Effect of exogenous PAF addition on IUI in male factor infertility

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Abstract

Male factor is the main or contributing cause in almost fifty percent of infertile couples.

Intrauterine insemination is a commonly performed procedure in cases of infertility. It is indicated in couples with male factor infertility.

A number of biochemical molecules in spermatozoa have been proposed as potential predictors of male fertility, one of them is platelet activating factor. Endogenous platelet activating factor (PAF) has been proved to have a role in the reproductive physiology. These functions include ovulation, sperm capacitation, acrosome reaction, fertilization, pre-implantation embryo development, implantation and parturtion. In this study, PAF addition during semen preparation was done. After this, the processed semen was used for intrauterine insemination. These steps were performed in couples with male factor infertility and the pregnancy rate was monitored. Platelet activating factor addition was done to enhance the motility and the fertilizing capacity of the spermatozoa and improve the pregnancy rates of intrauterine insemination and controlled ovarian hyperstimulation.

Keywords: Male factor infertility, Intrauterine insemination, Platelet activating factor, Semen processing.

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My work is dedicated to all the patients that were included in the study and I hope it is of use to many more.

List of abbreviations

CC -> Clomiphine citrate

CoA -> coenzyme A

COH-IUI -> Controlled ovarian hyperstimulation-intrauterine insemination

FSH -> Follicle stimulating hormone

GnRH -> Gonadotrophin-releasing hormone

GWCF -> Glass wool column filtration

hCG -> Human chorionic gonadotrophin

hMG -> Human menopausal gonadotropin

HP-FSH -> Highly purified-FSH

ICI -> Intracervical insemination

ICSI -> Intra-cytoplasmic sperm injection

IGF-I -> Insulin-like growth factor I

IUI -> Intrauterine insemination

IVF -> In-vitro fertilization

IVF-ET-> In-Vitro fertilization – Embryo transfer

BMI -> Body mass index

LH -> Luteinizing hormone

mRNA -> messenger RNA

NICE -> National Institute of Clinical Excellence

OH -> Ovarian hyperstimulation

OHSS -> Ovarian hyperstimulation syndrome

PAF -> Platelet activating factor

PAF-AH -> Platelet activating factor acetyl hydrolase

PR -> Pregnancy rates

RCT -> Randomised controlled trial

rec-FSH -> Recombinant-FSH

SCI -> Spinal cord injury

TMSC -> Total motile sperm count

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Introduction

Male fertility requires the production of an adequate concentration of normal mature spermatozoa with sufficient motility and ability to undergo capacitation and acrosome reaction so as to bind and penetrate the zona pellucida and finally achieve fertilization. Defects in any of these necessary steps can lead to male infertility. In couples that have failed to conceive after one year of regular unprotected intercourse, male factor causes amount to about 30% of these cases, and is a co-factor in an additional 20% of the cases. Thus, male factors are involved in 50% of the couples complaining of infertility. Male factor infertility is diagnosed after full history taking, physical examination and laboratory investigations. Unfortunately, the underlying cause for the abnormal semen analysis is not identified in many instances. In these cases, both empirical therapies and techniques such as intrauterine insemination (IUI), Invitro fertilization (IVF) and Intracytoplasmic sperm injection (ICSI) are often utilizied. The role of medical treatment in male factor infertility is an extremely controversial subject, aside from few clear cut conditions where medical or surgical treatment is generally recommended as hypogonadotrophic hypogonadism.

Intra-uterine insemination is the most commonly performed assisted reproductive technique procedure. IUI is indicated for couples with unexplained infertility, mild to moderate male-factor infertility or certain

female factors. Introduction of IVF technology opened the door for modern methods of sperm washing and processing. These techniques made IUI safe. Since that time intrauterine insemination remains a widely used treatment option for couples with infertility. The rational for performing IUI, is that the motile spermatozoa, are concentrated in a small volume and are injected directly into the uterus, bypassing the cervix, thus reaching easily the site of the released oocyte. The effectiveness of IUI for the treatment of infertility has been evaluated in several studies and the main debatable points have been whether results should be attributed to the technique of IUI as such or to the close monitoring of the cycle and/or to the use of ovarian stimulation.

The use of pharmacological adjuvants to enhance sperm function is a real possibility and a very attractive one. Application of truly effective adjuvants may prove to be a cost-effective approach prior to more invasive and expensive treatments such as IVF and ICSI.

Many researches have focused on the male reproductive physiology, in order to achieve more information, insight and better ability to treat male subfertility. A great number of factors have been discovered to have a role in helping the human spermatozoa to reach its fertilizing capabilities. Among them, platelet activating factor (PAF) was found to be present in the human sperm. It has been found to be one of the endogenous factors responsible for the regulation of spermatozoa's fertilization capacity. The exact mechanism of its action on spermatozoa is not clearly known.

PAF is a signaling phospholipid that has many additional properties other than platelet activation. The role of PAF in sperm functions has many facets. These include: enhancing sperm maturation, enhancing sperm motility and enhancing sperm capacitation and acrosome reaction. Platelet activating factor was also found to have a role in fertilization, preimplantation embryo development, implantation and parturition (Harper, 1989).

AIM OF THE WORK

The aim of this work is to evaluate the effect of PAF supplementation during semen preparation in couples with male factor infertility, undergoing intrauterine insemination and controlled ovarian stimulation.

Furthermore, we intend to review the literature on intrauterine insemination, platelet activating factor and their impact on fertility along with different semen processing techniques.

Chapter I

Platelet activating factor:

Introduction:

Platelet activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phosphorylcholine) belongs to a family of acetylated glycerophospholipids with a diverse spectrum of biological activities in a variety of cell types. It is well known that PAF is produced by various inflammatory cells such as basophils, neutrophils, eosinophils and vascular endothelial cells following appropriate stimulation, and that it is thought to be a chemical mediator of allergy and inflammation (Prescott *et* al, 1990). PAF is also found in normal tissues such as brain, stomach and kidneys (Tokumura *et* al, 1987, Sugatani *et* al, 1989, Camussi *et* al, 1989).

Benveniste et al (1972) first identified PAF 40 years ago when they found that it was a potent mediator of rabbit platelet aggregation in immunoglobulin E–stimulated basophils. 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 exists endogenously as a mixture of molecular species with structural variants of the alkyl moiety.

The C-16 species is predominant in human sperm (Sanwick *et* al, 1992).

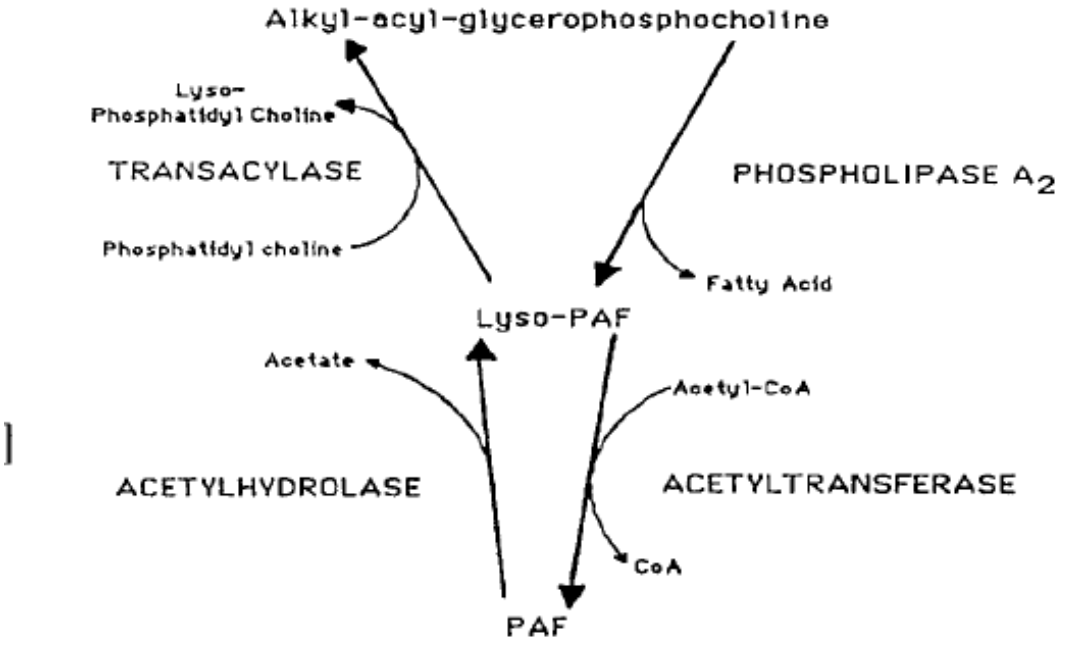
Arrata et al (1978) used ^{31}P nuclear resonance spectroscopy to suggest a role for phosphate esters in male infertility. Levine et al (1987) subsequently used ^{31}P nuclear resonance spectroscopy to demonstrate that PAF concentrations were higher in fertile men than in infertile men and that PAF was absent in semen samples from vasectomized men.

PAF seems to have an important role in reproduction by being involved in ovulation, sperm capacitation, fertilization, pre-implantation embryo development, implantation, and parturition (Harper, 1989). PAF, being present in human sperm, is one of the endogenous factors directly correlated with sperm motility, forward progression, and is responsible for the regulation of spermatozoa fertilization capacity (Minhas *et* al, 1991).

Although the exact mechanism or mechanisms for PAF action remain unclear, its importance for normal reproductive function is evident.

PAF synthesis and metabolism

Phospholipase A2 is present in human spermatozoa. It is calcium-dependent and catalyzes the formation of 1-Oalkyl- 2-lyso-sn-glycero-3 phosphocholine (lyso-PAF) from alkyl-acyl-glycerophosphocholine, an inert structural cell membrane component (Bennet *et* al, 1986). Lyso- PAF is biologically inactive. It can be acetylated by acetyl transferase using acetyl-coenzyme A (CoA) as an acetate donor to form 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphorylcholine (PAF). Lyso-PAF may also be acetylated by a CoA-independent arachidonyltransacylase to form alkyl-acyl glycerophosphocholine. Acetylhydrolase (PAF-AH) 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. The metabolic pathway for PAF synthesis is presented in the figure below (figure 1).



Acetyltransferase and acetylhydrolase are both present in mammalian spermatozoa and seminal fluid (Gujarati *et* al, 1987). Consequently, both the enzymes necessary for PAF activation and deactivation are present in spermatozoa and seminal fluid. Letendre et al (1992) suggested that acetylhydrolase might itself act as a sperm decapacitation factor. This is based on the observation that capacitation occurs in human spermatozoa without exogenous mediators following sperm removal from seminal fluid. 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 (Roudebush *et* al, 1990, 1993; Hellstrom *et* al, 1991; Angle *et* al, 1993). PAF may indeed be a biomarker for capacitation.

It is believed that a number of molecules may play an important role in the process of PAF synthesis and secretion. It has been previously reported that human spermatozoa synthesize PAF via the remodelling pathway that is stimulated by progesterone and the calcium ionophore, A23187, both of which are known to induce the acrosome reaction (Harper *et* al, 2006). In the pre-ovulatory follicle, estradiol ceases to be synthesized by granulose cells and is replaced by progesterone just prior to ovulation (Moor & Seamark, 1986). Progesterone and 17-hydroxyprogesterone cause an immediate increase in free cytosolic sperm calcium (Blackmore *et* al, 1990). It was reported that exposure of rabbit spermatozoa for 15 minutes to progesterone causes an increase in the synthesis and release of PAF (Minhas *et* al, 1993). A similar mechanism seems to occur in human spermatozoa (Ripps *et* al, 1993). Following exposure of human and rabbit spermatozoa to synthetic