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Original Research Article

Evaluation of the Role of Theophylline in Sperm Selection for Intracytoplasmic Sperm Injection (ICSI) in Men with Obstructive Azoospermia

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Abstract

Different strategies may be used to differentiate live immotile spermatozoa from dead ones, thus aiding in the selection of viable gametes for ICSI; Including Hyposmotic Swelling (HOS) test, Sperm tail flexibility test, Motility stimulant sperm challenge using Pentoxifylline. More recently Theophylline has been tested as a chemical tool for stimulating spermatozoa. This study was designed to compare ICSI outcome in two groups of 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. The present case control study included 22 obstructive azoospermic men. They were categorized into two subgroups according to method of selecting immotile but viable spermatozoa (Sperm tail flexibility test and Theophylline). 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, 15.5 versus 34.5 minutes (P<0.001). Median time for single sperm isolation was highly significant less in study group than in control group, 2.1 versus 5.21 minutes (P<0.001).Additionally, clinical pregnancy outcome was highly significantly higher in the study group than in the control group, P<0.001. Theophylline reduced significantly the time needed for sperm isolation from fresh testicular samples, upgraded embryo quality, increased significantly implantation rate in ICSI and increased significantly biochemical and clinical pregnancy outcome in ICSI procedures as an assisted reproductive technique carried out on azoospermic male patients.

Key words: Theophylline, obstructive azoospermia (OA), ICSI

INTRODUCTION

Azoospermia is defined as undetected spermatozoa in the semen sample following standard procedures as recommended by world health organization (WHO). When absence of spermatozoa in the wet preparation, an examination of the centrifuged sample (3000 X g or greater for 15 minutes) is recommended. If no sperm are observed in the centrifuged sample on at least two occasions is defined as azoospermia (WHO, 2010). Azoospermia affects about 1% of all men and 15% of infertile men (Gudeloglu and Parekattil, 2013).

Detailed medical and reproductive history, physical examination, hormonal analysis (FSH, testosterone) and genetic studies are all necessary to find the cause of azoospermia and in majority of cases they can also define the type of azoospermia (Anawalt, 2013).

Clinically, azoospermia may be approached as two

types. The first one is obstructive azoospermia (OA) which is due to obstruction in male genital tract. The second type, that is also more frequent, is non-obstructive Azoospermia (NOA)which is related to inadequate synthesis of sperm by testis (Anawalt, 2013).

Obstructive Azoospermia (OA) is less common than NOA and occurs in 15-20% of men with azoospermia and can be the result of: infections, trauma, surgery, radiation or congenital anomalies (Rizk et al., 2014; Schlegel, 2004).

In 1991, Intracytoplasmic sperm injection (ICSI) introduced for treatment of male infertility with low sperm concentration then extended to treat the severe male infertility as Azoospermia (Jungwith et al., 2012). The (TESE-ICSI), was first described by Deveoey *et al.* (1995), the origin of the sperm has no negative effect on the ICSI outcome but selection of spermatozoa is crucial step for successful ICSI (Esteves et al., 2011). The sperm motility is considered as a major sperm selection criterion for sperm viability and is applied by every embryologist during ICSI (Neri et al., 2014). Immobility of spermatozoa in testicular biopsy samples is common in respect to normal event so the embryologist was being encountered a dilemma when all spermatozoa were immotile (Esteves et al., 2011). Different strategies may be used to differentiate viable immotile spermatozoa from non-viable ones, thus aiding in the selection of viable gametes for ICSI (Kovacic et al., 2006); Including Hyposmotic swelling (HOS) test, Sperm tail flexibility test (STFT) (Soares et al., 2003; Oliveira et al., 2004), Motility stimulant sperm challenges using Pentoxifylline (Kovacic et al., 2006). More recently Theophylline has been tested as a chemical tool for stimulating spermatozoa (Henkel and Schill, 2003).

So the present study aimed to compare ICSI outcome (Fertilization rate, Embryo grading, Biochemical pregnancy rate, Implantation rate, Clinical pregnancy rate) in two groups of 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 was used in the second group.

PATIENTS AND METHODS

The present case control study included 22 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. 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 of sperm suspension for intracytoplasmic sperm injection (ICSI)**,** then they were categorized into two subgroups according to method of selecting immotile but viable spermatozoa for ICSI. The first group as control group(n=12) Sperm tail flexibility test in which the tip of the sperm tail was been 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(n=10) 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 (Daniel, 2009).

RESULTS

There was no significant difference in hormonal levels of FSH, LH, prolactin and testosterone, between control and study groups (P>0.05), as shown in table 1.

There was no significant difference in sperm count isolated for ICSI between control and study groups (P = 0.335). Highly significant difference was observed in total time needed for all sperm isolation in all patients and also in time needed for single sperm isolation between control and study groups (P<0.001), the time in either cases was shorter in study group, as shown in table 2.

Fertility parameters are shown in table 3. There was no significant difference in sperm count between control and study groups (P = 0.792) and also in the normal morphology sperm count (P = 0.666). Number of fertilized ooctye was significantly higher in study group than in the control group, 5.50 (3.50) versus 4.00 (2.25), (P = 0.025).No significant difference was encountered in number of injected ooctyes (P=0.550). Number of grade I embryos was significantly higher in study group than in control group, 4.50 (1.75) versus 0.50 (3.25), (P = 0.001). No significant difference was encountered in number of

Table 1. Hormone levels in control and study group classified according to type of azoospermia(Obstructive)

Hormone	Control group	Study group	P-value*
FSH	5.60 (3.65)	6.20 (1.35)	0.817
LH	5.50 (3.58)	4.90 (1.90)	0.304
PRL	8.10 (3.38)	7.10 (2.25)	0.174
Testosterone	20.95 (21.02)	23.00 (2.15)	0.091

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

Table 2. Relation between sperm count and time needed for sperm isolation in control and study group (Obstructive)

Characteristic †	Control group	Study group	P-value*
Sperm count for ICSI	7.00 (3.25)	7.00 (4.00)	0.335
Time for total sperms (minute)	34.50 (12.00)	15.50 (9.75)	<0.001
Time for single sperm (minute)	5.21 (0.68)	2.10 (0.50)	<0.001

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

Table 3. Fertility parameters in control and study group classified according to type of azoospermia (Obstructive)

Characteristic	Control group	Study group	P-value
Sperm number	32.00 (11.75)	26.50 (24.25)	0.792*
Normal Morphology sperm	17.50 (7.50)	15.00 (9.75)	0.666*
Number of fertilized ooctye	4.00 (2.25)	5.50 (3.50)	0.025*
Injected oocyte	6.00 (4.00)	7.00 (4.00)	0.550*
G1	0.50 (3.25)	4.50 (1.75)	0.001*
G2	0.0 (2.25)	0.0 (1.00)	0.490*
G3	0.0 (1.00)	0.0 (1.00)	0.876*
Cleaved embryos	4.00 (1.25)	5.00 (3.75)	0.009*
Transferred embryos	3.00 (1.25)	3.00 (1.00)	0.243*
Implanted embryos	0.50 (1.00)	1.0 (1.75)	0.075*
Fertilization rate	12.50 (33.33)	33.33 (43.75)	0.063*
Biochemical pregnancy rate	5/10	9/12	0.442**
Clinical pregnancy	0.00 (1.00)	1.00 (1.00)	0.007*

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

grade II and grade III embryos among all groups (P>0.05).Number of cleaved embryos was significantly higher in study group than that in control group,5.00 (3.75) versus 4.00 (1.25) , (P = 0.009).No significant difference was encountered in number of transferred embryos, implanted embryos, fertilization rate and biochemical pregnancy rate (P >0.05). However, clinical pregnancy outcome was significantly higher in study group than in control group, 1.00 (1.00) versus 0.00 (1.00), (P = 0.007).

DISCUSSION

In the present study, Addition of Theophylline resulted in significantly less searching time for sperm isolation. Ebner *et al*. in 2011 stated that addition of Theophylline resulted in significantly less time for sperm selection in the study as compared with in the untreated control group; this result is similar to the finding of the present study. Nordoff *et al*., in 2015 stated that incubation of immotile frozen thawed testicular spermatozoa with Theophylline was found to be effective in almost all patients treated (98.5%) and showed to have the additional benefits of shorter searching time which is in accordance with finding of the present study. In addition to that, Ebner *et al*. (2014) tested the addition of Theophylline to samples obtained from men with absolute asthenozoospermia and showed that within minutes, the addition of Theophylline led to an improvement in progressive motility such that numerous previously immotile sperm revealed fast progressive motility and this finding also supports the results of the present study.

Regarding fertilization, the present study showed insignificant difference in absolute number of fertilized ova between control and study groups. This result disagree with the findings of Wöber *et al*. (2015) and Ebner *et al*., (2011) who stated that addition of Theophylline to samples obtained from men with azoospermia resulted in significantly higher rate of fertilization in comparison with control group. However, regarding the present study, the median number of

fertilized ova in study group was higher than that of the control group. Probably this resulted from the relatively small size of study group and increasing the size may make the difference significant.

Increasing rate of fertilization is accompanied by increase in the number of embryos and this may increase the chance of positive clinical pregnancy outcome in ICSI procedures.

In the present study, the most important observation, probably, will be the highly significant difference in good quality embryo number (grade I) between control and study groups and this observation agrees with the finding of Ebner *et al*. (2011) and Wöber *et al*. (2015) who stated that addition of Theophylline resulted in significantly higher number of good quality embryo.

The explanation of this diversity in embryo quality is probably to the extremely shortened time of sperm selection following the use of Theophylline (SpermMobil GM 501) allowing mobile sperm identification and selection within a relatively shorter time and by this way made the isolated sperms able to avoid 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).

In the current study, the median absolute number of transferred embryos implantation rate and biochemical pregnancy rate were insignificantly different in study group when compared to that of the control group; however, 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*. (2015), Ebner *et al*. (2011) and Ebner *et al*. (2014) who stated that addition of Theophylline to patients with azoospermia resulted in significant rise in biochemical and clinical pregnancy out come in the study group than in the control group. So the addition of Theophylline (SpermMobil GM 501) resulted in significantly shortened time for sperm isolation form fresh testicular biopsy of azoospermic male patients and significant increases in good embryo quality and clinical pregnancy outcome following ICSI procedure.

CONCLUSION

Theophylline reduced significantly the time needed for sperm isolation from fresh testicular samples obtained from obstructive azoospermic males. Upgraded embryo quality increased significantly with the Theophylline. It further increased significantly the implantation rate, biochemical and clinical pregnancy outcome in ICSI procedures as an assisted reproductive technique carried out on azoospermic male patients.

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