

Improving intrauterine insemination pregnancy outcomes by enhancing sperm motility with platelet activating factor

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Abstract

Ten to 15% of reproductive age couples in the United States are not able to achieve a successful pregnancy and are considered infertile. Infertility affects men and women equally. Male fertility requires the production of an adequate number of morphologically normal spermatozoa with sufficient motility and the ability to undergo hyperactivation, capacitation, and the acrosome reaction in order to penetrate the oocyte's cumulus oophorus and bind to the zona pellucida for fertilization. Defects in any of these necessary events will lead to infertility. A number of endogenous factors are implicated in the regulation of spermatozoan fertility potential, including platelet-activating factor (PAF). PAF clearly plays a significant role in reproductive physiology. It influences ovulation, fertilization, preimplantation embryo development, implantation, and parturition. Exogenous PAF has been used to promote sperm motility, sperm capacitation and the acrosome reaction. Finally, the addition of PAF to sperm wash for use in an intrauterine insemination program results in significantly higher pregnancy rates.

Key words: Sperm, platelet activating factor, sperm wash, intrauterine insemination, pregnancy.

In many fertility centers drug induced ovulation induction (OI) followed by intrauterine insemination (IUI) has become standard therapy for non-tubal factor related infertility. As the initial treatment for infertile couples, IUI is commonly used and is a cost effective procedure. The success of IUI is dependent on the ovulation induction regimen as well as seminal parameters. Cumulative pregnancy rates for three cycles have approached those of a single IVF treatment when exogenous gonadotropins are combined with IUI.

Spermatozoa prior to any assisted reproductive technique (e.g. intrauterine insemination) must be first processed (e.g. density/gradient wash or swim-up). This processing of spermatozoa insures that the most normal motile population is used to help facilitate conception. For IUI procedures, semen washing is important as it removes prostaglandins, debris and white blood cells while also reducing the number of non-motile and morphologically abnormal sperm cells. Additionally, removal of seminal plasma also benefits the sperm by enhancing capacitating condi-

tions-resulting in the enhancement of sperm motility. A number of additives which improve sperm motility have been investigated, including but not limited: cAMP, human Follicular fluid, xanthine, caffeine, pentoxifyline and platelet activating factor (PAF). This review will focus only on PAF, primarily due to the author's experience, but also in that an advantage that PAF is a natural substance and is non-toxic as is many other motility stimulators (Henkel and Schill, 2003).

Platelet-activating factor (PAF; 1-O-alkyl-2-Oacetyl-sn-glycero-3-phosphorylcholine) has attracted increasing interest as one of the more important signaling mediators since it was first discovered more than 30 years ago on the discovery that PAF mediated rabbit platelet aggregation in immunoglobulin E-stimulated basophils (Benveniste *et al.*, 1992). Since then, numerous investigators have demonstrated that PAF is a unique signaling phospholipid that has pleiotropic biologic properties in addition to platelet activation (Hanahan, 1986; Braquet *et al.*, 1987). PAF clearly plays a significant

role in reproductive physiology. It influences ovulation, fertilization, preimplantation embryo development, implantation, and parturition (Harper, 1989). PAF exists endogenously as a mixture of molecular species with structural variants of the alkyl moiety, with the C-16 species predominating in human sperm (Sanwick *et al.*, 1992). Although the exact mechanisms of PAF action remain unclear, the importance of PAF for normal reproductive function is clear. Ultimately, PAF may provide us with a biomarker for normal sperm function.

Phospholipase A2 is present in human sperm. It is calcium dependent and catalyzes the formation of 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso-PAF, which is biologically inactive) from alkyl-acylglycerophosphocholine, an inert structural cellular membrane component (Bennet *et al.*, 1987). Lyso-PAF is then acetylated by acetyl transferase using acetyl-CoA as an acetate donor to form PAF. Lyso-PAF may also be acetylated by a CoA-independent arachidonyltransferase to form alkyl-acyl-glycerophosphocholine. Acetylhydrolase is the primary enzyme responsible for inactivating PAF by the removal of the acetate group from the sn-2 position, resulting in the reformation of lyso-PAF. Acetyltransferase and acetylhydrolase are both present in mammalian sperm and seminal fluid (Gujarit *et al.*, 1987). Consequently, both the enzymes necessary for PAF activation and deactivation are present in sperm. Acetylhydrolase may itself act as a sperm decapacitation factor (Letendre *et al.*, 1992). In fact, the data suggest that the elimination of acetylhydrolase during normal capacitation promotes PAF synthesis, which results in increased sperm motility and improved sperm-egg interactions (Hellstrom *et al.*, 1991; Roudebush *et al.*, 1990; Angle *et al.*, 1993; Roudebush *et al.*, 1993). PAF may indeed be a biomarker for capacitation, and PAF-hydrolase as a decapacitation factor (Zhu *et al.*, 2004).

PAF is localized to the sperm and is not present in seminal secretions (Kumar *et al.*, 1988; Parks *et al.*, 1990). PAF is found in the sperm of many mammalian species including the rabbit, mouse, bull, and boar (Kuzan *et al.*, 1990; Kumar *et al.*, 1988; Parks *et al.*, 1990; Mook *et al.*, 1998). PAF and its receptor are also present in various primate species (Roudebush *et al.*, 1998; Roudebush *et al.*, 2001; Roudebush *et al.*, 2002). Finally, PAF is present in human sperm (Minhas *et al.*, 1991).

PAF metabolism is affected by androgens, estrogens, and progesterone (Mguruma *et al.*, 1993; Ohshihe *et al.*, 1994). Androgenic hormones play an important role in male fertility and are decreased significantly during the nonbreeding season. PAF concentrations in rhesus monkey (*Macaca mulatto*)

sperm are directly correlated with sperm motility and forward progression (Roudebush *et al.*, 2002). The concentration of PAF in human sperm originally was found to be inversely related to sperm quality (Angle *et al.*, 1991). However, Roudebush and Purnell (2002) reported that the PAF content in human sperm, processed for use for in vitro fertilization (IVF), has a positive correlation with motility indices and pregnancy rates.

The addition of PAF to human sperm increases sperm motility, enhances sperm penetration of cervical mucus, and improves sperm penetration assay results (Naz and Minhas, 1995). To fertilize an oocyte successfully, sperm must be able to penetrate the outer layers investing the oocyte, including the cumulus cells and the zona pellucida. Many investigators have examined the effect of exogenous PAF on human sperm motility (Ricker *et al.*, 1989; Jarvi *et al.*, 1993; Krausz *et al.*, 1994). Exposure of sperm to PAF significantly improves sperm motility. Incubation of human sperm with PAF causes an increase in the number of spermatozoa that undergo the acrosome reaction (Krausz *et al.*, 1994; Lee *et al.*, 1997). PAF-treated spermatozoa fertilized oocytes at a higher rate than those treated with lyso-PAF (Fukuda *et al.*, 1994). A higher rate of blastocyst formation was also noted in embryos that resulted from PAF-treated spermatozoa. The formation of the pronuclei reportedly took place at a faster rate in oocytes that were injected with sperm, which were induced to undergo the acrosome reaction as compared with spermatozoa in which the acrosome was intact (Fukuda *et al.*, 1994).

Human sperm prepared for intrauterine insemination using a sperm wash medium supplanted with PAF results in significantly increased pregnancy rates (Roudebush *et al.*, 2004; Fig. 1). This finding has been subsequently verified by others and thus confirms our original results demonstrating the effectiveness of exogenous PAF supplementation in an IUI program (Grigoriou *et al.*, 2005; Fig. 1). It should be noted that in all studies the significant improvement in IUI pregnancy rates could only be observed in patients presenting with a normal semen analysis (Baka *et al.*, 2009). 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. We have recently reported on the presence and distribution of the PAF receptor in human sperm and our preliminary data demonstrate that distribution of the receptor is significantly altered in abnormal sperm (Roudebush *et al.*, 2000; Purnell and Roudebush, 2001). We have also

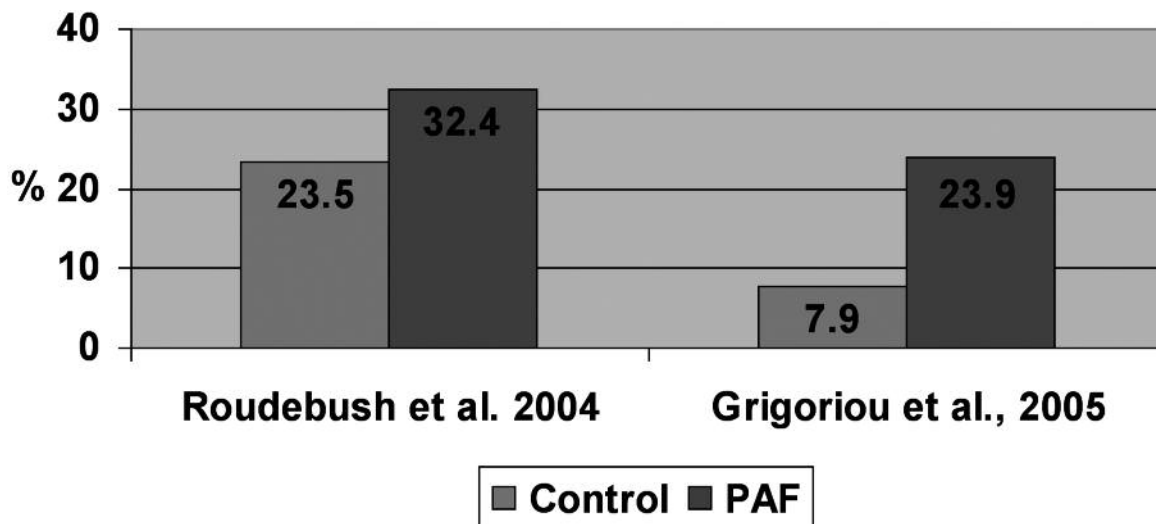


Fig. 1. — PAF IUI Pregnancy Rates

discovered that PAF receptor mRNA expression differs significantly between motile (high content) and nonmotile (low content) sperm (Purnell and Roudebush, 2001). The PAF plays a significant role in sperm function and may affect sperm motility and fertilization through a receptor-mediated control of intracellular calcium. The PAF has been shown to augment sperm capacitation (Sengoku *et al.*, 1993).

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

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