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METHODS FOR ENHANCEMENT OF SPERM FUNCTION

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1. ABSTRACT

From a review of recent advances in human reproduction, it is apparent that therapeutic approaches to male infertility have been revolutionized. While our understanding of sperm function at the molecular level is steadily increasing, the realization of consistent oocyte fertilization by mechanically bypassing natural barriers has given new perspective for future investigation. This chapter reviews current knowledge of adjuvants that enhance sperm function and lend themselves to clincal application. Each compound is presented with recent publications supporting proposed mechanisms of action. Specifically, follicular fluid, progesterone, pentoxifylline, platelet activating factor and other cytokines have been studied for their impact on the *in vitro* fertilization capacity of spermatozoa. Intracytoplasmic sperm injection (ICSI) has provided infertile couples with hope for successful reproduction without sperm donation. The precise mechanism that allows subsequent pronuclear formation and syngamy is currently unknown and experimental models are few. Adjuvants that can be used in conjunction with controlled ovarian stimulation and intrauterine insemination are prominent areas for further research as this would provide an alternative to the expense and risks of *in vitro* fertilization and embro transfer.

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2. INTRODUCTION

Infertility is a prevalent condition with significant societal and psychological impact. Thirty percent of couples seeking assistance for infertility are unable to conceive solely due to male related problems. Impaired sperm performance in conjunction with other female factors is noted in an additional twenty percent of infertile patients. Recent studies have documented a trend in declining semen quality world-wide(1-3). The potential for impaired function within the male complement is vast and reflected in the prevalence of male infertility.

Normal male fertility requires completion of a daunting sequence of elaborate processes including spermatogenesis, sperm transport, accessory gland function along with timely sperm deposition through adequate coitus. Sperm-oocyte interaction poses an additional set of impediments to successful reproduction but is a juncture most amenable to treatment with *in vitro* techniques. Invasive and non-invasive techniques to achieve fertilization are now routinely used in assisted reproduction programs. This article will address clinical application and basic research efforts in the pursuit of improving sperm function within assisted reproduction.

3. ENHANCEMENT OF SPERM-OOCYTE INTERACTION

Prior to acquiring fertilization ability the sperm undergoes complex morphological and biochemical changes(4). This process of capacitation allows hyperactivated motion, zona pellucida binding,

acrosome reaction and penetration. Once within the oocyte, decondensation is necessary for male pronuclear formation; thus completing the most essential goal of spermatozoa: delivery of genetic material to the oocyte.

Natural reproduction offers other obstacles requiring adequate sperm function. After vaginal deposition, a fraction of the total sperm enters the cervical mucus, potentially extending viability to four days. The fertilizing spermatozoa must traverse the female reproductive tract to reach the distal fallopian tube, the usual site of fertilization. In the absence of patent fallopian tubes or surgically correctable obstruction, these couples are constrained to *in vitro* fertilization for reproduction. In this environment, gamete interaction becomes dependent upon oocyte quality, sperm count, motility, morphology and ability to capacitate followed by acrosome loss(4, 5).

Fertilization hampered by oligozoospermia can be effectively overcome *in vitro* by employing contemporary sperm separation techniques followed by micro-insemination or intracytoplasmic sperm injection. Impaired sperm function, however, may be improved by the use of several adjuvants discussed in the following sections.

3.1 Follicular Fluid

Several studies have confirmed that follicular fluid enhances sperm motility, capacitation, acrosome reaction, penetration of the zona free hamster oocytes, and fertilization rates(6-9). Mbizvo *et al*. showed that follicular fluid treated sperm exhibited a threefold increase in hyperactivation when compared to non-treated human sperm(10). In the same investigation they noted that the portion of the follicular fluid extracted of steroids had no stimulatory effect on sperm hyperactivation. The steroid-rich fraction of the follicular fluid stimulated hyperactivation to the same degree as whole follicular fluid(10). Meizel's group demonstrated that a G-75 sephedex fraction of human follicular fluid (HFF) was capable of inducing a physiological acrosome reaction in human spermatozoa(11). Further characterization of this G-75 fraction with organic solvent extraction followed by reverse phase HPLC identified two peaks containing acrosome reaction inducing ability. The HPLC peak with highest acrosome reaction inducing ability was identified by gas chromatography electron ionization mass spectrometry and proton NMR spectroscopy to be progesterone. The second peak was identified as 17-hydroxyprogesterone (12).

Follicular fluid is pharmacologically complex and contains a number of constituents capable of affecting sperm performance. Human follicular fluid is known to contain glycosoaminoglycans, platelet activating factor, serum albumin, and xanthine derivatives which act as phosphodiesterase inhibitors to elevate intracellular cAMP. Thus, it is becoming increasingly evident that follicular fluid is a potent stimulator of sperm performance *in vitro* and, in fact, may be playing an important role *in vivo* in modulating sperm function as the spermatozoa encounter increasing concentrations of follicular fluid in proximity of the cumulus oophorus.

3.2 Progesterone

Progesterone and 17-hydroxyprogesterone have been shown to cause an immediate increase in free cytosolic Ca^{2+} in both capacitated and non-capacitated human sperm with the effect being triggered within a few seconds of hormone treatment(13, 14). Similarly, progesterone conjugated to larger proteins was equally effective yet incapable of rapid penetration of the sperm plasma membrane. Selective expression of a progesterone receptor on human spermatozoa has been recently suggested by fluorescence microscopy and flow cytometry(15). Kinetic analysis revealed most spermatozoa that bind progesterone complete the acrosome reaction and that samples with impaired progesterone binding also fail respond with Ca^{2+} influx. These studies represent the first evidence of a relationship between a novel, nongenomic progesterone receptor on the surface of sperm and sperm function.

Support for a therapeutic application of progesterone was found with our demonstration of significantly improved fertilization rates of rabbit oocytes (57% versus 16%, $p<0.001$) when sperm were treated with progesterone for 15 minutes prior to subzonal insertion. Furthermore, this 15-minute treatment was shown to increase both the synthesis and release of PAF from rabbit spermatozoa(16). A similar mechanism seems to be operative in human spermatozoa supporting an intermediary role for PAF in the progesterone stimulation of sperm function(17). We propose that spermatozoa coming in contact with progesterone in the female reproductive tract are stimulated to synthesize and secrete PAF. Secreted PAF then stimulates sperm function in an autocrine fashion, via a cascade of secondary mediators generated by phosphatidylinositol 4,5-bisphosphate breakdown and Ca^{2+} mobilization.

3.3 Platelet Activating Factor

Platelet activating factor (PAF) (1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine) belongs to a family of acetylated glycerophospholipids with a diverse spectrum of biological activities in a variety of cells types. Furthermore, many cell types possess the ability to synthesize PAF upon stimulation(18).

PAF has been identified as an endogenous component of spermatozoa from several species including human (19-22). Lipids from spermatozoa are extracted by the method of Bligh and Dyer(23). Separation and purification is achieved by thin layer chromatography. Biological PAF activity is confirmed by aggregation of washed rabbit platelets and quantification is achieved by organic phosphate analysis(19, 24).

Furthermore, spermatozoa possess the enzymatic complement to synthesize and metabolize PAF. Acetyltransferase, the enzyme necessary for PAF formation from lyso-PAF, and acetylhydrolase, the enzyme responsible for removing the acetate group from the PAF molecule and converting it back to its inactive form lyso-PAF, are both present(25). Acetylhydrolase activity has been demonstrated in human seminal plasma and a role for this enzyme as a decapacitation factor has been put forward(26). In preliminary experimentation in our laboratory we have noted a significant decrease in spermatozoal-acetylhydrolase activity upon capacitation, concurrent with an increase in endogenous PAF levels.

Following exposure of human and rabbit spermatozoa to synthetic PAF, flow cytometry revealed mobilization of Ca^{2+} in cells loaded with Indo-IAM(13). Progesterone and 17-hydroxyprogesterone cause an immediate increase in free cytosolic sperm calcium(14). We have previously reported that a 15-minute exposure of rabbit spermatozoa to progesterone (1μg/ml). produces both an increase in the synthesis and release of PAF(16). A similar mechanism seems to be operative in human spermatozoa(17, 27). The above studies suggest an intermediary role of PAF in the progesterone stimulation of sperm function and led us to propose that spermatozoa coming in contact with progesterone in female reproductive tract are stimulated to synthesize and secrete PAF. This secreted PAF, in an autocrine fashion, then stimulates sperm function via a cascade of secondary mediators(28).

Motility stimulation upon treatment of human spermatozoa has been documented utilizing both videomicrography and computer assisted motion analysis(29-31). Stimulation of penetration of cervical mucus and zona free hamster oocytes has also been reported upon treatment of human spermatozoa with synthetic PAF(32-34).

In animal studies, PAF treatment of murine and rabbit spermatozoa has been shown to significantly improve fertilization rates of oocytes *in vitro* utilization both conventional *in vitro* and microsurgical fertilization(35-40). Treatment of murine spermatozoa with PAF prior to insemination of oocytes had no detrimental effect on subsequent embryo development *in vitro* or *in vivo*. In addition, the reproductive efficiency of animals generated from embryos produced by PAF-treated sperm was not compromised as mating of these animals upon maturity resulted in the delivery of normal healthy pups(41).

PAF seems to be an intrinsic factor in spermatozoal function with roles in sperm transport, capacitation and fertilization of the oocyte. Embryonic PAF secretion and improved embryo viability with PAF treatment implicate PAF as a factor in development and implantation. Appropriately, studies are in progress to elucidate the complex functions of PAF in embryo development and implantation, as well as spermatozoal physiology and pharmacology.

3.4 Cytokines

Cytokines represent a spectrum of peptide hormones produced within the immune system and by other cell types in response to stimulation. Cytokines modulate spermatogenesis and sperm function. High concentrations of interferon-alpha and gamma (IFN) and tumor necrosis factor-alpha (TNF) have demonstrated deleterious effects on sperm motility and hamster penetration rates in the sperm penetration assay(42, 43). On the contrary, a recent report documented an increase in sperm count and motility in severely oligospermic patients receiving intramuscular injections of IFN-alpha(44).

Elegant work by Naz *et al.* (45) showed levels of Interleukin-6 (IL-6) to be significantly higher in the seminal plasma of infertile men when compared to fertile controls. Furthermore, *in vitro* incubation of sperm with IL-6 was shown by the same group to enhance motility parameters and penetration of zona free hamster oocytes(46). Thymosin alpha-1 has also been shown to enhance the capability of human sperm to penetrate zona free hamster oocytes and improved motility parameters. This effect was produced in spermatozoa from infertile males(47).

3.5 Pentoxifylline

Pentoxifylline is a methylxanthine inhibitor of phosphodiestrase and improves motility of both fresh and cryopreserved human spermatozoa(48, 49). Cyclic adenosine 3', 5' - monophosphate (cAMP). is a known regulatory element of axonemal motility, and by its actions on protein phosphorylation may be responsible for pentoxifylline's actions. Elevated cAMP levels as a consequence of pentoxyifylline treatment may be modulating other events such as capacitation and acrosome reaction(50).

Prior to clinical application, animal studies assessed the embryo toxicity of pentoxifylline using murine IVF and embryo transfer (ET) models. No differences in the fertilization rates or subsequent

rates of blastocyst formation were noted when pentoxifylline was washed out prior to insemination of murine oocytes. Pregnancy rates upon ET were also unaffected and offspring were normal(51).

The initial clinical application of pentoxifylline entailed exposure of the sperm to the adjuvant for a period greater than two hours prior to insemination of oocytes. In a small series of nine couples who had experienced up to three cycles of fertilization failure, five successful pregnancies were established with pentoxifylline-treated sperm(52). In subsequent studies, reducing sperm exposure to pentoxifylline to 30-40 minutes, gave significantly improved fertilization rates and fewer cases of fertilization failure. Significantly improved rates of fertilization and embryo yield continue to be observed in cases of severe oligoasthenozoospermia(53).

A 10-minute exposure of capacitated normozoospermic samples to pentoxyifylline (1 mg/ml) did not alter the percentage of motile cells but mean values of all other motion parameters were significantly different. Similar changes were also noted with asthenozoospermic samples. Significant increases in curvilinear velocity were noted for both types of specimens. Elevated curvilinear velocity was maintained in normozoospermic specimens one hour longer than asthenozoospermic specimens. Significant improvement in hyperactivation was also noted for both types of specimens(54). The improvements in motility for oligozoospermics are noted to be immediate and transient while for normozoospermics various motility parameters are sustained for periods ranging between one and four hours. Acrosome reaction ionophore challenge test (ARIC) measures the proportion of spermatozoa that respond to A23187 and demonstrates high sensitivity and predictive value for IVF(55). Pentoxifylline improves the fertilizing ability of spermatozoa in certain cases of acrosome reaction insufficiency. Prospective randomized studies have shown the ARIC test to be useful predictor of benefit for pentoxifylline treatment(56, 57).

Impaired sperm function has been associated with elevated levels of reactive oxygen species with a postulated mechanism being acrosomal membrane pertubation. The ensuing damage may cause the plasma membrane to lose its responsiveness to calcium influx signal that would otherwise trigger the acrosome reaction(58). Routine semen preparation may stimulate release of these damaging oxygen species from sperm cells. John Yovich and collegues at the PIVET Medical Center utilize the ARIC test in all cases of infertility as a diagnostic indicator of sperm function impairment. An ARIC score of less than 10% is repeated on a pentoxifylline-treated sample. Improved scores upon pentoxifylline treatment provides the basis for offering this adjuvant for intrauterine insemination (IUI), gamete intrafallopian transfer (GIFT) and IVF. Pregnancies have been generated from each of the above procedures utilizing pentoxifylline-treated sperm, resulting in the delivery of normal healthy infants. There is no increase in pregnancy wastage of fetal abnormalities when compared with matched controls undergoing assisted reproduction without pentoxifylline. More than 200 babies have been born from pentoxifylline-treated sperm without any increase in the incidence of congenital anomalies(57).

3.6 Intracytoplasmic Sperm Injection

Treatment of male infertility has been revolutionized by the advent of intracytoplasmic sperm injection (ICSI). The procedure was perfected in the early 90's by Professor Van Steirteghem's group in Belgium. In a very short period the technology has been applied in assisted reproduction clinics world-wide.

Further advances have led to the development of round spermatid nuclei injection (ROSNI), a procedure that does not even require the completion of spermatogenesis in the male. Success has been achieved by the injection of round spermatid nuclei into oocytes(59). Spermatozoa extracted from the epididymis (MESA) and testis (TESA) also can serve as a source of male genetic complement in ICSI applications.

A desire of practitioners in the field has been to determine which patients would gain the most benefit from the application of ICSI. A retrospective review of data by the senior author provides new insight to this dilemma. Over a ten month period, January through October 1995, patients enrolled in the ICSI program were divided into three groups: Group I was male factor defined by the existance of abnormalities in one or more semen analysis parameters according to WHO standards and no female pathology evident. Group II was defined as the female factor and consisted of couples with normal semen parameters, but one or more conditions of: elevated day three FSH, poor response to gonadotropin stimulation, advanced maternal age ($\geq$39 years) or poor quality oocytes. Group III was defined as a combination of both male and female factors. On examining clinical pregnancy rates per transfer procedure, data clearly showed significant differences. Group I had a clinical pregnancy rate (30/75) of 40%. Group II had a clinical pregnancy rate of (5/46) 10.9%. The lowest pregnancy rate was seen in Group III at (5/97) 5.2%. Interestingly, fertilization and cleavage rates in the three categories were not signifcantly different(60). Several groups are beginning to review their data in a similar fashion in order to develop sound patient counseling strategies.

4. PERSPECTIVE

As presented in this review, the use of pharmacologic adjuvants for enhancement of sperm function is quite real and very enticing. Application of these adjuvants may prove to be a cost effective approach prior to moving to more invasive and expensive treatments involving intracytoplasmic sperm injection. However, just as judicious selection of patients for ICSI will maximize the benefit to infertile patients, the role of adjuvants must be well defined to maximize therapeutic outcome for specific patient populations.

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