The Effects of Follicular Fluid and Platelet-Activating Factor on Motion Characteristics of Poor-Quality Cryopreserved Human Sperm

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Purpose: To evaluate the effects of follicular fluid and platelet-activating factor on sperm motion characteristics of cryopreserved oligospermic and normospermic samples.

Methods: Sperm motion characteristics were evaluated prior to cryopreservation, immediately after thawing and following incubation in human tubal fluid, follicular fluid, or 1- μ M platelet-activating factor cultures. Sixteen oligospermic samples and 20 normospermic samples were examined. Sperm motion characteristics were analyzed manually according to WHO criteria (1999) and also with an automated videomicrography system.

Result(s): Incubation in follicular fluid increased overall motility and the percentage of sperm with fast progressive motility in normospermic but not oligospermic samples. Incubation with platelet-activating factor increased overall motility and the percentage of sperm showing nonprogressive motility in both oligospermic and normospermic samples.

Conclusion(s): The stimulatory effects of culture in follicular fluid as seen in normospermic samples do not show a significant benefit in oligospermic cryopreserved samples. Platelet-activating factor and follicular fluid increase motility via different mechanisms. Incubation of oligospermic cryopreserved sperm with PAF increases the number of motile sperm, thereby enabling easier identification of viable sperm

for intracytoplasmic sperm injection in samples with severe asthenozoospermia.

KEY WORDS: Oligospermia; sperm cryopreservation; sperm motility; platelet-activating factor; follicular fluid.

INTRODUCTION

Cryopreserved sperm has been used in the treatment of infertility for more than 40 years (1). Despite continued refinement of methods of sperm cryopreservation, a reduction in motility of between 25%–75% is found after thawing (2). It is well established that the ability of cryopreserved sperm to fertilize an oocyte and achieve a pregnancy is greater if the postthaw motility percentage is high (3). The postthaw motility is related to the precryopreservation motility (4).

The application of sperm cryopreservation for infertile males has been complicated by the high loss of postthaw motility (5). However, with the development of intra cytoplasmic sperm injection (ICSI), where only one viable sperm per oocyte is needed (6), the cryopreservation and storage of poorer semen samples for future ICSI application has become a worthwhile treatment option. The optimisation of the postthaw motility of poor semen samples facilitates the selection of sperm during the ICSI procedure. It is a responsibility of the embryologist performing the ICSI to choose the most “viable” sperm to inject (6). The parameters the embryologist primarily relies upon to determine “viability” are motility and morphology (6). Higher numbers of motile sperm per sample allow a more stringent assessment of the morphology of sperm suitable for injection.

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One method that has been described to identify viable sperm in samples with very poor or no motile sperm present is the hypo-osmotic swelling test (HOS) (7). However, this test has been shown to be unreliable for cryopreserved sperm (8). The ability to improve postthaw sperm motility will benefit men with poorer semen parameters undergoing assisted reproduction treatment. A number of methods are commonly used to improve motility in postthaw cryopreserved semen from normospermic men. Sperm motion characteristics improve after incubation with methylxanthines (9), platelet-activating factor (PAF) (5), and vero cell coculture (10). Sharma *et al.* (1997), showed that postthaw motility of cryopreserved oligospermic samples could be increased by exposure to methylxanthines in culture. However, methylxanthines, including pentoxifylline and caffeine may exert mitogenic effects, and because these agents may be absorbed into the sperm, it raises safety concerns regarding their use (12). Compounds that sperm are exposed to in-vivo, such as PAF (13) and follicular fluid (FF), can stimulate sperm motion characteristics in normal, fresh semen samples (14). PAF receptors have been identified on individual sperm, localized to the midpiece and proximal head (15). This suggests a direct role for PAF in influencing sperm motility. The potential stimulatory effects of PAF and FF on sperm motion characteristics of oligospermic, cryopreserved semen have not been studied.

The aim of this study was to assess the effects on sperm motility parameters of human tubal fluid (HTF) medium alone, PAF and FF incubated with postthawed cryopreserved semen from oligospermic men.

MATERIALS AND METHODS

Subjects

Semen samples from male partners of couples being treated with in vitro fertilization were collected by masturbation after a period of 3–5 days abstinence. The samples were assessed according to WHO criteria (1999). FF was obtained from follicular aspirates at the time of oocyte collection from women undergoing in vitro fertilization treatment. This project was approved by the Hospital Authority of Hong Kong Ethics Committee, Approval No. (10) in PD/08/04/98.

Cryopreservation of Semen

Samples were diluted stepwise to a 1:1 concentration with Human Semen Preservation Medium

(HSPM) (17) and aliquoted into 1.8-ml vials. Cryopreservation vials were placed at 4°C for 10 min, then at –20°C for 10 min, and at –80°C for 10 min prior to being plunged into liquid nitrogen. Cryopreserved samples were thawed at 37°C and washed twice with Hepes buffered HTF to remove the cryoprotectant.

Sperm Culture

Samples were divided into three replicates and were resuspended to a concentration of 20×10^6 /ml (unless available were insufficient total sperm numbers, in which case the highest possible concentration was used). The samples were then incubated at 37°C with 5% CO₂ in a humidified incubator, for 2 h in one of the following media:

- a. HTF (Irvine Scientific, USA) medium with 10% synthetic serum substitute (Irvine Scientific, USA) (HTF)
- b. FF
- c. HTF with 1- μ M PAF (Sigma, USA)

Sperm Analysis

Five-microliter aliquots of each sample for each of the culture conditions were analysed by a computer-assisted motion analyzer (Hobson Sperm Tracker System Ltd., Sheffield, UK)

Percent motility (%mot), sperm concentration ($\times 10^6$ /ml), curvilinear velocity (VCL: the total distance travelled by a given sperm divided by the total time elapsed), straight line velocity (VSL: the straight line distance from the beginning of the sperm track divided by the total time elapsed), average path velocity (VAP), linearity (LIN: the ratio of curvilinear to straight line velocity), amplitude of lateral head displacement (ALH: the mean width of sperm head oscillation) and percent hyperactivated (criteria: VCL = 70- μ m/s, ALH > 5- μ m, LIN < 30%) were analysed. Two hundred sperm per aliquot were tracked in accordance with WHO guidelines (16).

The Hobson Sperm Tracker results for motility and sperm concentration were verified manually (16).

Statistical Analysis

Paired Student *t*-test was used to evaluate percentage changes in the motion characteristics. A value of *p* < .05 was considered significant. Semen samples in each group were studied individually to avoid any possible effects of pooling the samples.

RESULTS

Thirty-six semen samples, 16 oligospermic (5 with counts between $15\text{--}20 \times 10^6/\text{ml}$, 3 with counts between $10\text{--}15 \times 10^6/\text{ml}$, 4 with counts between $5\text{--}10 \times 10^6/\text{ml}$, and 4 with counts between $1\text{--}2 \times 10^6/\text{ml}$) and 20 normospermic samples, were analysed.

Computer-Assisted Sperm Analysis

The data derived from the Hobson Sperm Tracker for the postthaw oligospermic samples showed a high variation in sperm concentration and motility, both within the sample itself and when comparing it to the manual method (Table I). The differences in results were outside the 95% confidence interval for differences between two counts indicating a systematic error (16). Very slow progressive motility and non-progressive motility that were common in these samples were not detected by the program. Therefore the use of the sperm tracker for the samples used in this study was not considered appropriate, and the manual method was used to perform comparisons between the sample groups.

Analysis of Sperm Motility

Immediately after thawing, the mean percentage motility was significantly lower in oligospermic ($5.7\% \pm 5.9\%$) compared to normospermic ($18.9\% \pm 11\%$) samples ($p < .001$).

Following 1- or 2-h incubation in HTF alone, there was no significant change in the mean percentage motility in either the oligospermic or normospermic samples (Table II). Following 1-h incubation in FF there was no significant difference in the mean percentage motility in either the oligospermic or the normospermic samples (Table II). However, after 2-h incubation in FF the mean percentage motility in normospermic samples was significantly increased

Table I. Examples of Variation Within Repeated Analysis of the Same Sample and Between Manual and Computer-Assisted Semen Analysis (CASA) in postthawed Oligospermic Samples

	CASA 1		CASA 2		Manual	
	Conc.	Mot. %	Conc.	Mot. %	Conc.	Mot. %
Sample 14	4.1	0	7.2	6	22	2
Sample 19	5.2	10	8.1	0	25	1
Sample 21	1.0	1	4.1	9	20	30

Note. Conc.: sperm concentration; Mot. %: the percentage of motile sperm.

Table II. The Mean $\pm$ SD for % Motility Following Incubation in HTF, FF, or HTF with $1\text{-}\mu\text{M}$ PAF for 1 and 2 h

	Normospermic ($N = 20$)	Oligospermic ($N = 16$)
Postthaw	18.9 ± 11.1	5.7 ± 5.9
HTF (1 h)	21.5 ± 11.4	6.4 ± 6.1
HTF (2 h)	24.5 ± 13.0	6.7 ± 6.0
FF (1 h)	23.0 ± 10.9	6.5 ± 6.1
FF (2 h)	$29.3 \pm 11.6^*$	7.1 ± 5.7
PAF (1 h)	$29.7 \pm 12.8^*$	$12.1 \pm 7.3^{**}$
PAF (2 h)	$36.1 \pm 14.2^{***}$	$13.1 \pm 7.1^*$

* $p < .05$; ** $p = .001$; *** $p = .005$.

($18.9\% \pm 11.1\%$ vs. $29.3\% \pm 11.6\%$, $p < .05$). This increase was not seen in the oligospermic samples (Table II). After 1-h incubation in HTF with $1\text{-}\mu\text{M}$ PAF, there was a significant increase in the mean percentage motility in both oligospermic ($5.7\% \pm 5.9\%$ vs. $12.1\% \pm 7.3\%$, $p < .001$) and normospermic ($18.9\% \pm 11.1\%$ vs. $29.7\% \pm 12.8\%$, $p < .05$) groups. After 2-h incubation in HTF with $1\text{-}\mu\text{M}$ PAF, the mean percentage motility was significantly higher than the postthaw result in both oligospermic ($5.7\% \pm 5.9\%$ vs. $13.1\% \pm 7.1\%$, $p < .05$) and normospermic ($18.9\% \pm 11.1\%$ vs. $36.1\% \pm 14.2\%$, $p < .005$) groups, but was not significantly different from the result of 1-h incubation in HTF with $1\text{-}\mu\text{M}$ PAF (Table II).

There was no significant difference in the mean percentage motility between samples incubated for 1 h in FF compared to HTF (Table II). However after 2-h incubation in FF compared to HTF the percentage motility in normospermic ($29.3\% \pm 11.6\%$ vs. $24.5\% \pm 13.0\%$, $p < .01$), but not in oligospermic, sample was significantly increased (Table II). Incubation in HTF with $1\text{-}\mu\text{M}$ PAF for 1 h significantly increased the mean percentage motility compared to incubation with HTF alone in both oligospermic ($12.1\% \pm 7.3\%$ vs. $6.4\% \pm 6.1\%$, $p < .001$) and normospermic ($29.7\% \pm 12.8\%$ vs. $21.5\% \pm 11.4\%$, $p < .001$) samples. Incubation in HTF with $1\text{-}\mu\text{M}$ PAF for 1 h significantly increased the mean percentage motility compared to incubation in FF in both oligospermic ($12.1\% \pm 7.3\%$ vs. $6.5\% \pm 6.1\%$, $p < .02$) and normospermic ($29.7\% \pm 12.8\%$ vs. $23.0\% \pm 10.9\%$, $p < .05$) samples. After 2-h incubation in HTF with $1\text{-}\mu\text{M}$ PAF, the mean percentage motility was significantly higher in both oligospermic ($13.1\% \pm 7.1\%$ vs. $6.7\% \pm 6.0\%$, $p < .001$) and normospermic ($36.1\% \pm 14.2\%$ vs. $24.5\% \pm 13.0\%$, $p < .001$) samples compared to incubation in HTF alone. Normospermic samples incubated in FF for 2 h did not show any difference in the mean percentage motility compared to normospermic samples incubated in HTF with $1\text{-}\mu\text{M}$ PAF (Table II).

Table III. The Mean $\pm$ SD for % Fast Progressive Motility Following Incubation in HTF, FF, or HTF with 1- μ M PAF for 1 and 2 h

	Normospermic (<i>N</i> = 20)	Oligospermic (<i>N</i> = 16)
Postthaw	8.8 $\pm$ 8.3	0.6 $\pm$ 1.2
HTF (1 h)	11.1 $\pm$ 9.1	0.8 $\pm$ 1.5
HTF (2 h)	13.9 $\pm$ 9.9	1.4 $\pm$ 1.7
FF (1 h)	14.6 $\pm$ 8.7	2.5 $\pm$ 3.0
FF (2 h)	19.0 $\pm$ 9.7*	3.8 $\pm$ 3.9
PAF (1 h)	14.1 $\pm$ 9.2	1.9 $\pm$ 2.4
PAF (2 h)	14.1 $\pm$ 9.2	2.4 $\pm$ 2.4

**p* < .05.

In comparison, oligospermic samples incubated in HTF with 1- μ M PAF for 2 h did show significantly increased mean percentage motility compared to oligospermic samples incubated in FF for 2 h (13.1% $\pm$ 7.1% vs. 7.1% $\pm$ 5.7%, *p* < .01).

Analysis of Fast Progressive Motility

Incubation for 1 h in HTF alone, FF or HTF with 1- μ M PAF had no effect on fast progressive motility in either oligospermic or normospermic samples (Table III). After 2-h incubation in HTF alone and HTF with 1- μ M PAF again showed no effect on fast progressive motility (Table III). However FF incubation for 2 h resulted in a significant increase in the mean percentage of fast progressive motility for normospermic (8.8% $\pm$ 8.3% vs. 19.0% $\pm$ 9.7%, *p* < .05) but not for oligospermic samples (Table III).

Analysis of Slow Progressive Motility

Incubation for 1 or 2 h in HTF alone, FF or HTF with 1- μ M PAF had no significant effect on the mean percentage of slow progressive motility in either oligospermic or normospermic samples (Table IV).

Analysis of Nonprogressive Motility

Incubation in either HTF alone or in FF for 1 or 2 h had no effect on the mean percentage of non-

Table IV. The Mean $\pm$ SD for % Slow Motility Following Incubation in HTF, FF, or HTF with 1- μ M PAF for 1 or 2 h

	Normospermic (<i>N</i> = 20)	Oligospermic (<i>N</i> = 16)
Postthaw	7.7 $\pm$ 5.4	3.0 $\pm$ 3.9
HTF (1 h)	8.5 $\pm$ 5.5	3.1 $\pm$ 4.2
HTF (2 h)	8.7 $\pm$ 5.7	3.0 $\pm$ 4.1
FF (1 h)	6.4 $\pm$ 5.1	2.5 $\pm$ 2.7
FF (2 h)	5.5 $\pm$ 4.8	2.1 $\pm$ 2.2
PAF (1 h)	5.9 $\pm$ 4.1	3.0 $\pm$ 3.0
PAF (2 h)	6.7 $\pm$ 4.7	2.6 $\pm$ 2.7

Table V. The Mean $\pm$ SD for % Nonprogressive Motility Following Incubation in HTF, FF, or HTF with 1- μ M PAF for 1 or 2 h

	Normospermic (<i>N</i> = 20)	Oligospermic (<i>N</i> = 16)
Postthaw	2.5 $\pm$ 1.8	2.1 $\pm$ 1.9
HTF (1 h)	2.1 $\pm$ 1.9	2.5 $\pm$ 2.2
HTF (2 h)	2.2 $\pm$ 1.2	2.4 $\pm$ 2.2
FF (1 h)	2.1 $\pm$ 2.5	1.6 $\pm$ 1.5
FF (2 h)	1.9 $\pm$ 1.8	1.3 $\pm$ 1.3
PAF (1 h)	10.9 $\pm$ 4.6*	8.6 $\pm$ 3.8**
PAF (2 h)	13.5 $\pm$ 4.8*	8.2 $\pm$ 3.4*

p* < .05; *p* < .001.

progressive motility in either oligospermic or normospermic samples (Table V). Incubation in HTF with 1- μ M PAF for 1 h resulted in a significant increase in the mean percentage of nonprogressive motility of both oligospermic (2.1% $\pm$ 1.9% vs. 8.6% $\pm$ 3.8%, *p* < .001) and normospermic (2.5% $\pm$ 1.8% vs. 10.9% $\pm$ 4.6%, *p* < .05) samples. Incubation in HTF with 1- μ M PAF for 2 h showed a significant increase in the mean percentage of nonprogressive motility, in oligospermic (2.1% $\pm$ 1.9% vs. 8.2% $\pm$ 3.4%, *p* < .05) and normospermic (2.5% $\pm$ 1.8% vs. 13.5% $\pm$ 4.8%, *p* < .05) samples but there was no difference between the effect of PAF in 1-h incubation compared to 2-h of incubation (Table V).

DISCUSSION

Most studies that have used computer-assisted semen analysis have studied normal semen that has undergone percoll separation to minimize the number of nonmotile sperm in the prepared sample (10,14) or else have examined semen with poorer motility but normal counts (5). The lack of reliability seen in our results may have been because of the combination of very low counts and the high percentage of immotile sperm in these samples. It is not possible to analyse nonprogressive motility with the Hobson Sperm Tracker, but this type of motility is significant when assessing severe male factor infertility where ICSI is indicated. Because computer analysis was unreliable, we manually assessed the samples in our study.

Our results showed that incubating postthawed cryopreserved sperm in HTF medium had no effect on motility characteristics. These data are consistent with the results of previous studies (11,14). FF has previously been shown to increase the motility and the number of sperm showing fast progressive motility (14), and these findings were also confirmed by our

data. However, this stimulatory effect was not seen in oligospermic samples.

PAF, on the other hand, exerted a stimulatory effect on both oligospermic and normospermic samples with a maximal effect at 1 h. These results support the findings of Hellstrom *et al.* (1991), on normospermic semen donors, who showed PAF to have a stimulatory effect on motility with a maximal response at 1- μ M with 1-h incubation. There are no previous data on the effect of PAF on oligospermic samples. Normospermic samples showed a significant increase in sperm with nonprogressive motility when incubated with PAF and a significant increase in fast progressive motility when incubated with FF. These results suggest that PAF and FF both exert a stimulatory effect but they promote different motility characteristics. Fabri *et al.* (1998) showed that FF increased motility, curvilinear velocity and the maximum amplitude of head displacement, but decreased linearity. On the other hand, Hellstrom *et al.* (1991) showed PAF to have a stimulatory effect on motility, on straight line velocity, and on linearity. This supports the suggestion that PAF and FF may have different modes of action. The motion parameters that changed after incubation with FF are associated with sperm entering the hyperactivated state (10). It could be hypothesised that the motion characteristics associated with PAF incubation do not relate to the hyperactive state, but instead result from a direct effect on the sperm's ability to move. This hypothesis is supported by the evidence that PAF receptors on sperm are located predominantly on the proximal head and at the mid-piece, which is the mitochondria-rich region of the sperm (15). These findings provide insight into the biology of sperm motility, and further studies that explore the intracellular pathway of PAF in individual sperm may identify other factors that limit or enhance sperm motility.

To date, our knowledge of methods which may improve sperm motility is based mainly on studies of good quality sperm (9,11,14). Our results clearly show that good quality sperm and poor quality sperm do not respond to motility stimulants in the same manner. Therefore, we can not assume that factors which improve motility in good quality sperm will be of benefit to poor quality samples.

The most important finding in this study was that incubation with PAF increased the motility in sperm samples with severe asthenozoospermia. This is seen in many oligospermic samples following cryopreservation. Incubation with PAF therefore has an important clinical application. For samples with little or no

motility, incubation with PAF may allow the identification of sufficient numbers of motile sperm to make selection of viable sperm for ICSI possible. This is particularly important as the other possible method of detecting live sperm with severe asthenozoospermia (the HOS test) has been shown to be unreliable in cryopreserved sperm (8).

In summary, whereas incubation with FF does not have a significant stimulatory effect on poor quality cryopreserved sperm, incubation with PAF increases the number of sperm that show motility in both poor and good quality cryopreserved sperm.

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REFERENCES

1. Bunge RG, Sherman JK: Fertilizing capacity of frozen human spermatozoa. *Nature* 1953;172:767
2. Richter MA, Haning RV Jr., Shapiro SS: Artificial donor insemination: Fresh versus frozen semen; the patient as her own control. *Fertil Steril* 1984;41:277-281
3. Clarke GN, Bourne H, Hill P, Johnston WIH, Spiers A, McBain JC, Baker HWG: Artificial insemination and in-vitro fertilization using donor spermatozoa: A report on 15 years of experience. *Hum Reprod* 1997;12(4):722-726
4. Sidhu RS, Sharma RK, Kachoria S, Curtis C, Agarwal A: Reasons for rejecting potential donors from a sperm bank program. *J Assist Reprod Genet* 1997;14(6):354-360
5. Hellstrom WJG, Wang R, Sikka SC: Platelet-activating factor stimulates motion parameters of cryopreserved human sperm. *Fertil Steril* 1991;56(4):768-770
6. Kahraman S, Tasdemir M, Tasdemir I, Vicdan K, Özgür S, Polat G, Islk AZ, Biberoglu K, Vanderzwalen P, Nijs M, Schoysman R: Pregnancies achieved with testicular and ejaculated spermatozoa in combination with intracytoplasmic sperm injection in men with totally or initially immotile spermatozoa in the ejaculate. *Hum Reprod* 1996;11(6):1343-1346
7. Casper RF, Meriano JS, Jarvi KA, Cowan L, Lucato ML: The hypo-osmotic swelling test for selection of viable sperm for intracytoplasmic sperm injection in men with complete asthenozoospermia. *Fertil Steril* 1996;65(5):972-976
8. Esteves SC, Sharma RK, Thomas AJ Jr., Agarwal A: Suitability of the hypo-osmotic swelling test for assessing the viability of cryopreserved sperm. *Fertil Steril* 1996;66(5):798-804
9. Sikka SC, Hellstrom WJG: Functional evaluation and motility parameters of Pentoxifylline-stimulated cryopreserved human sperm. *ARTA* 1991;1:309-314
10. Pearlstone AC, Chan SYW, Tucker MJ, Wiker SR, Wang C: The effects of Verco (Green monkey kidney) cell co-culture on the motility patterns of cryopreserved human spermatozoa. *Fertil Steril* 1993;59(5):1105-1111

11. Sharma RK, Kohn S, Padron OF, Agarwal A: Effect of Artificial stimulants on cryopreserved spermatozoa from cancer patients. *J Androl* 1997;157:521–524
12. Lander N, Soloway AH, Minton JP, Rawal BP, Gairola CC: Potential metabolic mutagens of caffeine and various methylxanthines. *J Pharm Sci* 1988;77:955–962
13. Minhas BS, Kumar R, Rickre DD, Robertson JL, Dodson MG: The presence of platelet-activating factor- like activity in human spermatozoa. *Fertil Steril* 1991;372–378
14. Fabbri R, Porcu E, Lenzi A, Gandini L, Marsella T, Flamigni C: Follicular fluid and human granulosa cell cultures: Influence on sperm kinetic parameters, hyperactivation and acrosome reaction. *Fertil Steril* 1998;69(1):112–117
15. Reinhardt JC, Cui X, Roudebush WE: Immunofluorescent evidence of the platelet-activating factor receptor on human spermatozoa. *Fertil Steril* 1999;71(5):941–942
16. World Health Organization Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th Ed, Cambridge UK, Cambridge University Press
17. Mahadevan M, Trounson AO: Cryopreservation of human semen. *In* Artificial Insemination by Donor, Melbourne, Brown Prior Anderson, 1980, pp 19–32