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Effects of Pentoxifylline on Sperm Motion Characteristics in Normozoospermic Men Defined by a Computer-aided Sperm Analysis

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ABSTRACT

This study was conducted to determine whether pentoxifylline has an *in vitro* effect on sperm motion characteristics in normozoospermic men. The subjects of the study were 15 male volunteers. After the spermatozoa were washed with mBWW medium, the sperm suspension was divided into two aliquots: one was treated with 1 mg/ml pentoxifylline, the other was used as a control. The sperm motion characteristics were examined by an HT-M2030 at 30, 60, 120, 180, 240 and 300 min during continuous exposure to the drug. As results, pentoxifylline increased the curvilinear velocity and the lateral head displacement. However, it did not affect sperm motility, the straight line velocity. Pentoxifylline may improve sperm fertilizing ability by altering the characteristics of sperm motion, not by increasing the number of motile spermatozoa.

Key words: *Human, Spermatozoa, Motility analyzer, Pentoxifylline*

The prognosis for infertile couples has been improved dramatically with the development of assisted-reproductive techniques. Althought severe male factor infertility can be overcome by intracytoplasmic sperm injection (ICSI), an improvement in conventional in vitro fertilization (IVF) technique is still important, because more children are born by IVF than ICSI. Sperm motility has been considered to be one of the most important parameters in evaluating the fertilizing ability of a man, and it is closely related to the fertilization rate in IVF[2] or to the pregnancy rate following artificial insemination[29]. Therefore, any treatment that enhances sperm motility or motion in IVF may be of clinical value in the treatment of male infertility.

Pentoxifylline (PF), a methylxanthine phosphodiesterase inhibitor, is well documented as a stimulant of sperm motility. PF has been clinically used to stimulate sperm motility in various situations, e.g. cryopreserved human semen for therapeutic donor insemination[21], electroejaculation in conjunction with the gamete intra fallopian transfer (GIFT) procedure[23], IVF severe male infertility[26] and obstructive azoospermia where spermatozoa were retrieved from the epididymides[12]. The use of PF in IVF has been considered safe on the basis of some reports considering the prognosis of newborns[18,20,32].

The most well-documented effect of PF on human spermatozoa concerns motility[1,14,22]. More recently other effects such as enhancement of sperm capacitation[6,8,13,15,19,26,28], the acrosome reaction[5,11,25,33], and protection against the effects of reactive oxygen species[10] have been reported.

It is postulated that PF inhibits cyclic adenosine monophosphate (cAMP) phosphodiesterase activity[9]. The increased intracellular cAMP influences sperm motion by enhancing endogenous adenosine triphosphate (ATP) utilization[24]. Several studies have suggested that PF increases the number of progressively motile spermatozoa, resulting in an increase of sperm motility[6,8,15], whereas others have suggested that its effect is limited to an increase in sperm velocity[13,19,26,28]. These paradoxical results occur in part because most investigations so far have reported only the gross examination of spermatozoa, without making any objective analysis of sperm motion characteristics. We therefore carried out a study to determine the effects of PF on sperm motion characteristics at various time intervals, using a computer-aided sperm analyzer, HTM-2030 (Version 5.40Y, Hamilton Thorn Research, Danvers, MA).

MATERIALS AND METHODS

Preparation of Spermatozoa

Fifteen semen samples were obtained after at least 3 days of abstinence from 15 healthy volunteers with normal semen parameters according to the World Health Organization[31]. The ejaculate was incubated at 37°C until liquefaction was complete. The spermatozoa were washed twice (100g, for 5 min) in modified Biggers-Whitten-Whittingham medium supplemented with 5 mg/ml human serum albumin (Fr V., Sigma Chemical

Co., St. Louis, MO) (mBWW medium), and resuspended to make a final concentration of 30×10^6 /ml. The sperm suspension was then divided equally into two aliquots: one was supplemented with a stock solution of PF (Trental®, Sigma) to make a final concentration of 1 mg/ml, which is the most widely used concentration in clinical applications of PF. The other was used as a control. The sperm suspensions were incubated at 37°C (5% CO_2 in air) for up to 300 min.

Sperm Motion Analysis

Sperm motion analysis by the HTM-2030 was performed using a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) on droplets of PF-treated and on control samples at 30, 60, 120, 180, 240 and 300 min. The conditions employed for sperm motion analysis were similar to those reported by Burkman[3]: analysis duration of 0.67 sec (20 frames); minimum contrast, 7; minimum size, 6; "slow cells" were accepted as motile (slow gate = 5 μm/sec); HTM magnification factor, 2.13; low-size gate, 0.4; high-size gate, 1.6; low-intensity gate, 0.4; high-intensity gate, 1.6. A heated stage inside the HTM-2030 maintained the sample temperature at 37°C.

At least 200 motile spermatozoa from 3 different fields were observed in each sample and the following parameters were examined: percentage of motile spermatozoa (sperm motility), straight line velocity (VSL; velocity calculated from the beginning to the end of a digitized sampling interval), curvilinear velocity (VCL; velocity calculated from the sum of track point-to-track point velocity), linearity (LIN; indicates track straightness, 0% = circular track, 100% = straight path) and lateral head displacement (ALH; distance the spermatozoa head swing from side to side).

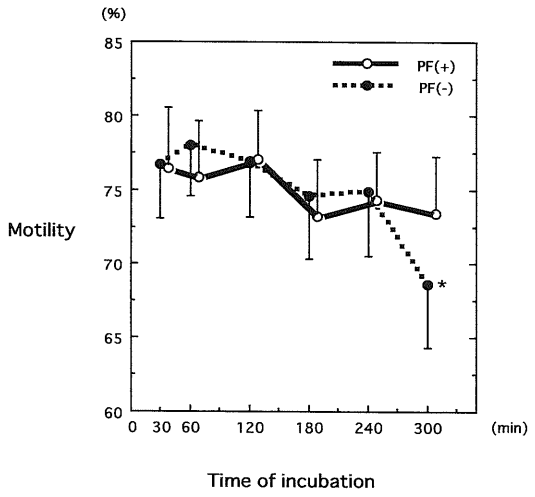

Fig. 1. Percentage of motile spermatozoa (sperm motility) was not changed by addition of PF until 240 min, then a significant decrease was observed in the control at 300 min. (n=15)
*Statistically significant differences from that of PF(+) ($p<0.05$)

Statistical Analysis

Data were analyzed using a StatViewII statistical package (Abacus Concepts, Inc., Berkeley, CA). Sperm motion parameters in PF-treated and control sample were compared by Wilcoxon's t-test and the difference was considered as significant at a 5% level. The sperm motility was transformed by arcsin before the analysis.

RESULTS

Sperm motility was not changed by the addition of PF until 240 min, but there was a significant decrease in the control sperm motility at 300 min (Fig. 1). The VSL was unaffected at all times after

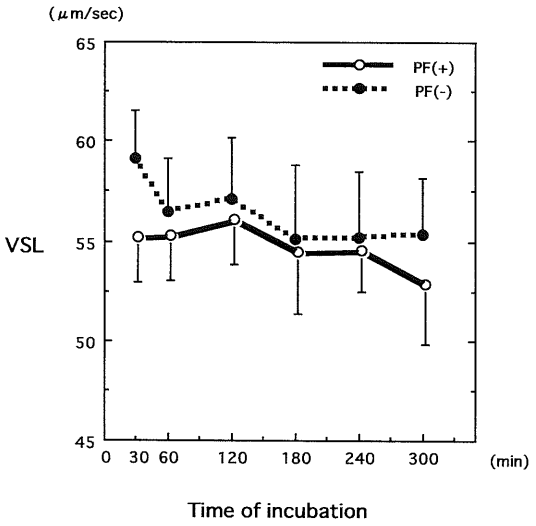

Fig. 2. Straight line velocity (VSL) was unaffected after addition of PF. (n=15)

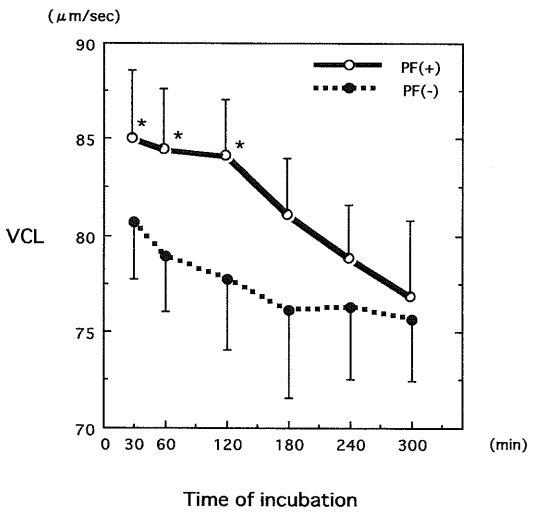

Fig. 3. Curvilinear velocity (VCL) was significantly increased by addition of PF until 120 min. (n=15)
*Statistically significant differences from that of PF(–) ($p<0.05$)

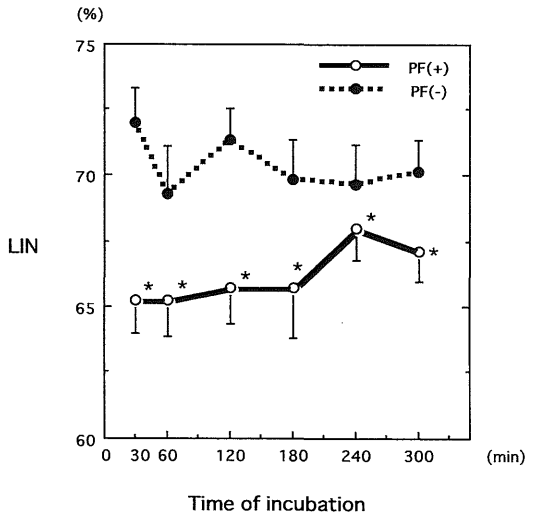

Fig. 4. Linearity (LIN)(=VSL/VCL) was significantly decreased by addition of PF at all times. (n=15)
*Statistically significant differences from that of PF(–) ($p< 0.05$)

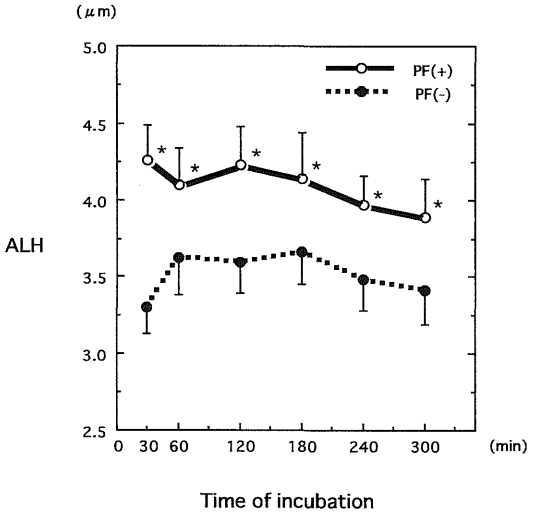

Fig. 5. Lateral head displacement (ALH) was significantly increased by addition of PF at all times. (n=15)
*Statistically significant differences from that of PF(–) ($p< 0.05$)

addition of PF (Fig. 2). In contrast, VCL was higher in PF-treated samples than in control samples at all times and a significant difference in VCL was observed from 30 min to 120 min (Fig. 3), resulting in a reduction in the LIN in PF-treated samples at all times (Fig. 4). The sperm motility, VSL and VCL in the control and PF-treated samples decreased gradually at all times studied (Fig. 1, 2, 3), but the LIN was not changed in either samples up to 300 min (Fig. 4).

The ALH was significantly enhanced by the PF at all times (Fig. 5). The ALH in the control sample increased significantly from 30 min to 60 min and decreased gradually after 180 min. In the PF-treated sample, however, the ALH was not changed until 180 min (Fig. 5).

DISCUSSION

There is controversy as to whether sperm motility determined by a Computer-Aided Sperm Analysis (CASA) shows a significant difference following treatment with PF when compared with control samples. Tesarik et al[26], Pang et al[19], Lewis et al[13] and Tournaye et al[28] observed no change in the sperm motility after treatment with PF in normozoospermic men. In contrast, Fuse et al[8], McKinny et al[15] and Centola et al[6] reported that the sperm motility of PF-treated samples from normozoospermic men was maintained well up to 120 min or more, resulting in a significant difference from controls. Interestingly, Lewis et al[13] observed a significant difference in sperm motility in asthenozoospermic men, that was not observed in normozoospermic men. In the present study, there was no difference in the sperm motility between the PF-treated and control samples until 240 min of incubation, then the PF-treated samples showed higher motility than the control samples at 300 min.

These results, including the present one, cannot simply be compared with other reports because experimental conditions such as sperm treatment before adding PF (washing or swim-up), sperm concentration in the suspension and medium used, final concentration of PF and incubation (=observation) period differ among the reports. In addition, the computerized system used for examination of the sperm motion was not the same. Using CASA, however, there are no reports claiming an obvious increase in the sperm motility after treatment with PF, in contrast to several studies in which sperm motility was determined by a conventional microscopic examination. Therefore, we can conclude that under some experimental conditions PF can prevent a decrease in the sperm motility after long periods of incubation. The reported increase in sperm motility in conventional microscopic examinations may be due to subjective factors resulting from the increased VCL as described below.

Controversy also remains as to the effect of PF on the VSL. On the whole, a significant difference in the VSL was reported in those reports in which a difference in sperm motility was observed. However, Moohan et al[16] reported a difference in the VSL but no difference in the sperm motility of PF-treated samples in normozoospermic men. We observed no significant difference in the VSL between the PF-treated and control samples up to 300 min.

In contrast to the sperm motility and the VSL, almost all reports observed a significantly higher VCL in PF-treated samples in both normozoospermic and asthenozoospermic men. The increasing of the effect of PF on VCL is important because

among the sperm motion characteristics evaluated by CASA, VCL was reported to have a very strong correlation with the fertilization rate in IVF[7,13,30]. In the present study, an obvious difference in the VCL was observed at the first examination (= 30 min incubation) and the difference remained up to 120 min. McKinney et al[15] observed a significant increase in the VCL in the sperm samples incubated for 1 hr with PF compared with the samples before adding PF, and Tesarik et al[26] observed a difference in the VCL between the PF-treated and control samples in a very short incubation such as 10 min. Therefore, it is apparent that PF immediately increases sperm VCL, and maintains VCL higher than in control samples. Consequently, the LIN that is expressed by VSL/VCL decreased or was unchanged in most of the reports. In the present study, the LIN was significantly lower in the PF-treated samples because the VSL was not changed. The ALH was significantly higher in the PF-treated samples than in the control samples, which is consistent with all previous reports. These findings indicate that PF does not increase the linear velocity of sperm, rather the curved component.

In the present study, we analyzed the sperm motion from 30 min to 300 min because there are different opinions as to the duration of the effects of PF on spermatozoa. Yovich et al[32] reported that the fertilization rates in IVF were higher in spermatozoa exposed to PF for 30 min than in those exposed for 60–90 min, and they postulated that the loss of efficacy over long periods is due to an exhaustion of energy substrates or to a limited intracellular energy supply. On the other hand, Lewis et al[13] reported that the sperm VCL was increased significantly from 30 min to 240 min by exposure to PF. As to the reason for the longer efficacy of PF, they postulated that PF is simply acting competitively with intracellular cAMP phosphodiesterase. Calogero et al[4] reported that PF increases sperm intracellular cAMP content for 240 min. Although we did not examine any biochemical parameters of spermatozoa, the present data suggest that the effects of PF on sperm motion may continue for at least 120 min and for as much as 300 min.

It is difficult to directly relate the present results to the reported clinical usefulness of PF in the treatments of male infertility[17,20,27,32]. Our observations suggest, however, that PF may facilitate the sperm in passing through the zona pellucida and entering the oocyte, by increasing its VCL and ALH. In recent years, the effects of PF not only on sperm motion characteristics but also on the sperm acrosome reaction have been reported[5,11,25,33]. We are therefore planning to investigate the effects of PF on the acrosome reaction in patients with male infertility.

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