Embryonic Implantation Outcome Using Semen from Men with Obstructive and Non-Obstructive Azoospermia: Comparative Study.
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Key words: Azoospermia, ICSI Outcome, Embryo Implantation.

Abstract:
Background: Infertility affects about 20% to 28% of the general population. Primary and secondary infertility account for 80% and 20% of infertility cases respectively. It has been shown that the major male factor infertility include azoospermia, oligospermia, asthenospermia, and teratospermia or combination of these factors.
Objectives: The objective of the study was to compare intracytoplasmic sperm injection (ICSI) and embryo implantation outcomes using testicular sperm extraction (TESE) in obstructive and non-obstructive azoospermic men. The male patients were divided into two groups depending on the etiology of azoospermia. Group one (n=35) had an obstructive azoospermia (OAZ) and group two (n=42) had a non-obstructive azoospermia (NOA). Both groups underwent TESE-ICSI program.
Materials and Methods: The patients were divided into two groups depending on the etiology of azoospermia. Group one had OAZ (n=35) and group two had NOA (n=42). Seminal fluid analyses, reproductive hormones assay, bilateral differential testicular biopsies, testicular sperm extraction and ICSI were performed. The study was carried out at IVF Institute for Embryo Research and Infertility Treatment and the Departments of Obstetrics/Gynecology, Urology and Physiology/ Medical College, Baghdad University Teaching Hospital. The hypo-osmotic swelling test was used to check sperm viability prior to the ICSI procedure. The pregnancy and embryo implantation were checked by Beta-HCG test and ultrasound examination 12 and 35 days after embryo transfer respectively to report viable pregnancies.
Results: Concentration of follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL) was significantly higher in NOA group compared to the OAZ group. The testosterone level and the testicular volume were significantly higher in the OAZ group compared to NOA group. The results of the study showed that the ICSI rate (fertilization rate), cleavage rates, and the number of transferred embryos were significantly higher in the OAZ group compared to NOA group. The pregnancy rate were similar between both groups. The embryo implantation rate per embryo transfer was significantly lower in NOA group compared to the OAZ group.
Conclusions: The type of azoospermia in humans have a significant adverse impact on embryo implantation. The percent of viable fetuses was significantly affected in the NOA compared to OAZ male patients. These findings indicate that viable and morphologically normal looking spermatozoa under high power dissecting microscope are not necessarily genetically normal in non-obstructive azoospermic patients.
الخلاصة

نتائج زرع الأنجج عند استخدام السائل المنوي للرجال المصابين باللانطفية بسبب
انسداد أو عدم انسداد القنوات المنوية: دراسة مقارنة سريرية

стьيد الآثاري، على رضا، منذر البر زنجي، شهيلة الأطرجي، زيد الثانم، وفاء العمير

لاستقصى المعهد الاختبائي واجبات الأنجج، مستشفى بغداد التعليمي، قسم التسائي والتوليد والبوليا، كلية الطب. جامعة بغداد

تراوح نسبة انتشار العقم بين الأزواج بشكل عام ما بين 20% حتى 28%، وتشمل العقم الأولي
80% والعمم الثاني 20% من الحالات. وبناءً على الدراسات أن عامل العقم الذكري تشمل حالات
إعدام النطف (اللانتيفية)، فئة النطف، التدفقات الخلقية للنطف، الشوهات الخلقية، مشاركة أكثر
من عدم العقم في حالة العقم الذكري.

كان البندف من هذه الدراسة مقارنة نتائج عمليات الحقن المجهري للحيوان المنوي داخل
سيتوبلازما البويبة (ICSI)، ومعدلات انغ desea (زرع) الأنجج في حالات مرضا العقم المصابين
باللانتيفية. تم تقسيم المرضى إلى مجموعتين. ضمت المجموعة الأولى المصابين بانسداد القنوات
المنوية وعددهم 35 مريضاً. في حين ضمت المجموعة الثانية المرضى المصابين بانعدام النطف
لا إنسدادي وعددهم 42 مريضاً. تم إجراء عملية الخزعة الطبيعية للخصية لاستخراج النطف
و عملية الحقن المجهري للاقل المجموعتين. إضافة إلى ذلك، تم استخدام تحميل
بروجستيرون المهيمنة (Duphasone) والبروجستيرون الفموية (Cyclogest 400 mg)
والبرينيدنولون (Prednisolone)

لوجد رابط بين تراكيز البرولاكتين وFSH وLH كانت عالية في المجموعة النلا إنسدادية. مقارنة مع
المجموعة المصابية بانسداد القنوات المنوية وبدلاً للاحتقائية معتبة.

كان تركيز هرمون التستيسترون أعلى وحجم الخصية أكبر في المجموعة المصابية بانسداد القنوات
المنوية في المجموعة المتصابية بانسداد القنوات المنوية بعملية إحفاش مصابة باللانتيفية. وبناءً على
ناتج البحث وجود زيادة (ICSI) إضافة إلى زيادة عند الإنجج المنمو (أداء إحتقائي) داخل
الرحم في المجموعة المتصابية بانسداد القنوات المنوية بمجموعة اللاحقا إنسدادية. إن
معدل الحمل كان متشابهاً في المجموعتين بينما كان معدل الحمل قياسياً عند الإنجج التي تم
غرتها داخل الرحم مرتفعاً وبدلاً للاحتقائية معتبة في المجموعة المتصابية بانسداد القنوات
المنوية قياسياً بالاحقا إنسدادية.

يستنتج من هذه الدراسة أن سبب اللانتيفية في الذكور يلعب دوراً فعالاً في مدى القدره الزرعية
(الانغ desea) للنجج الناتجة عن عمليات الحقن المجهري. إن وجود نطفة (حيامن) حياة ذات
أشكال طبيعية أتى مع عمليات الحقن المجهري لدى المرضى المصابين بانعدام النطف الشاهد
على نجاح الأنجج الناتجة منها، فكلما حاولت إعداد أنجج لها قدرة طبيعية
للانغ desea في داخل الرحم إذ أن هؤلاء المرضى يعانون من اللانتيفية، بحال الأمر
في عمليات تكون النطف في الخصية.

الكلمات الدلالة: اللانطفية، انسداد الانتصاب المنوي، الاختبائي المجهري الإجباري، زرع الأنجج البشري.
Introduction:
Infertility affects about 20 to 28% of general population. Primary and secondary infertility account for 80% and 20% of the infertility cases, respectively (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 (MFI) include azoospermia, asthenospermia, teratospermia, severe oligospermia, immunological infertility or a combination of these anomalies.

Patients with OAZ are characterized by small volume of ejaculate, normal testicular size and reproductive hormone concentrations. Non-obstructive azoospermic men have defective spermatogenesis. Testicular sperm extraction (TESE) is used for sperm aspiration in OAZ and NOA patients in whom sperm could not be obtained by percutaneous epididymal sperm aspiration (PESA) or microscopic epididymal sperm aspiration (MESA) procedures (4-6). In severe oligospermia, asthenospermia, teratospermia, NOA and OAZ, the conventional in vitro fertilization (IVF) technique is not advisable due to the low sperm count and motility in addition to high morphologically abnormal sperm including round-headed sperm in the infertile semen. The fertilization rates of these abnormal sperm are very poor and result in the production of abnormal preimplantation embryos. The treatment of choice for such MFI is TESE, PESA or MESA combined with ICSI technique (7-8).

In cases of NOA, the testicular volume is small and the concentrations of the reproductive hormone are abnormal. The chance of viable sperm retrieval is less compared to OAZ cases. The success of viable sperm aspiration depends on the severity of germ cell damage in NOA men. It has been shown that despite the severe defect in spermatogenesis in NOA men, bilateral differential testicular biopsies and TESE techniques demonstrate the presence of focal areas of normal spermatogenesis in seminiferous tubules. In these cases, viable normal sperm are available for ICSI-TESE treatment (7). The use of the hypo-osmotic swelling test (HOST) to examine the viability of the testicular sperm cells prior to ICSI was found to be a useful technique for identifying viable spermatozoa from non-viable sperm for ICSI program (9-10).

The objective of the study was to compare the TESE-ICSI outcome in terms of embryo implantation and fetal development in OAZ and NOA male patients.

Materials and Methods:
The mean age of the male patients was 38.6 years with a range from 27 to 50 years. The mean age of the female partner was 30 years and the duration of the infertility was from 2-8 years. The women had normal ovulatory cycles with normal reproductive hormone concentrations. The thyroid hormones and cortisol hormone concentrations were also normal. The major problem diagnosed in these couples was azoospermia.

Testicular volume, size and consistency were examined by ultrasound and palpation. A Minividus machine was used to measure reproductive hormone concentrations in the male and female patients. Seminal fluid analyses and testicular biopsies were performed in all the azoospermic men. The patients were divided into two
groups depending on the etiology of azoospermia. Group one (n=35) had OAZ and group two (n=42) had NOA. Both groups were involved in TESE-ICSI and embryo transfer (ET) program. The hypo-osmotic swelling test (HOST) was used to check sperm viability prior to ICSI procedure. The Beta-HCG was checked 12 days after ET and double checked after two days. The embryo implantation per embryo transfer was examined by ultrasound 4-5 weeks following ET to report viable pregnancies with active heart beating. The routine technique of ICSI and embryo transfer is described in detail elsewhere (9-12). Statistical analysis of the data was performed by using Student t-test and Chi-square analysis. Data were presented as mean with standard deviation. The level of statistical significance was defined as a P value less than 0.05 (13).

Results:

The concentration of FSH in the OAZ group was significantly lower compared to the NOA group (7.04 IU/l versus 19.25 IU/l, P<0.001). The concentration of the LH hormone in the NOA group was significantly higher than the OAZ group (10.74 IU/l versus 5.06 IU/l, P < 0.001). The concentration of the testosterone hormone in the OAZ group was significantly higher compared to the NOA group (6.85 versus 3.28 ng/ml, P <0.001). The prolactin hormone concentration in the NOA group was significantly higher compared to the OAZ group (11.05 ng/ml versus 7.50 ng/ml, P <0.001). The testicular size in the OAZ group was significantly larger than that of NOA group (24.45 cm³ versus 13.28 cm³ respectively, P <0.01). The reproductive hormone concentrations and the testicular size in the obstructive and non-obstructive infertile male patients are shown in table 1.

The results of the study with regard to ICSI outcome in OAZ and NOA men are shown in table 2. The ICSI rate (fertilization rate by ICSI procedure), embryo cleavage rate and the number of transferred embryos per woman were significantly higher in the OAZ group versus NOA group. The pregnancy rate was 42.9% in OAZ group and 35.7 in NOA group. The percentage of viable fetuses per patient was significantly lower in the NOA group compared to the OAZ group respectively (30.1% versus 41.7%, P<0.01, Table 2).

Discussion:

The significantly higher concentration of FSH hormone in the NOA group compared to the OAZ group may be due to decreased germinal cell mass and reduction in the function of Sertoli cells which results in defective spermatogenesis (5, 14). It was shown that elevated FSH levels in the absence of genetic syndrome in NOA cases indicate spermatogenic maturation arrest, tubular fibrosis or Sertoli cell only syndrome with focal areas of spermatogenesis (7, 15). The damage to 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 (16-17). The significant increase in the concentration of LH hormone in the NOA group compared to the OAZ group may be due to a severe degree of Leydig cell depletion and/or a genetic defect in spermatogenesis (18). The significantly lower concentration of testosterone hormone in the NOA group versus the
OAZ group is an indication of suppressed function of Leydig cells which control LH hormone secretion by the negative feedback control mechanism (15). Prolactin hormone concentration was significantly higher in the NOA group compared to the OAZ group. This elevated level of prolactin 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, FSH, testosterone secretions and infertility. It has been found that marked hyperprolactinemia inhibits sperm production in male patients and results in azoospermia or severe oligospermia (19).

The significant bilateral reduction in testicular size in the NOA group compared to the OAZ group may be secondary to Leydig cell hypofunction and reduction in testosterone production. Non-obstructive azoospermic men are characterized by small testicular size, reduced testosterone and increased 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, 8).

In the NOA group, the reduction in the ICSI rate, embryo cleavage rate and the number of embryos with transferable quality may be due to defects in the process of spermatogenesis, which affect quality and viability of sperm cells. These patients showed abnormal concentrations of FSH and LH in their blood. Low fertilization rate in the NOA group may also be due to failed oocyte activation or incomplete and abnormal decondensation of the sperm head inside the oocyte (20-21). Other workers reported similar observations in NOA men when nonviable sperm were injected into the oocytes, which are consistent with the data of the present study (22-23).

The development of viable pregnancy was significantly better in the OAZ group compared to NOA group. This indicates that the embryo implantation potential in the OAZ group is superior to that of NOZ group (24-26). Palermo et al (21) 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 sperm, which clearly indicate that it is possible to have an offspring by bypassing testicular sperm maturation, acrosome reaction, binding to the zona pellucida and fusion with oolemma.

In spite of a higher frequency of genetic anomalies in infertile men, these men could be treated with TESE-ICSI technique without a significant increase in abnormal outcomes of offspring. It is 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. The loss of the fetuses following embryo implantation may be attributed to defect in sperm, oocyte and/or embryo (27-28). The endometrial filter does not allow implantation of abnormal embryos and usually these abnormal embryos result in early abortions unless the endometrial filter is malfunctioning (29).

In other studies we reported that the outcome of ICSI in terms of pregnancy and embryo implantation were improved in the NOA patients who underwent
differential bilateral testicular biopsies (DBTB) and TESE-ICSI compared to TESE-ICSI without DBTB to localize viable sperm and/ or spermatid available for ICSI procedure (4, 9, 27-28). These studies agree with the results of the present study.

It was concluded from the results of the present study that the non-obstructive azoospermia in men have detrimental impact on fetal development. The significant reduction in fetal development that observed in the non-obstructive azoospermic group compared to obstructive azoospermic group indicates that the spermatogenesis and genetic normality of the sperm are essential factors for the development of normal gestation sac and viable fetuses in women.

**Acknowledgments:**
The authors thank the Ministry of Health and the Ministry of Higher Education and Scientific Research for their financial support. We are also grateful to the Director of Baghdad Teaching Hospital for his kind assistance and support.

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**References**


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Table 1. Concentration of reproductive hormones and testicular volume in the obstructive and non-obstructive azoospermic men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-obstructive azoospermic group</th>
<th>Obstructive azoospermic group</th>
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Table 2. Comparison between Intracytoplasmic sperm injection and embryo transfer outcomes in obstructive and non-obstructive azoospermic men

<table>
<thead>
<tr>
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<th>Group 1 (42)</th>
<th>Group 2 (35)</th>
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<tr>
<td>Number of patients</td>
<td>42</td>
<td>35</td>
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<tr>
<td>FSH (IU/l)</td>
<td>19.3 +/- 2.1</td>
<td>7.0 +/- 1.1*</td>
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<tr>
<td>LH (IU/l)</td>
<td>10.7 +/- 1.3</td>
<td>5.1 +/- 1.6*</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>3.3 +/- 1.2</td>
<td>6.9 +/- 1.4*</td>
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<tr>
<td>Prolactin (ng/ml)</td>
<td>13.1 +/- 2.2</td>
<td>7.5 +/- 1.6*</td>
</tr>
<tr>
<td>Testicular Size (cm³)</td>
<td>13.3 +/- 0.1</td>
<td>24.7 +/- 0.1**</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of the mean (SEM)
*P < 0.001 significant difference between groups.
** P < 0.01 significant difference between groups.
<table>
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<tr>
<th>Parameters</th>
<th>Obstructive Group*** (OAZ)</th>
<th>Non-Obstructive Group (NOA)</th>
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<tr>
<td>No. of Patients</td>
<td>35</td>
<td>42</td>
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<tr>
<td>No. of Oocytes</td>
<td>175</td>
<td>220</td>
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<tr>
<td>ICSI Rate (%)</td>
<td>74.3</td>
<td>62.7*</td>
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<td>Embryo Cleavage (%)</td>
<td>88.5</td>
<td>60.9**</td>
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<td>ET/patient</td>
<td>2.7</td>
<td>1.5*</td>
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<td>Pregnancy Rate /patient (%)</td>
<td>42.9</td>
<td>35.7</td>
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<td>Viable Fetuses/Patient (%)</td>
<td>41.7</td>
<td>30.1**</td>
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*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