

Study of Theophylline Role in Sperm Selection for Intracytoplasmic Sperm Injection (ICSI) in Men with Non-Obstructive Azoospermia

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Abstract

Background:

In order to improve ICSI outcome, viable sperms should be selected and for that purpose a lot of procedure were tested including Hyposmotic swelling (HOS) test, Sperm tail flexibility test, Motility stimulant sperm challenge using Pentoxifylline. Nowadays, Theophylline has been investigated as a chemical substance for stimulating spermatozoa.

Aim of the study:

This study was designed to study ICSI outcome in non-obstructive azoospermic men subjected to testicular sperm extraction who will be classified into two groups. Sperm selection for ICSI by Sperm tail flexibility test is used for the first group and chemical selection by Theophylline will be used in the second group.

Patients and Methods:

The present case control study included 18 non-obstructive azoospermic men; all of them underwent ICSI procedure at The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies\AL-Nahrain University and extended from September 2016 to May 2017. They were divided into two subgroups according to way of selecting immotile but viable spermatozoa (Sperm tail flexibility test and Theophylline).

Results:

The median time needed for sperm selection in all patients in the study group was highly significant less than that needed for the control group, 9.50 versus 37.00 minutes ($P < 0.001$). Median time for single sperm isolation was highly significant less in study group than in control group, 1.84 versus 4.75 minutes ($P < 0.001$). Additionally, clinical pregnancy outcome was high significantly higher in the study group than in the control group, $P < 0.001$.

Conclusion:

Theophylline reduces significantly the time needed for sperm isolation from fresh testicular samples, made better embryo quality, raised significantly implantation rate in ICSI and increased significantly biochemical and clinical pregnancy outcome in ICSI procedures as an assisted reproductive technologies carried out on azoospermic male patients.

Key words: Theophylline, non-obstructive azoospermia, ICSI

INTRODUCTION

Infertility is a common problem among newly married couple and may result in emotional breakdown. It is defined as the inability to achieve pregnancy within 12 month of regular unprotected intercourse and it affects 15% of cases of reproductive aged couples^(1, 2). The causes of infertility be related to female factor, male factor, a combination, or may be unexplained. About tow third of cases are linked to male factors⁽³⁾.

Azoospermia is seen in about 1% of all men and 15% of infertile men⁽⁴⁾. It is defined as undetected spermatozoa in the semen sample following standard procedures as recommended by world health organization (WHO). When absence spermatozoa in the wet preparation, an examination of the centrifuged sample (3000 X g or greater for 15 minutes) is recommended. To define azoospermia there must be no sperm in the centrifuged sample on at least two occasions⁽⁵⁾.

To define the cause of azoospermia, full medical and reproductive history, clinical examination, hormonal analysis (FSH, testosterone) and genetic studies are all

necessary and in most cases, the type of azoospermia can also be defined⁽⁶⁾. According to etiology azoospermia is divided into three primary categories⁽⁴⁾: Pretesticular, Testicular and Post-testicular.

However, from clinical point of view, azoospermia may be regarded as two types. The first one is obstructive azoospermia (OA) which is due to obstruction in male genital tract. The second type, which is also more common, is non-obstructive Azoospermia (NOA) which reflect inadequate synthesis of sperm by testis⁽⁶⁾.

OA can be the result of: infections, trauma, surgery, radiation or congenital anomalies^(7, 8). Non-obstructive azoospermia can be due to: genetic problems, drugs, congenital anomalies like varicocele and undescendent testis, radiation, and other less frequent factors such as genital injuries and heat, which may harm the process of sperm synthesis and maturation^(7, 9).

In 1991, Intracytoplasmic sperm injection (ICSI) was introduced for management of male infertility with low sperm concentration then extended to treat the sever male

infertility as Azoospermia⁽¹⁰⁾ The origin of the sperm has no negative impact on the ICSI outcome, for that reason the testicular sperm extraction (TESE) is an important and effective method for retrieval of immature sperm cells in cases of azoospermia. The first description about (TESE-ICSI) was made by Deveoey *et al.* in 1995.⁽¹¹⁾ In this method that can be performed as an outpatient procedure using local anesthesia, a small incision is made over the testis and samples of testis tissues are extracted and sent for lab. To obtain more sperm from the testicles in patients with non-obstructive azoospermia, sometimes it is necessary to do multiple TESE; however, multiple TESE has shown to be associated with inflammatory changes and stable devascularization of the testis⁽⁶⁾. For that reason, considering strategies which increase the chance of sperm retrieval from the first operation will bring better offer the patient to avoid repeated operation with associated complications. Selection of spermatozoa is a vital step for ICSI to be successful. The sperm motility is regarded as a main sperm selection criterion for sperm viability and is utilized by every embryologist at the time of ICSI⁽¹²⁾. It is often to realize immobile spermatozoa in testicular biopsy samples and this made the embryologist in a dilemma when all spermatozoa were immotile.⁽¹¹⁾ Different methods may be carried out to differentiate viable immotile spermatozoa from non-viable ones, thus aiding in the selection of viable gametes for ICSI⁽¹³⁾; including Hypotonic swelling (HOS) test, Sperm tail flexibility test (STFT)^(14, 15), Motility stimulant sperm challenges using Pentoxifylline⁽¹³⁾. More recently Theophylline has been tested as a chemical tool for stimulating spermatozoa⁽¹⁶⁾.

For that reason, the present study aimed to evaluate ICSI outcome (Fertilization rate, Embryo grading, Biochemical pregnancy rate, Implantation rate, Clinical pregnancy rate) in two groups of non-obstructive azoospermic men subjected to testicular sperm extraction. Sperm selection for ICSI by Sperm tail flexibility test is used for the first group and chemical selection by Theophylline will be used in the second group.

PATIENTS AND METHODS

The present case control study included 18 non-obstructive azoospermic men all of them underwent ICSI procedure at The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies\AL-Nahrain University and extended from September 2016 to May 2017. They were divided into two subgroups according to method of selecting immotile but viable spermatozoa (Sperm tail flexibility test and Theophylline). Each patient was thoroughly examined and then subjected to the investigation. The following procedures were undertaken: Patient preparation for testicular sperm extraction (TESE) procedure, Sperm Extraction procedure, Preparation of testicular spermatozoa, processing sperm suspension for intracytoplasmic sperm injection (ICSI), Sperm Selection for ICSI. Sperm tail flexibility test in which the tip of the sperm tail was slightly touched by the ICSI injecting pipette and the sperm which shows the slight movement irrespective of head movement was considered as viable

and further used for ICSI whereas the second method used in the study group for the selection of immotile normal morphological viable spermatozoa, pretreated with a ready-to-use Theophylline solution (GM501 SpermMobil, Gynémede) serving as an activating substance technique.

STATISTICAL ANALYSIS

Data were collected, summarized, analyzed and presented using three statistical software programs: the statistical package for social science (SPSS version 22), Microsoft Office Excel 2013 and MedCalc 2014. Categorical variables were presented as number and percentage whereas numeric variables were presented either as mean and standard deviation (SD) or median and interquartile range (IQR), according to the results of Kolmogorov-Smirnov test of normality distribution for numeric variables. The association between categorical variables was assessed using Chi-square test. Comparison of mean values between two groups was carried out using either independent samples-t test or Mann-Whitney U test. P-value was considered significant when it was equal to or less than 0.05⁽¹⁷⁾.

RESULTS

There was no significant difference in sperm count isolated for ICSI in both groups ($P=0.321$). Total search time needed for all sperm isolation in all patients was significantly shorter in the study group than that in the control group, 9.50 (5.00) versus 37.00 (12.50) minutes, ($P<0.001$). Additionally, search time needed for single sperm isolation was significantly shorter in the study group than that in the control group, 1.84 (0.81) versus 4.75 (0.81), ($P<0.001$), as shown in table 1. Overall, there was no significant difference in the serum level of FSH, LH, prolactin and testosterone between control and study groups ($P>0.05$), as shown in table 2.

Regarding parameters of fertility the following results were obtained: There was no significant difference in sperm count, normal morphology sperm count and number of fertilized oocyte between control and study groups ($P>0.05$), as shown in table 3; however, number of injected oocytes was significantly lower in the study group than the control group, 5.00 (1.75) versus 7.00 (1.50), ($P=0.009$); the Number of grade I embryos was significantly higher in the study group than in the control group, 2.50 (2.75) versus 2.00 (1.50) ($P=0.024$), but neither the number of grade II embryos nor the number of grade III embryos was significantly different between study and control groups ($P>0.05$); there was no significant difference in number of cleaved and transferred embryos; however, the number of implanted embryos was significantly higher in the study group than in the control group ($P<0.001$); rate of fertilization was significantly higher in the study group than that of the control group, 41.67 (29.17) versus 0.00 (0.00) ($P=0.001$); biochemical pregnancy rate also was significantly higher in the study group than that of the control group ($P=0.001$) and clinically pregnancy rate significantly higher in the study group than that of the control group ($P=0.001$), as shown in table 3.

Table 1: Sperm count and time needed for sperm isolation in control and study

Characteristic †	Control group	Study group	P-value*
Sperm count for ICSI	8.00 (3.00)	5.00 (1.75)	0.321
Time for total sperms (minute)	37.00 (12.50)	9.50 (5.00)	<0.001
Time for single sperm (minute)	4.75 (0.81)	1.84 (0.81)	<0.001

†Values were expressed as median (inter-quartile range); *Mann Whitney U test.

Table 2: Hormonal level in control and study groups

Hormone	Control group	Study group	P-value*
FSH	11.30 (9.95)	11.70 (12.94)	0.689
LH	7.65 (10.15)	8.02 (9.22)	1.000
PRL	10.19 (13.78)	10.64 (6.23)	1.000
Testosterone	2.50 (7.00)	2.65 (1.90)	0.531

†Values were expressed as median (inter-quartile range); *Mann Whitney U test.

Table 3: Fertility parameters in control and study groups

Characteristic	Control group	Study group	P-value
Sperm number	14.00 (4.50)	18.50 (4.50)	0.061*
Normal Morphology sperm	8.00 (2.50)	9.00 (2.75)	0.491*
Number of fertilized oocyte	5.00 (2.50)	3.50 (3.00)	0.750*
Injected oocyte	7.00 (1.50)	5.00 (1.75)	0.009*
G1	2.00 (1.50)	2.50 (2.75)	0.024*
G2	1.00 (2.00)	0.0 (0.75)	0.155*
G3	0.0 (0.50)	0.0 (0.00)	0.193*
Cleaved embryos	3.00 (1.50)	3.00 (3.00)	0.464*
Transferred embryos	3.00 (1.00)	3.00 (1.00)	0.572*
Implanted embryos	0.0 (0.00)	1.0 (0.00)	<0.001*
Fertilization rate	0.00 (0.00)	41.67 (29.17)	0.001*
Biochemical pregnancy rate	1/10	8/8	0.001**
Clinical pregnancy	0.00 (0.00)	1.00 (0.00)	0.001*

†Values were expressed as median (inter-quartile range); *Mann Whitney U test; ** corrected Chi-square test.

DISCUSSION

The main finding of the present study is that adding Theophylline made searching time for sperm isolation significantly lower. This result is similar to the finding of Ebner *et al.* in 2011⁽¹⁸⁾ who stated that addition of Theophylline significantly lowered searching time for sperm selection in the study group than in the control group. Shorter searching time for sperm isolation was also reported by Nordoff *et al.*, in 2015⁽¹⁹⁾. Moreover, Ebner *et al.* in 2014⁽²⁰⁾ investigated the addition of Theophylline to samples taken from male patients with absolute asthenozoospermic and observed that within minutes, Theophylline resulted in improved sperm progressive motility so numerous previously immotile sperm revealed fast progressive motility and this finding also is in accordance with the results of the current study.

The present study aimed also at investigating the role of adding Theophylline on fertility parameters. In this regard, the present study showed nodifference in absolute number of fertilized ova between the two groups enrolled, however fertilization rate was significantly higher in study group than in the control group. This finding is in accordance with the findings of Wöber *et al.*⁽²¹⁾, in 2015 and Ebner *et al.*, in 2011⁽¹⁸⁾ who stated that addition of Theophylline to samples taken from male patients with azoospermia resulted in significantly better fertilization rate in study group than in comparison with control group. Higher fertilization rate is associated with increase in the number of embryos and this may yield better chance for positive clinical pregnancy outcome in ICSI procedures.

One of the major observation in the present study is the significant higher number of good quality embryo number (grade I) in study group than in control groups and this is similar to that of Ebner *et al.*, in 2011⁽¹⁸⁾ and Wöber *et al.*, in 2015⁽²¹⁾ who stated that addition of Theophylline resulted in significantly higher number of good quality embryo.

The explanation of this observation concerning embryo quality is probably to the profoundly shortened time of sperm selection following the use of Theophylline (SpermMobil GM 501) permitting mobile sperm identification and selection within shorter time and hence protected the isolated sperms from longer duration of oxidative stress. Washing, centrifugation, and the incubation of sperm in different culture media may result in increased production of ROS (Walczak-Jedrzejowska *et al.*, 2013)⁽²²⁾.

In the present study, the median absolute number of transferred embryos was not significantly different between the two groups, even though, implantation rate was significantly higher in study group than that in the control group. This result is similar to the findings of Ebner *et al.*, in 2011 that made use of Theophylline (SpermMobil GM 501) in enhancing sperm mobility⁽¹⁸⁾.

In the present study, also, the rate of biochemical pregnancy was highly significantly higher in the study group than in the control group. Additionally, clinical pregnancy outcome, measured by ultrasound determined numbers of gestational sacs and fetal hearts, was highly significantly higher in the study group than in the control group. These findings are in accordance with the findings of Wöber *et al.*, in 2015⁽²¹⁾, Ebner *et al.*, in 2011⁽¹⁸⁾ and Ebner *et al.*, in 2014⁽²⁰⁾ who stated that addition of Theophylline to patients with azoospermia resulted in significant rise in biochemical and clinical pregnancy outcome in the study group than in the control group.

By conclusion the addition of Theophylline (SpermMobil GM 501) made time for sperm isolation from fresh testicular biopsy of azoospermic male patients significantly shorter and made significant increase in good embryo quality and in clinical pregnancy outcome following ICSI procedure.

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