCUSSION

In this retrospective study, ICSI cycles were preformed using epididymal and testicular retrieved sperms. The incentive was to develop the best infertility management strategy for azoospermic patients. Motility of the sperms has proven to play an important role in ICSI outcome. In the present study it was observed that in most testicular biopsies spermatozoa were present in which a few displayed sluggish, twitching type of motility, which were further used for ICSI. Similar to other studies.[11]

In the present study motile as well as immotile spermatozoa were used for microinjection. Once the mature oocyte were microinjected, fertilization was observed by the presence of 2-pronuclear development (2-PN). Following results were significant: i) The number of 2- PN retrieved in the subjects where ICSI was preformed with motile sperms retrieved through PESA were significant raised (p<0.05), than those where motile sperms were retrieved through biopsy. These results are similar to other studies.[12,13] ii) Fertilization rate was significantly raised (p<0.05), in ICSI cycle where motile sperms from PESA were taken as compare to non-motile sperms from PESA. These results are similar to study carried out by Mansour[13] who also compared the outcome of ICSI with sperms retrieved through PESA and Biopsy[13] iii) Cleavage rate were significantly raised (p<0.05),in ICSI cycles where motile sperms retrieved from biopsy were used.

Pregnancy rate were higher in ICSI cycles where motile sperms retrieved through biopsy were used as compare to when motile sperms retrieved from PESA were used. Live birth rate was significant raised (p<0.05), in subjects undergoing ICSI with motile sperms retrieved through biopsy as compare to motile sperm retrieved through PESA. Abortion rate was raised in subjects undergoing ICSI with motile sperms retrieved through PESA as compared to motile sperms retrieved through biopsy, similar to other studies.[14]

CONCLUSION

No matter what site of sperm retrieval is used (epididymis or testes), the sperm motility has great significance as pregnancy and live birth rates are significantly raised in subjects where motile sperms retrieved through biopsy where used for ICSI.

ACKNOWLEDGEMENT

The authors thank Mr. Najam Khan and Maj. Gen Masood Anwar for their guidance for completing this research paper.

REFERENCES

1. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Landsec J, *et al*. Incidence and main cause of infertility in a resident population (1,850,000) of three French regions (1988–1989). Hum Reprod 1991;6:811–6.
2. Jarrow JP. Seminal vesicle aspiration of fertile men. J Urol 1996;56:1005–7.
3. Tounaye H, Verhayen G, Nagy P, Ubaldi F, Goosens A, Silber S. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? Hum Reprod. 1997;12(1):80–6.
4. De Croo I, Van Der Elst J, Everaert K, De Sutter P, Dhont M. Fertilization, pregnancy, and embryo implantation rates after ICSI in cases of obstructive and non obstructive azoospermia. Hum Reprod 2000;15:1381 – 8.
5. Sousa M, Cremades N, Silva J, Oliveria C, Ferraz L, Teixeria da Silva J, *et al.* Predictive value of testicular histology in secretory azoospermic subgroups and clinical outcome after microinjection of fresh and frozen – thawed sperm and spermatid. Hum Repord 2002;17:1800–10.

6. Ethics Committee of the American Society for Reproductive Medicine.Child-Rearing Ability and the Provision of Fertility Services. Fertil Steril 2004;82(Suppl 1): S208–11.
7. Report on varicocele and infertility. Fertil Steril. 2004;82(Suppl 1):S142–5.
8. Tournaye H, Silber SJ, Nagy ZP, Liu J, Devroey P, Van Steirteghem A. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital absence of vas deferens. Fertil Sertil 1994;61:1045–51.
9. Nagy Z, Devroey P, Silber S, Van Steirteghem A, Liu J. Using ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa gives rise to comparable results after sperm injection. Fertil Sertil 1995;63:808–15.
10. World Health Organization. WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. New York: Cambridge University Press; 1999.
11. Tournaye H, Devroey Silber SJ, Van Steirleghen A. Liv J. Normal pregnancies resulting from testicular extraction and intraytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Sertil 1996;66:110–7.
12. Friedler S, Raziel A, Schachter M, Strassburger D, Bern O, Ron-El R. Outcome of first and repeated testicular sperm extraction and ICSI in patients with non-obstructive azoosperima. Hum Reprod 2002;17:2356–61.
13. Mansour RT, Aboulghar MA, Serour GI, Fahmi I, Ramazy AM, Amin Y. Intracytoplasmic sperm injection using microsurgically retrieved epididymal and testicular sper. Fertil Sertil 1997;65:566–72.
14. Balaban B, Urman B, Sertac A, Alatas C, Aksoy S, Mercan R and Nuhoglu A. *In-vitro* culture of spermatozoa induces motility and increases implantation and pregnancy rates after testicular sperm extraction and intracytoplasmic sperm injection. Hum Reprod 1999;14:2808–11.

Address for correspondence:

Dr. Shazia Ali, Department of Physiology, Islamic International Medical College, Peshawar Road, Rawalpindi, Pakistan.
Cell: +92-300-5001789
Email: alishazia259@gmail.com