Evaluation of the Effect of adding Pentoxifylline to processed semen samples on ICSI Outcome in Infertile Males with Mild and Moderate Asthenozoospermia in a Prospective Crossover Study

Thesis

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By

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Abstract

The aim of the study was to evaluate the effect of Pentoxifylline used in preparation of semen samples that will be used for intacytoplasmic sperm injection (ICSI) in infertile men complaining of mild and moderate asthenozoospermia in comparison to semen samples without PTX preparation on the outcome of ICSI. The study was carried out on 30 infertile patients where pentoxifylline was used for semen processing prior to oocyte injection, another 30 infertile patients where no pentoxifylline was used in semen processing and 60 infertile patients where crossing over of the semen sample was done further subdividing it into 2 subgroups in which the first half of the semen sample was incubated with pentoxifylline and the second half of the sample was not incubated with pentoxifylline and the wife's oocytes were divided on these 2 samples. The results showed that pentoxifylline has a significant positive effect on ICSI outcome in cases of mild and moderate asthenozoospermia as regards the fertilization rate, the embryos quality and the pregnancy rates without any significant embryotoxic effect or significant increase in the abortion rate. Moreover, the results are more solid when using prospective crossing over of the semen sample in each patient i.e. each patient acts as his own control.

Key words: Pentoxifylline, ICSI, Asthenozoospermia, Semen Processing, Crossing Over.

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List of Abbreviations

PTX, PX or PF: Pentoxifylline.

ICSI: Intracytoplasmic sperm injection.

OHSS: Ovarian hyperstimulation syndrome.

AZS: Asthenozoospermia.

PCD: Primary ciliary dyskinesia.

KS: Kartagener.

Oocytes retr: Number of oocytes retrived.

Oocytes inj: Number of oocytes injected.

Fert.Oocyt: Number of fertilized oocytes.

Embro no: Total number of embryos.

G1: Number of good embryos.

G2: Number of fair embryos.

G3: Number of bad embryos.

ET: Total numbers of embryos transferred.

Sacs no: Number of embryo sacs.

Impl rate: Implantation rate.

Introduction
&
Aim of The Work

Introduction

Pentoxifylline (or Pentoxyphlline / PTX) is a chemical that belongs to the family of methylxanthines. One pharmacological effect is the relaxation of vascular smooth muscles and therefore is prescribed in diseases associated with circulatory disturbances (e.g. intermittent claudication). It has been speculated that in men with idiopathic infertility testicular circulation might be disturbed and would be improved by PTX ***(Heite, 1979)***. However there is neither evidence for circulatory disturbances in idiopathic infertility nor is there proof for any clear therapeutic effects of PTX as an infertility regimen ***(Wang et al. 1983; Shen et al. 1991)***.

PTX is known to increase spermatozooal intracellular levels of 3'5'- adenosine monophosphate (c-AMP) in vitro which plays a role in sperm motility ***(Tash and Means, 1983)***. It is also thought that PTX enhances sperm motility in samples with poor progressive motility by increasing intracellular adenosine triphosphate (ATP) ***(Garbers et al. 1971)***.

Apart from the oral application, PTX has been used as an in vitro additive in IVF in order to improve fertilization rates ***(Tournaye et al. 1995)***. The typical protocol for PTX use involves a 30 minutes preincubation of prepared sperm with stimulant (PTX at 1-5 mmol/l). Sperm is then washed to remove the stimulant and is used immediately for ova fertilization ***(Mitchell, 2005)***.

A prospective randomized controlled study was done to determine whether the use of PTX would improve the IVF rate and outcome in couples with male factor infertility and previous failure of fertilization in vitro. It found that a significantly higher fertilization rate occurred in the group were oocytes were inseminated with spermatozoa treated with PTX compared with controls. The study concluded that PTX improves the fertilization rate and outcome in couples with male factor infertility and poor fertilization rates and did not suggest any increase in teratogenicity or evidence of congenital malformations in pregnancies following IVF cycles where PTX was used ***(Rizk et al. 1995)***

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No extensive studies were done that included the use of PTX or verify its effect on the outcome of ICSI in cases of mild and moderate asthenozoospermia, although extensive studies were done on its effect in cases of severe male factor or severe asthenozoospermia particularly in IVF cycles. Some studies have found that the application of PTX increases the sperm motility ***(Dimitridou et al. 1995)*** and fertilization rate ***(Yovich et al. 1990; Tarlatzis et al. 1995)***, while others have observed no differences in sperm parameters and fertilization rates after treatment with PTX ***(Tournaye et al. 1994; Fountain et al. 1995)***. It has also been found that adding PTX to thawed testicular spermatozoa increases the number of progressively motile sperms, when compared with culture in vitro alone ***(Thomas et al. 2001; Gonzalez et al. 2003)***.

Aim of The Work

The study aims to evaluate the effect of Pentoxifylline used in preparation of semen samples that will be used for intacytoplasmic sperm injection (ICSI) in infertile men complaining of mild and moderate asthenozoospermia in comparison to semen samples without PTX preparation on the outcome of ICSI as regards, fertilization rate, embryo quality, embryo implantation rate, pregnancy rate and abortion rate, in order to determine the benefit of using PTX in all semen samples for ICSI regardless of the degree of asthenozoospermia.

Review of Literature

IntraCytoplasmic Sperm Injection (ICSI)

I. HISTORY OF ICSI

Interest in the initial types of micro-manipulation procedures, such as zona drilling and partial zona drilling (PZD), evolved because of the disappointing results of standard invitro fertilization (IVF) for the severe male factor patients. In these procedures, a physical opening is created in the zona pellucida by using chemical "drilling" or by making a microscopic mechanical incision. In subzonal injection (SUZI), the micro-injection of spermatozoa into the peri-vitelline space (between the zona pellucida and the plasma membrane), gained popularity for severe male factor infertility because typically only 3 to 4 sperms were inserted per oocyte. The high rate of polyspermy, *(a lethal condition involving the entrance of more than 1 sperm into the egg)* with PZD and SUZI was finally overcome with ICSI, which requires the injection of only a single sperm per egg ***(Lamb and Lipshultz, 2003***.

Reports began appearing in scientific journals in 1992 of consistently successful treatment outcomes following the clinical application of ICSI. The reports were initially made by the group of workers of the Dutch-speaking Brussels Free University led by Professor Andre Van Steirteghem ***(Palermo et al., 1992; Meniru, 2001)***.

This procedure bypasses some of the physiologic events, such as capacitation and the acrosome reaction, that are normally required for fertilization in-vivo. In general, ICSI has allowed couples with male factor infertility to achieve pregnancy outcomes that are comparable with those of couples with non-male factor infertility using IVF treatment ***(Yao and Schust, 2002)***. It was also suggested that intracytoplasmic sperm injection (ICSI) could be used to treat all forms of male infertility ***(Palermo et al., 1995)***. The couples with a short history of infertility must be assured that assisted reproductive techniques are recommended only if natural ways of conception isn't possible ***(Kohn and Schill, 2002)***.

A number of operative techniques have now been developed for the recovery of spermatozoa from the testis and other parts of male genital tract they include; percutaneous epididymal sperm aspiration (PESA), micro epididymal sperm aspiration (MESA) and testicular sperm aspiration (TESA) and testicular sperm extraction (TESE) ***(Meniru, 2001)***.

TESE/ICSI has been applied to functional azoospermia and represents an extraordinary treatment advance for one of the most severe forms of male factor infertility. Men previously regarded as sterile can now establish a pregnancy with TESE/ICSI ***(Sharlip et al., 2002)***.

Clinical pregnancy rates between 11% and 49% per cycle have been reported for TESE/ICSI in functional azoospermia. It is possible to perform ICSI with cryopreserved testicular sperm, and several studies suggest that pregnancy rates are not compromised ***(Küpker et al., 2000)***.

Testicular sperm extraction (TESE) associated with ICSI gives patients suffering from non-obstructive azoospermia (NOA) the possibility of becoming a father. The success rate of TESE based on sperm recovery is approximately 50%, and the commonly used non-invasive parameters are not predictive enough. Only the invasive testis biopsy has a good prognostic value ***(Koscinski et al., 2005)***.