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

Submitted in partial fulfillment for the M.D. in Andrology & STDs

By

Ahmad Raef Sadek Ali Sadek Mohammad Sadek (M.Sc.)
Kasr Al-Ainy Faculty of medicine
Cairo University

Under supervision of

Prof. Dr. Bahgat Aly Metawae
Professor of Andrology & STDs
Faculty of Medicine – Cairo University

Prof. Dr. Medhat Kamel Amer
Professor of Andrology & STDs
Faculty of medicine – Cairo University

Dr. Hossam Al Din Hosny Ahmad
Lecturer of Andrology & STDs
Faculty of medicine – Cairo University

Faculty of Medicine
Cairo University

2012
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.
Acknowledgement

First and foremost, thanks to Allah, the most beneficial and most merciful.

I would like to express my deep thankfulness and gratitude to Dr. Bahgat Aly Metawae, Professor of Andrology and STDs, Faculty of Medicine, Cairo University for his valuable advices and support throughout the work.

I am greatly honored to express my sincere appreciation to Dr. Medhat Kamel Amer, Professor of Andrology and STDs, Faculty of Medicine, Cairo University for his continuous, sincere and valuable help and support in every detail of this study.

Many thanks and respect to my dear teacher Dr. Hossam Al Din Hosny Ahmad, Lecturer of Andrology and STDs, Faculty of Medicine, Cairo University for his continuous help, generous advice throughout this work and his guidance to me.

Special thanks and gratitude to all the staff of Adam International hospital for their kind guidance and great help during the preparation of the work.

Finally, I would also like to dedicate this work to my dear mother, my brothers, my beloved wife and my sons and to the one I miss the most and cherish his memory my late beloved and dear father Dr. Raef Sadek.
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of figures</td>
<td>4</td>
</tr>
<tr>
<td>List of abbreviations</td>
<td>5</td>
</tr>
<tr>
<td>Introduction &amp; Aim of The Work</td>
<td>6</td>
</tr>
<tr>
<td><strong>Review of Literature</strong></td>
<td></td>
</tr>
<tr>
<td>• ICSI</td>
<td>12</td>
</tr>
<tr>
<td>• Asthenozoospermia</td>
<td>101</td>
</tr>
<tr>
<td>• Pentoxifylline</td>
<td>121</td>
</tr>
<tr>
<td><strong>Patients and methods</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>148</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>172</td>
</tr>
<tr>
<td><strong>Summary</strong></td>
<td>182</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>188</td>
</tr>
<tr>
<td><strong>Arabic Summary</strong></td>
<td>239</td>
</tr>
</tbody>
</table>
List of Figures

Fig.1: Injection of the oocyte *(Abington Reproductive Medicine, Gladstone, New Jersey, 2000)*

Fig.2: Grades of embryos *(Van Steirteghem et al., 1997).*

Fig.3: Sperm tail structure.

Fig.4: Molecular structure of pentoxifylline.

Fig.5: Four distinct patterns of embryo fragmentation.

Fig.6: Graph illustrating Pregnancy within group I.

Fig.7: Graph illustrating Pregnancy within group II.

Fig.8: Graph illustrating Abortion within group I.

Fig.9: Graph illustrating Abortion within group II.

Fig.10: Bar chart comparing fertilization rate between group IIIA and group IIIB.

Fig.11: Bar chart comparing parameters of group IIIA and group IIIB.
**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 retrieved.

**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 spermatozoal 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 \textit{(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 \textit{(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 \textit{(Rizk et al. 1995)}.
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).
II. INDICATIONS FOR ICSI

ICSI has been proposed as a treatment for severe male infertility, borderline male infertility, non male factor infertility and unexplained infertility. In registry reports, ICSI is used in more than half of assisted reproduction procedures with fresh non donor oocytes or embryos (Centers for Disease Control and Prevention, 2005; Andersen et al., 2006).

I-Severe male factor infertility

ICSI is the first intervention for severe male factor infertility to achieve pregnancy rates that are equivalent to IVF pregnancy rates for non-male factor infertility (Van Rumste et al., 2000).

(A) Ejaculated spermatozoa:

- Oligozoospermia.
- Asthenozoospermia (beware for 100% immotile sperms).
- Teratozoospermia ($\leq 4\%$ normal morphology by strict criteria- beware for globozoospermia).
- High titres of antisperm antibodies.
- Repeated fertilization failure after conventional IVF-ET.
- Autoconserved frozen sperm from cancer patients.
- Ejaculatory disorders (eg, retrograde ejaculation).

(B) Epididymal spermatozoa:
- Congenital bilateral absence of the vas deferens.
- Young syndrome.
- Failed vasoepididymostomy.
- Failed vaso-vasostomy.
- Obstruction of both ejaculatory ducts.

(C) Testicular spermatozoa:
- All indications for epididymal spermatozoa.
- Failure of epididymal sperm recovery because of fibrosis.
- Azoospermia caused by primary testicular failure (eg. maturation arrest, Sertoli-Cell Only "SCO").
- Necrozoospermia.

(The ESHRE Capri Workshop Group 2006).

Azoospermia is defined as complete absence of the sperms in the ejaculate. All samples with absent sperms should be centrifuged to detect the presence of sperms in the sediment. Centrifugation should be done at more than 3000 rpm for 15 minutes. Samples should not be considered as azoospermic except after repeated search in the sediments after centrifugation (WHO, 1999).
The prevalence of azoospermia in the general population is estimated at 2%. The incidence of azoospermia in an infertility clinic population is about 20%. Azoopermia may be classified as being obstructive or non-obstructive in nature. Approximately one third of patients will have obstructive azoospermia, while the remainder will have non-obstructive azoospermia (NOA) (Steven et al., 1999).

In patients with azoospermia, ICSI can be performed with epididymal sperm aspiration (Tournaye et al., 1994), or with testicular sperm obtained by testicular aperm extraction (Devroey et al., 1994), or by fine needle aspiration (Bourne et al., 1995).

In some patients with non-obstructive azoospermia, such as partial germ cell aplasia or incomplete maturation arrest, ICSI can be carried out with testicular spermatozoa; it may be required to search for hours to find motile testicular spermatozoa in order to inject the metaphase-2 oocytes and spermatozoa will only be found in about half of the patients with non-obstructive azoospermia. (Van Steirteghem et al., 1998).
The etiology of NOA includes chromosomal abnormalities, Cryptorchidism and Anorchia, Endocrinal Abnormalities, Trauma, Torsion testis, Orchitis, Irradiation, Drugs and chemicals, Varicocele, Ejaculatory problems (Vogt et al., 1996).

In the recent decade, a progress has been made in the understanding of the genetics of men with azoospermia and the treatment modalities for these patients. Assisted reproductive techniques can help most of the patients, but there are several genetic abnormalities that must be considered before decision making for treatment of their infertility (Sadeghi-Nejad and Farrokhi, 2007).

The genes critical for spermatogenesis are located on the long arm (q) of the Y chromosome. This region is referred to as the AZF, as the most severe phenotype associated with its deletion is azoospermia. The AZF region has three non overlapping loci-AZFa, AZFb, and AZFc- deletions of which are associated with spermatogenic failure (Dada et al., 2004). Yq microdeletions are the most prevalent cause of spermatogenic failure in men with azoospermia or severe oligozoospermia. Infertile men with azoospermia or severe oligozoospermia should undergo karyotyping and testing for Yq microdeletions (Bhasin, 2007).
Y chromosome microdeletions seem to be responsible for severe bilateral testicular damage that can be phenotypically expressed by unilateral cryptorchidism as well as by idiopathic infertility (Forestà et al., 1999).

Klinefelter syndrome is the most common sex-chromosome disorder; it affects approximately one in every 660 men. This syndrome is characterized by the presence of one or more extra X chromosomes, and the karyotype 47, XXY is the most prevalent type (Bojesen and Gravholt, 2007).

The presence of spermatogonia type Ad (dark) in testicular biopsy at surgery (orchidopexy) is an excellent prognostic parameter for future fertility. Cryptorchidism boys lacking these cells will develop infertility despite successful orchidopexy at an early age (Hadziselimovic et al., 2007).

The endocrine causes of azoospermia include abnormalities of the adrenal gland, disorder of the hypothalamic pituitary gonadal axis (HPGA), hypogonadotropic hypogonadism and androgen receptor defects (Wu, 1983).

Testicular biopsy is one of the common forms of trauma. Testicular biopsy may leave inflammatory changes, haematoma and vascular injury, which may lead to permanent devascularization and parenchymal fibrosis (Amer et al., 2000).