Original Article

Clinical results of the intra-cytoplasmic sperm injection with surgically retrieved sperms in azoospermic men at Sindh Institute of Reproductive Medicine, Karachi

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Abstract

Objective: To analyse the result of intra-cytoplasmic sperm injection (ICSI) by using surgically retrieved sperms in the azoospermic male.

Methods: Eight (9 cycles) azoospermic men were given the intra-cytoplasmic sperum injection after sperms were collected through surgical retrieval either by percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE) at the Sindh Institute of Reproductive Medicine (SIRM), Karachi, which is an assisted reproductive technology centre. Fertilisation, cleavage and pregnancy rates were calculated in their spouses with surgical sperm collection (SSC) and intra-cytoplasmic sperm injection. The study related to the period between September 2007 and February 2009. Statistical analysis were done using SPSS version 11.0.

Result: After the intra-cytoplasmic sperm injection procedure, fertilisation rate of 72.72% and cleavage rate of 78.12% were achieved in the eight patients. A total of 3 (33.33%) clinical pregnancies were obtained through the transfer of embryo from surgical sperm collection in the azoospermic men.

Conclusion: Intra-cytoplasmic sperm injection with surgical sperm collection proved to be the only successful treatment for men with azoospermia. It gave 33.33% chances of fatherhood in men who were previously labelled infertile. Results were found to be promising and are expected to improve with time and experience.

Keywords: Male infertility, Surgical sperm collection, Assisted reproduction Azoospermia (JPMA 62: 448; 2012).

Introduction

Infertility continues to be a critical condition. The proportion of couples seeking medical treatment for infertility is estimated at 4-17%.[1-3] The incidence of male infertility is increasing all over the world, including Pakistan.[4] In general, 15-20% of infertile men are azoospermic.[1,5] Male infertility is still a problem. In Pakistani social setup which is characterised by male domination, it becomes more important to find a solution for male infertility.[4] Worldwide assisted reproductive techniques have changed the whole concept of infertility management since the birth of the first In Vitro Fertilization (IVF) baby in 1978.[6] The subsequent advent of intra-cytoplasmic sperm injection (ICSI) has revolutionised the concept of male infertility management, and ICSI has emerged as the treatment of choice for azoospermic and idiopathic male-factor infertility.[1] This has changed the world of men who had been labelled infertile, to conceive before the first successful ICSI in 1992.[7] In the majority of men with idiopathic oligozoospermia or azoospermia, a cause cannot be identified. Treatment options include ICSI using sperm from epididymal aspiration, intratesticular sperm or elongated spermatids obtained from testicular biopsy.[1] In Pakistan, sperm donation is not an option to be considered due to a conflict with the belief system within the Muslim population.[4]

As only one viable sperm per oocyte is required for ICSI, men with occasional sperm present within the genital tract can be offered ICSI. As the success of ICSI has become evident, the number of patients entering ICSI programmes has increased manifold.[4] However, ICSI is expensive and is associated with chromosome aneuploidy. Hence, men considering ICSI should be offered genetic counselling by experts in the field.[1] Finally, if ICSI is used to manage the infertile state of the male, the major burden of procedures and drug exposure falls on the female.

The current study involved 8 patients (9 cycles, one patient had two cycles as the first cycle had failed) who opted for ICSI through surgically retrieved sperms, during the first year of SIRM. It needs to be emphasised that ICSI for the treatment of severe male-factor infertility, especially azoospermia, is particularly important in Muslim countries because the use of donor spermatozoa is usually forbidden.

Patients and Methods

In this prospective case series, the patients were

selected through Convenience Sampling from the SIRM outpatient department. A complete history was taken to assess for risk factors that may have led to azoospermia, such as cryptorchidism bilateral inguinal hernia procedures, or family history of cystic fibrosis. Thorough physical examination was carried out, with special attention to the volume of the testes, presence of epididymal dilatation and the existence of the vasa deferens or tumours. For patients with azoospermia, semen samples, obtained by masturbation after at least 48 hours of abstinence, were analysed three times or more. The spin-down method was also performed for initially azoospermic diagnosed samples, and those cases were excluded from the study in which few sperms were found during pallet examination.

Initially, 53 azoospermic males were selected. Serum hormone levels testosterone, LH and FSH were advised to determine whether the patient had gonadotropin deficiency (low testosterone and low or inappropriately normal LH and FSH), primary testicular failure (low testosterone, elevated LH and FSH), selective spermatogenic failure (normal testosterone and LH, elevated FSH), or androgen resistance (high testosterone, elevated LH). From among the 53 azoospermic males, FSH and LH levels were done in 52 (98.9%), one patient brought a three years old report of FSH and LH which were normal. He was not willing to have them repeated due to financial constraints. A total of 21 (40%) patients presented with ancillary evaluation record, such as post-ejaculate urine analysis, transrectal ultrasound and scrotal ultrasound examination which helped to determine the presence of ejaculatory duct obstruction, scrotal masses or to detect small testis tumour. These reports were examined by the urologist and repeated only if there was a strong indication.

Prior to sperm retrieval, initial evaluation of the females was carried out to confirm that they had adequate ovarian reserves to undergo assisted reproduction for the ICSI procedure.

After the initial evaluation of the females, hormonal analyses and urological evaluation, diagnostic SSC by percutaneous epididymal sperm aspiration (PESA) or, testicular sperm aspiration (TESA) and testicular sperm extraction (TESE) was offered. Of the initial 53 patients, 33 agreed to proceed with PESA, TESA or TESE, but sperms were surgically retrieved in only 16 patients as the rest were not willing for further treatment because of financial constrains.

After the surgical retrieval of sperm, detailed counselling and the attainment of informed consent, ICSI was offered to the 16 couples. Pros and cons of treatment options were discussed and they were guided to remain away from ineffective interventions. ICSI with SSC sperms was not found advisable in 2 patients either because of bad morphology sperms or the presence of only round spermatids. Six patients had domestic problems and did not continue with the treatment. Hence, ICSI with surgically retrieved sperms was performed in eight patients (nine cycles). Of them, five patients (six cycles) required PESA for sperm retrieval. TESE was required in three cases (three cycles); in which sperm retrieval was performed through open testicular biopsy.

After initial assessment, female partners were treated for ovarian stimulation. In four patients, suppression with injection Buserelin subcutaneous (gonadotrophin releasing hormone agonist GnRHa) was started on day 21 of the preceding cycle (long protocol). In the other four, suppression and stimulation simultaneously (flare up) was started on day 2 of the menstrual cycle. In one of these, GnRh antagonist was used on day 8, 9, 10 of controlled ovarian hyper stimulation to prevent leutinisation.

In patients with long protocol, after the suppression of hormone levels (FSH, LH, serum estradiol and serum progesterone) and ultrasonic confirmation of inactive ovaries and endometrium, human menopausal gonadotrophin (HMG) or recombinant FSH (rFSH) or Pure FSH was started for ovarian stimulation.

Monitoring was performed by serial transvaginal ultrasound and estradiol levels. Once the follicle reached 1.7-2.0 cm size, human chorionic gonadotrophin (HCG) was given for leutinisation. After 32-34 hours, ultrasound (transvaginal) directed follicle aspiration (UDFA) was done.

The ova were picked and washed in the media and were incubated at 37°C in 5% CO2 environment for 1-2 hours prior to cumulus removal. The oocytes were denuded from the cumulus cells using hyaluronidase 100 IU/ml and mechanical aid of fine Pasture pipettes. The denuded oocytes were then placed in the fresh culture medium and allowed to further mature for 2-4hours prior to ICSI. Oocytes at maturation stage of metaphase ll were selected for injection. Sperm retrieval was performed either by PESA or TESE. The goals of surgical sperm retrieval are: (1) to obtain the best quality sperms possible; (2) to retrieve an adequate number of sperms for both immediate use and for cryopreservation, and (3) to minimise damage to the reproductive tract.

All the sperms obtained surgically (either by PESA or TESE) destined for ICSI were incubated for 4-6 hours.

All percutaneous epididymal sperm aspirations were performed by a urologist under local anaesthesia.

The procedure was preferred because it can be performed without surgical scrotal exploration, it can be repeated easily at a low cost, and it does not require an operating microscope or expertise in microsurgery. The testis was stabilised and the epididymis was held between the surgeon's thumb and the forefinger. A 21-gauge needle was carefully directed through the skin of the scrotum into the caput of the epididymis. While applying negative pressure, the tip of the needle was pushed within the substance of the epididymis until a cloudy fluid was observed in the needle tubing. The aspirate was then flushed with 0.5ml of buffered culture medium and was examined under a microscope. Next, 5.0ml of culture media was added to the aspirate, and centrifuged twice at 1500 RPM for 5 minutes each. The supernatant fluid was removed and pellet was re-suspended in 0.3 to 1.0 ml of culture media in a 5.0 ml Falcon tube. Finally, the tube was incubated at 37ºC in a CO2 incubator until needed for ICSI.

Testicular sperm extraction was used in cases of obstructive azoospermia. Following local anaesthesia with 1% lidocaine, an incision was made in the scrotal skin and the tunica albuginea was opened for obtaining a large volume of testicular tissue. This technique may create potential devascularisation problems, because of the limited blood supply of testicle course underneath the tunica albuginea of the testis before it penetrates into the testicular parenchyma.

The excised testicular tissue were placed in a Falcon tube containing 2 ml of the buffered culture medium. The tissue was then shredded into small pieces with sterile needles and examined under a microscope for the presence of live sperms. The TESE sample was then processed just like PESA samples.

ICSI was performed with microinjection equipment installed on an inverted microscope. The microinjection dish was prepared by adding several 5-µL droplets of Human Tubular Fluid (HTF) buffered media. The prepared sperms (1-2 µL) were added to the drops of 50% polyvinylpyrolidone (PVP) solution to slow down the sperms' forward progression. The oocytes were placed into the droplets of the medium. Each denuded oocyte was then rinsed a couple of times in the culture media. A normal looking sperm was rendered immobilised by crushing the tail between the injection pipette and dish. A holding pipette in a position held the oocyte so that the polar body was at 6 or 12 O'clock position. The freshly immobilised spermatozoon was aspirated from the tail end into an injection pipette. The injection pipette was pushed through the zona pellucida to puncture the oolema. Rupture of the oolema was confirmed by aspiration of a small volume of the oocyte cytoplasm into the injection pipette and then spermatozoa were released into the oocyte cytoplasm.

After microinjection, oocyte were rinsed and placed in equilibrated culture medium with 10% human serum albumin (HSA). The oocytes were incubated and observed for signs of fertilisation 16 to 18 hours after injection. The cleavage of fertilised oocytes was assessed about 24 hours after fertilisation.

Most of the embryos were judged for quality and the best 2-3 were selected for transfer. In eight cases, embryo transfer was done on day 2, while ET was done on day 3 in one patient. K- jets 7019 SIVF cook catheter or K- soft 5000 (for more difficult cervical cannulation) was used for ET. The leftover embryos were cryopreserved by vitrification (fast freezing procedure).

Progesterone vaginal pessary was started from the day of egg retrieval and hCG 2500 IU (02 doses) injection were given 48 hours apart from the day of ET for the luteal phase support to those patients who were not at risk for the development of ovarian hyper stimulation syndrome (OHSS).

Serum ß-H.C.G was checked 15 days after the embryo transfer for the confirmation of pregnancy. Ultrasound scan was performed after one week of positive serum ß-hCG report to confirm the number of gestational sacs.

Statistical analyses were performed using SPSS version 11.0. Frequencies, means and standard deviations (SDs) were calculated.

Results

A total of 8 patients were selected for the ICSI cycle with the surgically retrieved sperms from September 2007 to February 2009. The female age ranged from 24 to 35 years with a mean of 28.22 ± 3.22 years. The total number of stimulated cycles was 9 as one patient had undergone two cycles.

Demographic characteristics of patients with azoospermia (Table-1), SSC outcome (Table-2) as well as the demographic characteristics of eight females having nine ICSI cycles (Table-3) were noted. Three women had hysterosalphingography for tubal patency without knowing the seminal fluid analysis of the husband from among initial 53 couples.

In 6 cycles, sperms were collected by PESA and by TESE in 3. All cycles responded well on ovarian stimulation and went through UDFA.

The number of ova collected was 79, (an average of 8.78 ova per aspiration). They were checked for maturity and 44 (55.69%) of them were found to be at metaphase II stage that was selected for injection. The remaining 35 ova

Table-1: Demographic characteristics of patients (male) with azoospermia (n=53).

Age	19 years -42 years *(27-58 ±5.02)
Duration of infertility	08 month- 22 years *(32.42 ± 3.2)
No: of Marriages	4-Jan
Past medical surgery	05 (9.433%)
Diabetes mellitus	1
Hypertension	1
Hypothyrodism	1
Asthma	1
Fatty Liver	1
Past medical surgery	07(13.20%)
Nephrectomy	3
Muscle Biopsy	1
Urethral dilatation	1
Testicular biopsy	1
Gunshot at pelvic region	1
Addiction: Smoking, Naswar, Chalia, Pan, Gutka	39 (73.5%)
H/O cystic fibrosis	01 patient
Hormonal profile of husband	
+ Follicle stimulating hormone	02-39 mIU/ml *(12.58 ±9.70)
Leutinising hormone	01-31 mIU/ml *(6.88 ± 5.34)

* (Mean ± Standard deviation).
+ Sperms were also retrieved surgically in four azoospermic patients who had FSH

Table-2: SSC outcome in male with azoospermia (n=53).

Details of SSC outcome	**No of patients & percentage**
SSC (PESA/ TESA OR TESE)	
Proceed	33 patients (62.26%)
Not Proceed	20 patients (37.73%)
Diagnostic SSC positive	16 patients (48.48%)
SSC procedure performed	33 patients (62.26%)
PESA	16 patients (48.48%)
TESE	14 patients (42.42%)
TESA	03 patients (9.09%)
Out come of SSC procedure	
PESA	
Sperms retrieved	12 patients
Sperms not retrieved	04 patients
TESA	
Sperms retrieved	03 patients
Sperms not retrieved	-
TESE	
Sperms retrieved	08 patients
Sperms not retrieved	06 patients
ICSI proceed with SSC	08 patients (09 cycles)

SSC = Surgical sperm collection
PESA = Percutaneous epididymal sperm aspiration
TESA = Testicular sperm aspiration
TESE = Testicular sperm extraction.

were immature. Normal fertilisation rate (2PN) of 72.72% was achieved, whereas overall fertilisation rate (2PN + 1PN) of 79.54% was observed.

The embryo cleavage rate was 78.12% whereas the failed cleavage rate was 21.88%. All patients (8 patients; 9 cycles) went through embryo transfer. In eight cases (8 cycles) ET was done on day 2, while ET was done on day 3 in one patient (1 cycle). Subsequently, 19 (out of 25) embryos were transferred, giving an average of 2.11 ET per patient.

Table-3: Summary of results.

Female Age	24years- 35years *(28.22 ± 3.2)
Duration of infertility	9months - 17 years *(5.98 ± 4.75)
Infertility	
Primary	07
+ Secondary	02
Miscarriage	01 (50%)
Preterm delivery (twin)	01 (50%)
Ovarian stimulation protocol	
Long GnRH agonist	04 cycles
GnRH antagonist	01 cycle
Flare-up protocol	04 cycles
Duration of ovarian stimulation, days	12 days-16 days
Total dose of Gonadotrophins,	150 IU – 450 IU
No: of follicle > 16mm	6ova -21 ova a (11.89 ± 4.859)
Total retrieved oocyte	79 ova
Immature (G1, M I)	35
MII	44
Normal fertilization rate (2 Pn)	32 (72.72%)
Abnormal fertilization rate	12 (27.27%)
0 PN	11
1 PN	01
Cleavage Rate	25 (78.12%)
Grading of Embryo	
Grade 1	09 (36%)
Grade 2	11 (44%)
Grade 3	05 (20%)
No: of Embryo transferred per patient	2.11%
Total No: of Embryo transferred (all patients)	19
Pregnancy rate	03 (33.33%)
Pregnancy rate per ET	15.78%

+Two patients had previous history of ICSI with SSC, one ended up into miscarriage and the second patient had history of twin preterm delivery.

Clinical pregnancies were achieved in 03 cycles. In two of them, sperms were collected by PESA and in one by TESE. Overall pregnancy rate per cycle was 33.33%. The pregnancy rate per embryo transfer was 15.78%.

Discussion

Since male-factor infertility is increasing, there is an immense demand for effective treatment. Even in an overpopulated country like Pakistan, childlessness is still a personal tragedy. There is actually a need of balanced reproduction.[4] During the last decade, numerous studies have evaluated the effectiveness of various treatments for infertility.[8] As a result, assisted reproductive techniques are developing every day and giving better results. Intra cytoplasmic sperm injection (ICSI) is the most successful mirco-manipulation technique for treating male-factor infertility.[4] The technique was used initially in veterinarian practice. The first pregnancy in humans was reported in

July 1992; although this procedure was first applied to human gametes in 1988.[9] ICSI has given many couples the chance of biological parenthood.

In many infertility centres, intra-cytoplasmic sperm injection (ICSI) with epididymal or testicular spermatozoa is a routine treatment for men with azoospermia. The use of testicular spermatozoa for ICSI has, since its introduction in 1992, been very successful in enabling men with azoospermia to achieve genetic fatherhood.[10] The first successful pregnancy was achieved after testicular sperm extraction (TESE) from patients suffering from obstructive azoospermia.[11] The same year, a pregnancy was reported after a simplified fine needle aspiration technique, called testicular sperm aspiration (TESA).[12] In cases of non-obstructive azoospermia (NOA) the outcome is more unpredictable and the sperm recovery rate lower than in obstructive cases.[13,14]

Our study demonstrated that both PESA and TESE are the methods of choice for obtaining adequate numbers of spermatozoa from patients with azoospermia, as well as both are simple techniques requiring minimal equipment.

There are only a few published reports where the physiological consequences of different sperm recovery techniques have been studied. The procedures are often repeated and their consequences for the testis are still unknown. An earlier study detected persistent devascularised focal lesions by ultrasonography in nine out of 14 men (64%) six months after the TESE procedure.[15] Intra-testicular lesions diagnosed as resolving post-operative haematomas were found by ultrasound in 11% of the patients and in 7% of aspirated testes 3 months after TESA. The subjective discomfort was mostly mild and none of the lesions were permanent. Serum FSH and testosterone levels did not change after the procedure. There were three patients with borderline antisperm antibodies (ASA) formation, but none were classified as ASA-positive. Testicular sperm aspiration seems to be a safe method for sperm retrieval with minimal physiological consequences in men with obstructive and non-obstructive azoospermia.[11]

Our study discusses the experience of the first year of ICSI with surgically retrieved sperms at SIRM. After counselling of patients regarding treatment options, both the expected increase in cycle fecundity and treatment expense, 09 ICSI cycles were started on stimulation regimen with HMG (according to the SIRM'S protocol). Average numbers of ova collected per patient (8.78) were optimal. To prevent OHSS, too many follicle and eggs should be avoided.

The only criterion for a successful attempt of ICSI are the presence of few motile or live sperms somewhere in the male reproductive tract, which can be retrieved for injection. Sperms were retrieved surgically either by PESA or TESE.

To optimise the possibility of achieving successful sperm recovery, multiple[16] and bilateral[17] testicular sampling is recommended. Some groups have emphasised that TESE is a superior technique,[17,18] while others have reported TESA to be almost as effective as the more invasive TESE procedure.[14,19] Microsurgical TESE is a novel approach to the identification of focal areas with sperm production and in two prospective studies, was considered more effective and less traumatic than conventional TESE.[20,21] Multiple gun-needle biopsy is a promising new sperm recovery technique described in men with both obstructive and non-obstructive azoospermia.[22]

Fertilisation rate of 60-70% is obtained with ICSI once the injection procedure has been optimised as reported by pioneer workers.[23,24] The fertilisation rate of 72.72% achieved at SIRM is quite promising for a new centre. An earlier study reported 64% fertilisation in cases of PESA, Microsurgical Epidymal Sperm Aspiration (MESA) or TESE,[4] while another reported fertilisation rates of 77%, and 75% in cases of ICSI with TESE and PESA sperms respectively.[25]

Triplet pregnancy is alarming, especially in regions where neonatal care is scarce and expensive. The number of embryos transferred must be discussed with the couple and one must try to keep it at two. The average number of ET per patient was 2.1 in our series.

Out of the 9 cases in which sperm were retrieved in PESA or TESE, three women became pregnant, giving pregnancy rate of 33%, which is encouraging and comparable with other reports.[4]

The findings of our study show that the minimally invasive techniques of PESA and TESE can be successfully performed to retrieve adequate numbers of spermatozoa for ICSI. Our results also demonstrated that the fertilisation rates, embryonic development and cleavage rates are satisfactory with TESE/ICSI and PESA/ICSI. It must improve with time as the working team gains more experience.

Limitations:

Limitations of the study included a small number of patients with financial constraints which prevented the carrying out of some investigations.

Conclusion

The result of the report and the data obtained from other published studies clearly show that the ICSI with SSC has revolutionised the treatment of the male-factor

infertility due to azoospermia. ICSI procedure achieved fertilisation rate of 72.72% and cleavage rate of 78.12%; thus ICSI proved to be the only successful treatment for men with azoospermia, giving them 33.33% chances of fatherhood.

With continued analysis of sperm parameters, embryo development, and implantation rates, the treatment outcome of ICSI could only get better.

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