Effect of sperm treatment with exogenous platelet-activating factor on the outcome of intrauterine insemination

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Objective: To evaluate the effect of sperm treatment with exogenous platelet-activating factor (PAF) on IUI clinical pregnancy rate.
Design: Prospective randomized study.
Setting: Assisted Reproduction Unit, 2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, Athens, Greece.
Subject(s): Fifty-two couples with unexplained infertility, candidates for IUI.
Intervention(s): Sperm treatment with an exogenous mixture of PAF (final concentration, 10^{-7} mol/L) in sperm-washing medium, direct swim-up technique of sperm preparation, a maximum of six IUI cycles per couple with or without PAF treatment.
Main Outcome Measure(s): Clinical pregnancy rate (pregnancies confirmed by ultrasonography per hundred cycles).
Result(s): The overall clinical pregnancy rate after a maximum of six IUI cycles was significantly higher when sperm was treated with PAF compared with the rate after the direct swim-up technique (23.07% vs. 7.92%).
Conclusion(s): Treatment of sperm with exogenous PAF might improve the clinical outcome of IUI in cases of unexplained infertility. (Fertil Steril® 2005;83:618–21. ©2005 by American Society for Reproductive Medicine.)

Key Words: Platelet-activating factor, intrauterine insemination, unexplained infertility, spermatozoa

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-*sn*-glycerol-3-phosphorylcholine) is an important phospholipid mediator that is produced by a variety of cells (1). Apart from platelet activation, PAF has numerous other effects (2, 3); among them, its involvement in ovulation, sperm capacitation, fertilization, preimplantation embryo development, implantation, and parturition indicates its essential role in reproduction (4).

Platelet-activating factor is present in human sperm, being one of the endogenous factors responsible for the regulation of spermatozoa fertilization capacity (5). The exact mechanism of its action on spermatozoa is not clearly known, but it is probably mediated by a specific receptor (6); the expression, presence, and distribution of this receptor have been found altered in abnormal spermatozoa (7). The PAF content in human spermatozoa has a significantly positive relation not only to spermatozoa motility but to the pregnancy outcome as well (8); treatment of human sperm with exogenous PAF stimulates sperm motility rate and fertilization potential (9, 10).

Ten to fifteen percent of infertility cases are characterized as unexplained, meaning that clinical and laboratory investigation of the couple remains nonsignificant. Intrauterine insemination is usually included in the management of these cases, before IVF/intracytoplasmic sperm injection treatment is adopted. The aim of the present study was to evaluate the effect of exogenous PAF treatment on IUI clinical outcomes in cases of morphologically normal sperm, for which relevant reports are rare in the medical literature (11).

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MATERIALS AND METHODS

The study included 52 couples with unexplained primary infertility presenting at the Assisted Reproduction Unit of the 2nd Department of Obstetrics and Gynecology, University of Athens, Greece, between May 2002 and October 2003. All the female study subjects met the following criteria: duration of infertility ≥1 year, regular menstrual cycle of 26–32 days, ovulatory basal body temperature chart, midluteal serum P levels ≥32 ng/mL, levels of FSH, LH, androstenedione, T, DHEAS, and PRL within normal range on cycle day 3, normal thyroid function tests, nonsignificant results from transvaginal ultrasound scan, normal hysterosalpingogram results, and nonsignificant findings at diagnostic laparoscopy. All the male study subjects had normal semen analysis parameters on two occasions, according to World Health Organization criteria. The Institutional Review Board of Aretaieion Hospital approved the study, and study subjects consented before participation.

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The first-step treatment decision for all couples was IUI in an ovarian stimulation cycle. In all the cases, clomiphene citrate (100 mg daily) was administered on days 3–7 of the cycle. Ovarian response was monitored by serial ultrasound scans, and when the mean diameter of the leading follicle was ≥18 mm, hCG (10,000 U) was administered. Intrauterine insemination was arranged to be performed 34–38 hours after the hCG injection. According to a method of blocked randomization (4 consecutive blocks of 10 followed by 1 block of 6), the study couples were allocated to two groups: in group A-PAF (26 couples), the sperm for IUI was treated with PAF, whereas in group B-nonPAF (26 couples), the direct swim-up technique was used. If pregnancy was not achieved, a similar IUI cycle was arranged. After completing three consecutive IUI cycles with no success, group A failures had a maximum of three new IUI cycles with direct swim-up sperm preparation (group A-nonPAF), whereas group B failures had a maximum of three new IUI cycles with PAF treatment of the sperm (group B-PAF). A positive pregnancy outcome was recorded when a gestational sac containing a fetal pole was observed by ultrasound scan. Statistical analysis was performed with the appropriate use of Fisher's exact test, Student's *t*-test, and Kruskal-Wallis test.

In groups A-PAF and B-PAF, sperm were washed free of seminal fluid by centrifugation (400 × *g*, 10 minutes) and were treated with an exogenous mixture of PAF (final concentration, 10^{-7} mol/L) in sperm-washing medium (Sydney IVF Sperm Medium; Cook, Sydney, Australia). Synthetic PAF (Calbiochem-Novabiochem, La Jolla, CA) was stored in a stock solution (1×10^{-6} mol/L) of chloroform and methanol (1:4). Before use, 0.1 mL of stock PAF was dried under a steam of nitrogen in siliconized tubes and dissolved in 1.0 mL of sperm-washing medium. After an exposure period of 15 minutes, the sperm was washed free of unbound PAF and suspended in sperm-washing medium. In groups A-nonPAF and B-nonPAF, the direct swim-up technique with the use of sperm-washing medium (Cook) was adopted. Inseminated sperm was standardized to a volume of 0.5 mL and a count of 20×10^6 progressively motile spermatozoa.

RESULTS

The mean (±SD) age of female study subjects was 30.6 ± 3.1 years and 31.8 ± 4.1 years in groups A and B, respectively (nonsignificant difference). Male study subjects were aged 34.1 ± 5.3 years and 33.2 ± 3.3 years in groups A and B, respectively (nonsignificant difference). Table 1 shows the sperm parameters in all groups, before and after preparation; inseminated sperm was successfully standardized to a volume of 0.5 mL and a count of 20×10^6 progressively motile spermatozoa in all cycles. Table 2 shows the number of IUI cycles and the pregnancies achieved per attempt in all four groups. Discontinuation from the study included two subjects from group B-nonPAF, two from group B-PAF, and two from group A-nonPAF.

The clinical pregnancy rate (clinical pregnancies per hundred cycles) was 22.22%, 8.57%, 24.39%, and 6.45% in groups A-PAF, B-nonPAF, B-PAF, and A-nonPAF, respectively. In the first three IUI attempts, clinical pregnancy rates were significantly higher in cases in which sperm were treated with PAF (A-PAF vs. B-nonPAF, $P=.02$). A similar significant increase was observed in the subsequent three IUI attempts (B-PAF vs. A-nonPAF, $P=.04$), as well as in the total of the six attempts (A-PAF and B-PAF vs. A-nonPAF and B-nonPAF, $P=.002$). Within the two groups A and B, sperm treatment with PAF resulted in a significant increase of clinical pregnancies (A-PAF vs. A-nonPAF, $P=.04$; B-PAF vs. B-nonPAF, $P=.02$). The order of IUI attempts did not influence the outcome (clinical pregnancies, $P=.36$).

TABLE 1

Sperm parameters before and after preparation in the study groups.

	Before preparation	After preparation
Group A-PAF		
Concentration ($\times 10^6$/mL)	61.98 ± 19.04	72.57 ± 17.99
Progressive motility (%)	60.04 ± 9.71	68.80 ± 7.85
Group B-nonPAF		
Concentration ($\times 10^6$/mL)	56.35 ± 23.03	62.45 ± 18.35
Progressive motility (%)	58.51 ± 10.98	80.07 ± 8.42
Group B-PAF		
Concentration ($\times 10^6$/mL)	59.85 ± 20.77	69.97 ± 19.30
Progressive motility (%)	62.09 ± 10.08	69.90 ± 8.98
Group A-nonPAF		
Concentration ($\times 10^6$/mL)	63.29 ± 16.46	68.67 ± 15.86
Progressive motility (%)	62.12 ± 9.12	83.81 ± 4.81

Note: Values are mean ± SD.

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