

## Embryo Implantation In Intracytoplasmic Sperm Injection-Stimulated Cycle Using Testicular And Epididymal And Ejaculated Sperm From Azoospermic, And Severely-Teratospermic Men.

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### Summary:

**Background:** The infertility affects about 20% to 28% of Iraqi population and the primary and secondary infertility cover 80% and 20% of infertility cases respectively. It has been shown that the major male infertility factors include oligospermia, astheno-spermia, teratospermia and azoospermia.

**Objectives:** The objective of this study was to compare the fertilizing capacity, in vitro embryonic developmental rate and embryo implantation following the use of epididymal, testicular, and ejaculated sperm in azoospermic and severely teratospermic men. **Patients and Methods:** The males in experiment one were divided into three groups, severely teratospermic group (STSG, n=44), azoospermic-epididymal group (ASEG, n=35) and azoospermic-testicular group (ASTG, n=40). In experiment two the azoospermic patients were divided into two groups, obstructive (OASG, n=35) and non-obstructive (NASG, n=42). Both groups were underwent testicular extraction and intracytoplasmic sperm injection (TESE-ICSI) treatments.

**Results:** Concentration of FSH, LH, prolactin was significantly higher in non-obstructive group compared to obstructive group ( $P < 0.001$ ). The concentrations of testosterone and the volume of the testes were significantly higher in the obstructive group versus non-obstructive group ( $P < 0.01$ ). Percentages of the fertilizable oocytes and the number of the transferred embryos per patient in the ASTG group were significantly lower compared to STSG and ASEG groups. The pregnancy and implantation rates were not significantly different in the STSG, A<sub>s</sub>SEG, and ASTG groups. ICSI rate and embryo developmental rate and the number of the transferred embryos per patient were significantly lower in the non-obstructive group (NASG) compared to the obstructive group (OASG) Pregnancy and viable fetus percentages were similar between both groups ( $P > 0.05$ ).

**Conclusions:** Sources of sperm retrieval found to have no effect on embryo implantation and pregnancy rates when viable sperm are available for ICSI. Pregnancy and viable gestation sac percentages were not affected by the etiology of azoospermia in either obstructive, or nonobstructive with focal areas of spermatogenesis were present in testes of azoospermic men.

Key wards: ICSI Outcome, Embryo Implantation, azoospermic and teratospermic men.

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### Introduction:

Infertility affects about 20 to 28% of the population and primary and secondary infertility cover 80% and 20% of the infertility cases, respectively, in Iraq (1-2). It has been shown that infertility might affect 15 to 25% of couples in the United State (3). The important causes of male factor infertility include azoospermia, astheno-spermia, teratospermia, severely oligospermi or combination of these anomalies. In defective spermatogenesis and obstructive

azoospermic cases, the routine in vitro fertilization (IVF) was found to be unsuccessful. The treatments of choice for such male infertility cases are intracytoplasmic sperm injection (ICSI) technique combine with percutaneous epididymal sperm aspiration (PESA), microscopical epididymal sperm aspiration (MESA) or testicular sperm extraction (4).

Male patients with obstructive azoospermia are characterized by small volumes of ejaculates, normal testicular volumes and normal concentrations of gonadotropins (5). In cases of non-obstructive azoospermia, the testicular volumes are small and the concentrations of the reproductive hormone are abnormal. In the cases of non-obstructive

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azoospermia, the chance of viable sperm retrieval is less than in the cases of obstructive azoospermia and the success of viable sperm aspiration depends on the severity of germ cell damage (6). It has been shown that despite the severe defect in spermatogenesis in non-obstructive azoospermic men, bilateral differential testicular biopsies and testicular sperm extraction (TESE) techniques demonstrate the presence of viable sperm available for ICSI treatment (7). The use of the hypo-osmotic swelling test (HOST) to examine the viability of the testicular, epididymal and ejaculated sperm cells prior to performing ICSI was found in animals and humans to be a useful technique for identifying viable spermatozoa for intracytoplasmic sperm injection (4,8).

The objective of the experiment one of the present research was to compare the fertilizing capacity, in vitro embryonic developmental rate (percentages) and embryo implantation following the use of ejaculated sperm from severely teratospermic men (STSG), sperm retrieved by percutaneous epididymal sperm aspiration (PESA) in azoospermic patients (ASEG) and testicular sperm extraction in azoospermic men (ASTG). The objective of experiment two was to compare the TESE-ICSI outcome using sperm from obstructive and non-obstructive azoospermic infertile male patients.

## Materials And Methods:

### Experiment one

The total number of the infertile male patients involved in experiment one of this study was 119. The mean age of the male patients was 38.59 years with a range from 27 to 51 years. The mean age of the female partner was 33 years and the mean duration of the infertility was 8.35 years. The women had normal ovulatory cycles. Reproductive hormone concentrations (FSH, LH, Prolactin, Progesterone and estradiol) were normal. The thyroid hormones and cortisol hormone concentrations were also normal. The major problem diagnosed in these couples was male factor infertility. Testicular volume and size were examined by ultrasound and palpation. A MiniVidus Machine was used to measure reproductive hormones in the male patients. Testicular biopsies and seminal fluid analyses were performed in all the males involved in this study. In the first experiment of this study, male patients were assigned randomly depending on the sources of spermatozoa into three groups. The number of the male patients in the first group was 44. Their semen was characterized by severe terato-spermia (STSG), and these patients were gone failed in vitro fertilization (IVF) trials. The number of the male patients in the second group was 35. Their semen was characterized by azoospermia and these patients were assigned to the azoospermic epididymal group (ASEG). They were treated by percutaneous epididymal sperm aspiration (PESA) and ICSI

program. The number of male patients in the third group was 40 and their semen was characterized by azoospermia and named azoospermic testicular group (ASTG). These males were assigned to testicular sperm extraction (TESE) and ICSI program. The hypo-osmotic swelling test (HOST) was used to check sperm viability prior to ICSI procedure. The routine technique of ICSI-ET is described in details elsewhere (4,7,9).

### Experiment Two

The total number of the male patients involved in the experiment two was 77 azoospermic men. The mean age of the men was 36.38 years and the mean age of the females was 32.49 years and the mean duration of infertility was 9.60 years. The male patients were divided into two groups according to the type of azoospermia. The first group had obstructive azoospermia and named obstructive azoospermic group (OASG). The number of the male patients in OASG was 35. The second group was without obstructive azoospermia and named non-obstructive azoospermic group (NASG). The number of the male patients involved in the NASG was 42. All male patients were received TESE-ICSI treatment.

Seminal fluid analyses and fructose level assays of the seminal plasma were performed for all men in both groups. Hormonal analyses were performed for all patients to measure reproductive hormone concentrations (Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), testosterone and prolactin). Thyroid hormones were also measured in the blood of all patients in both groups. Testicular volume was examined by ultrasound and palpation. Bilateral differential testicular biopsies were performed to all the patients.

The fertilization, in vitro embryo growth, embryo implantation and pregnancy rates were recorded following oocyte retrieval and ICSI treatments (7,9,10).

The pregnancy rate per patient with embryo transfer was checked by the B-HCG test two weeks after embryo transfer. Viable fetal sac percent per pregnant women (fetal heart beat) was examined four to five weeks following embryo transfer.

Statistical analysis of the data was performed by using ANOVA. Data were presented as mean (standard error or the mean). Student t-test and Chi-square test were used for analysis of the data. The level of statistical significance was defined as when the P value was less than 0.05 (11).

### Results

The reproductive hormone concentrations and the size of the testes in the obstructive and non obstructive infertile male patients are shown in table 1. The concentration of FSH was 7.04 (1.08) mIU/ml in the obstructive azoospermic group compared to 19.25 (2.10) mIU/ml in the nonobstructive group. The

statistical differences between both groups were significant ( $P < 0.001$ ). The concentration of the LH hormone in the nonobstructive azoospermic group was significantly higher than the obstructive group (10.74 (1.30) mIU/ml versus 5.06 (1.58) mIU/ml,  $P < 0.001$ ). The concentration of the testosterone hormone in the obstructive azoospermic group was significantly higher ( $P < 0.001$ ) compared to the non-obstructive azoospermic group (6.85 (1.37) versus 3.28 (1.24) ng/ml). The prolactin hormone

concentration in the non-obstructive azoospermic group was significantly higher ( $P < 0.001$ ) than the obstructive azoospermic group (11.05 (2.16) ng/ml versus 7.50 (1.85) ng/ml). The size of the testes in the obstructive azoospermic group was significantly larger ( $P < 0.01$ ) than the non-obstructive azoospermic group (4.45 (0.11) cm versus 3.28 (0.13) cm)

**TABLE 1. The Concentration of reproductive hormones and the size of testes in the non obstructive (NASG group) and obstructive (OASG group) azoospermic men.**

Parameters	Non-obstructive azoospermic group	Obstructive azoospermic group -
Male patient No,	42	35
FSH (mIU/ml)	19.25 (2.10)	7.04 (1.08)*
LH (mIU/ml)	10.74 (1.30)	5.06 (1.58)*
Testosterone (ng/ml)	3.28 (1.24)	6.85 (1.37)*
Prolactin (ng/ml)	11.05 (2.16)	7.50 (1.85)*
Testicular Size (cm)	3.28 (0.13)	4.45 (0.11)**

\* $P < 0.001$  highly significant difference between groups. \*\*  $P < 0.01$  highly significant difference between groups. Data are mean with standard error of the mean (SEM).

The results of the ICSI treatment following the injection of ejaculated (STSG), epididymal (ASEG), and testicular (ASTG) sperm into the cytoplasm of the oocytes are shown in table 2. The total number of the oocytes recovered from the women in the STSG, ASEG and ASTG was 298, 246 and 268 oocytes respectively. There were no significant differences in the number of oocytes recovered per patient in the studied three groups ( $P > 0.05$ ). The ICSI rate (percentage of fertilized oocytes with two polar bodies and two pronuclei) was significantly lower in the ASTG group compared to other groups ( $P < 0.05$ , 60.82 versus

68.70, 60.82). The in vitro embryonic developmental rate was not significantly different among the three studied groups ( $P > 0.05$ ). The number of embryos transferred per patient was significantly lower in the ASTG compared to the other groups ( $P < 0.05$ , 3.07 versus 3.77, 4.07). The pregnancy rate was 40% in the ASTG, 47.73% in the STSG and 48.57% in the ASEG ( $P > 0.05$ ). The embryo implantation rate (viable fetal sac percentage per pregnant women) was not significantly different between the STSG, ASEG and ASTG (34.09%, 31.43%, 35%, respectively,  $P > 0.05$ ).

**TABLE 2. The ICSI outcome in the severely teratospermic group (ejaculated sperm), azoospermic epididymal group (epididymal sperm) and azoospermic testicular group (testicular sperm).**

Groups	Ejaculated sperm**	Epididymal sperm	Testicular sperm
Patient No.	44	35	40
Oocytes No	298.	246	268
Oocytes/patient	6.77	7.02	6.70
ICSI rate (%)	73.83	68.70	60.82*
Embryo growth (%)	81.82	78.11	75.46
Embryo transferred per patient (NO.)	4.09	3.77	3.07*
Pregnancy rate (%)	47.73	48.57	40
Viable fetal sac (%) (Implantation Rate)	34.09	31.43	35

\* $P < 0.05$  significantly different from other groups. \*\*One set of male twins was born in this group.

A comparison of the results of ICSI outcome was performed between the obstructive and non-obstructive azoospermic men following TESE and ICSI-ET treatment and the results are shown in table 3. The number of the retrieved oocytes in the non-obstructive group (NASG) was 220 and in the obstructive group (OASG) were 175. The percentage of the fertilized oocytes in the nonobstructive azoospermic group was significantly decreased ( $P < 0.05$ ) compared to the obstructive azoospermic group (62.73 versus 74.29). The rate of in vitro embryo development was significantly increased ( $P < 0.01$ ) in the obstructive group compared to the non-obstructive group (88.46

versus 60.87). The percentage of the embryos transferred was significantly higher ( $P < 0.05$ ) in the obstructive group than the non-obstructive group (81.74 versus 75). The number of the embryos transferred in the non-obstructive group was significantly lower ( $P < 0.05$ ) compared to the obstructive group (1.50 versus 2.68 respectively). The pregnancy rate per patient with embryo transfer was 42.86% in the obstructive group versus 35.71% in the non-obstructive group ( $P > 0.05$ ). The percentage of viable fetal sac per pregnant woman five weeks following embryo transfer was not significantly different between the obstructive and non-obstructive azoospermic groups ( $P > 0.05$ ).

**TABLE 3. Intracytoplasmic sperm injection and embryo transfer outcome in obstructive and non-obstructive azoospermic men.**

Parameters	Obstructive group***	Non-obstructive group
Patient No.	35	42
Oocytes No.	175	220
ICSI rate (%)	74.29	62.73*
Embryo growth (%)	88.46	60.87**
Embryos transfered (%)	81.74	75.0*
No. embryo transferred per woman	2.68	1.50*
Pregnancy rate (%)	42.86	35.71
Viable fetal sac (%)	37.14	30.95

\* $P < 0.05$  significantly different from corresponding group. \*\* $P < 0.01$  significantly different from corresponding group. \*\*\* One set male twins was born in this group.

**Discussion:**

The significantly greater concentration of FSH hormone in the non-obstructive azoospermic group compared to the obstructive azoospermic group may be due to decreased germinal cell mass and reduction in the function of Sertoli cells which results in defective spermatogenesis (5, 12). It was shown that the elevated FSH levels in the absence of genetic syndrome in non-obstructive azoospermic cases indicate spermatogenic maturation arrest, tubular fibrosis or Sertoli cell syndrome with focal areas of spermatogenesis (7, 13). The damage in the function of Sertoli cells results in decreased secretion of inhibin which controls FSH release by the negative feedback mechanism and this causes an increase in FSH levels (14, 15). The significant increase in the concentration of LH hormone in the nonobstructive azoospermic group compared to the obstructive azoospermic group may be due to a severe degree of Leydig cell depletion and or a genetic defect in spermatogenesis (16). The significantly lower concentration of testosterone hormone in the non-obstructive azoospermic group versus the obstructive group is an indication of suppressed function of Leydig cells which control LH hormone secretion by the negative feedback control mechanism (13). Prolactin hormone concentration was significantly higher in the non-obstructive azoospermic group compared to the obstructive azoospermic group. This elevated level of prolactin hormone may be due to stress and/or abnormality in the function of prolactin secreting cells in the anterior pituitary gland. Hyperprolactinemia in the human causes hypogonadotropic hypogonadism with decreased LH and FSH secretions and infertility. It has been found that marked hyperprolactinemia inhibits sperm production in male patients and results in azoospermia or severe oligospermia (17).

The significant bilateral reduction in testicular size in the non-obstructive azoospermic group compared to the obstructive azoospermic group may be secondary to Leydig cell hypofunction and reduction in testosterone production. Nonobstructive azoospermic men are characterized by small testicular size, reduced testosterone and increased in FSH and LH levels. Conversely, obstructive azoospermic men usually have normal testicular size, normal concentrations of FSH and LH hormones but reduced ejaculate volume (5, 6).

The percentage of fertilized oocytes in the ASTG (testicular sperm) was significantly decreased compared to the ASEG (epididymal sperm) and STSG (ejaculated sperm). This may be due to reduce sperm viability in the ASTG and/or abnormality in sperm ultrastructure (4). Other investigators (18) reported 63.7% and 72.9% ICSI rates using testicular and epididymal sperm, which confirm our results

(we observed 60.82% in testicular group and 68.70% in the epididymal group). The rate of embryo development in vitro was not significantly different among the three groups (ASTG, ASEG, STSG), which indicates that they have similar potential for in vitro embryonic development (19). The increased number of embryos transferred per patient in the STSG compared to the testicular group may be due to an increase in the total number of the retrieved oocytes in the this group. It may also be due to increased numbers of the fertilizable oocytes since only viable sperm was used in ICSI procedure.

The pregnancy and implantation rates were not significantly different among the three groups, which indicates that the origin and source of sperm retrieval have no effect on embryo implantation and pregnancy rate. Similar data were reported by other workers, which agree with the results of the present study (20).

A comparison was made between the ICSI outcome of non-obstructive and obstructive azoospermic infertile men. The ICSI and in vitro embryo development rates were significantly higher in the obstructive azoospermic group compared to the non-obstructive group. This may be due to the good quality of the sperm that were retrieved in the obstructive azoospermic group. In the non-obstructive azoospermic group, the cause may be due to defects in spermatogenesis, which affect quality and viability of sperm cells. Low fertilization rate in the non-obstructive azoospermic group may also be due to failed oocyte activation or incomplete and abnormal decondensation of the sperm head inside the oocyte (21-23). Other workers reported similar observations in azoospermic men when unviable sperm were injected into the oocyte, which are consistent with the data of the present study (4). Number of the embryos transferred per woman in the obstructive group was significantly higher than the nonobstructive group. This may be due to the significantly higher fertilization rate and in vitro embryonic development in the obstructive azoospermic group.

There were no significant differences in the pregnancy rate between the obstructive and nonobstructive groups, which indicate that the implantation potential of both groups are similar. Palermo et al (19) reported that the positive ICSI outcome is not dependent on sperm concentration, morphology or motility in men who have severe impairment in sperm concentration, motility and morphology. Successful fertilizations have been achieved by ICSI using immature testicular and epididymal

sperm, which clearly indicate that it is possible to have a offspring by bypassing testicular and epididymal sperm maturation, sperm acrosome reaction, binding to the zona pellucida and fusion with oolema (24). Palermo, et al (18) reported that in spite of a higher frequency of genetic anomalies in infertile men these men could be treated with ICSI technique without a significant increase in adverse outcomes of offspring. Steirteghem, et al. (20) reported that the outcome of several thousands of ICSI cycles in terms of fertilization, embryo cleavage and implantation was similar to that for conventional IVF in couples with tubal or idiopathic infertility.

It was concluded from the results of the present study that the sources of sperm, either ejaculated, epididymal or testicular sperm used for intracytoplasmic sperm injection have no effect on embryo implantation and pregnancy rates. Similar results were observed in obstructive and nonobstructive azoospermic men, which indicate that the etiology of azoospermia has no effect on embryo implantation potential and the development of viable pregnancies.

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