

# Platelet-activating factor significantly enhances intrauterine insemination pregnancy rates in non-male factor infertility

William E. Roudebush, Ph.D., Andrew A. Toledo, M.D., Hilton I. Kort, M.D.,  
Dorothy Mitchell-Leef, M.D., Carlene W. Elsner, M.D., and Joe B. Massey, M.D.

Reproductive Biology Associates, Atlanta, Georgia

**Objective:** To determine the efficacy of treating semen specimens with platelet-activating factor (PAF) before IUI.

**Design:** Prospective randomized double-blinded study of PAF treatment of sperm for patients with a history of infertility undergoing IUI.

**Setting:** Private infertility center.

**Intervention(s):** Patients had ovulation induction therapy with clomiphene citrate (CC) or gonadotropin, two IUIs per month with PAF treatment.

**Main Outcome Measure(s):** Clinical pregnancy rates.

**Result(s):** There was a significant difference in IUI pregnancy rates per cycle between control (10/56; 17.9%) and PAF (14/47; 29.8%) treatment groups in the normal male study arm. There was a significant difference in cumulative IUI pregnancy rates between control (10/35; 28.6%) and PAF (14/26; 53.9%) patient groups in the normal male study arm. There was no significant difference in IUI pregnancy rates per cycle between control (12/124; 9.7%) and PAF (14/119; 11.8%) treatment groups in the male factor study arm. There was no significant difference in cumulative IUI pregnancy rates between control (12/46; 26.1%) and PAF (14/38; 36.8%) patient groups in the male factor study arm. There was a significant difference in overall cumulative IUI pregnancy rates between control (21/81; 25.9%) and PAF (27/64; 42.2%) patient groups.

**Conclusion(s):** The inclusion of PAF into the IUI sperm wash procedure significantly improves pregnancy rates. However, the significant improvement can only be shown to affect men presenting with normal semen parameters. (Fertil Steril® 2004;82:52–6. ©2004 by American Society for Reproductive Medicine.)

**Key Words:** Platelet-activating factor, intrauterine insemination, sperm, pregnancy

Received August 11, 2003;  
revised and accepted  
November 25, 2003.

Presented in part (O-222)  
at the 58th Annual Meeting  
of the American Society for  
Reproductive Medicine,  
Seattle, WA, October 12–  
17, 2002.

Reprints requests: William  
E. Roudebush, Ph.D.,  
Reproductive Biology  
Associates, 1150 Lake  
Hearn Drive, Suite 400,  
Atlanta, Georgia 30342  
(FAX: 404-256-8376;  
E-mail: roudebush@  
rba-online.com).

0015-0282/04/\$30.00  
doi:10.1016/j.fertnstert.2003.  
11.057

In many centers drug-induced ovulation induction followed by intrauterine insemination (IUI) has become standard therapy for nontubal factor-related infertility. The initial treatment for infertile couples is IUI and is commonly used (1). The success of IUI is dependent on the ovulation induction regimen (2), as well as seminal parameters (3). Cumulative pregnancy rates for three cycles have approached those of a single IVF treatment when gonadotropins are combined with IUI (4). Commonly, because of lower patient costs, complexity and risks of high order multiple pregnancy (i.e., triplets or more), clomiphene citrate (CC) is initially used. In our center the typical treatment regimen involves three CC/

IUI cycles followed by gonadotropin/IUI for up to three cycles. Failed IUI therapy subsequently results in consideration of IVF therapy. Silverberg et al. (5) have shown that two IUI procedures per cycle have improved pregnancy rates, although this continues to be controversial (6).

Male fertility requires production of an adequate number of morphologically normal sperm with sufficient motility and the ability to undergo capacitation and the acrosome reaction to penetrate the oocyte's cumulus oophorus and zona pellucida for fertilization. Defects in any of these necessary events may lead to subfertility or infertility. Recently, for example, chromatin integrity of sperm was found to be related to IUI pregnancy rates (7).

A number of endogenous biochemical factors have been attributed to regulate the fertility potential of spermatozoa, for example, platelet-activating factor (PAF). Platelet-activating factor is a unique and novel signaling phospholipid that has pleiotropic biologic properties in addition to platelet activation. Since its discovery in the early 1970s this novel compound has been implicated in a variety of reproductive functions including fertilization, implantation, and parturition (8). The exact mechanism is uncertain, yet its importance in normal fertility is significant.

Platelet-activating factor is present in human spermatozoa (9), and its endogenous content has a significant and positive relationship with motility and pregnancy rate (10). Exogenous PAF has been used to stimulate human sperm motility (11) and IVF and embryo development rates in rabbits (12). A preliminary study (13) demonstrated the improvement of IUI pregnancy rates after a short-term exposure of sperm to PAF.

In the present study, our objective was to determine the effect of exogenous PAF on IUI pregnancy rates in couples presenting with normal and abnormal semen analyses. The study was a prospective randomized double-blinded study of PAF treatment of sperm for patients with a history of infertility undergoing IUI. Patients were randomized (blind number draw from computer-generated random number table) into either receiving PAF treatment or serving as controls for up to three IUI cycles. Furthermore, couples were categorized into either normal or male factor groups.

## MATERIALS AND METHODS

### Study Population and Management

Healthy, infertile patients with nontubal factor infertility were made aware of the study and informed of the study design with the possibility of improved results based on a small clinical trial (13). The PAF treatment option was not made available outside the current study. Possible risks were discussed and informed consent was obtained under guidelines approved by the Western Institutional Review Board (Seattle, WA). Inclusion criteria were as follows: basal FSH level <15 mIU/mL, evidence of a normal uterine cavity, and no contraindication to pregnancy. Infertility diagnoses included anovulatory, endometriosis, idiopathic, tubal (single or fibroids), cervical factor, and male factor. Male factor infertility patients were classified as such if they failed to meet one or more reference standards (14). Couples were permitted to enter the study at any point in a series of IUI treatments. Couples (normal male and male factor study groups) were randomized into one of two study groups (group 1, control; group 2, PAF) from January 2001 to December 2002. Once patients were assigned to the respective treatment group, only the RBA Andrology Laboratory staff was aware of assignment. Patients were subsequently treated in each consecutive cycle with the same regimen (i.e., control or PAF augmented). No clinical staff (physician or nurse) was informed of assignment until the study was

completed. Cycle stimulation was controlled by CC or gonadotropins. In CC-controlled cycles, 50–150 mg of CC was given for 5 cycle days. Timing of IUI was based on LH surge or ultrasound-timed hCG administration. In cycles managed by gonadotropins, stimulations were started on cycle day 3 with 75–225 IU daily (with dosages individually titrated based on patient age and previous response). Ultrasound (one to four ultrasound follicular studies per IUI cycle) monitoring with E<sub>2</sub> (Access2, Beckman Coulter, Inc., Brea, CA) observation was performed until the lead follicle was at least 18 mm in all but exceptional cases. Preovulatory LH surge timing was monitored by urine LH levels. The IUI was performed 12–18 hours and again in 36–38 hours after hCG injection. Patients taking CC who experienced a spontaneous LH surge had only one IUI performed 24 hours later.

### Semen Analysis

Semen specimens were permitted to liquefy for 30–60 minutes at 37°C. Sperm concentration and motility, before and after treatment, were evaluated by computer-assisted semen analysis (IVOS v10.9i, Hamilton-Thorne Research, Beverly, MA) operating at a sampling frequency of 60 Hz. All analyses were performed using MicroCell-20 Micron (Conception Technologies, San Diego, CA) counting chambers. A total of five random areas were selected and evaluated by the IVOS system at 37°C.

### Semen Processing and PAF Exposure for IUI

#### Control Group

Semen specimens were processed (400 g; 12 minutes) through a 90% density silane-coated silica suspension (1:1; Promotor; CERES Fertility, San Diego, CA), washed with 4 mL of sperm wash medium (InVitroCare, San Diego, CA), centrifuged (300 g; 8 minutes) and resuspended with 0.5 mL of PureSperm-Wash (Nidacon International AB, Goteborg, Sweden).

#### PAF Treatment Group

Semen specimens were processed (400 g; 12 minutes) through a 90% density silane-coated silica suspension (1:1; Promotor), resuspended in 10<sup>-7</sup>M PAF in sperm wash medium (InVitroCare) and incubated for 15 minutes at 37°C. After incubation, sperm were washed (300 g; 8 minutes) free of PAF and resuspended in 0.5 mL of PureSperm-Wash (Nidacon).

### IUI and Pregnancy Outcomes

Washed sperm preparations were inseminated with an IUI Catheter (Lifetek Medical, Inc., Portage, WI). Pregnancy outcomes were determined first by  $\beta$ -hCG serum levels (Access2, Beckman Coulter, Inc.) and confirmed by ultrasonography (fetal heartbeat = a positive outcome).

### Sample Size and Statistical Analysis

Power estimates based on published IUI pregnancy rates (5%–16%) and preliminary data reveal that a minimal sample size of 83 IUI cycles per male factor and 40 IUI cycles per normal male treatment group is required for alpha to equal

TABLE 1

Platelet activating factor-intrauterine insemination patient demographics.

Demographic	n
Male age (y)	
Normal study arm	Control, 36.4 ( $\pm$ 3.01); PAF, 35.5 ( $\pm$ 4.02)
Male factor study arm	Control, 38.5 ( $\pm$ 6.01); PAF, 36.0 ( $\pm$ 5.94)
Female age (y)	
Normal study arm	Control, 36.2 ( $\pm$ 4.16); PAF, 35.9 ( $\pm$ 4.93)
Male factor study arm	Control, 35.8 ( $\pm$ 4.54); PAF, 34.1 ( $\pm$ 4.40)
Cycle stimulation	
Clomiphene citrate	53 (32.1%)
Gonadotropin	112 (67.9%)
Infertility etiology (primary)	
Anovulatory	35 (24.1%)
Endometriosis	12 (8.3%)
Idiopathic	8 (5.5%)
Tubal	2 (1.4%)
Cervical factor	4 (2.8%)
Male factor	84 (57.9%)
Estradiol levels day of hCG (gonadotropin cycles only)	mean ( $\pm$ SEM)
Control	1,122.65 ( $\pm$ 150.9)
PAF	801.80 ( $\pm$ 126.0)

Roudebush. Platelet-activating factor and IUI pregnancy. *Fertil Steril* 2004.

0.05 and to show a difference with 80% power ( $\beta = 0.80$ ). A 50% increase in pregnancy rate after treatment to the IUI protocol was used. Data were analyzed by  $\chi^2$ . Statistical calculations were performed with SigmaStat for Windows, version 2.03 (Jandel Scientific Corporation, San Rafael, CA).

## RESULTS

A prospective comparison of pregnancy rates was performed after sperm treatment with or without PAF, at the time of semen washing just before IUI. Patient demographics are presented in Table 1. There were no significant differences in patient demographics between the pregnant and nonpregnant groups or control and PAF treatment groups. A total of 165 patients

TABLE 2

Platelet activating factor-intrauterine insemination pregnancy rate per cycle.

IUI cycle	n
Normal male study arm	
Control	10/56 (17.9%) <sup>a</sup>
PAF	14/47 (29.8%) <sup>a</sup>
Male factor study arm	
Control	12/124 (9.7%)
PAF	14/119 (11.8%)

<sup>a</sup>Significantly different,  $P < .05$ .

Roudebush. Platelet-activating factor and IUI pregnancy. *Fertil Steril* 2004.

TABLE 3

Cumulative Platelet activating factor-intrauterine pregnancy rates per patient.

Patient group	n
Overall	
Control	22/81 (27.2%) <sup>a</sup>
Clomiphene citrate	11/31 (35.5%)
Gonadotropin	11/50 (22.0%) <sup>b</sup>
PAF	28/64 (43.8%) <sup>a</sup>
Clomiphene citrate	8/22 (36.4%)
Gonadotropin	17/40 (52.5%) <sup>b</sup>
Normal male study arm	
Control	10/35 (28.6%) <sup>c</sup>
PAF	14/26 (53.9%) <sup>c</sup>
Male factor study arm	
Control	12/46 (26.1%)
PAF	14/38 (36.8%)

<sup>a,b,c</sup>Similar superscripts are significantly different,  $P < .05$ .

Roudebush. Platelet-activating factor and IUI pregnancy. *Fertil Steril* 2004.

enrolled in the study, of these 3 (1.8%) converted to IVF and 17 (10.3%) never followed through with IUI therapy.

Patient pregnancy rates are presented in Table 2 (per cycle) and Table 3 (cumulative). Cumulative IUI pregnancy rate by number of attempts according to treatment group for all patients (normal and male factor) are presented in Figure 1. In couples presenting with normal semen characteristics the cycle-specific pregnancy rate was 29.8% (14/47) when PAF was used. This was significantly higher ( $P < .05$ ) when compared with the control (10/56; 17.9%). Cumulative pregnancy rates were significantly higher ( $P < .05$ ) in the PAF treatment group (14/26; 53.90%) than the control group (10/35; 28.6%). There was no significant difference in IUI pregnancy rates per cycle between control (12/124; 9.7%) and PAF (14/119; 11.8%) treatment groups in the male factor study arm. There was no significant difference in cumulative IUI pregnancy rates between control (12/46; 26.1%) and PAF (14/38; 36.8%) patient groups in the male factor study arm. There was a significant difference ( $P < .05$ ) in overall cumulative IUI pregnancy rates between control (22/81; 27.2%) and PAF (28/64; 43.8%) patient groups.

TABLE 4

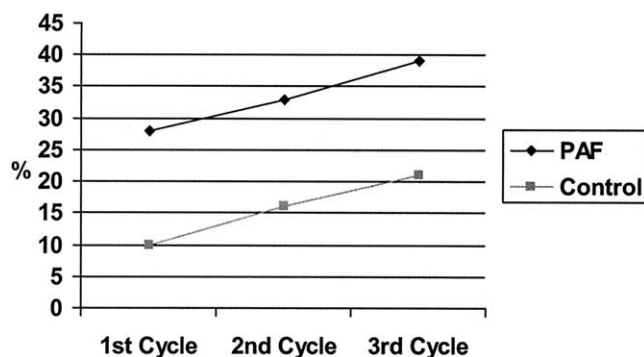
Platelet activating factor-intrauterine insemination multiple pregnancy rate.

	Control	PAF
Twins	3/22 (13.6%)	5/28 (17.9%)
Triplets	2/22 (9.1%)	0 (0.0%)
Quadruplets	2/22 (9.1%)	2/28 (7.1%)
Total multiple rate	7/22 (31.8%)	7/28 (25.0%)

Roudebush. Platelet-activating factor and IUI pregnancy. *Fertil Steril* 2004.

**FIGURE 1**

Cumulative intrauterine insemination (IUI) pregnancy rate by number of attempts according to treatment group for all patients (normal and male factor). PAF = platelet-activating factor.



Roudebush. Platelet-activating factor and IUI pregnancy. *Fertil Steril* 2004.

There was no significant difference in multiple pregnancy rates between the control and PAF treatment groups (Table 4).

## DISCUSSION

The inclusion of PAF to IUI sperm wash procedure improves pregnancy rates. However, this significant improvement could only be demonstrated in cases where the semen analysis was normal. This confirms our original results demonstrating the effectiveness of exogenous PAF supplementation in an IUI program (13).

Treatment of sperm in male factor patients with PAF showed an increase in pregnancy rates, albeit not statistically significant. It is possible that sperm in these individuals are incapable of responding to the exogenous PAF due to poor PAF receptor levels and, or faulty PAF receptors in the sperm (15). The PAF antagonists will inhibit the motility, acrosome reaction and hamster oocyte penetration in exposed sperm, thus suggesting the presence of receptors for PAF (16, 17). We have recently reported on the presence and distribution of the PAF receptor in human sperm (18) and our preliminary data demonstrate that distribution of the receptor is significantly altered in abnormal sperm (19). We have also discovered that PAF receptor mRNA expression differs significantly between motile (high content) and non-motile (low content) sperm (15). In addition we have found that sperm with abnormal motility have different PAF receptor mRNA sequences (20). The PAF plays a significant role in sperm function and has recently been reviewed (21). It may affect sperm motility and fertilization through a receptor-mediated control of intracellular calcium. The PAF has been shown to augment sperm capacitation (22, 23).

We believe that the beneficial effect of PAF on IUI pregnancy rates is due to PAF binding to its receptor on the

**TABLE 5**

Ongoing platelet activating factor-intrauterine insemination pregnancy rate.

Stimulation	Pregnancy rate
Clomiphene citrate	12/31 (38.7%)
Gonadotropin	40/94 (42.6%)
Overall	52/125 (41.6%)

Roudebush. Platelet-activating factor and IUI pregnancy. *Fertil Steril* 2004.

cells' surface, initiating intracellular calcium release, enhancing sperm capacitation and cell motility, thus enhancing fertilization rates. Sperm with defective or low numbers of PAF receptor may not respond to PAF (endogenous or exogenous), thus having poor motility (22). Additional studies will elucidate the reproductive significance of PAF activity and PAF's mechanism of action in spermatozoa. Other possible factors may attribute to the high pregnancy rates observed in this study. For example, in most cases we performed two IUI's per cycle, which has been demonstrated to improve pregnancy outcome (5).

Additional clinical studies are warranted to further establish the use of PAF therapy for patient's undergoing IUI therapy for infertility treatment. In particular, larger numbers of male factor infertility patients will determine the significance of PAF-IUI therapy for these individuals. To summarize, exposure of sperm to PAF can significantly increase IUI pregnancy rates.

## APPENDUM

On February 10, 2003, we initiated the routine washing of semen with PAF before IUI. Our overall ongoing pregnancy rate since then is 41.6% and is presented in Table 5.

*Acknowledgments:* The authors express their sincere gratitude and thanks to the staff and physicians (Daniel Shapiro, M.D., David Keenan, M.D., and Scott Slayden, M.D.) at Reproductive Biology Associates for patient recruitment and laboratory work without whose assistance would have made this study impossible.

## References

- Garceau L, Henderson J, Davis LJ, Petrou S, Henderson LR, McVeigh E, et al. Economic implications of assisted reproductive techniques: a systematic review. *Hum Reprod* 2002;12:3090-109.
- Ecochard R, Mathieu C, Royere D, Blache G, Rabilloud M, Czyba JC. A randomized prospective study comparing pregnancy rates after clomiphene citrate and human menopausal gonadotropin before intrauterine insemination. *Fertil Steril* 2000;73:90-3.
- Pasqualotto EB, Daitch JA, Hendin BN, Falcone T, Thomas AJ, Nelson DR, et al. Relationship to total motile sperm count and percentage motile sperm to successful pregnancy rates following intrauterine insemination. *J Assisted Reprod Genetics* 1999;16:476-82.

4. Matorras R, Diaz T, Corcostegui B, Ramon O, Pijoan JI, Rodriguez-Escudero FJ. Ovarian stimulation in intrauterine insemination with donor sperm: a randomized study comparing clomiphene citrate in fixed protocol versus highly purified urinary FSH. *Hum Reprod* 2002;8:2107-11.
5. Silverberg KM, Johnson JV, Olive DL, Burns WN, Schenken RS. A prospective, randomized trial comparing two different intrauterine insemination regimens in controlled ovarian hyperstimulation cycles. *Fertil Steril* 1992;2:357-61.
6. Cantineau AE, Heineman MJ, Cohlen BJ. Single versus double intrauterine insemination in stimulated cycles for subfertile couples: a systematic review based on a Cochrane review. *Hum Reprod* 2003;18:941-6.
7. Duran EH, Morshedi M, Taylor S, Oehninger S. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. *Hum Reprod* 2002;17:3122-8.
8. Harper MJK. Platelet-activating factor: a paracrine factor in preimplantation stages of development? *Biol Reprod* 1989;40:907-13.
9. Minhas BS, Kumar R, Ricker DD, Robertson JL, Dodson MG. The presence of platelet activating factor-like activity in human spermatozoa. *Fertil Steril* 1991;55:372-6.
10. Roudebush WE, Purnell ET. Platelet-activating factor content in human spermatozoa: predicting pregnancy outcome. *Fertil Steril* 2000;74:257-60.
11. Ricker DD, Minhas BS, Kumar R, Robertson JL, Dodson MG. The effects of platelet activating factor on the motility of human spermatozoa. *Fertil Steril* 1989;52:655-8.
12. Roudebush WE, Fukuda AI, Minhas BS. Enhanced embryo development of rabbit oocytes fertilized in vitro with platelet-activating factor treated spermatozoa. *J Assisted Reprod Genet* 1993;10:91-4.
13. Wild MD, Roudebush WE. Platelet-activating factor improves intrauterine insemination outcome. *Am J Obstet Gynecol* 2001;184:1064-5.
14. WHO Laboratory Manual. The examination of human semen and sperm-cervical mucus interaction. 4th ed. Prepared by WHO Special Programme for Research Development and Research Training in Human Reproduction: Cambridge University Press, Cambridge, UK 1999.
15. Purnell ET, Roudebush WE. Platelet-activating factor activity (ligand and receptor transcript) content in sperm: motile versus nonmotile. In: Robaire E, Chemes H, Morales CR, eds. *Andrology in the 21st century*. Proceedings of the VIIth International Congress of Andrology. Englewood, NJ: Medimond Publishing Company, Inc., 2001:71-6.
16. Sengoku K, Tamate K, Takaoka Y, Ishikawa M. Effects of platelet-activating factor on human sperm function in vitro. *Hum Reprod* 1993;8:1443-7.
17. Minhas BS. Platelet-activating factor treatment of human spermatozoa enhances fertilization potential. *Am J Obstet Gynecol* 1993;168:1314-7.
18. Reinhardt JC, Cui X, Roudebush WE. Immunofluorescent evidence for the presence of the platelet-activating factor receptor in human spermatozoa. *Fertil Steril* 1999;71:941-2.
19. Roudebush WE, Wild MD, Maguire EH. Platelet-activating factor receptor expression in human spermatozoa: differences in mRNA content and protein distribution between normal and abnormal spermatozoa. *Fertil Steril* 2000;73:967-71.
20. Roudebush WE, Mayorov V, Adkison LR, Slayden SM, Shapiro DB, Elsner CW, et al. Abnormal sequence of the platelet-activating factor-receptor in nonmotile spermatozoa. *Fertil Steril* 2002;76:P-460.
21. Levine AS, Kort HI, Toledo AA, Roudebush WE. A review of the effect of platelet-activating factor on male reproduction and sperm function. *J Androl* 2002;23:471-6.
22. Wu C, Stojanov T, Chami O, Ishii S, Shimizu T, Li A, et al. Evidence for the autocrine induction of capacitation of mammalian spermatozoa. *J Biol Chem* 2001;276:26962-8.
23. Toledo AA, Mitchell-Leef D, Elsner CW, Slayden SM, Roudebush WE. Fertilization potential of human sperm is correlated with endogenous platelet-activating factor content. *J Assist Reprod Genet* 2003;20:192-5.