Effect of Pentoxifylline on Human Sperm Preparation

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INTRODUCTION

Infertility is currently not an uncommon problem in Thailand. In Siriraj Hospital the incidence of infertility in couples was found to be about 14.6%[1], the cause initially unknown. The management of infertility varies. Some of the patients require only very simple treatment but the others require treatment involving higher assisted reproduction techniques. In natural conception, only the sperm which has good motility and normal morphology can fertilize the egg.[2] So, recovery of such a healthy sperm is one of the most important procedures prior to the selection of the treatment option. Currently, there are many techniques available for the recovery of healthy sperm and the technique to be employed depends on the quality of the semen sample. For example, for harvesting healthy sperm from the sperm with poor motility, the Percoll gradient technique is the method of choice.[3-5] However, even though a high number of sperm can be recovered using the Percoll gradient technique, the sample still contains both motile and non-motile sperms. Because the non-motile sperm can not accomplish fertilization, the high number of sperm recovered from the Percoll technique would seem to have no benefit. The most updated assisted reproductive technique, intracytoplasmic sperm injection, was developed to assist fertilization with poor quality of live non-motile sperm. But in semen sample containing non-motile sperm, it is difficult to distinguish live from dead sperm when the semen sample is prepared using the simple technique. Therefore the goal of this study was to modify a simple sperm swim-up technique for use in recovering the motile sperm. The modification was adding 3.3 mmol pentoxifylline solution into a step of the sperm swim-up procedure. If pentoxifylline had a positive effect on the semen samples, at the end of the procedure, a high number of healthy sperm should be recovered in a short period of time.

MATERIALS AND METHODS

The study was an experimental study and was performed at the Infertility Unit, Department of Obstetrics and Gynecology, Siriraj Hospital. All the semen samples were from the husband that had not received any treatment with sex hormones or pentoxifylline for at least three months before the study. The semen samples were divided into two groups and each sample had a volume of 1 ml. The semen samples from the first group were prepared using a normal swim-up preparation technique.[4-5] For the second group, 1 ml of 3.3 mmol pentoxifylline was added to the semen samples and incubated at 37 °C for thirty minutes. After the incubation, the semen samples were prepared using the same technique as was used fro the first group of samples. Five minutes after the swim up procedure, the upper 0.5 ml of supernatant was collected and analyzed. The samples were analyzed for sperm concentration, motility and morphology. The criteria for semen analysis were based on those criteria for semen analysis were based on those criteria standardized by the World Health Organization.6 Sperm morphology was described microscopically after nigrosin and eosin staining. The data were analyzed statistically and comparisons of the data were based on were paired t-test and Wilcoxon Signed-ranks test.

RESULTS

Thirty pairs of semen sample were studied. The average age of the husbands was 32.8 years old ranging from 26 to 43 years old. According to the World Health Organization criteria, the semen analysis of those thirty samples appeared to be normal in volume, motility, vitality, concentration, and morphology (Table 1).

After completing the sperm preparation process, the supernatants were collected and analyzed. The semen samples, at 5 minutes after preparation, in the group prepared by adding pentoxifylline had a significantly higher sperm concentration than did those in the group using the conventional preparation technique (Table 2). However, if the swim up time was delayed for more than ten minutes, the sperm concentrations of the two groups of samples were not significant difference (Table 3).

The sperm motility and morphology after the swim up procedure were also studies. The data showed that the sperm in the group with added pentoxifylline had significantly greater motility than did the normal preparation ($p < 0.05$) as shown in table 4. This increase included all gradings of sperm motility as defined by the World Health Organization, grade A to grade C.[6] Fifty to seventy percent of normal sperm were found the study. The number of sperm with normal morphology was also greater in the pentoxifylline group than the conventional-prepared group (Table 5). However, both techniques resulted in producing a higher number of sperm with normal morphology than the standard level defined by the World Health Organization. Among abnormal sperm morphologies found, small head sperm was the most common.

DISCUSSION

In the past, infertility caused by male factors was a very difficult problem. The three most common male factor disorders are a low sperm concentration (oligospermia), poor sperm motility (asthenospermia), and abnormal sperms morphology (teratospermia). These abnormal sperm are unable to fertilize the ovum naturally. Currently though, these

problems are able to be corrected using the micromanipulation technique. This technique requires great skill and the procedure itself is very expensive so it should be the last choice of treatment. Furthermore, many techniques were developed and applied for recovery of healthy sperm. Of these techniques, administration of pentoxifylline to the husband was one of the trials done. Pentoxifylline is commonly used to treat periheral vascular disease and low cerebral blood flow.[13] Pentoxifylline administration has also been proven to have a positive effect on sperm motility, enhancing sperm motility and helping achieve normal fertilization.[7-12] But pentoxifylline has many adverse effects such as potentiation for antihypertension and/or hypoglyce-mia when taking orally.[13] To avoid these side effects this study was designed to use pentoxifylline in vitro.

The technique involved mixing pentoxifylline solution with semen samples and incubated in test tube for thirty minutes. The mixture was then used for sperm swim-up technique as described earlier. Five minutes after preparing the samples, the sperm concentration of the sperm in samples that were prepared using the pentoxifylline technique was higher than that in samples prepared using the conventional technique, 2.53 million sperms/ml vs 1.18 million sperms/ml respectively ($p < 0.001$). However, when the preparation was left standing longer than fifteen minutes, the sperm concentrations of both samples were not different regardless of technique ($p > 0.05$). This was because the sperm samples we used in the study were all normal, and usually the sperm concentration will reach equilibrium within thirty minutes. Beyond this time the sperm concentration will not be different. At five minutes after preparation the sperm motility was also compared (Table 4), and it was found that the motility of the sperm prepared by pentoxifylline technique was greater than that of samples prepared by the convention method, 95.23% and 91.17% respectively ($p < 0.05$). From these results, it could be concluded that pentoxifylline had a direct effect on the sperm motility to the ability to harvest more sperms than via the conventional method. Furthermore, the sperm morphology was also studied. More sperms with normal morphology were found in the sample prepared using the pentoxifylline technique than in the samples of the conventively ($p < 0.001$). In the conventionally prepared samples, sperm with poor motility but normal morphology, were not able to swim up to the layer of the test tube. But in the pentoxifylline-prepared samples, sperms with poor motility but good morphology were activated and then able to swim up the test tube.[10-11]

The action of pentoxifylline on the sperm lasts for a few hours, enough time to complete intrauterine insemination and in in vitro fertilization. During the process of intracytoplasmic sperm injection (ICSI), live sperms are also necessary. But in samples of non-motile sperm, it is difficult to separate live sperm from the one that recently died. Therefore when pentoxifylline is applied to the sperm preparation, it will be able to enhance motility of those sperms result in proper selection of sperm to be injected to the egg.[8,12] However, more studies are needed to conclude this efficacy of pentoxifylline on the sperm from the poor semen samples.

CONCLUSION

Pentoxifylline can improve the sperm motility even in in vitro preparation. The increase of sperm motility leads to the ability to harvest samples with a higher sperm concentration and higher percentage of morphologically normal sperm in a short period of time.

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Table 1. General data of the husbands and their semen samples.

	Mean ± SD	Range
Age (years)	32.80 ± 3.97	26-43
Semen analysis		
Volume (ml)	2.95 ± 0.85	2.20 - 5.20
Motility (%)	66.30 ± 9.91	52 - 58
Vitality (%)	69.43 ± 8.86	55 - 85
Concentration (10^6/ml)	69.52 ± 37.6	21.87- 167
Normal morphology (%)	50.63 ± 6.35	40 - 75

Table 2. Sperm concentration after 5 minutes swim-up in samples prepared using the conventional technique and the pentoxifylline technique.

	Sperm concentration (million/ml)		
	Mean ± SD	Median	p value*
Non pentoxifylline	1.18 ± 2.75	0.30	
Pentoxifylline	2.53 ± 5.67	0.80	< 0.001

*Wilcoxon Signed-ranks test ($p < 0.05$, significant)

Table 3. Sperm concentration after swim up at different time intervals.

Swim up time (minutes)	Sperm concentration difference between group C and group P mean ± SD (million/ml)	p value
5	0.052 ± 0.92	0.009
15	0.540 ± 0.98	0.058
30	1.040 ± 2.13	0.093
60	0.770 ± 1.59	0.092

Group P = Pentoxifylline-prepared samples
Group C = conventionally-prepared samples
Significant if p value < 0.05

Table 4. Sperm motility after 5 minutes swim up in pentoxifylline and non pentoxifylline technique.

	Sperm motility (Percent)		p value*
	Mean ± SD	Interval	
Non-pentoxifylline	91.17 ± 10.81	87.13, 93.22	
Pentoxifylline	95.23 ± 5.14	93.20, 97.26	0.014

*paired t-test ($p < 0.05$, significant)

Table 5. Number of sperm with normal morphology after 5 minutes swim-up in pentoxifylline and non pentoxifylline technique.

	Normal sperm morphology (Percent)		p value*
	Mean ± SD	Interval	
Non pentoxifylline	52.67 ± 6.63	50.19, 55.15	
Pentoxifylline	63.20 ± 6.45	60.78, 65.62	0.001

*paired t-test ($p < 0.05$, significant)