Original Article

THE EFFECT OF PENTOXIFYLLINE IN SEMEN PREPARATION FOR INTRAUTERINE INSEMINATION

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ABSTRACT

Objective: Pentoxifylline (PX) is a methyxanthin derivative that influences the sperm motility characteristics. and has been reportedly effective in preserving sperm motility in vitro. This study was undertaken to evaluate whether addition of pentoxifylline to the previously standardized in-vitro treatment of semen improves the percentage of pregnancies after homologous IUI.

Methodology: The study involved 110 infertile couples (66 classified infertile for male factors and 44 for other factors) who underwent a total of 300 cycles of homologous IUI: 202 for male factor infertility and 98 for other factors.

Results: Out of the 202 cycles performed for male factor infertility, 122 underwent the standard preparation of semen, pregnancy rate were 11.5%. While 80 had a semen preparation with pentoxifylline. The pregnancy rate were 27.5% better with pentoxifylline preparation ($P<0.05$). Abortions and malformations were equally distributed in the standard treatment and in the pentoxifylline group.

Conclusion: Our results demonstrate that pentoxifylline may be used for improving the male infertility treatment program.

KEY WORDS: Intrauterine insemination (IUI), Pentoxifylline, Semen Preparation.

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INTRODUCTION

Pentoxifylline (PX) is a methyxanthin derivative in the same pharmacologice group as caffeine that inhibits the breakdown of cyclin adenosine monophosphate (cAMP). This generates cellular glycolysis and endogenous adenosine triphosphate (ATP) Production that influences the sperm motion characteristics.[1,2] In general, PX has been reportedly effective in preserving sperm motility in vitro, also when administered orally to the asthenozoospermic patients.[3-6]

One of the major causes of male factor infertility is related to asthenozospermia, particularly severe cases, which may influence the pregnancy success rates following assisted reproductive techniques.[7] Therefore, a potential pitfall exists for these infertile men where their only infertility problem resides in sperm motility parameters. Thus, improvement of sperm motility with application of PX may not only be beneficial for intracytoplasimic sperm injection (ICSI) programs, but also in vitro fertilization (IVF), and intra-uterine insemination (IUI) cycles[3] mainly in association with

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intrauterine insemination (IUI). In fact, most of the reports deal with in-vitro fecundation[8] and little is known about the effectiveness of pentoxifyline in IUI. Sperm motility is thought to play an extremely important role for positive results in IUI, and even more important is the ability to maintain motility over time as insemination and ovulation may not coincide exactly.[9]

Since pentoxifylline treatment significantly increases the sperm capacity to undergo the acrosome reaction in response to both natural and artificial stimuli,[10] there is a reasonable concern about possible untimely occurrence of extensive acrosome reactions. Therefore a retrospective clinical trial was planned in order to evaluate whether addition of pentoxifylline to the previously standardized in-vitro treatment of semen improves the percentage of pregnancies after homologous IUI.

METHODOLOGY

The study involved 110 steril couples, 66 of whom were classified infertile for male factor and 44 for other factors, who underwent a total of 300 cycles of homologous IUI at the academic infertility center during eight months. In the middle of this period the standard method for the preparation of semen was modified at one single step, that is the supplementation of pentoxifylline. The purpose of the trial was to compare the pregnancy rate in the first four months, when semen was prepared in the absence of pentoxifylline, with the pregnancy rate in the last four months, when pentoxifylline addition became a routine step in semen preparation. During this period some patients were treated only with or without pentoxifyline supplementation, but 24 of them, who were under treatment just when the preparation of spermatozoa was modified, underwent both laboratory procedures in different cycles. The significance of pentoxifyline addition during the preparation of semen for IUI was therefore better expressed by pregnancy rate per cycle than per patient. Among the 66 infertile men, 42 underwent the standard treatment without pentoxifylline and 48 were subjected to pentoxifylline addition. Out of the forty two, 32 were asthenozoospermic with average cell number 85×10^6/ml (25-200), rapid progressive motility 25% (1-45), sluggish progressive motility 9% (1-29), and rapid progressive motility after treatment 53.9% (20-85); then were oligoasthenozoospermic with average cell number 15×10^6/ml (10-20), rapid progressive motility 32.8% (15-45), sluggish progressive motility 2% (0-10), and rapid progressive motility after treatment 54.4% (27-78). Out of the 48 patients whose semen was treated with pentoxifylline, 34 were asthenozoospermic with average cell number 62×10^6/ml (30-15), rapid progressive motility 23-6% (0-40), sluggish progressive motility 11.9% (0-37), and rapid progressive motility after treatment 63.7% (35-90); Fourteen were oligoasthenozoospermic with average cell number 15×10^6/ml (10-20), rapid progressive motility 18.5% (0-38), sluggish progressive motility 13.8% (0-50) and rapid progressive motility after treatment 64.7% (35-100). As to morphology, no sample was teratozoospermic.

Semen collection and preparation: All subjects were requested to abstain from any sexual activity for 3-4 days before semen collection. Semen specimens were obtained by masturbation and collected in a wide-mouthed, sterile, plastic container. The ejaculate was allowed to liquefy at room temperature and was then analyzed using a Makler counting chamber according to the World Health Organization guidelines.[11]

Standard Treatment: Spermatozoa were separated through a discontinuous Percoll gradient. Isotonic Percoll was prepared by diluting 45 ml of Percoll (Pharmacia, Uppsala, Sweden) with 5 ml 10×concentrated EBSS (Life Technologies Ltd, Paisley, Scotland), 4.5 ml 5% human albumin, 2.4 mg sodium lactate, one ml one M Hepes (Life Technologies Ltd, Paisley, Scotland). Gradients were made with 2.5 ml 80% Percoll (4ml

isotonic Percoll in one ml EBSS 1X) overlayered by 2.5 ml 40% Percoll (2 ml isotonic Percoll in 3 ml EBSS 1X). in the last step 2.0 ml of freshly collected semen were layered on top of 40% Percoll and then centrifuged at 600 g for 20 min to obtain a pellet of functionally normal spermatozoa.

Pentoxifylline treatment: Pentoxifylline (Sigma, St Louis, MO, USA) was dissolved (1 mg/ml) in one Ham's F-10 Medium (ICM Biomedicals, Costa Mesa, CA, USA). An aliquot of one ml of freshly collected semen was mixed with one ml of pentoxifylline solution and incubated at 37°C 10 minutes at the end of the incubation the sample was layered on a Percoll gradient and centrifuged as previously describe.

Whatever the treatmen, the pellet was resuspeded in 0.2 ml of Irvine washing medium (Irvine Scientific, Santa Ana, CA, USA) re-evaluated, and inseminated with a Kremer-Delafontaine catheter (Laboratoire CCD, Paris, France) 36 hour after human chorionic gonadotrophin (HCG; 10,000IU) injection.

Statistical Analysis: X^2 test was applied as appropriate for comparison of percentage. A P value<0.05 was taken to be indicative of a statistically significant difference.

RESULTS

In eight months 300 cycles of IUI were performed, 202 for male factor inferiility, 98 for other factores: anovulation (n=42), endometriosis (n=4), idiopathic (n=42). As shown in Table-I, 122 out of the 202 cycles performed for male factor infertility underwent the standard preparation of semen and were followed by Fourteen pregnancies (pregnancy rate= 11.5%), while 80 had a semen preparation with pentoxifylline addition and were followed by 22 pregnancies (pregnancy rate = 27.5%), with a significant difference between the two methods (P<0.05).

Out of the 98 cycles carried out for factors different from male infertility, 20 underwent the standard preparation of semen and were followed by four pregnancies (pregnancy rate= 20.0%), while 78 had pentoxifylline addition and were followed by eighteen pregnancies (pregnancy rate = 23.1%). The difference between the two groups was not significant.

Abortions were equally distributed in the standard treatment group (n=6) and in the pentoxifylline group (n=8).

DISCUSSION

Pentoxifylline improves the motility of spermatozoa, but several conflicting reports concerning the quality of motility have been published, Pentoxifylline has been found, to have no effect on the number of progressively motile spermatozoa in normozoospermic samples but increases the number of progressively motile spermatozoa in asthenozoospermic samples.[8,12-15]

Other authors suggest that pentoxifylline effect is limited to an increase in sperm velocity in asthenozoospermic samples[16-18] and that it has no effect on the VSL (straight line velocity), while having a dramatic and consistent effect on VCL (curvilinear velocity).[14,19] In contrast, some studies have demonstrated an increase of sperm velocity. It has also been reported that linearity does

Table-I: Pregnancy rates per cycle (%) in couples affected by male factor undergoing intrauterine insemination

	Male factor	*Other factors*	*Overall*
Standard treatmen	(14/122)	(4/20)	(18/142)
PTF treatmen	27.5 (22/80)	23.1 18/78)	25.3 (40/158)
P-value	P<0.05	NS (18/78)	NS (40/158)

NS= not significant

not increase in parallel with an increase in VSL,[20] that pentoxifylline induces forward acceleration, augmentation of lateral head excursions, and intensification of flagellar beat[19] and also that it significantly improves sperm forward progressive motility.[21]

Apart from the real effect induced by pentoxifylline, it has been reported that in IUI sperm velocities are significantly lower in conceptual that in non-conceptual cycles.[22]

What is even more uncertain about pentoxifylline is the demonstration of its role in pregnancy rate improvement when patients are inseminated with pentoxifylline treated semen. Recently it has been shown that in an IVF programme the fertilization success and pregnancy rate are unrelated to pretreatment of semen with the drug and that there is no therapeutic advantage to using pentoxifylline in IVF for male factor infertility.[23,24]

One might reasonably expect that the advantages of using pentoxifylline in IUI are even less than in IVF. In fact, the drug seems to sensitize the spermatozoa to undergo acrosome reaction for both natural and artificial stimuli.

For this reason the potential beneficial action of pentoxifylline on sperm motility could be undermined by the adverse effect of inducing a premature massive acrosome reaction of the prepared spermatozoa as soon as they are inseminated.[25]

We have shown that the treatment of oligoasthenozoospermic semen with pentoxifylline leads to a significant increase in the pregnancy rate after IUI, while treatment of normaozoospermic samples does not cause any significant increase. Therefore spermatozoa which have no motility defect does not benefit from pentoxifylline treatment. We have also found that pentoxifylline has no effect on the number of progressively motile spermatozoa in normozoospermic samples.[13,14,23]

An impairment of early zygote development using standard concentrations of pentoxifylline was demonstrated in an experimental IVF model in mice, gusting that after drug administration spermatozoa should be carefully washed out before in-vitro insemination.[21] Our standard technique of semen preparation does not include washing steps after pentoxifylline treatment, though leading to positive results with IUI. At present it cannot be stated whether spermatozoa become free of pentoxifylline after centrifugation through Percoll gradients and where possible remnants of the drug are completely removed during their migration in the female genital tract.

After the successful fertilization of a human occyte by microinjection of a testicular spermatozoon, almost all problems related to male infertility seemed to be resolved.[24-26] Our experience enables us to suggest that couples affected by male factor sterility should be allowed a chance with IUI: in our study three attempts are usually performed before an oligoasthenozoospermic patient is recommended to undergo the much more expensive microinjection treatments. It is our opinion that patients whose ejaculate reaches at least this minimal threshold should be considered for IUI.

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