Laser Assisted Zona Thinning Versus the Conventional Mechanical Method for Intracytoplasmic Sperm Injection in ICSI Programs

"A Randomized Controlled Trial"

Thesis

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Contents

List of Abbreviations

AMA	:	Advanced maternal age.
Arf	:	Argon fluoride gas laser.
ART	:	Assisted reproductive technologies
ATP	:	Adenosine triphosphatase.
BcB	:	Brilliant cresyl blue stain.
BMI	:	Body mass index.
C ICSI	:	Conventional ICSI.
COC	:	Cumulus oocyte complex.
COH	:	Controlled ovarian hyper stimulation.
D3EQ	:	Day 3 embryo quality.
DFC	:	Dense Fibrillar Component.
D-OPU	:	Day of ovum pick up.
E2	:	Estradiol.
ER: YAG	:	Erbium yttrium aluminium garnet laser.
FC	:	Fibrillar Center.
FP	:	Fragmentation pattern.
FSH	:	Follicle stimulating hormone.
G6PDH	:	Glucose 6 phosphate dehyrogenase.
GC	:	Granular Component.
GnRH-a	:	Gonadotrophin- releasing hormone agonist.
GV	:	Germinal vesicle.
HCG	:	Human chorionic gonadotrophin.
HMG	:	Human menopausal gonadotrophins.
HO- YSGG	:	Holmium yttrium Scandian gallium garnet.
ICSI	:	Intracytoplasmic sperm injection.
IGF	:	Insulin like growth factor.
IGF B	:	Insulin growth factor binding protein.
InGaAsp	:	Indium gallium arsenic phophorus.
IVF-ET	:	In Vitro Fertilization -Embryo Transfer.
KrF	:	Lrypton fluoride.
LA ICSI	:	laser assisted intracytoplasmic sperm injection.

List of Abbreviations (Cont.)

LH	:	Leutinizing hormone
MFD	:	Multiple follicular developments.
ND: YAG	:	Neodymium yttrium aluminium.
NICE	:	National Institute Health and Clinical Excellence.
NPBs	:	Nucleolar precursor bodies
OI	:	Ovulation induction.
OR	:	Odds ratio.
PB	:	Polar body.
PGD	:	Pregentic diagnosis.
PVP	:	Polyvinylpyrrolidone
PZD	:	Partial zonal dissection
SE	:	Standard error
SUZI	:	Sub zonal insemination
TSH	:	Thyroid stimulating hormone.
WHO	:	World Health Organization.
Xecl	:	Xenon chloride .
ZP	:	Zona pellucida.
Z-Score	:	Zygote scoring.

List of tables

List of tables (Cont.)

List of figures

List of figures (Cont.)

List of figures (Cont.)

Introduction

The birth of Louise Brown after in vitro fertilization in 1978 was a wonderful step forward, but the field has been dogged by a number of difficulties. The success of assisted reproductive technology depends upon the intricate relationship between the transferred embryo and the endometrium ***(Bider et al., 1997).***

Intracytoplasmic sperm injection (ICSI), a method of in vitro fertilization (IVF) in which a single sperm is introduced directly to the cytoplasm of a mature oocyte**.** ICSI has become a routine method of fertilization if male factor infertility is evident and the treatment is also used in instance of non male factor infertility ***(Ebner T, 2004).***

Since its inception, ICSI has been performed by the mechanical penetration of the zona pellucid and the oolemma by a glass needle through which the sperm is injected into the cytoplasm. This technique has several drawbacks. These include mechanical damage to cytoplasmic membrane, the cytoskeleton and the meiotic spindle that occurs during membrane penetration and sperm deposition (***Ebner T, 2001).***

Although the effectiveness of this procedure has been clearly demonstrated it's associated with oocyte degeneration rates 5% to 19%. The reasons for this degeneration are unclear. Observation of ZP of oocyte by scanning revealed no zona fragmentation during procedure and the injection hole closes immediately after the needle is withdrawn, with injection site being nearly undetectable 15 minutes later. (***Schwartz p et al, 1996).***

In fact, oocyte degeneration and abnormal fertilization constitute the principle reasons for the cancellation of assisted reproductive technique cycles. Additionally, some oocytes are very fragile and the zona pellucida (ZP) can be very resistant to penetration, resulting in sudden breakage of membrane during ICSI (***Liu J, 1995).***

To overcome this problem, several groups have developed laser assisted ICSI technique. Lasers have already been used as convenient and safe tools in assisted hatching and pre implantation genetic diagnosis (***Rienzi et al, 2001).***

Laser assisted ICSI (LA - ICSI) featuring micro opening or drilling of the ZP prior to ICSI, allows the insertion of the sperm injection needle with less distortion of the oocyte and may therefore be less traumatic (***Rienzi et al, 2001)*** which will reduce the degeneration rate of human oocytes and increase embryo development rates in patients who had experienced prior ICSI failure caused by poor oocytes survival. (***Demirol A, 2006).***

Several groups have reported similar results in selected patients with histories of poor ICSI outcomes for which only limited numbers of oocytes were available (***Abdlmassih S, 2002).*** In addition, when used to overcome fertilization problems, laser assisted ZP thinning prior to routine ICSI both improved oocytes survival and increase the hatching rate in vitro (***Moser M, 2004).***

First reported a pregnancy using laser-assisted ICSI in a couple with four previous conventional ICSI failures and poor oocyte survival **(*Rienzi et al, 2001*).** They noted minimal oocyte deformation with the use of laser-assisted ICSI, and survival of 8 of the 13 metaphase II oocytes retrieved and injected. Two small randomized studies of patients with a previous history of high rates of oocyte degeneration (>20%) following ICSI, or who produced oocytes with fragile oolemmas, yielded dramatic statistically significant reductions in oocyte degeneration rates (***Abdlmassih S, 2002)*** (***Rienzi et al, 2001*)**. 0.5% versus 16% and 2% versus 14%, respectively) and improved embryo quality with the use of laser-assisted ICSI compared to conventional ICSI **(Yanagida K et al 1999).**

The objective of this study was to evaluate the benefits of laser-assisted ICSI among patients undergoing in vitro fertilization with ICSI. We describe results of the randomized trial examining laser-assisted ICSI including comparisons of oocyte fertilization and degeneration rates and blastocyst formation rates and biochemical pregnancy rate.

Aim of the Work

This study carried out in infertile women who subjected to treat therir infertility by ICSI comparing laser assisted zona thinning prior to ICSI versus conventional ICSI with following outcome:

1ry outcome: successful chemical pregnancy by qualitative B-HCG test.

2nd outcome: successful fertilization, cleavage, blastocyst formation, decrease degeneration and fragmentation.

Research question: is laser zona thinning prior to ICSI will improve oocyte survival, fertilization, blastocyst formation, decrease degeneration of oocytes and increase pregnancy rate?

Research hypothesis:

Laser assisted ZP thinning prior to ICSI will improve oocytes survival, increase successful fertilization, cleavage and blastocyst formation and will increase pregnancy rate.

Null hyothesis :

Laser zona thinning prior to ICSI will not significantly improve pregnancy rate or decrease degeneration of oocytes or increase fertilization rate and blastocyst formation rate.

Intracytoplasmic Sperm Injection (ICSI)

History:

Interest in the initial types of micro-manipulation procedures, such as zona drilling and PZD, evolved because of the disappointing results of standard 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 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 and a problem 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 of 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 is thought to bypass 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).***