International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

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

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Abstract

To assess if laser zonal thinning prior to ICSI will improve oocyte survival, fertilization, blastocyst formation, decrease degeneration of oocytes and increase pregnancy rate or not. It was a randomized controlled trial conducted at Specialized Fertility Reproductive Science Centre (Dar El Maraa) and included 364 women who were subjected to treat their infertility by ICSI, over a 2-year period, between September 2013 and September 2015. Participants were randomly assigned following simple randomization procedures (computerized random numbers) into 2 treatment groups (conventional ICSI) control group and (laser ICSI) treatment group. Results of the trial were assisted including comparisons of oocyte fertilization and degeneration rates and blastocyst formation rates and biochemical pregnancy rate. 364 cycles were done and inseminated in accordance of the study design. The mean fertilization rate was 78.9% for C ICSI (conventional ICSI) and 75 % for LA ICSI (laser ICSI), the mean blastocyst formation rate 45.2% in C ICSI and 44.4 % for LA ICSI, the mean oocyte degeneration rate of 12.6% for C ICSI and 11.4 % for LA ICSI, mean embryo cell number on day 3 was 5.5 % for C ICSI and 5.7 % on day 3 for LA ICSI, mean degree of embryo fragmentation was 10.6% on day 3 for C ICSI and 9% on day 3 for LA ICSI. Mean pregnancy rate in C ICSI 95% and 97 % for LA ICSI with no significant difference were observed for any of the measured outcomes variables.

Keywords

Intracytoplasmic sperm injection – Laser

International Journal of Obstetrics and Gynaecology Research (IJOGR)
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http://www.ijogr.com/

I. 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 [1].

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 [2].

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 [3]. 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 [4].

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 [5].

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 [6]. 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[6]. This 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 [7]. Several groups have reported similar results in selected patients with histories of poor ICSI outcomes for which only limited numbers of oocytes were available [8]. 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 [9].

First reported was a pregnancy using laser-assisted ICSI in a couple with four previous conventional ICSI failures and poor oocyte survival [6]. They noted minimal

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

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 [6,8,10].

II. Patients and Methods

A randomized controlled trial was conducted at specialized fertility reproductive science centre (Dar El Maraa) comparing the efficacy of laser assisted zona thinning versus conventional mechanical method for intracytoplasmic sperm injection in ICSI program.

Time: From September 2013 to September 2015.

Study Population: The study included 364 women who subjected to treat their infertility by ICSI at fertility reproductive science centre (Dar El Maraa), according to the following **eligibility requirements**:

Inclusion Criteria:

1. Maximum basal FSH 10 mIU/ml.
2. autologous IVF-embryo transfer cycles.
3. No uterine abnormality or communicating hydrosalpinx.
4. Eligible diagnoses will include: tubal diseases (except communicating hydrosalpinx), endometriosis, male factor infertility, or unexplained infertility

Exclusion Criteria:

1. History of medical chronic disorder e.g., DM, SLE.
2. Patients who failed to achieve any ovulation, or cryopreserved all of their embryos at the pronuclear stage of development.
3. Elevated FSH level.

Ethical consideration:

Institutional review board (IRB) approval: The ethical scientific committee for approving the study discussed the protocol, and informed consent was obtained before participation.

Consent procedure: The Investigator made certain that an appropriate informed consent process was in place to ensure that potential research subjects, or their authorized representatives, were fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Investigator obtained the written,

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The Investigator retained the original signed informed consent form.

Subject Confidentiality: All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the site would not include unique personal data to maintain subject confidentiality.

Randomization: For allocation of the participants, a computer-generated list of random numbers was used and was kept in the infertility clinic computer and with research supervisors. Participants were randomly assigned following simple randomization procedures (computerized random numbers) to 2 treatment groups (C ICSI) control group and (LA ICSI) treatment group. Group assignments were allocated according to a computer-generated randomized series, were kept in sealed envelopes and were known to embryologist. Treatment assignments were coded on embryo culture dishes.

Intervention:

1) Controlled ovarian stimulation and oocyte retrieval:

- Down regulation in mid luteal phase of pretreatment cycle using Triptorelin acetate (Decapeptyl 0.5 mg SC, ferring Gmbh Germany)
- Ovarian stimulation with highly purified HMG IM injection 150 iu/day, menotropin, ferring, USA and the doses of the drugs was adjusted to age, body weight, and ovarian size by TV U/s and ovarian response.
- Human chorionic gonadotropin (choriomon 5000 iu IM injection, IBSA institute biochimique SA) was administrated when three or more follicles are at least 18 mm in largest diameter
- Transvaginal follicular aspiration was performed 24 - 36 hours later.
- The oocytes were classified according to maturity. the cumulus - corona complexes were removed by exposure to a hyaluronidase type 4 solution at concentration of 80 iu/ml
- The denuded oocytes were incubated in IVF -50 medium (scandianvian IVF science AB , Sweden)

2) Semen preparation: Before laser treatment the human sperms from the patient undergoing IVF were suspended in human tubal fluid medium containing 10% polyinylpyrolidine (PVP - ICSI-100, scandianvian IVF science AB, Sweden) diluted in IVF- 50 was used to immobilize the spermatozoa. The immobilized sperms were

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

aspirated in injection pipette for microinjection into the oocytes.

3) Laser assisted zona thinning and ICSI: For LA-ICSI, an oocyte was held with the polar body in the 12-o'clock position. The ZP was thinned by approximately 70% at the 3-o'clock position (where the injection needle is to penetrate) using a laser pulse 100-µ sec in duration (ZILOS-tk class I laser, Hamilton Thorne Research, Beverly, MA, USA). An ICSI needle was introduced into the oocyte through the pre-thinned region of the ZP so, oocytes were injected by injection pipette through this channel easily the overall process was termed LA-ICSI, and ICSI was performed. On the other hand the control group where conventional ICSI was done as described elsewhere and was termed the C-ICSI. In both C-ICSI and LA-ICSI the sperm injection is done carefully to limit the risk of oocyte damage, and oolemma reaction is not observed.

4) Outcome (Evaluation of oocyte integrity, fertilization, embryo development, and pregnancy)*:* Fertilization and degeneration of injected oocytes and number of cells /embryo and fragmentation rate were monitored 16-18 hours after ICSI and on day 3. Fertilized embryos were transferred to drops of fresh cleavage medium. At day 5, embryo quality was graded into five levels based on the percentage of fragmentation and the number and size of blastomeres. Two or three embryos selected for embryo transfer. To artificially prepare the endometrium, E2 valerate (4-6 mg/day; Progynova, Schering, Bayer, New Zealand) was administrated and progesterone (100 mg/day; Samil Pharm. Co., Ltd., Seoul, Korea) was injected when the endometrium attained 8 mm in thickness. Pregnancy was evaluated by measurement of the serum β-HCG level 14 days after embryo transfer and a chemical pregnancy were defined by positive serum B-HCG.

Outcome Measures:

Primary outcome: Successful biochemical pregnancy, defined as positive serum pregnancy test performed 14 days after embryo transfer.

Secondary outcomes: Successful fertilization, cleavage and blastocyst formation and degeneration rate and fragmentation rate and quality of embryo.

Sample Size Justification:

The required sample size has been estimated using the Power Analysis and Sample Size software (PASS©) version 11 (NCSS, LLC. Kaysville, Utah, USA).

The primary outcome measure is the proportion of patients becoming pregnant as diagnosed biochemical. A Data from previous study stated that ZP thickness is important in analysis of the results of IVF programs, since they observed that when sperm is normal the thickness of the ZP of fertilized oocytes (16.6 + 3.2) is significant

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

lower than that of the ZP of oocyte that were not fertilized (18.9 + 4.0) [11].

It has been estimated that a sample size of 182 patients in each study group would achieve an 80% power (β-error = 0.2) to detect an effect size (w) of 0.35. The test statistic used has been the two-sided Pearson χ^2-test with 2 degrees of freedom and significance has been targeted at the 95% confidence level (α-error = 0.05).

III. Results

A randomized controlled trial was conducted at specialized fertility reproductive science centre (Dar El Maraa) 346 women were included in this study following informed written consent comparing the efficacy of laser assisted zona thinning versus conventional mechanical method for ICSI from September 2013 to September 2015.

Patients were randomly divided into two groups 182 in each group:

1) Conventional ICSI as control group.
2) LA ICSI (laser assisted ICSI) as treatment group.

Patient age ranged from 19 to 40 years (mean = 31.2 ± 5.6 SD) for conventional ICSI (mean = 30.3 ± 4.8 SD). The BMI ranged from 22 to 43.The mean BMI was 28.8±4.3, the mean duration of infertility was7.8±4.4 years. The majority of couples were diagnosed with mixed factor for infertility (35.1 %, 30.2%). Other diagnoses included endometriosis, tubal factor, PCO, ovulation disorder and unexplained infertility. 364 cycles were done and inseminated in accordance of the study design. The mean fertilization rate was 78.9% for conventional ICSI and 75 % for LA ICSI, the mean blastocyst formation rate 45.2% in C ICSI and 44.4 % for LA ICSI, the mean oocyte degeneration rate of 12.6% for C ICSI and 11.4 % for LA ICSI, mean embryo cell number on day 3 was 5.5 % for C ICSI and 5.7 % on day 3 for LA ICSI, mean degree of embryo fragmentation was 10.6% on day 3 for C ICSI and 9% on day 3 for LA ICSI. Mean pregnancy rate in C ICSI 95% and 97 % for LA ICSI with no significant difference were observed for any of the measured outcomes variables.

The results of multivariable binary logistic regression analysis for the relation between the fertilization technique and occurrence of pregnancy as adjusted for the patient's age. After adjustment for the effect of age, there was no statistically significant relation between the fertilization technique and occurrence of pregnancy (odds ratio, 1.03; 95% CI, 0.68 to 1.56; p-value, 0.883).

Table 1-2, show the mean levels of FSH, LH, TSH hormones were (6.4±1.8,7.5±2.8,3.1±0.4) mIU/ml respectively, the mean level of estradiol hormone was49.9±25.1 pg/ml. The antral follicular count (AFC) ranged from 3 to 20 follicle, the mean number was 9.9±3.4 follicles. The duration of ovarian stimulation ranged from 8 to 21 days, the mean duration was 12.1±2 days. The mean dose of HMG was

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

49.5±13.4 ampoules, the mean time to oocyte retrieval was14.1±2days.

Table (3) shows no statistical significance between 2 groups as regard Number of retrieved, injected, fertilized, and cleaved oocytes, and number of produced blastocysts. The fertilization rate of the C ICSI group was higher than that of LA ICSI group, 78.9% versus 75.0% respectively (P = 0.126) and the cleavage rate was higher in the C ICSI group than in the LA ICSI group, 66.7% versus 63.6% respectively (*P*<0.473*)*, the blastocyst formation rate was higher in C ICSI than that of LA ICSI 45.2 versus 44.4 respectively (*P*<0.983*)*, the degeneration rate on day 3 was 12.6% for C ICSI while slightly lower in LA ICSI 11.4%. Fragmentation on day 3 10.6% in C ICSI and 9% in LA ICSI, the difference was not statistically significant. Biochemical pregnancy was slightly higher in LA ICSI than C ICSI but difference was statistical insignificant 52.2 % for C ICSI and 53.3 % for LA ICSI.

Table 7 shows the results of multivariable binary logistic regression analysis for the relation between the fertilization technique and occurrence of pregnancy as adjusted for the patient's age.

After adjustment for the effect of age, there was no statistically significant relation between the fertilization technique and occurrence of pregnancy (odds ratio, 1.03; 95% CI, 0.68 to 1.56; p-value, 0.883).

IV. Discussion

Laser technology seems to be a promising tool that has been introduced in assisted reproduction treatment in recent years. Lasers have been applied in assisted reproduction with the purpose of manipulation of the ZP of embryos to perform assisted hatching [12-14]**.** Also be used for preimplantation genetic diagnosis and microdissection of the ZP with a laser system simplifies subsequent polar body biopsy or removal of blastomeres because of the advantage of using a single needle for the procedure [15-17].

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 [6]. This 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 [7]**.** Several groups have reported similar results in selected patients with histories of poor ICSI outcomes for which only limited numbers of oocytes were available [8]**.**

First reported was a pregnancy using laser-assisted ICSI in a couple with four previous conventional ICSI failures and poor oocyte survival [6]**.** 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 [6,8]**.** Despite being well designed, evaluation of the results of these two previous randomized studies is problematic because both used individual oocytes rather than patients as the unit of analysis. These 'unit of analysis' errors are common - a recent systematic review identified 'unit of analysis' errors in 82% of clinical trials examined. The use of multiple observations per patient results in unpredictable bias in treatment effects estimates, and an exaggerated apparent sample size leading to spuriously low p-values [18].

We found no evidence that pre-drilling through the zona pellucida prior to insertion of the ICSI needle improves oocyte survival or embryo quality, contrary to the conclusions of two smaller prior studies [8]**.** Rates of oocyte degeneration and fertilization, cell numbers and fragmentation rates and blastocyst formation were similar regardless of ICSI treatment. Treatment effect on oocyte degeneration rates suggests that even if degeneration rates are lower with laser-assisted compared to conventional ICSI, the reduction is unlikely to be statistically significant as suggested by the earlier studies.

Patient selection may have played a role in the difference between our results and those of the earlier reports. The earlier reports included patients selected based on a history of ICSI with high rates of oocyte degeneration [8] or fragile oolemmas ***(Rienzi L, 2004)*****,** while ours was based on unselected patients undergoing ICSI. It is therefore possible that a subset of patients may benefit from laser micromanipulation, even if there are no significant benefits to the general patient population.

Same result in a study [19], Oocytes retrieved from 59 patients scheduled for ICSI were randomly divided into equal treatment and control groups. Outcome variables (oocyte fertilization and degeneration, embryo cell numbers and fragmentation on days 2 and 3, and compaction and blastocyst formation rates) were compared between treatment and control groups by paired-sample t-test. Subgroup analysis was performed according to zona pellucida and oolemma breakage patterns.

No significant differences between treatment and control groups were observed for any of the measured outcome variables. However, fragile zonae pellucidae were associated with significantly poorer embryo quality, and fragile oolemmas that broke easily upon insertion of the injection needle were associated with a significantly higher oocyte degeneration rate. Nevertheless, there were also no between-treatment differences in clinical outcomes within these patient subpopulations.

In a year 2000 a prospective randomized trial [20] included 103 patients less than 37 yearrs with no previous implantation failure-comparing zona thinning by laser versus conventional ICSI stated that zona thinning by non-contact diode laser has no benfit to that population.

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

We do not believe that operator experience was a factor in our inability to distinguish a beneficial effect of laser micromanipulation. The lack of any detectable learning curve supports this contention. The embryologist performing ICSI in this study were highly skilled and had years of experience performing ICSI and working successfully with the laser for embryo manipulation procedures such as assisted hatching and embryo biopsy. Embryologist performing the procedure felt that it was relatively simple and could be performed quickly, although even with practice the entire process of insemination was still more time consuming with the addition of the laser micromanipulation. They also agreed that the compression of the oocytes during the ICSI procedure was dramatically reduced in the laser-assisted group, as previously reported [6].

As we found no clinical benefit to drilling through the zona with a laser before sperm injection, we also found no evidence of harm as a result of the laser micromanipulation of oocytes. Oocytes inseminated by laser-assisted ICSI were as likely to be fertilized successfully and had similar preimplantation development as oocytes inseminated by conventional ICSI. This study therefore provides evidence for the safety of laser micromanipulation of human oocytes via the comparable preimplantation development of embryos derived from both the study and control groups.

The question of safety is always an important point when introducing a new technique. The technical parameters of the laser system that we used are not associated with harmful effects (because it is a laser that emits waves that are not absorbed by nucleotides). However, the laser beam does generate a heat effect that can indeed damage cells depending on the energy level/pulse duration and the distance between the center of the laser beam and the cell membrane. The energy level/pulse duration used in our study was very low (5-8 times lower than that used for assisted hatching especially because a maximum distance was maintained in the perivitelline space).

However, to ensure the maximum safety at all times the greatest care was exercised during the procedure. For instance, great attention was always paid so that the distance between the perivitelline space and oolemma was the maximum at the point of laser drilling. In addition, the innermost layer of the ZP (~0.5 μm) was kept intact and very short pulse duration (_2 ms) was applied, which prevented any visible sign of oolemma reaction even at the last laser shot when performing LA-ICSI.

The high precision of the laser system enabled us to create a channel on the ZP with a very narrow opening just wide enough to permit the passage of the ICSI needle [21], especially if it is not large enough to permit evacuation of the embryos from the ZP. A small hole may strangulate the embryo and may be a phenomenon probably associated with increased incidence of monozygotic twinning [22-23].

Because of this fact, we made an effort to drill the smallest possible hole that permitted the passage of the ICSI needle. Characteristics of

the zona pellucida, or possibly the oolemma, may indicate patients more likely to benefit from laser-assisted ICSI. It is well known that breakage patterns of the zona and oolemma can differ greatly between oocytes. Oocyte compression during the ICSI procedure is greatest in those eggs with zonae or oolemmas that are particularly difficult to penetrate. Laser drilling through a difficult zona pellucida eliminates the dramatic compression of the oocyte that would otherwise result from ICSI of such eggs. Fragile oolemmas have been reported to be associated with higher rates of oocyte degeneration [24].

However, it is more difficult to imagine a benefit of laser-assisted ICSI for a fragile oolemma. So we suggest to do further study to assest the benefit of laser assisted ICSI according to breakage pattern of zona and oolemma.

This study suggests that the routine use of laser assisted zona thinning prior to ICSI is of no benfit to general population contrary to previous reports.

We suggest to do further study to assets the benefit of laser assisted ICSI in subgroup of patients according to breakage pattern of zona and oolemma in order to define a selective group, which may benefit better from the procedure.

V. Conclusion

It was found no evidence that pre-drilling through the Zona pellucida prior to insertion of the ICSI needle improves oocyte survival or embryo quality. Rates of oocyte degeneration and fertilization, cell numbers and fragmentation rates and blastocyst formation were similar regardless of ICSI treatment.

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

VI. References

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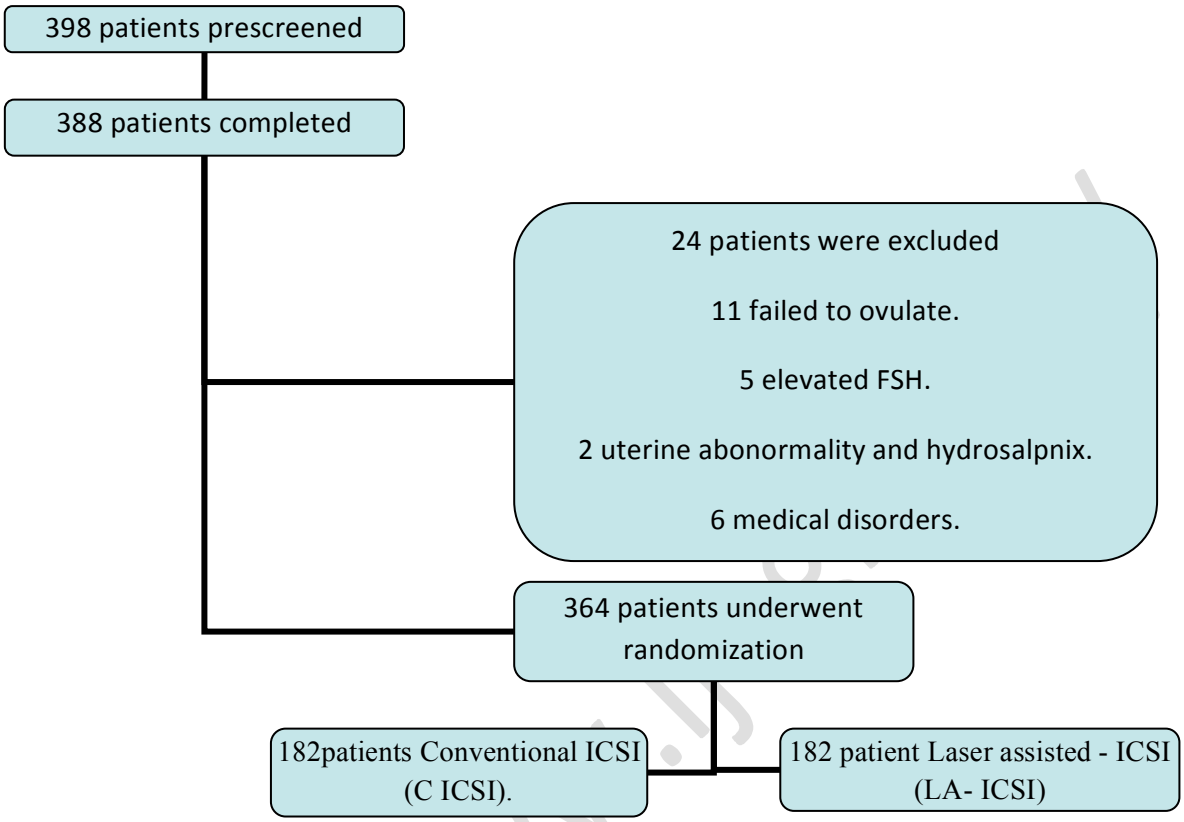
International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/



International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

Table (1): Mean age in the two study groups

Variable	C - ICSI (n=182)	LA- ICSI (n=182)	p-value
Age (years)	31.2 ± 5.6	30.3 ± 4.8	0.100
Age category:			
<35 yr	138 (75.8%)	147 (80.7%)	0.592
≥35 yr	44 (24.1%)	35 (19.2%)	
Indication for IVF			0.514
Mixed	64 (35.1%)	55 (30.2%)	
Tubal factor	53 (29.1%)	43 (23.6%)	
Male factor	27 (14.8%)	38 (20.8%)	
Unexplained infertility	36 (19.7%)	44 (24.1%)	
Sex selection	2 (0.01%)	2 (0.01%)	

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

Table (2): Descriptive statistics for the whole study population: as regard hormonal profile & ovarian stimulation

Variable	N	Min.	Max.	Mean	SD	Median	IQR
FSH level, mIU/ml	85	1.4	10	6.4	1.8	6.1	5 - 8
LH level, mIU/ml	85	2.1	14	7.5	2.8	7.3	5.7 - 9.5
Prolactin level, ng/ml	85	2.7	35	15.1	6.2	14.7	11 - 18
TSH level, mIU/l	85	2.1	4.2	3.1	0.4	3.1	2.9 - 3.4
E2 level, pg/ml	85	2.7	107	49.9	25.1	45	33.5 - 64.4
Antral follicle count (AFC)	85	3	20	9.9	3.4	10	7.5 - 12
Duration of ovarian stimulation , days	85	8	21	12.1	2	12	11-13
HMG dose, ampoules	85	24	78	49.5	13.4	50	38-58
Time to oocyte retrieval, days	85	10	23	14.1	2	14	13-15

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

Table (3): Number of retrieved, injected, fertilized, and cleaved oocytes, and number of produced blastocyts & outcome measures in the two groups.

Variable	C-ICSI (n=182)	LA-ICSI (n=182)	p-value
Number of retrieved oocytes	12(9 to 15)	15 (12 to 18)	0.232
Number of injected oocytes	9 (7 to 11)	11 (9 to 13)	0.118
Number of fertilized oocytes	7 (5 to 9)	8 (6 to 10)	0.335
Number of cleaved oocytes	6 (4 to 8)	7 (6 to 9)	0.219
Number of blastocycts	4 (2 to 6)	5 (3 to 6)	0.225
Fertilization rate (%)	78.9 (66.7 - 85.7)	75.0 (66.7 - 100)	0.126
Cleavage rate (%)	66.7 (50.0 to 75.0)	63.6 (58.3 to 71.4)	0.473
Blastocyst formation rate (%)	45.2 (25.0 to 60.0)	44.4 (35.7 to 50.0)	0.983
Biochemical pregnancy	95 (52.2%)	97 (53.3%)	0.916§
Degeneration rate (%)	48 (12.6)	43 (11.4)	0.63
Cell number on day 3	5.5	5.7	0.38
Fragmentation on day 3	10.6	9	0.16

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

Table (4): Comparison of the outcome measures associated with laser-assisted ICSI or conventional ICSI in patients aged <35 or ≥35 years

	Variable	Conventional ICSI (n=126)	Laser-assisted ICSI (n=147)	p-value¶
<35 yars	Fertilization rate (%)	75 (66.7 to 85.7)	73.3 (66.7 to 80)	0.**002**
	Cleavage rate (%)	66.7 (50 to 75)	63.2 (58.3 to70.4)	0.674
	Blastocyst formation rate (%)	44.9 (25 to 66.7)	45.5 (35.7 to 50.0)	0.923
	Variable	**Conventional ICSI (n=56)**	**Laser-assisted ICSI (n=35)**	**p-value¶**
≥35 years	Fertilization rate (%)	80 (66.7 to 86.6)	72.7 (65.2 to 80)	**0.024**
	Cleavage rate (%)	66.7 (56.3 to 80)	66.7 (60 to 73.2)	0.803
	Blastocyst formation rate (%)	47.5 (17.7 to 60)	42.9 (34.1 to 50)	0.778

Data are presented as median (interquartile range).

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

Table (5): Comparison of the biochemical pregnancy rate associated with laser-assisted ICSI or conventional ICSI in patients aged <35 or ≥35 years

Age category	Biochemical pregnancy	Conventional ICSI (n=182)	Laser-assisted ICSI (n=182)	Total	p-value¶
<35 years	Negative	57 (45.2%)	70 (47.60%)	127	0.716
	Positive	69 (54.8%)	77 (52.40%)	146	
	Total	126	147	273	
≥35 years	Negative	30 (53.6%)	15 (42.9%)	45	0.391
	Positive	26 (46.40%)	20 (57.10%)	46	
	Total	56	35	91	

Data are presented as number (%).
Fisher's exact test.

Table (6): Odds ratio, relative risk, and number needed to treat for the occurrence of positive biochemical pregnancy with the laser-assisted ICSI group referenced to the conventional ICSI group

Index	Estimate	95% CI	p-value
Odds ratio (OR)	1.05	0.69 to 1.58	0.834
Relative risk (RR)	1.02	0.84 to 1.24	0.834
Number needed to treat (NNT)	91	8.8 (NNH) to 10.9 (NNT)	

NNH, number needed to harm; 95% CI, 95% confidence interval.
z-test.

International Journal of Obstetrics and Gynaecology Research (IJOGR)
Vol. 3 (2016) No.2, pp. 221-239
http://www.ijogr.com/

Table (7): Multivariable binary logistic regression analysis for the relation between the fertilization technique and occurrence of pregnancy as adjusted for the patient's age

Variable	B	SE	Wald	p-value	Odds ratio	95% CI for odds ratio
Fertilization technique (LA ICSI=1, C-ICSI=0)	0.03	0.21	0.02	0.883	1.03	0.68 to 1.56
Age (>35 years=1, <35 years=0)	-0.11	0.24	0.21	0.644	0.89	0.55 to 1.44
Constant	0.12					

LA-ICSI, laser-assisted ICSI; C-ICSI, conventional ICSI.

B, regression coefficient; SE, standard error; Wald, Wald statistic.