Embryo Grading and Pregnancy Outcome in Women of Patients with Azoospermia Following ICSI

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Abstract

Background:

Azoospermia is defined as the lack of sperm in male ejaculate and is suggested to be present in about 1 % of all males and to account for 10-15% of the causes of infertility in men**.** The development of intracytoplasmic sperm injection (ICSI) as an effective therapy. Embryo grading has gained special attention in published literature regarding its predictive role in pregnancy outcome following ICSI; however, substantial controversy is present that justified the carrying out of the present study.

Objective:

To study the association between embryo quality and pregnancy rate in women of males with azoospermia.

Patients,Materials and Methods:

This prospective cohort study included 60 patients with azoospermia, 15 male with obstructive type and 45 males with non-obstructive type.Those patients were randomly selected form the population of infertile couples who regularly visits the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies and from Kamal Al-Samarrai Hospital -Center for Infertility Treatment and IVF.

Results

Pregnancy was encountered in 7 patients (23.33%). It was not statistically associated with type of azoospermia (P=0.388), also it was not affected by age and serum hormonal level**,** but highly determined by good quality of embryo. Quality of embryo was graded from 1 to 4. The lower the grade, the better the quality of the embryo was. Quality of embryo was not significantly correlated with type of azoospermia, age and hormonal levels.

Conclusions

Good quality of embryo is a significant predictor of positive pregnancy outcome in ICSI and the quality of embryo is not affected by age of male patient or type of azoospermia.

Key words: **Azoospermia, ICSI, embryo grading**

Introduction:

Azoospermia is defined as the lack of sperm in male ejaculate and is suggested to be present in about 1 % of all males and to account for 10-15% of the causes of infertility in men[(1)]. It is classified into two major forms: either obstructive or non-obstructive. The presence of organic obstruction in the reproductive male pathways distal to the testes is the pathognomonic pathophysiology in obstructive type [(2)]. Non-obstructive azoospermia (NOA) is often regarded as a non-medically manageable reason of infertility in men. These patients, who make up to 10% of global infertile male subjects, have abnormal spermatogenesis as with consequent azoospermia. The establishment of *in vitro* fertilization using intracytoplasmic sperm injection (ICSI) as a mainstay treatment modality made a number of these male patients to be successful biologic fathers a child via surgically retrieved sperm from the testis. The obstacle, however, is to facilitate their spermatogenic activity to enable the acquisition of sperm in their ejaculate or to improve the opportunity of a successful sperm retrieval from the testis for ICSI[(3)]. An accurate diagnosis of azoospermia and thorough evaluation of the patient to diagnose the cause of infertility are needed to guide appropriate treatment options and to identify the associated cost advantages, risks and hope for treatment success.The development of intracytoplasmic sperm injection (ICSI) as an effective therapy for profound male factor infertility has become a definite treatment for the majority of male reproductive tract

abnormalities [1].The outcome of assisted reproductive techniques has been shown to be affected by several paternal and maternal factors as well as factors related to embryo quality. The quality of embryo has been shown to be a significant determinant of outcome of pregnancy by several authors; while others found no significant association between clinical pregnancy outcome and embryo quality following assisted reproduction [4]. It has been postulated by several authors that sperm quality may negatively affects embryo quality and implantation rate who stated that low fertilization and cleavage rates, significantly impaired embryo quality and blastocyst formation were also observed, especially when testicular sperm from OA and NOA patients was used for ICSI[5].

Patients, Materials and Methods:

This prospective cohort study included 60 patients with azoospermia, 15 male with obstructive type and 45 males with non-obstructive type. Those patients were randomly selected form the population of infertile couples who regularly visits the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies and from Kamal Al-Samarrai Hospital center of infertility treatment and IVF (Baghdad / Iraq). Testicular biopsies of azoospermic patients were obtained and fixed with modified Zenker (fixative) and then processed routinely to produce paraffin blocks. From each paraffin block, 2sections of 5 µm thickness were taken: one section was stained with H&E to study histopathological changes and the other was stained immunohistochemically for MCL-1 monoclonal antibody. All male patients were subjected to hormonal assays including: FSH, LH,

testosterone and prolactin, whereas female partners were subjected to assessment of FSH, LH, progesterone and estradiol. Embryo quality was graded from 1 to 4 score in which grade 1 score indicated the best quality of embryo and with increasing score the quality get less good according to Veeck's scoring system[4].Statistical analysis was performed using SPSS version 22. Data were presented as number and percentage for categorical variables or as mean ±SD for numerical variables. For the analysis of numeric variables, independent student test was used in case of normal distribution and Mann Whitney U test in case of non-normal distribution. Chi-square test and Fischer Exact tests were used to study association between any two categorical variables. Correlation and regression analysis was used between immunohistochemical and histological scoring variables. The level of significance was considered at 0.05.

Results:

General characteristics of the Azoospermic men and their female partners are shown in tables 1 and 2.

Mean serum hormonal levels with corresponding ranges were presented in table 3 and figure 1. Comparisons revealed the following: serum FSH, LH and prolactin were significantly higher in the non-obstructive group whereas serum testosterone was significantly lower in the non-obstructive group.

Table 2: General characteristics of female partners

Characteristic	Group 1	Group 2	Total	P
Age (mean±SD) years	29.00 ±4.61	27.60 ±6.12	28.30 ±5.37	0.485*
Infertility type (Primary/Secondary)	12/3	15/0	27/3	0.244†
Duration (mean±SD) years	7.20 ±2.78	5.60 ±4.27	6.40 ±3.64	0.234*
Weight (mean±SD) kg	68.07 ±8.72	66.73 ±9.51	67.40 ±8.99	0.692*
FSH (mean±SD) IU/ml	7.04 ±1.76	7.19 ±1.08	7.11 ±1.43	0.785*
LH (mean±SD) IU/ml	5.51 ±1.46	5.93 ±1.09	5.72 ±1.29	0.381*
Progesterone (mean±SD) IU/ml	15.89 ±3.81	15.73 ±3.71	15.81 ±3.70	0.912*
Estradiol (mean±SD) IU/ml	157.93 ±59.05	115.00 ±44.71	136.47 ±55.90	0.042**

Group 1: Female partners of males with obstructive azoospermia; Group 2: Female partners of males with non-obstructive azoospermia; *Independent sample t-test; † Fischer Exact test; ** Mann Whitney U test

Table 1: General characteristic of the study sample

Characteristic	Obstructive (N = 15)	Non-Obstructive (N = 45)	Total (N = 60)	P*
Age (mean ±SD) years	34.07 ±6.82	33.07 ±7.84	33.32 ±7.55	0.661 †
Smoker N (%)	4 (26.7)	15 (33.3)	19 (31. 7)	0.755*
Varicocele N (%)	3 (20.0)	8 (17.8)	11 (18.3)	1.000*
RGUTI N (%)	3 (20.0)	1 (2.2)	4 (6. 7)	0.073*
Chemical Toxin N (%)	2 (13.3)	7 (15. 6)	9 (15.0)	1.000*
Physical Toxin N (%)	3 (20.00)	10 (22.2)	13 (21. 7)	1.000*
Congenital anomaly N (%)	1 (6.7)	1 (2.2)	2 (3.3)	1.000*

†Independent samples t-test*Corrected Chi-square test; RGUTI: recurrent genitourinary tract infections

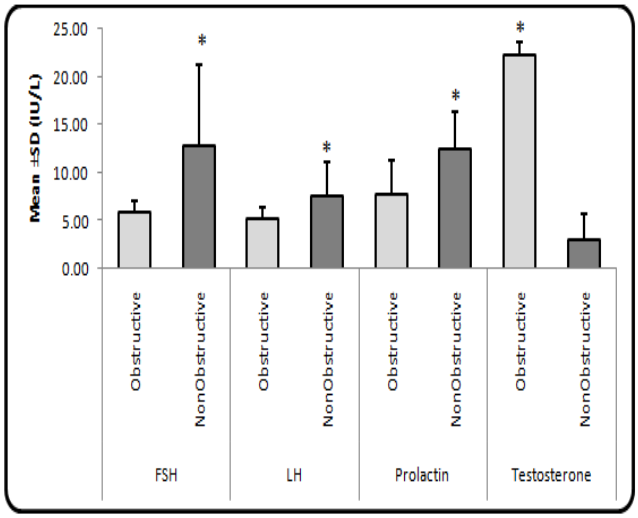

Figure 1: Bar chart showing comparison of mean serum hormonal levels between obstructive and obstructive azoospermia groups

Sperms were successfully isolated from 30 patients (50%) and the isolation rate was significantly higher in the obstructive azoospermia group than in the non-obstructive azoospermia group, 100% versus 33.3% (P<0.01). Sperm isolation rate was not affected by age of the patient, but it was significantly related to lower FSH, LH and prolactin and higher testosterone level, as shown in table 4.

Table 4: Factors predicting positive sperm isolation

Characteristic	Positive (N = 30)	Negative (N = 30)	P
Obstructive /non-obstructive (N)	15/15	0/30	<0.001*
Age (mean ±SD)	33.40±8.22	33.23±6.96	0.933**
FSH (mean ±SD)	9.09±8.29	13.10±6.93	0.001†
LH (mean ±SD)	6.49±3.89	7.52±2.64	0.028†
Prolactin (mean ±SD)	10.03±4.41	12.69±3.70	0.014**
Testosterone (mean ±SD)	12.60±9.92	3.13±2.82	0.001†

*Chi-square test; **Independent samples t-test; † Mann Whitney U test

Table 3: Comparison of mean serum hormonal levels between obstructive and non-obstructive azoospermia patients

Hormone	Group	Mean ± SD	Range	P
FSH	Obstructive	5.96±1.06	4.5 - 8.1	0.001*
	Non-Obstructive	12.81±8.37	2.4 - 38.6	
	Total	11.10±7.84	2.4 - 38.6	
LH	Obstructive	5.29±1.18	3.2 - 7.2	0.017*
	Non-Obstructive	7.58±3.63	1.6 - 18	
	Total	7.01±3.34	1.6 - 18	
Prolactin	Obstructive	7.75±3.62	4.7 - 20	<0.001†
	Non-Obstructive	12.56±3.76	0.55 - 21.4	
	Total	11.36±4.25	0.55 - 21.4	
Testosterone	Obstructive	22.20±1.39	20.1 - 24.6	<0.001*
	Non-Obstructive	3.09±2.58	0.86 - 15.5	
	Total	7.87±8.67	0.86 - 24.6	

*Mann Whitney U test; †Independent samples t-test

Successful fertilization was seen in all cases with positive sperm isolation, in other words fertilization rate was 100% with regard to sperm isolation. Quality of embryo was graded from 1 to 4. The lower the grade, the better the quality of the embryo was as shown in figures 2, 3 and 4. Quality of embryo was not significantly correlated with type of azoospermia, age and hormonal levels (table 5).Among those 30 patients with positive fertilization, pregnancy was encountered in 7 patients (23.33%). It was not statistically associated with type of azoospermia (P=0.388), also it was not affected by age and serum hormonal level, but highly determined by good quality of embryo (table 6).

Table 5: Factors predicting embryo quality

Characteristic	r	P
Type of azoospermia	0.321	0.073*
Age	-0.044	0.818
FSH	0.053	0.782
LH	0.052	0.787
Prolactin	0.210	0.265
Testosterone	-0.343	0.064

*Kindall's Tau_B correlation, †Spearman correlation

Table 6: Factors predicting positive pregnancy outcome

Characteristic	Positive (N = 7)	Negative (N = 16)	P
Obstructive/non-obstructive N	5/2	10/13	0.388*
Age (mean ±SD)	32.86 ±6.69	33.57 ±8.76	0.825†
FSH (mean ±SD)	7.46 ±5.26	9.58 ±9.05	0.750†
LH (mean ±SD)	6.33 ±4.79	6.54 ±3.70	0.432†
Prolactin (mean ±SD)	8.64 ±3.81	10.45 ±4.57	0.280†
Testosterone (mean ±SD)	17.01 ±10.12	11.26 ±9.68	0.091†
Embryo quality	r= -0.413		0.021**

*Corrected Chi-square test; **Kindall's Tau_b correlation; † Mann Whitney U test

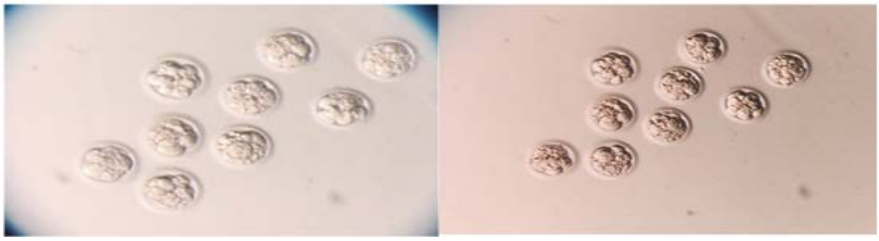

Figure 2: Example of grade 1 embryo

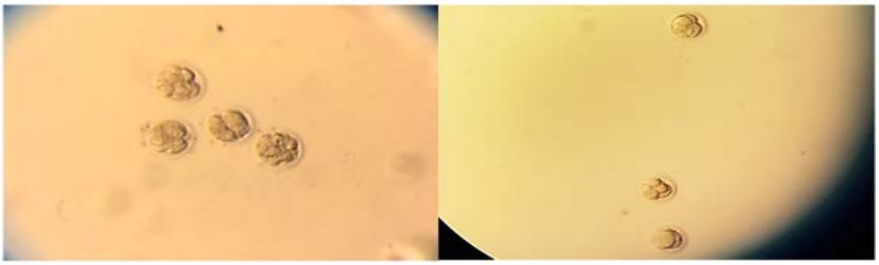

Figure 3: Example of grade 2 embryo

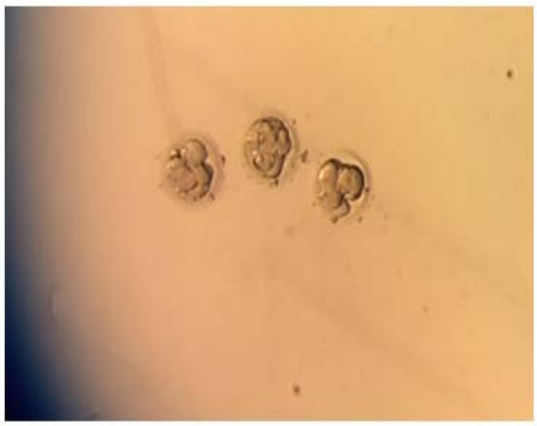

Figure 4: Example of grade 3 embryo

Discussion:

The present study showed that Sperms were successfully isolated from 30 patients (50%) and that the isolation rate was significantly higher in the obstructive azoospermia group than in the non-obstructive azoospermia group, 100% versus 33.3% (P<0.01); these results were comparable to the finding of other studies in which sperm retrieval rate was higher in obstructive azoospermia in comparison with non-obstructive type (61%) [(6)]. It was also, similar to the present study finding, stated by some authors that testicular sperm recovery from azoospermic males with all diagnoses was high (70 to 100%) except non-obstructive azoospermia (31%)[(7)]. In the current study, sperm isolation rate was not affected by age of the patient, but it was significantly related to lower FSH, LH and prolactin and higher testosterone level and also it was significantly determined by higher histological and MCL 1 scores. We agreed with Kalsi *et al*., 2015[(8)] that age of the patient has no significant effect on sperm retrieval rate; however we disagree with him in that FSH level had no significant impact on rate of sperm retrieval. In accordance with the present study findings, it was found that sperm isolation was significantly affected by FSH, LH and testosterone level[(9)].

The current study pointed to the finding that embryo quality was not significantly related to type of

azoospermia whether obstructive or non-obstructive. Similar finding was recorded by Demir *et al*., (2012)[10]; however we disagree with the finding of other authors that the OA group had better overall quality embryos than the NOA group [11]. It has been postulated by several authors that sperm quality may negatively affects embryo quality and implantation rate who stated that low fertilization and cleavage rates, significantly impaired embryo quality and blastocyst formation were also observed, especially when testicular sperm from OA and NOA patients was used for ICSI[5]. A possible explanation for this may be that testicular spermatozoa are less mature and subsequently less competent to fertilize than the ejaculated ones, since the final steps of sperm maturation take place in the epididymis. Moreover, studies have shown a higher incidence of chromosomal aneuploidies in testicular spermatozoa from NOA patients as compared to OA patients [12], which may explain, at least partly, the lower embryo development rates observed.

Several studies showed that there is no statistically significant difference in clinical pregnancy rates between the two [13] and this result solidify the finding of the present study which also found no significant difference in clinical pregnancy rate between obstructive and non-obstructive azoospermia rate. In accordance with the result of the present study it was found that there was no differences were noted in clinical pregnancy rate between obstructive azoospermia and non-obstructive azoospermia groups[7]. In contradiction to the finding of the current study, rate of fertilization was significantly lower with non-obstructive azoospermia[7].It has also been recorded that fertilization and clinical pregnancy rates was lower in non-obstructive azoospermia[14]; However a meta-analysis of surgical sperm retrieved in azoospermic patients concluded that

sperm origin does not affect assisted fertilization cycle outcome[15].Another similar result to our finding has been registered by Tsai *et al*., in 2015[16] who found no significant difference in clinical pregnancy rate between the two groups.

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