

## EFFECT OF PENTOXIFYLLINE ADDED TO FREEZING MEDIA ON SUBSEQUENT POST-THAW HYPOOSMOTIC SWELLING TEST AND OTHER SEMEN PARAMETERS

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This study evaluated the effects of pentoxifylline (PTX) on post-thaw semen parameters as well as the hypoosmotic swelling (HOS) test. Fourteen samples were evaluated for volume, count, motility, % grade A sperm, and HOS test. Two aliquots were frozen, one in freezing medium and the other in a 3 mM solution of PTX and freezing medium. Both groups were frozen in liquid nitrogen vapors for 30 min. Thawing was performed at 37°C for 15 min, followed by a wash with 2 parts 0.5% HSA/MHTF to 1 part sample. Pellets were resuspended in 0. MHTF and then evaluated as described above. In addition, motility was evaluated 2 h post-thaw. Following freeze-thaw, the mean motile densities were similar ( $17.5 \times 10^6$  motile/mL vs.  $20.4 \times 10^6$  motile/mL for PTX and control, respectively). Two hours post-thaw, the PTX group had a mean sperm motility of 31.3% vs. 37.7% for the control group ( $p > .05$ ). There were no significant differences in % grade A sperm in PTX (13.0%) vs. control (12.0%). Similarly, HOS scores did not improve following cryopreservation (43.0% and 50.6% for PTX and control, respectively). Thus, no improvement was found by freezing sperm with PTX.

**Keywords** pentoxifylline, sperm, acrosome, freezing, hypoosmotic swelling

The hypoosmotic swelling (HOS) test is used to evaluate the functional integrity of the sperm membrane [5]. Very poor pregnancy rates (PRs) were achieved following in vitro fertilization-embryo transfer (IVF-ET) and in vivo techniques when the male partner had a HOS score of <50% [1, 2]. Furthermore, cryopreservation significantly decreased HOS scores as well as conventional semen parameters [3]. Pentoxifylline (PTX) enhances sperm motility, acrosome reaction, and fertilizing ability of human sperm [6-9, 12]. There is evidence that PTX suppresses reactive oxygen species (ROS) release [10]. A study by Wang et al. [13]

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demonstrated an increase in post-thaw motility with the addition of PTX to the freezing medium. However, Wang et al. did not evaluate the effect of PTX on the HOS test.

This study was initiated to further evaluate the effects of PTX on both conventional semen parameters as well as the HOS test.

## MATERIALS AND METHODS

*Sperm Collection and Evaluation.* Ejaculates ( $n = 14$ ) were collected from normal donors, via masturbation, and allowed to liquify for 20–30 min. They were then evaluated according to World Health Organization (WHO) standards [14] for volume, count, percent motility, percentage of grade A sperm, and functional integrity of the sperm membrane as determined by the HOS test [5].

*Semen Preparation.* After evaluation, specimens were divided into two aliquots and treated as follows: Group 1 (control) was cryopreserved in freezing medium (Irvine Scientific Cat #9971) only; and group 2 (PTX) was cryopreserved in 3 mM solution of PTX and freezing medium (1 mg PTX/mL final concentration). Both groups were frozen by placing the vials in liquid nitrogen vapors for 30 min followed by a plunge into liquid nitrogen. After at least 2 weeks, the samples were thawed at 37°C for 15 min. The samples were washed using two parts 0.5% HSA/modified HTF to one part sample for 5 min at 300g and resuspended in 0.5 mL/MHTF. The washed samples were evaluated for count, motility, % grade A sperm, and HOS test. In addition, motility was reevaluated after 2h.

*Statistical Analysis.* The data were analyzed using Friedman's nonparametric analysis of variance (ANOVA).

## RESULTS

The mean post-thaw count and percent motility was  $48.3 \times 10^6/\text{mL}$  and 36.3% for the PTX group and  $48.3 \times 10^6/\text{mL}$  and 42.2% for the control. Motile densities were also similar:  $17.5 \times 10^6$  vs.  $20.4 \times 10^6$  motile/mL for PTX and control, respectively ( $p > .05$ ). Two-hour post-thaw motilities were similar as well: 31.3% for the PTX group and 37.7% for the control group ( $p > .05$ ). The percent grade A sperm was also similar in the PTX (13.0%) and the control (12.0%) groups ( $p > .05$ ). HOS scores did not improve with the addition of PTX (43.0%) compared to the control group (50.6%). No significant differences were found between the control and PTX groups in percent motility, percent grade A sperm, or 2-h motility following cryopreservation (Friedman's nonparametric ANOVA).

## DISCUSSION

The addition of PTX to the freezing medium did not improve post-thaw sperm quality and no improvement was seen in post-thaw HOS test scores. In fact, the mean PTX HOS score was considered abnormal, whereas the control group was still in the normal range [2].

In this study, a 3 mM solution of PTX was used. This is the most common concentration used in clinical practice for increasing sperm motility and inducing the acrosome reaction [4, 8, 11]. However, Yovich [9] suggests that a higher concentration of PTX, up to a 10 mM solution, is required to complete ROS release suppression. The toxicity of PTX at such a concentration has yet to be determined. It is possible that freezing sperm with a higher concentration of PTX may indeed improve post-thaw sperm quality.

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