Effect of Pentoxifylline on Immotile Testicular Spermatozoa

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Purpose: *A model for differentiating live and dead sperm cells during intracytoplasmic sperm injection (ICSI) is proposed.*
Methods: *We used pentoxifylline, a phosphodiesterase inhibitor known to enhance sperm motility, to initiate motility in testicular spermatozoa. Ten immotile testicular sperm samples were divided into two parts for examination of sperm motility with and without pentoxifylline treatment at 30, 60, and 90 min.*
Results: *The samples without pentoxifylline remained immotile even after 90 min of incubation: the addition of pentoxifylline initiated sperm motility in all samples: 51.8 ± 10.2, 64.4 ± 9.4, and 70.8 ± 8.9% (mean ± SD) at 30, 60, and 90 min, respectively.*
Conclusions: *That pentoxifylline may be used to differentiate live testicular spermatozoa during ICSI, which may improve fertilization and pregnancy rates, is suggested.*

KEY WORDS: pentoxifylline; testicular spermatozoa; intracytoplasmic sperm injection.

INTRODUCTION

It has been well documented that in couples with obstructive and nonobstructive azoospermia, testicular sperm extraction (TESE) with intracytoplasmic sperm injection (ICSI) will allow the development of viable embryos and establishment of viable pregnancies (1,2). Testicular biopsy-extracted spermatozoa are often immature and immotile. The vitality of testicular spermatozoa is high, despite their lack of motility. The problem associated with the use of immotile sperm is that the viability of the single spermatozoon used for injection is not determined, although most of this sperm population was proved to be alive (3). Furthermore, the use of immotile testicular sperm in ICSI may result in decreased fertilization (4,5). The most practical and accurate way of choosing live sperm for ICSI is to initiate sperm motility.

The present study was designed to determine the effectiveness of pentoxifylline, a 3′,5′-nucleotide phosphodiesterase inhibitor known to enhance sperm motility (6), for initiating motility in testicular spermatozoa in an effort to propose a method for differentiating live and dead testicular sperm cells during ICSI.

MATERIALS AND METHODS

Sperm Sampling and Preparation

Between August 1995 and May 1996, 154 TESE procedures were performed in couples referred for ICSI treatment with the indications of nonobstructive azoospermia, total necrozoospermia in the ejaculate, and obstructive azoospermia where no spermatozoon was retrieved from the epididymis. Ten immotile testicular sperm samples obtained as described previously (1,2) were included in the study. In one case, TESE was performed to find live spermatozoa, because viability testing of the ejaculate, done by Eosin Y staining, revealed total necrozoospermia. For the other nine cases, TESE was performed due to nonobstructive azoospermia.

The technique of TESE began with a small incision being made in the scrotal skin and carried through the peritoneal tunica vaginalis. Then a small piece of testicular tissue was extruded through a small incision made in the tunica albuginea. The excised tissue was placed in a Falcon tube containing 1 ml HEPES-buffered Earle's medium. The piece of testicular tissue was then progressively divided into small segments and gently crushed between microneedles in a petri dish containing HEPES-buffered medium to obtain a sperm suspension. Then the suspension was divided into two parts for examination of sperm motility with

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and without pentoxifylline (Sigma Co., St Louis, MO; 3.6 m*M* final concentration) treatment at 30, 60, and 90 min of incubation at 37°C under an atmosphere of 5% CO_2 and air. In every sample, 50 to 100 spermatozoa as available were evaluated according to the presence of motility at 30, 60, and 90 min of incubation by the same observer, to whom no information about the samples was given. Comparisons were made using analysis of variance (ANOVA).

RESULTS

The sperm motility in pentoxifylline-treated and -untreated samples was calculated. After 90 min of incubation without pentoxifylline, six sperm samples remained immotile, while in four samples there were a few spermatozoa showing a very slight twitching motion. On the other hand, the addition of pentoxifylline increased the sperm motility significantly ($F = 522.85$, $P < 0.0001$) in all samples: 51.8 ± 10.2, 64.4 ± 9.4, and 70.8 ± 8.9% (mean ± SD) at 30, 60, and 90 min, respectively. In the case where there was no live and motile spermatozoon in the ejaculate, after TESE the vitality testing revealed 80% live sperm, and the 90-min pentoxifylline treatment initiated sperm motility in 77% of the testicular sperm population. The effect of pentoxifylline on individual sperm samples is shown in Table I.

DISCUSSION

The key to obtaining better results with ICSI is to inject live spermatozoa. Total lack of motility makes the selection of live spermatozoa difficult. Determination of sperm viability is an invaluable prerequisite in conducting ICSI. Testicular spermatozoa are often immature and immotile. Although incubating the sperm sample for a time may initiate motility in some of the spermatozoa, there is usually no dramatic improvement in motility. And it is not rare that we have to use immotile sperm for ICSI.

For cases with a total lack of motility in the testicular sperm sample, pentoxifylline can be used to initiate motility of live spermatozoa, if incubation under embryo culture conditions does not help. Pentoxifylline, a 3′,5′-nucleotide phosphodiesterase inhibitor, induces sperm motility by prolonging the availability of cAMP in the cell (6), which requires additional work. The dramatic improvement in sperm motility detected in this study allows us to speculate that pentoxifylline is used more efficiently by immature cells such as testicular spermatozoa. Although, the hypoosmotic swelling test is another option for differentiating live spermatozoa, a method for initiating sperm motility may be more accurate. In patients with conditions such as axonemal defects (immotile cilia syndrome), enzymatic defects (e.g., protein–carboxyl methylase deficiency), functional sperm-tail defects, and total teratozoospermia, pentoxifylline will probably not be useful. For these cases, evaluating membrane integrity by the hypoosmotic swelling test may be indicated for choosing live spermatozoa.

As long as the cause of immotility is not a structural anomaly such as those aforementioned and tail stump syndrome, pentoxifylline can be used to differentiate live testicular spermatozoa by restoring motility, which may improve the fertilization and pregnancy rates in ICSI treatment.

Table I. Effect of Pentoxifylline (PTX) Treatment on Immotile Testicular Spermatozoa

	Sperm motility (%)			
		Treated with PTX		
Sample No.	Untreated, 90 min*	30 min**	60 min[a,**]	90 min[a,*,**]
1	0	47	71	80
2	8	63	75	81
3	6	62	69	71
4	0	49	58	66
5	4	42	64	71
6	5	60	72	78
7	0	64	72	77
8	0	41	59	63
9	0	36	44	52
10	0	54	60	69

[a] Not significant.
* $P < 0.0001$.
** $P < 0.01$.

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