

Study of pentoxifylline effects on motility and viability of spermatozoa from infertile asthenozoospermic males

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ABSTRACT

Background: The quality of semen is one of the major parameters in male infertility. Pentoxifylline, a methylxanthine derivative, is an agent primarily used in the treatment of intermittent claudication and other vascular disorders. Studies have shown that pentoxifylline enhances the quality and quantity of sperms. In this study, we have investigated the *in vitro* effects of pentoxifylline on viability and motility of spermatozoa in samples of infertile oligoasthenozoospermic males. **Materials and Methods:** In this observer-blinded clinical trial, semen samples of 25 infertile oligoasthenozoospermic males were collected in Alzahra Educational Medical Center of Tabriz University of Medical Sciences from August 2010 to August 2012. After the isolation of spermatozoa by the swim-up method, they were randomized into four groups in ISM₁ environment: The controls treated normally: Group 1 treated by pentoxifylline at a dose of 50 µg/ml, Group 2 treated by pentoxifylline at a dose of 100 µg/ml, and Group 3 treated by pentoxifylline at a dose of 200 µg/ml. Sperm viability and motility were compared among the groups on 45 min, 24 h, 36 h, and 48 h intervals. **Results:** Mean percentages of live sperms were 98.40%, 51.40%, 20.60%, and 6.00% in control group and 98.40%, 69.20%, 38.60%, and 14.60% in Group 3 on the mentioned intervals, respectively. This mean percentage decrease of live sperms was significantly lower in Group 3 comparing with that of other groups ($P = 0.01$). Mean percentages of motile sperms were 54%, 8.40%, 2.80%, and 0% in control group; and 54%, 16%, 4.80%, and 1.40% in Group 3 on the mentioned intervals, respectively. There was not a significant difference between the four groups in this regard ($P = 0.19$). **Conclusion:** Pentoxifylline can enhance the viability of sperm of infertile oligoasthenozoospermic males with no significant effect on its motility.

Key words: Asthenozoospermia, pentoxifylline, sperm motility

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INTRODUCTION

Infertility means failure in pregnancy in spite of frequent sexual intercourse without contraception for 1 year which shows decrease in the ability of fertility and breeding.¹ Based on studies, 15–20% of couples suffer from infertility.² About 45–50% of infertility reasons resulted from male problems and simultaneous male female problems only exists in some cases.³

The primary male examination should be rapid, noninvasive, and inexpensive, including history taking, physical examination, estimating the size of testicles, seminal, and hormonal analysis.^{4,5} Low sperm motility or low sperm number, or both of them, consist the most important infertility cases of men.⁶ Three major influential factors on men infertility are decrease in sperm number, including

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azoospermia and oligospermia, asthenospermia, and abnormal sperm morphology.⁷

Sperm examination should be done macroscopically in terms of seminal volume and microscopically in terms of concentration and motility and morphology.⁸ In sperm motility investigation, clinically speaking percentage of rapid progressive motile sperms is more valuable.⁹

Assisted reproductive technology (ART) is used to increase fertility through approximating spermatozoa and ovule.¹⁰ It is possible to mention to controlled ovarian hyperstimulation combined with intrauterine insemination, *in vitro* fertilization, and intracytoplasmic sperm injection (ICSI) of ART methods which most of the infertile couples use.¹¹ In a meta-analysis study, the success rate of fertility for couples in a cycle of using ART was 42.7% and in three cycles of using ART, it was 57.9%.¹² Disadvantages of ART methods include multiple pregnancies, and also increase in the possibility of premature birth, low birth weight, and perinatal mortality. In addition, possibilities of genital, musculoskeletal, cardiovascular, and gastrointestinal disorders increase while implementing ART methods.¹³

Pentoxifylline is a derivative of xanthine, which decreases blood viscosity by increasing deformability of red blood cells and is used to symptomatic relief of intermittent thrombosis,¹⁴ and its usage has been effective in therapy of cardiovascular diseases, cerebrovascular diseases, other diseases related to local blood flow abnormalities (vascular),^{15,16} and reduction of ischemic effects.¹⁷ Common side effects of using pentoxifylline are nausea, vomiting, headache, dizziness, and rare complications such as angina, arrhythmias, shortness of breath, and hypotension. The incidence rate of complications has been reported as one-third in studies.¹⁸

In patients suffering oligoasthenozoospermia, implementing hormonal therapies had low effects, suggesting the need for more discussions.¹⁹ It is possible to increase sperm motility using pentoxifylline *in vitro* conditions.²⁰ Results of the previous studies show that pentoxifylline has a desirable effect on sperm motility,²¹ but to reach definite results in this field, we need further investigations. The objective of this study is to investigate the effect of pentoxifylline in infertile men suffering oligoasthenozoospermia on motility and viability of sperm in the culture medium.

MATERIALS AND METHODS

In this observer-blinded clinical trial registered on Iranian Registry of Clinical Trials (IRCTs) site (IRCTID: IRCT201110175942N1), semen samples of 25 infertile oligoasthenozoospermic males were collected in Alzahra Educational Medical Center of Tabriz University of

Medical Sciences – where is the main referral center of infertility in Northwest of Iran – from August 2010 to August 2012. Inclusion criteria were: Age among 25–40, primary infertility during recent 1–2 years, sperm motility <50% in the progressive type or <25% in rapidly progressive type, not receiving oral pentoxifylline for 9 h before sampling (considering drug half-life), sperm number <20 million in a milliliter, and lack of sexual relationship during recent 3–7 days. Exclusion criteria were azoospermia, seminal infections, history of contact with chemicals and radiation, history of trauma to testicles, varicocele, hydrocele, undescended testis or its corrective surgery and vasectomy reversal surgery, other mental or physical diseases, and also implementing other ART methods.

In this study, sample volume determined as 25 specimens based on sperm motility and considering $\alpha = 0.05$ and minimum 5% increase in motility of rapidly progressive sperms in studied samples and 80% power. After a complete explanation of objectives and methods of study and privacy of information and getting written informed consent, 25 people were randomly included into the study using Randlist Software version 1.2 (Randlist Ins., Chicago, USA).

Samples were collected in sterile containers by masturbation method. Only one seminal sample was taken from each person. In case of spoiling or loss of sample for any reason, the person was asked to refer again to give another sample in next 3–7 days without performing any sexual intercourse. After getting the samples, they were rapidly put in the incubator 37°C for complete liquefaction of samples. After preparing the sample using standard method and isolation of spermatozoa with swim up method, samples were randomly divided into four groups in ISM1 environment: Samples in three groups influenced by three different pentoxifylline doses whereas one group (control group) was kept without pentoxifylline. In Group 1, pentoxifylline 50 µg/ml; in Group 2, pentoxifylline 100 µg/ml; and in Group 3, pentoxifylline 200 µg/ml was added. A code was assigned for each group, and samples were kept in same conditions in all four groups and were investigated based on recommended guidelines according to the World Health Organization manual.²² The person evaluating samples reported results based on the code and was unaware of grouping method. The percentage of live and motile rapidly progressive sperms was determined and compared in 45th min and 24th, 36th, and 48th h. The desirable result in this study was considered as vitality of sperm and increasing minimum 5% sperm motility in rapidly progressive type.

The collected information was analyzed via SPSS™ version 15 statistical software (SPSS Ins., Chicago, USA) and intra- and inter-group comparisons were done by repeated-measures analysis. Data have been presented as mean ± standard deviation and frequency and percentage. Statistical significance level was considered as $P \leq 0.05$.

RESULTS

Mean age of participants in this study was 31.52 ± 2.4 years. The percentage of live sperms in our studied groups in mentioned time intervals is presented in Table 1.

Mean percentage of live sperms had reduced significantly in all four groups through time ($P < 0.05$). Mean reduction rate of live sperm percentage during investigation interval was significantly lower in 200 μg group than that of other three groups ($P = 0.01$). This rate did not show statistically significant difference among other groups ($P = 0.12$).

The percentage of motile rapidly progressive sperms in studied groups during investigated time intervals is shown in Table 2.

Mean of motile sperms during investigated time interval did not have statistically significant difference among four groups ($P = 0.19$).

DISCUSSION

In this study, the effect of various pentoxifylline doses (50, 100, 200 μg in ml) on sperm viability and mortality rate in infertile men suffering oligoasthenozoospermia was investigated in culture medium. Based on this, pentoxifylline with 200 $\mu\text{g}/\text{ml}$ dose significantly increased viability rate of sperms in infertile men suffering oligoasthenozoospermia. During 45 min to 48 h after incubation, different doses of pentoxifylline did not have a significant effect on sperm

motility rate (percentage of rapidly progressive cases), and there was not a significant difference with control group from this perspective.

In a study about the effect of pentoxifylline on viability and motility rate of sperm before and after freezing, using pentoxifylline before freezing has led to a reduction in viability rate, and increase in motility rate of sperm and after freezing has led to increase in viability and decrease in motility of sperm.²³ In another study on semen of dog, the highest viability rate of sperm has been in freezing condition alongside with 1 mM pentoxifylline.²⁴

Since the reduction in seminal lifetime is one of the destructive factors on sperm fertility, by increasing sperm viability time, fertility power also increases. In another study, the effect of pentoxifylline on increasing horse seminal lifetime has been shown.²⁵ In another study, rate of sperms with normal morphology had increased with pentoxifylline at 400 mg twice a day and acrosomal reaction has also increased.²⁶

In the current study, pentoxifylline leads to increase the viability of sperms, but no significant effect was observed on motility rate of sperms. Also in a study, adding 3 mM pentoxifylline during incubation has not caused any change in sperm motility.²⁷ In a study on horse, 3.5 mM pentoxifylline has not had any effect on motility of sperms but has significantly increased ratio of live and powerful sperms.²⁸

Table 1: Rate of alive sperm in study groups at different time of sperm analysis

Times: Groups:	After 45 minutes mean \pm SD (minimum, median, maximum)	After 24 hours mean \pm SD (minimum, median, maximum)	After 36 hours mean \pm SD (minimum, median, maximum)	After 48 hours mean \pm SD (minimum, median, maximum)
Control group	98.40 \pm 8.00 (60, 60, 100)	51.40 \pm 24.90 (5, 50, 90)	20.60 \pm 17.52 (0, 20, 60)	6.00 \pm 10.31 (0, 0, 40)
Group 1(50mcg/ml)	98.40 \pm 8.00 (60, 60, 100)	50.40 \pm 21.89 (5, 50, 90)	18.60 \pm 17.75 (0, 15, 75)	5.40 \pm 11.17 (0, 0, 50)
Group 2 (100mcg/ml)	98.40 \pm 8.00 (60, 60, 100)	51.40 \pm 23.87 (5, 50, 90)	21.00 \pm 19.26 (0, 20, 70)	6.20 \pm 11.11 (0, 0, 50)
Group 3 (200mcg/ml)	98.40 \pm 8.00 (60, 60, 100)	69.20 \pm 22.58 (20, 75, 100)	38.60 \pm 22.80 (0, 40, 80)	14.60 \pm 18.54 (0, 0, 60)

Results are given as mean \pm SD, minimum, median and maximum. $P < 0.05$

Table 2: Rate of motile (rapid progressive) sperm in study groups at different time of sperm analysis

Times: Groups:	After 45 minutes mean \pm SD (minimum, median, maximum)	After 24 hours mean \pm SD (minimum, median, maximum)	After 36 hours mean \pm SD (minimum, median, maximum)	After 48 hours mean \pm SD (minimum, median, maximum)
Control group	54.00 \pm 11.55 (20, 60, 70)	8.40 \pm 9.65 (0, 5, 35)	2.80 \pm 10.11 (0, 0, 50)	0.00 \pm 0.00 (0, 0, 0)
Group 1 (50mcg/ml)	54.00 \pm 11.55 (20, 60, 70)	7.72 \pm 9.35 (0, 5, 35)	1.12 \pm 2.76 (0, 0, 10)	0.40 \pm 2.00 (0, 0, 10)
Group 2 (100mcg/ml)	54.00 \pm 11.55 (20, 60, 70)	8.60 \pm 10.36 (0, 5, 40)	1.12 \pm 2.76 (0, 0, 10)	0.40 \pm 2.00 (0, 0, 10)
Group 3 (200mcg/ml)	54.00 \pm 11.55 (20, 60, 70)	16.00 \pm 12.58 (0, 10, 50)	4.80 \pm 7.29 (0, 0, 25)	1.40 \pm 4.21 (0, 0, 20)

Results are given as mean \pm SD, minimum, median and maximum. $P < 0.05$

In the current study, pentoxifylline did not have a significant effect on sperm motility. Also in another study, in low doses such as 0.01 mM, pentoxifylline had a positive effect on sperm motility but in higher doses such as 10 mM had reverse effect on sperm motility.²⁹ In another study, pentoxifylline at 1.76 mM dose significantly increased sperm motility rate and also fertility rate and clinical pregnancy were significantly higher in the intervention group.³⁰ Furthermore, in another study adding pentoxifylline has led to an increase in sperm motility.²¹

A study on effect of pentoxifylline on sheep sperms has mentioned to increase in sperm viability and preserving spermatic membrane after 30 min and has not had significant effect on sperm motility.³¹

Totally, most of the studies have shown that using pentoxifylline lead to an increase in fertility ability of oligoasthenozoospermic persons through increasing stability and viability of sperm, increasing motility of sperm, or both factors. Therefore, it seems that it is possible to use pentoxifylline as an adjuvant drug in therapy of infertility.³²⁻³⁶

CONCLUSION

In this study, pentoxifylline at 200 µg/ml dose significantly increased sperm viability in infertile men suffering oligoasthenozoospermia. Therefore, implementing it in testicular sperm extraction or percutaneous epididymal sperm aspiration methods in ICSI helps the improvement of results of this therapy, especially in cases where ovules were immature and would remain in culture medium for hours before microinjection for maturation of ovule. In this study, pentoxifylline did not have statistically significant effect on sperm motility rate of infertile men suffering oligoasthenozoospermia.

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Conflicts of interest

There are no conflicts of interest.

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