

## Outcomes of Intracytoplasmic Sperm Injection in Obstructive and Non-Obstructive Azoospermia

Hamid Hussein Alkelaby<sup>1\*</sup>, Sahib Yahya Almurshidi<sup>2</sup> Raed Talib Madlool<sup>3</sup>

1 Urology Department/ College of Medicine/ University of Kerbala/ Kerbala/ Iraq

2 Urology Department/ Medical College/ Kufa University/ Kufa/ Iraq

### Abstract

**B**ackground: infertile couples due to male factor reasons present with azoospermia as a cause of their infertility can positively benefit from intracytoplasmic sperm injection (ICSI) for fathering genetics offspring's.

**Aim of study:** to compare the intracytoplasmic sperm injection outcomes in obstructive and non-obstructive azoospermic men.

**Materials and methods:** A retrospective study compromised 42 couples seeking for treatment due to azoospermia as a cause of infertility. 19 men out of 42 had obstructive azoospermia (OA) and the remainder 23 man had non-obstructive azoospermia (NOA). All those males underwent surgical sperm retrieval techniques either testicular sperm extraction (TESE) in all NOA cases and some OA cases or percutaneous epididymal sperm aspiration (PESA) in the rest of OA cases. Sperms retrieved surgically were used either fresh or after cryopreservation for ICSI and the main outcomes; fertilization, cleavage and pregnancy rates and embryo quality were compared statistically. Data were expressed as mean  $\pm$ SD and number and percent, and groups were compared by compares of two independent means. Differences were considered significant at  $p < 0.05$ .

**Results:** the results revealed significant reduction ( $p < 0.05$ ) in the fertilization rate in couples with NOA (38.2%) compare to (59.5%) for OA group, as well as a significant drop in cleavage (80.6% vs 68.9%) and pregnancy (31.57% vs 13.04%) rates was also shown in NOA group. The mean number of embryos ( $5.88 \pm 3.46$  vs  $3.33 \pm 2.54$ ,  $p < 0.017$ ) obtained by ICSI and percent of good quality embryos (55.2% vs 31.37%,  $p < 0.014$ ) were significantly higher in OA group.

**Conclusion:** type of azoospermia has greater effect on the success of ICSI cycles. The immaturity of testicular sperm in NOA can affect fertilization, embryo development and pregnancy rate.

**Key words:** ICSI, azoospermia, testicular sperm extraction and epididymal aspiration.

### Introduction

Infertility is regarded as clinical and public problem because it touches not only the health system but also the social life. It is defined as the inability of sexual active couples to have pregnancy after twelve months of regular unprotected coitus<sup>(1)</sup>.

Male infertility is reflected when recognizable female foundations of childlessness are excluded and semen parameters miscarry to realize WHO criteria<sup>(2)</sup>. Reproductive dysfunction in male is the solitary or influential cause of infertility in about 50% of couples making

the medical assessment for managing infertility<sup>(3)</sup>.

Prevalence of azoospermia, the complete nonappearance of spermatozoa in the ejaculate after centrifugation, is in approximately 1% of all men and up to 15% of infertile men<sup>(4)</sup> It is categorized into two widely different subgroups: 1. Obstructive azoospermia (OA); when normal spermatogenesis is preserved but there is mechanical block for sperm passage due to acquired or congenital causes<sup>(5)</sup> 2. Non-obstructive azoospermia (NOA); abnormal sperm production as a result of testicular and pre-testicular causes

\*For Correspondence: E-Mail [hamid\\_mutlage@yahoo.com](mailto:hamid_mutlage@yahoo.com)

mostly genetic defects such as Klinefelter syndrome, Y-chromosome microdeletions<sup>(6)</sup>. Many of those azoospermic male preserve sperm creation at variable levels in the testes and it is not essentially to imply sterility on them<sup>(7)</sup>.

Etiologies of azoospermia compromise three major groups: pretesticular, testicular, and post testicular. Differentiation among these can be applied sometimes by measuring testicular size, assessment of seminal volume or measuring hormonal levels<sup>(8)</sup>. Genetic factors explain up to 21-29% of azoospermia causes, whereas 12-41% of azoospermic cases are idiopathic and most likely due to unknown genetic factors<sup>(9)</sup>.

With the introduction of intracytoplasmic sperm injection (ICSI) as a standard treatment modality a number of these men successfully fathering a child through surgically retrieved sperm from the testis<sup>(10)</sup>. The first pregnancy by ICSI with testicular sperm from men with OA was achieved in 1993<sup>(11)</sup>. Sperm retrieval techniques (SRTs) are surgical methods that have been developed to obtain spermatozoa from the epididymides and testicles of azoospermic men seeking fertility treatment<sup>(7)</sup>.

An essential part of fertility management in ART clinics is the cryopreservation of sperm, especially important in treatment of couples with azoospermic male factor following surgical sperm retrieval procedures<sup>(12)</sup>. Nowadays, the use of frozen-thawed testicular or epididymal sperm in ICSI has become an effective and standard method for treating obstructive and non-obstructive azoospermia<sup>(13)</sup>.

## Materials and Methods

Retrospective study was performed at Al-Sadder Medical City, Fertility center Undersupervision of Urology Department in Kufa Medical College over two years from January 2013 to January 2015. The study population comprised (42 couples) with primary infertility diagnosed with

azoospermia attending the fertility center seeking fertility treatment.

The female partners were examined and evaluated by gynecologists while the male examination and assessment was done by the urologists. The diagnosis was reconfirmed by two separated SFA of 2-3 months interval according to WHO criteria (1999). Hormonal profile including (FSH, LH, testosterone and prolactin) was recorded from patient case sheet. No exclusion was applied to the etiology of the azoospermia or to the number and outcome of previous fertility treatments.

(23 patients) were of non-obstructed type and underwent TESE /ICSI cycles after cryopreservation of the surgically retrieved sperms, the rest (19 patients) were of obstructed type and PESA "Fresh or frozen" or TESE with ICSI was done for them. All testicular sperm extraction and epididymal sperm aspiration were performed by urologist under general anesthesia.

Number of Oocytes, number of embryos transfer and quality of embryo of each group was recorded. Main outcome measure; fertilization, cleavage and pregnancy rates and quality of embryo were statically analyzed.

### *Testicular sperm extraction (TESE) processing:*

The testicular tissue was taken directly to the lab, then minced and shredded into small pieces using two sterile bended syringes; a small drop was taken and examined under a light microscope for the presence of sperm; when found analysis was made including the count of sperm and motility percentage. Medium " HEPES buffered Ham's F10 (Beernem, Belgium) containing different tissue cells "after removal of squeezed tissues" was collected in 15 ml Conical tube (Falcon, USA), and then the sample aspirate was centrifuged at 3000 rpm for 10 min. The supernatant was discarded leaving just 0.3 to 0.5 mL of the fluid above the pellet, and then mixes the pellet well with the media. The tube was incubated for 45 min in 37°C and 5%

CO<sub>2</sub>" and the sperm if found were cryopreserved in LN<sub>2</sub> and used for ICSI after thawing at day of oocytes retrieval.

*Epididymal sperm aspiration (PESA) processing:*

Epididymal aspirates were diluted with sperm medium; a small drop was taken and examined for presence of sperms, if more epididymal specimens were taken, samples of similar quality were shared together for processing<sup>(14)</sup>. Sperms were prepared by centrifugation swim up; the pellet was mixed well with the media and kept at 37°C for 30 min and after incubation, the sperm obtained after processing either used directly for sperm injection or may be eventually cryopreserved.

*Cryopreservation of epididymal and testicular sperm:*

Testicular or epididymal suspension was transferred to 1.8 ml labeled container "CryoTube™ Vial, Thermo scientific, Denmark". Suspension was mixed gently with equal volumes of cryoprotectant media (Sperm Freeze™, Beernem, Belgium) by adding drop by drop of the media to the suspension within the vial to minimize hyperosmotic stress, while continuously shaking the tube. The mixture was kept for 10 min. at room temperature for equilibration and Freeze vertically for 15 min. just above the level of LN<sub>2</sub>; finally vials were plunged into liquid nitrogen (-196°C) until day of oocytes retrieval to be used after thawing for ICSI.

*Intracytoplasmic sperm injection:*

All females underwent controlled ovarian hyperstimulation using GnRH agonist or antagonist, in combination with human menopausal gonadotropin, and/ or recombinant human FSH. Motile sperm that appear morphologically normal was selected for immobilization. MII oocyte was hold in place by holding pipette, Polar body was positioned at 6 o'clock in order to reduce the likelihood of destructive the meiotic spindle<sup>(15)</sup>, and the injector with the immobilized spermatozoa inside it was

introduced in the cytoplasm of MII oocyte at the 3 o'clock position. Assessment of fertilization was made 16-18 hour after ICSI (at the morning of the day following injection) by checking the number of polar bodies and pronuclei.

*Embryo transfer (ET):*

morphological evaluation of embryos 24-48 hours after oocytes retrieval was done including; the presence of first cleavage, blastomeres symmetry and the magnitude of fragmentation<sup>(16)</sup>.

Good quality embryos (grade I) which have even and equal size blastomeres with no or negligible cytoplasmic fragments were selected first for transfer followed by (grade II); embryo with equal size blastomeres, minor cytoplasmic fragments. Then embryos with unequal sized blastomeres and no more than 50% fragments (grade III) are transferred while other embryos with upward grading are neglected. A maximum of three or four embryos (best three or four embryos selected for transfer) were transferred 48 to 72 hours after oocyte retrieval<sup>(17, 18)</sup>. Pregnancy was established by the elevated levels of serum hCG concentrations (positive pregnancy test) at least 12 days following ET which was done at private labs by commercial kits since most of couples living in different governorate far away from the center.

## Results

In total of 42 ICSI cycles evaluated in this study, PESA was performed in 14 patients with OA and TESE was performed in 28 patients (5 patients had OA and 23 patients had NOA). There were no significant differences between OA and NOA when demographic characteristics of couples were assessed for both male and female age and infertility duration ( $p > 0.05$ ). On the other hand here was significant increase in serum level of FSH ( $5.4 \pm 2.8$  vs  $16.3 \pm 8.9$ ,  $p < 0.001$ ) and LH ( $3.9 \pm 1.2$  vs  $6.2 \pm 2.3$ ,  $p < 0.02$ ) in NOA patients, while non-significant reduction in serum level of

testosterone hormone was detected in (1). NOA group ( $p > 0.9$ ) as described in table

**Table 1.** Clinical characteristics of study group.

| Variables                    | OA           | NOA       | P value  |        |
|------------------------------|--------------|-----------|----------|--------|
| Male age(yrs)                | 30.1±5.25    | 34.2±9.44 | .1162    |        |
| Female age(yrs)              | 24.9±4.92    | 29.9±8.10 | .073     |        |
| Duration of infertility(yrs) | 4.7±2.23     | 6.1±4.63  | .0879    |        |
| Hormonal profile             | FSH          | 5.4±2.8   | 16.3±8.9 | 0.001* |
|                              | LH           | 3.9±1.2   | 6.2±2.3  | 0.02*  |
|                              | Testosterone | 4.3±1.2   | 3.1±1.8  | 0.9    |

OA; obstructive azoospermia, NOA; non-obstructive azoospermia, FSH; follicle stimulating hormone, LH; luteinizing hormone.

\* Significant difference ( $p < 0.05$ ).

The main ICSI outcomes between two groups were summarized in table (2); no significant differences were revealed in the no. of retrieved oocytes and no. of injected MII oocytes between two groups ( $p > 0.05$ ), while the mean no. of 2PN was significantly higher in OA group than NOA ( $6.58 \pm 3.33$  vs  $3.26 \pm 2.86$ ,  $p < 0.001$ ). The fertilization rate was also significantly higher in OA patients (59.5% vs 38.2%,  $p < 0.009$ ), as well as there was significant drop in cleavage (80.6% vs 68.9%,  $p < 0.02$ ) and pregnancy (31.57% vs 13.04%,  $p < 0.009$ ) rates in NOA group. In table (3) comparison of embryo quality between OA and NOA groups was described in brief, significant increase in mean no. of embryos ( $5.88 \pm 3.46$  vs

$3.33 \pm 2.54$ ,  $p < 0.017$ ) was obtained by ICSI in couples with OA. No. of transferred embryos was significantly higher in OA ( $3.37 \pm 1.63$  vs  $2.04 \pm 1.65$ ,  $p < 0.015$ ) for NOA. Grade I embryos was significantly higher in OA group 52(55.2%) than 20(31.37%) for NOA group ( $p < 0.014$ ). Regarding grade II embryos there was no significant differences ( $p > 0.05$ ) in number and percentage between the two groups and was 39(37.9%) and 24(36.4%) for OA and NOA respectively, correspondingly; the percent of grade III and upward or bad quality embryos was significantly reduced in OA group (9"6.8%" vs 16" 22.7%",  $p < 0.012$ ).

**Table 2.** The ICSI outcomes between obstructive and non-obstructive azoospermia regardless the underline cause.

| Variables           | OA(19)          | NOA(23)         | P value |
|---------------------|-----------------|-----------------|---------|
| Retrieved oocytes   | 237(12.47±5.26) | 258(11.22±4.47) | 0.4189  |
| No. of MII          | 211(88.4)       | 208(80.6)       | 0.1604  |
| No. of 2PN          | 125(6.58±3.33)  | 75(3.26±2.86)   | 0.001*  |
| Fertilization rate% | 59.5            | 38.2            | 0.009*  |
| Cleavage rate%      | 80.6            | 68.9            | 0.029*  |
| Pregnancy rate%     | 31.57 (6)       | 13.04(3)        | 0.009*  |

2PN; two pronuclear stage, MII; metaphase II oocyte. \* Significant difference ( $p < 0.05$ ).

**Table 3.** Embryo quality in obstructive and non-obstructive azoospermia.

| Variable                            | OA (n=19)      | NOA (n=23)    | P value |
|-------------------------------------|----------------|---------------|---------|
| No. of embryos                      | 100(5.88±3.46) | 60(3.33±2.54) | 0.017*  |
| Transferred embryos                 | 64(3.37±1.63)  | 47(2.04±1.65) | 0.015*  |
| No. of grade I embryo               | 52(55.2%)      | 20(31.37%)    | 0.014*  |
| No. of grade II embryo              | 39(37.9%)      | 24(36.4%)     | 0.151   |
| No. of grade III and upward embryos | 9(6.8%)        | 16(22.7%)     | 0.021*  |

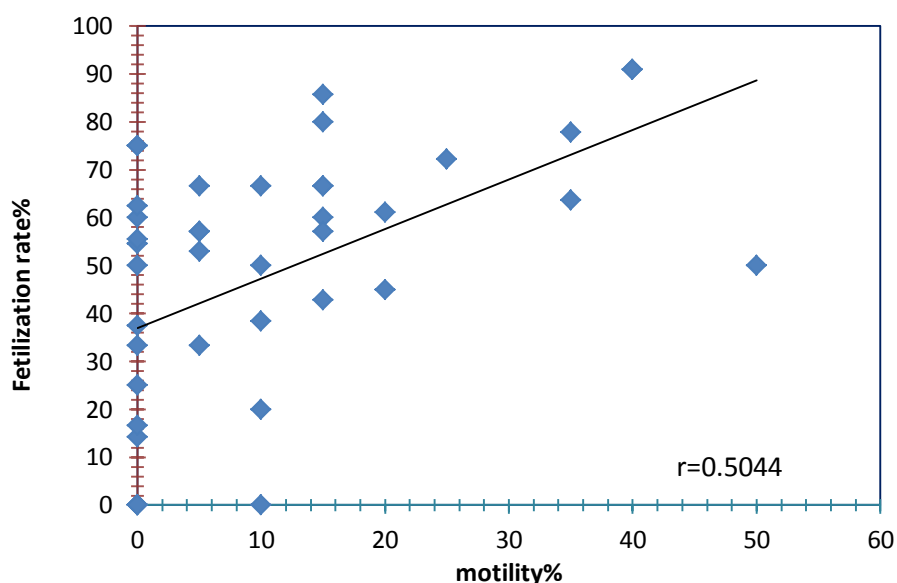
\* Significant difference ( $P < 0.05$ ).

There was positive correlation between motility of surgically retrieved sperm in

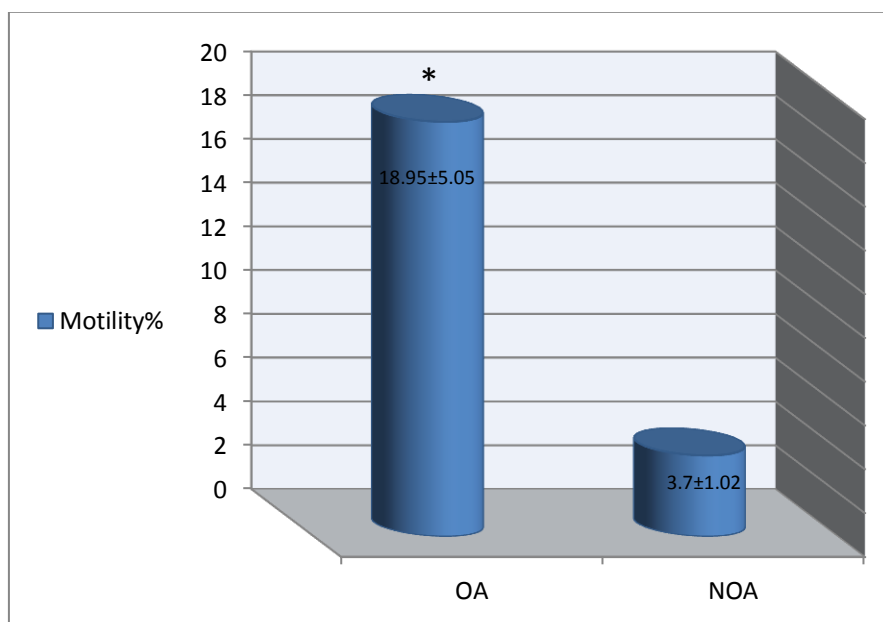
azoospermic men and fertilization rate following ICSI ( $r=0.5044$ ) as it has been

shown in figure (1), Correspondingly; figure (2) show that sperm from patients with OA had highly significant increase in

motility percent when compared to sperm motility in patients with NOA ( $18.95 \pm 5.05$  vs  $3.07 \pm 1.02$ ,  $p < 0.001$ ).



**Figure 1.** Correlation between sperm motility in azoospermic patients and fertilization rate.



**Figure 2.** Comparison between motility of surgically retrieved sperm in cases of obstructive and non-obstructive azoospermia.

### Discussion

Before the innovation of ICSI many infertile couples with sever male factor infertility have no chance to acquire conception. Later on, the introduction of ICSI has changed the management of severe male factor infertility radically and

become a milestone in their treatment. Very soon, patients with the most severe form of male infertility, azoospermia, having the hope to be genetic fathers by using their own gametes with the development of sperm surgical retrieval techniques; epididymal in cases of OA and testicular in all cases of NOA and

some cases of OA with or without sperm cryopreservation.

Azoospermia is an imaginative term mean lack of sperm in the ejaculate and divided into two widely diverse subgroups; OA when there is block of excretory ducts with normal sperm production and NOA when abnormal or absent spermatogenesis is present<sup>(19)</sup>. The quality and viability of the sperm may be related to the underlining cause of azoospermia and site of sperm retrieved<sup>(20)</sup>.

The data of present study publicized highly significant drop in the fertilization, cleavage and pregnancy rates in couples with NOA, similar results were found in other research's<sup>(21,22)</sup>, this reduction may be related to the quality of sperm used in injection since all sperm used for ICSI in NOA patients were of testicular origin and sperm reach their maturity in the epididymis rather than testis<sup>(23)</sup>, on the other hand; some authors had been report that patients with NOA shows higher levels of chromosomal abnormality and Y chromosome micro deletion, therefore; samples taken from testicular biopsies had more DNA-damaged sperm and the success rate are lower in those patients<sup>(24)</sup>. Support these facts; the significantly higher concentration of gonadotropins (FSH and LH) and lesser concentration of testosterone hormones in the NOA group compared to the OA group. These abnormalities may be due to decreased germinal cell mass and reduction in the function of Sertoli cells which results in defective spermatogenesis including spermatogenic maturation arrest, tubular fibrosis or Sertoli cell only syndrome with focal areas of spermatogenesis<sup>(25)</sup>. The impairment of the function of Sertoli cells results in decreased secretion of inhibin hormone that controls FSH release by the negative feedback mechanism and this causes an increase in FSH levels of Sertoli cells which results in defective spermatogenesis<sup>(26)</sup>.

On the other hand, sperm from patients with extremely faulty spermatogenesis

may have a greater affinity to carry deficits, such as that related to the centrioles and genetic material, which eventually affects the competency of the sperm to stimulate the egg and activate the establishment and development of a typical zygote and a viable embryo as found in sperm from NOA patients<sup>(27,28)</sup>.

Furthermore, all NOA patients underwent sperm cryopreservation following TESE in contrast to OA patient in which (42%) of them had fresh PESA and the other frozen PESA or TESE and some studies revealed a negative effect of freezing on sperm quality by several damaging processing<sup>(29)</sup> and increasing sperm DNA fragmentation<sup>(30)</sup>.

In addition to freezing, a detrimental effect on sperm may occur during thawing process especially in rapid thawing rate and during first few hours<sup>(31)</sup>. Some authors reported that using frozen testicular sperm for oocyte injection will result in lower fertilization rate and overall ICSI outcomes due to effect of freezing on severely defected sperm<sup>(32, 33)</sup>. Cormier and Bailey; has shown that the fertilization capacity of the frozen-thawed sperm may be decreased due to the triggering of the signal pathway that lead to capacitation by freezing and thawing of the spermatozoa<sup>(34)</sup>.

Another point of this study is the impact of embryo quality and cleavage rate on implantation rate. Sakkas and Gardner concluded that embryo quality which is largely based on embryo cleavage rate and morphology a vital factor affecting the clinical consequence of ART<sup>(35)</sup>; similarly others considered the quality of embryos appears to be the standard basics of achievement embryo implantation and the finest prognostic factor for pregnancy in ICSI cycles<sup>(36, 37)</sup>. For this; growth of morphological grading system for embryo led to progress in the pregnancy rate and decreases the incidence of multiple pregnancy in ART cycles<sup>(38)</sup>, so the selection of best quality embryo to be

transferred to the uterus have great chance for implantation<sup>(39)</sup>.

## Conclusions

Intracytoplasmic sperm injection is effective methods for treating couples with sever male factor infertility even azoospermic patients in combination with surgical sperm retrieval techniques (percutaneous epididymal aspiration and testicular sperm extraction). Type of azoospermia (obstructive or non-obstructive) play an important role in the outcomes of ICSI regardless the underlining pathology. Significantly lower ICSI results were achieved when sperm from patients with non-obstructive azoospermia were used in contrast to sperms from patients with obstructive azoospermia.

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