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Platelet-activating factor content in human spermatozoa and pregnancy outcome

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Objective: To determine whether platelet-activating factor (PAF) content in human spermatozoa from an isolated population is related to fertilization and pregnancy outcome.

Design: Prospective analysis of PAF content in human spermatozoa after a Percoll gradient wash and its relation to fertilization and pregnancy outcome.

Setting: University-based reproductive genetics laboratory.

Subject(s): Couples undergoing assisted reproduction.

Intervention(s): Lipids extracted from Percoll gradient spermatozoa were quantitated for PAF content by a specific radioimmunoassay.

Main Outcome Measure(s): The relation between spermatozoa-derived PAF levels and motility, concentration, morphology, and fertilization and pregnancy rates were determined by using regression analysis and the Student *t*-test.

Result(s): Radioimmunoassay and regression analysis showed a significant and positive relation between PAF content in human spermatozoa and concentration and motility indices and implantation rate. Patients who became pregnant had a significantly higher PAF content in the spermatozoa used (7.285 pmol/10^6 cells) than did patients who did not become pregnant (2.990 pmol/10^6 cells).

Conclusion(s): The PAF content in human spermatozoa has a significant and positive relation with motility and concentration indices and implantation rate. Pregnancy rates but not fertilization rates may be predicted by measuring PAF levels in an isolated subpopulation of human spermatozoa. (Fertil Steril® 2000;74:257–60. ©2000 by American Society for Reproductive Medicine.)

Key Words: Human spermatozoa, platelet-activating factor, fertilization, pregnancy

Male fertility requires production of an adequate number of normal mature spermatozoa with sufficient motility and the ability to undergo capacitation and the acrosome reaction so that they bind and penetrate the zona pellucida for fertilization. Defects in any of these necessary characteristics can lead to male infertility.

Several endogenous factors, such as platelet-activating factor, are thought to regulate the fertility potential of the spermatozoa. Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine) is a unique and novel signaling phospholipid that has pleiotropic biological properties in addition to platelet activation. Since its discovery in the early 1970s, this novel compound has been implicated in various reproductive functions, including fertilization, implantation, and parturition. The exact mechanism is uncertain, yet its importance in normal fertility is substantial.

Platelet-activating factor (PAF) plays an important role in mammalian reproduction (1). Minhas et al. (2) examined PAF content in spermatozoa after different methods of processing (e.g., swim-up and pellet washing) but did not compare normal and abnormal specimens. The PAF content in human spermatozoa was originally reported to have an inverse relation with asthenozoospermia; however, that study used the entire ejaculate and did not examine the different subpopulations of spermatozoa (e.g., motile vs. nonmotile) (3).

Of note, in other mammalian spermatozoa, such as that of the pig, PAF content appears to have a positive relation with fertility status of the male (4). High-fertility boar spermatozoa have a substantially greater PAF content than does low-fertility boar spermatozoa. The PAF

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content in squirrel monkey spermatozoa is substantially higher during the breeding season than the nonbreeding season (5). Testosterone, estrogen, and progesterone affect PAF metabolism (6, 7).

Exogenous PAF will increase the motility rate [e.g., forward progression (8)] but will not improve motion characteristics [e.g., lateral head displacement (9)] in exposed human spermatozoa. The mechanism of action of PAF on spermatozoa is not well known. The mechanism of action of PAF in other cell types is mediated by a specific receptor (10, 11). The presence and distribution of the PAF receptor on human spermatozoa was recently elucidated (12), and its distribution is greatly altered in abnormal forms (13).

The relation between PAF activity in human spermatozoa and fertilization and pregnancy outcome is not known. Therefore, our goal was to determine the relation of PAF content in human spermatozoa that was processed for use in assisted reproduction to fertilization and pregnancy outcome. A second objective was to correlate PAF content in spermatozoa used for IVF with processed spermatozoa variables (concentration, motility, and morphology).

MATERIALS AND METHODS

Specimen Collection

Human spermatozoa were obtained during routine Percoll (Sigma Chemical Co., St. Louis, MO) processing and IVF at the Southeastern Fertility Center, Mount Pleasant, South Carolina. The Medical University of South Carolina Institutional Review Board approved the study. Patients were undergoing routine IVF (100,000 normal, motile cells were used per insemination) with no assisted fertilization (i.e., micromanipulation) or frozen embryo transfer. A positive pregnancy outcome was recorded when a fetal heart beat was observed on ultrasonography. Spermatozoa were washed and quick-frozen in phosphate-buffered saline with 20% glacial acetic acid and transported on ice to the Medical University of South Carolina.

Lipid Extraction

Endogenous lipids were extracted by using the method of Bligh and Dyer (14). Briefly, chloroform and methanol (3:2 vol/vol) were added to the spermatozoa suspension and mixed for 1 minute every 10 minutes for 1 hour. After extraction, the samples were centrifuged (2,000 $\times$ *g* for 5 minutes) and lipids were obtained from the organic phase after addition of chloroform and distilled water (1.1:0.9 vol/vol). The organic phase was evaporated to dryness under a gentle stream of nitrogen, dissolved in assay buffer (0.1% sodium azide and 0.05% Tween 20 in 50 mM sodium citrate; pH 6.3), and stored at -20°C.

Radioimmunoassay

Endogenous PAF levels were determined by using a PAF-specific radioimmunoassay (^{125}I) according to the manufacturer's instructions (NEN Research Products; DuPont, Boston, MA). Briefly, primary antibodies were added to tubes containing the extracted spermatozoa lipid samples or assay buffer. Tubes were mixed and incubated for 15 minutes at room temperature. Secondary antibodies and tracer were added, and the solution was mixed and incubated for 24 hours at room temperature. After centrifugation (2,000 $\times$ *g* for 30 minutes), the supernatant was decanted and the tubes were blotted and counted. The standard curve was calculated by using regression analysis (logit value of normalized percent bound versus log of ng PAF assayed). The extracted PAF content is expressed as pmol/10^6 spermatozoa.

Radioimmunoassay Performance

The sensitivity of the PAF radioimmunoassay was approximately 0.1 pmol. Intraassay (multiple replicates in a single assay) reproducibility was 3.53 ($\pm$0.420) pmol and interassay (multiple replicates in multiple assays) reproducibility was 2.88 ($\pm$0.134) pmol.

Statistical Analysis

Data were analyzed by using regression analysis and the Student *t*-test. Statistical calculations were performed by using SigmaStat for Windows, version 2.03 (Jandel Scientific Corp., San Rafael, CA).

RESULTS

Thirty-nine spermatozoa samples were assayed for the presence of PAF, and PAF was detected in all spermatozoa samples. The PAF content in the Percoll-isolated spermatozoa population ranged from a low of 0.002 pmol/10^6 cells (this patient's partner did not become pregnant) to a high of 33.564 pmol/10^6 cells (this patient's partner became pregnant). The mean ($\pm$SE) concentration of PAF in the spermatozoa for all samples was 4.752 $\pm$ 1.044 pmol/10^6 cells.

Regression analysis revealed a significant relation (R^2= 0.0952; F=4.314; $P<.05$) between PAF content and spermatozoa concentration. The relation between PAF content and spermatozoa motility was also significant (R^2=0.207; F=10.701; $P<.01$). However, the relation between PAF content and spermatozoa morphology was not significant (R^2=0.0226; F=0.949; P=.336).

Regression analysis revealed a significant relation (R^2=0.501; F=7.039; $P<.05$) between PAF content in spermatozoa and implantation rate. The relation between PAF content in spermatozoa and pregnancy outcome was also significant (R^2=0.108; F=4.467; $P<.05$).

The mean ($\pm$SE) PAF content in spermatozoa was 7.285 $\pm$ 2.113 pmol/10^6 cells in patients who became pregnant (16 of 39 [41%]) (23 of 39; 59%) and 2.990 $\pm$ 0.857 pmol/10^6 cells in patients who did not become pregnant. The amount of PAF in the spermatozoa from pregnant patients was significantly higher ($P<0.05$) than that in the spermatozoa from the nonpregnant group.

DISCUSSION

Platelet-activating factor is present in human spermatozoa (2), and its level was reported to have an inverse relation with asthenozoospermia. We found that the PAF content in a subpopulation of spermatozoa isolated for use for assisted reproduction was positively related to concentration and motility indices, implantation rate, and pregnancy outcome. These findings are similar to those reported in other species.

The amount of PAF in porcine spermatozoa depends on the fertility status of the animal (4). The PAF content of spermatozoa is twofold higher in high-fertility male pigs (7.3 pmol/10^6 cells) than in low-fertility male pigs (3.6 pmol/10^6 cells). This reduction in PAF content in spermatozoa is similar to that observed in the squirrel monkey, which is a seasonal breeder (5). In this New World nonhuman primate, PAF levels are significantly higher during the breeding season (6.84 pmol/10^6 spermatozoa) than during the nonbreeding season (1.45 pmol/10^6 spermatozoa). A calcium-dependent phospholipase A_2 is present in human, mouse, hamster, guinea pig, and ram spermatozoa (15).

Phospholipase A_2 catalyses the formation of lyso-PAF [1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine] from alkyl-acyl-glycerophosphocholine (an inert component of the structural cellular membrane). Lyso-PAF can either be acetylated by a lyso-PAF-acetyl transferase (with acetyl-coenzyme A as the acetate donor) to form PAF or acylated by a coenzyme A–independent arachidonyl transacylase to form alkyl-acyl-glycerophosphocholine. Platelet-activating factor acetyl hydrolase is responsible for removal of the acetate group from the PAF molecule, resulting in the reformation of the biologically inactive lyso-PAF. Lyso-PAF-acetyl transferase and PAF-acetyl hydrolase are both present in human spermatozoa (16).

Thus, the enzymes necessary for PAF metabolism are present in spermatozoa. Metabolism of PAF is affected by testosterone (6) and by estrogen and progesterone (7). Additional studies are needed to compare circulating hormone levels with PAF and PAF-acetyl hydrolase content in spermatozoa.

The PAF-acetyl hydrolase is also present in seminal plasma and is believed to have a role as a decapacitation factor (17). Removal of acetyl hydrolase during the capacitation process may promote PAF synthesis or use, which in turn would allow spermatozoa motility to increase (2, 8, 18–20). Platelet-activating factor stimulates capacitation and the acrosome reaction and its effects are a calcium-dependent process (21).

Platelet-activating factor plays an important role in the fertilization process; it enhances fertilization rates in mouse and rabbit oocytes (2, 19, 21). Enhanced embryo development has also been reported in rabbit oocytes that were fertilized in vitro with PAF-exposed spermatozoa (21). Thus, the higher PAF content observed in pregnant women in our study may have resulted in optimal embryonic quality available for implantation.

Platelet-activating factor antagonists inhibit motility; acrosome reaction; and, in hamsters, oocyte penetration in exposed spermatozoa (18, 21). In addition, these antagonists inhibit fertilization (2, 18). These data indicate the presence of a PAF-specific receptor in spermatozoa, which has been confirmed by immunofluorescence microscopy (12). Therefore, PAF seem to bind to cell surface receptors on spermatozoa, initiating the formation of inositol triphosphate and diacylgylcerol and increasing intracellular calcium (5, 11). As a secondary messenger, calcium may regulate spermatozoa function by modulating the activity of molecules that transduce intracellular signals, which in turn influence spermatozoa motility.

The highest concentration of PAF receptor is found at the neck and midpiece regions of the spermatozoa. The midpiece is the location of the mitochondria and is essential for spermatozoa motility. The neck is the location of the proximal centriole, which plays a critical role in preimplantation embryo development (22). Platelet-activating factor may have a stimulatory effect on centriole-intact spermatozoa, enhancing their fertilization success and resulting in improved development rates. Additional studies are warranted to elucidate the role of PAF in spermatozoa selection in the fertilization process and the effect of PAF on postfertilization preimplantation development.

Before ART, spermatozoa must be processed (e.g., by Percoll washing or swim-up). Processing of spermatozoa facilitates fertilization by ensuring that the most normal motile population is used. The amount of PAF in processed human spermatozoa is positively related to pregnancy outcome. Measurement of PAF levels in spermatozoa before assisted reproduction may help predict outcome. The reproductive significance of PAF activity in spermatozoa and the role of PAF in the establishment of pregnancy require further study.

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