

GYNAECOLOGY

Can Pentoxifylline-Supplemented Cryoprotectant Improve Human Sperm Motility After Cryopreservation?

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Sperm cryopreservation or sperm banking is the prolonged maintenance of sperm in the frozen state at extremely low temperature. The technique is proposed to help infertile couples who have male problems, and patients undergoing treatments that may affect their fertility. After the discovery of frozen frog semen in snow by Spallanzani 200 years ago.⁽¹⁾ Sperm cryopreservation technique has been developed continuously from freezing in dry ice to freezing in liquid nitrogen.

However, freeze-thaw procedures can damage the spermatozoa. The cryoinjury results in decreased post-thaw motility and viability that affects fertilizability of spermatozoa. Pentoxifylline, 2-deoxyadenosine, and caffeine were shown to improve sperm motility and viability when added to the sperm medium.⁽²⁾ Pentoxifylline is the most popular sperm stimulant used at the optimal concentration of 2.5-3 mM.⁽³⁻⁶⁾ Early use of pentoxifylline with frozen-thaw spermatozoa was reported in 1988 with the promising results.⁽⁷⁾

Most studies have reported the benefit of pentoxifylline as a post-thaw stimulant of the frozen sperm. Very few studies showed the effect of pentoxifylline when added in the cryopreserved medium.^(6,8,9) Pentoxifylline may act as a cryoprotectant agent in addition to a sperm stimulant. This study was

designed to show the effect on sperm post-thaw motility of the pentoxifylline-supplemented cryoprotectant.

Materials and methods

Thirty-one specimens of ejaculated human semen with normal characteristics [$>50\%$ motile spermatozoa, $>20 \times 10^6$ spermatozoa/ml and $>30\%$ normal morphology; World Health Organization (WHO) 1992]⁽¹⁰⁾ were obtained from normal donors at the Infertility Clinic in Songklanagarind Hospital from March to October 2002. Semen samples were split into three equal aliquots after semen analysis and allocated to the following groups: semen alone, semen with cryoprotectant medium (made in-house), and semen with pentoxifylline-supplemented cryoprotectant (3 mM of pentoxifylline, Sigma, St. Louis, USA).

Cryopreservatinon

Semen specimens were cryopreserved with liquid nitrogen vapour technique according to the following methodology. The semen specimen was added to an equal volume of cryoprotective medium or pentoxifylline-supplemented cryoprotective medium drop by drop. The mixtures were left for 10 minutes at room temperature and aspirated into 0.25 ml straws.

The straws was then placed in the vapour phase of liquid nitrogen (10 cm above the liquid level) for 30 minutes before being submersed quickly into the liquid nitrogen. The specimens were stored for 3-7 days, and were then quickly thawed by rolling on a 37°C stage warmer for 30 seconds. Thawed specimens were examined for progressive motility of the spermatozoa. Progressive motility was scored independently by 2 observers who were blinded to the treatment. Percentages of spermatozoa with progressive motility were compared across treatments using the generalized estimating equations method for linear regression.

Results

Demographic data of sperm donors are shown in Table 1. The mean age of sperm donors was 28.4±5.7 years, the mean volume of semen was 2.71±0.83 ml and the mean sperm density was 116.9±58.5 million/ml. The mean percentage of sperm motility (95% CI) classified as grade A or B and C or D before cryopreservation were 61.7 and 38.3, respectively. Cryoprotectant was found to improve post-thaw progressive motility from a mean of 10.2% (95%CI 8.5-11.9%) for semen alone to 22.0% (95% CI 20.3-23.8%) for semen with cryoprotectant medium. Pentoxifylline-supplemented cryoprotectant could improve post thaw progressive motility to 29.3% (95% CI 27.5-31.0%) (P< 0.0005 in all 3 pairwise comparisons) (Table2).

Table 1. Demographic data of sperm donors

	Min	Max	Mean	Standard deviation
Age (years)	22	41	28.4	5.7
Volume (ml)	2	4	2.71	0.83
Normal Morphology (%)	33	75	52.84	14.38
Sperm density (million/ml)	45	289	116.9	58.5

Table 2. Mean percentage of sperm motility (95% confidence interval), according to grade A or B and C or D, adjusted for observers

Condition	Grade of sperm motility	
	A and B	C and D
0. Fresh semen	61.7 (60.0-63.5)	38.3 (36.5-40.0)
1. Post-thaw after freezing without cryoprotectant	10.2 (8.5-11.9)	89.8 (88.1-91.5)
2. Post-thaw after freezing with cryoprotectant	22.0* (20.3-23.8)	78.0 (76.2-79.7)
3. Post-thaw after freezing with pentoxifylline-supplemented cryoprotectant	29.3** , *** (27.5-31.0)	70.7 (69.0-72.5)

* P < 0.0005 for compare between group 1 and group 2

** P < 0.0005 for compare between group 1 and group 3

*** P < 0.0005 for compare between group 2 and group 3

Discussion

Cryoinjury is always a problem for sperm freezing. Without a good procedure, frozen-thaw specimen may lose nearly all progressive motile spermatozoa necessary for fertilization. In addition to a well-controlled freezing program, cryoprotectant or cryopreserved medium can decrease the loss and ameliorate the injury. However, frozen-thawed specimens still have poor performance when compared to fresh specimens. Sperm stimulating agents are used to improve sperm performance for clinical use.

Methylxanthines have been shown to stimulate the motility of cryopreserved sperm after a short period of incubation.⁽¹¹⁻¹⁶⁾ The methylxanthines can inhibit cyclic adenosine monophosphate (cAMP) phosphoesterase resulting in an increase in intracellular cAMP concentration. The increase in intracellular cAMP concentration may stimulate the sperm motility. Some other agents such as caffeine and 2-deoxyadenosine can stimulate sperm function as well.⁽¹⁷⁾ However, those agents and its metabolites may, at least in vitro, have mutagenic effects.⁽¹⁸⁾ Therefore, some stimulating agents may have limited role in clinical use.

Pentoxifylline, a trisubstituted methylxanthine derivative, is recently developed and originally used as an oral agent for the treatment of claudication in patients with peripheral vascular diseases. Pentoxifylline has been used successfully in the treatment of oligoasthenospermic male.^(16,19) The main mechanisms of action of pentoxifylline are (i) inhibition of phosphodiesterase, (ii) inhibition of calcium efflux and (iii) antagonism of the adenosine receptor.⁽²⁰⁾ The first pregnancy resulted from pentoxifylline-treated spermatozoa using pronuclear stage embryo transfer (PROST) was reported in 1988.⁽⁷⁾ In that instance pentoxifylline was used to stimulate a sperm preparation as part of in vitro fertilization (IVF). Up to the present, there have been no birth defects noted in live births from pentoxifylline-treated sperm.⁽¹⁹⁾ Pentoxifylline has been tested at levels up to 450 mg/kg/day in laboratory animals with no significant teratogenic effects.

Most previous studies reported significantly improved sperm performance after being incubated with pentoxifylline post-thaw.^(2,3,21) The stimulating effect could last as long as 24 hours-enough for clinical use. Considering the antioxidative effect of pentoxifylline,⁽²²⁾ pentoxifylline-supplemented cryoprotectant may decrease the cryoinjury associated with the freezing process. However, the results from earlier studies are still controversial. Wang and co-workers found better post-thaw sperm motility with cryoprotective additives like pentoxifylline and platelet activating factor.⁽²³⁾ Brennan and Holden reported the benefit of pentoxifylline-supplemented cryoprotectant at lower concentration(1mM).⁽⁶⁾ The post-thaw acrosome reaction response may be better with the prefreeze use of pentoxifylline.⁽⁸⁾ In contrast, Check et al. found no improvement in post-thaw sperm motility and hypoosmotic swelling test after freezing with pentoxifylline.⁽⁹⁾ Our study showed some benefit with pentoxifylline addition in cryoprotective medium. However, more studies may be needed to compare the benefit with post-thaw use in human sperm. Moreover, pregnancy result will be the ultimate validation for prefreeze use of pentoxifylline.

In summary, this study demonstrates the benefit of pentoxifylline-supplemented cryoprotective medium. At the 3 mM concentration of pentoxifylline, the additive could improve the post-thaw sperm motility significantly. However, more studies are needed before application in clinical use.

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References

1. Sherman JK. Cryopreservation of human semen. In: Keel BA, Webster BW eds. CRC Handbook of the Laboratory Diagnosis and Treatment of Infertility. 2nded. Boston: CRC Press, 1990: 229-59.
2. Sharma RK, Tolentino MV Jr, Agarwal A. Sperm kinematics of cryopreserved normozoospermia specimens after artificial stimulation. *Urology* 1996;47: 77-81.
3. Sharma RK, Tolentino MV Jr, Thomas AJ Jr, Agarwal A.

- Optimal dose and duration of exposure to artificial stimulants in cryopreserved human spermatozoa. *Urology* 1996; 155: 568-73.
4. Centola GM, Cartie RJ, Cox C. Differential responses of human sperm to varying concentrations of pentoxifylline with demonstration of toxicity. *J Androl* 1995; 16: 136-42.
 5. Johnston RC, Mbizvo MT, Summerbell D, Kovacs GT, Baker HW. Relationship between stimulated hyperactivated motility of human spermatozoa and pregnancy rate in donor insemination. *Hum Reprod* 1994;9: 1684-7.
 6. Brennan AD, Holden CA. Pentoxifylline-supplemented cryoprotectant improves human sperm motility after cryopreservation. *Hum Reprod* 1995; 10 : 2308-12.
 7. Yovich JM, Edirisinghe WR, Cummins JM, et al. Preliminary results using pentoxifylline in a pronuclear stage tubal transfer (PROST) program for severe male factor infertility. *Fertil Steril* 1988;50:179-81.
 8. Esteves SC, Sharma RK, Thomas AJ, Agarwal A. Cryopreservation of human spermatozoa with pentoxifylline improves the post-thaw agonist-induced acrosome reaction rate. *Hum Reprod* 1998;13:3384-9.
 9. Check DJ, Kiefer D, Katsoff D, Check JH. Effect of pentoxifylline added to freezing media on subsequent post-thaw hypoosmotic swelling test and other semen parameters. *Arch Androl* 1995;35:161-163
 10. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rded. Cambridge: Cambridge University Press, 1992.
 11. Cai XQ, Marik JJ. Improving penetrating capacity of spermatozoa with poor motility by addition of caffeine at coincubation with zona-free hamster ova. *Fertil Steril* 1989;51:719-21.
 12. Aitken RJ, Best FSM, Richardson DW, et al. Influence of caffeine on movement characteristics, fertilizing capacity and ability to penetrate cervical mucus of human spermatozoa. *J Reprod Fertil* 1983;67:19-27.
 13. Barkay J, Bartoov B, Ben-Ezra S, et al. The influence of in vitro caffeine treatment on human sperm morphology and fertilizing capacity. *Fertil Steril* 1984;41:913-8.
 14. Aaberg RA, Sauer MV, Sikka A, et al. Effects of extracellular ionized calcium, diltiazem, and cAMP on motility of human spermatozoa. *J Urol* 1989;141:1221-24.
 15. Ree JM, Ford WC, Hull MG. Effect of caffeine and of pentoxifylline on the motility and metabolism of human spermatozoa. *J Reprod Fertil* 1990;90:147-56.
 16. Hammitt DG, Bedia E, Rogers PR, et al. Comparison of motility stimulants for cryopreserved human semen. *Fertil Steril* 1989;52:495-502.
 17. Tash JS, Means AR. Regulation of protein phosphorylation and motility of sperm by cAMP and calcium. *Biol Reprod* 1982;26:745-63.
 18. Harrison RF, Sheppard BL, Kalisz M. Observations on the motility, ultrastructure and elemental composition of human spermatozoa incubated with caffeine. *Andrologia* 1980;12:434-43.
 19. Yovich JM, Edirisinghe WR, Cummins JM, et al. Influence of pentoxifylline in severe male factor infertility. *Fertil Steril* 1990;53:715-22.
 20. Lanzafame F, Chapman MG, Guglielmino A, Gearon CM, Forman RG. Pharmacological stimulation of sperm motility. *Hum Reprod* 1994;9:192-99.
 21. Sharma RK, Kohn S, Padron OF, Agarwal A. Effect of artificial stimulants on cryopreserved spermatozoa from cancer patients. *J Urol* 1997;157:521-24.
 22. Yovich JL. Pentoxifylline: actions and applications in assisted reproduction. *Hum Reprod* 1993;8:1786-91.
 23. Wang R, Sikka SC, Veeraragavan K, Bell M, Hellstrom WJ. Platelet activating factor and pentoxifylline as human sperm cryoprotectants. *Fertil Steril* 1993;60:711-5.