Predictors of intracytoplasmic sperm injection (ICSI) outcome in couples with and without male factor infertility

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Summary

Aim: To find out the predictors of ICSI outcome.

Methods: Forty-three pregnancies in 100 consecutive ICSI cycles.

Results: Every 1,000 pg/ml increase in hCG-day E2 (OR = 0.46, CI: 0.25-0.83, p = 0.01) and 1% decrease in the rate of normal sperm morphology (OR = 0.81, CI: 0.67-0.98, p = 0.03) caused a significant decrease in clinical pregnancy rate and live birth rate (respectively, OR = 0.5, CI: 0.32-0.96, p = 0.03, OR = 0.66, CI: 0.5-0.86, p = 0.002) while every increase in the number of good quality embryos transferred caused a two-time increase in the clinical pregnancy rate (OR = 2.1, CI: 1.2-4, p = 0.01). On the other hand, every increase in the number of four-cell cleavage embryos (OR = 1.02, CI: 1.002-1.04, p = 0.03) and hCG-day endometrial thickness (OR = 1.6, CI: 1.15-2.24, p = 0.005) were found to increase the live birth rate. Implantation rate (m = 8.3 ± 14.6) was significantly lower in cases with leucocytospermia (n = 33) compared to cases without leucocytospermia (n = 67, m = 17.4 ± 24.6, p = 0.02).

Conclusion: Leucocytospermia, hCG-day E2 level and endometrial thickness, normal sperm morphology, and number of good quality embryos are predictors of implantation, clinical pregnancy and live birth rate following ICSI.

Key words: ICSI; Outcome; Infertility; Semen parameters; Predictors.

Introduction

Today, intracytoplasmic sperm injection (ICSI) is used with high success rates in male factor infertility. Due to the high fertilization and implantation rates reported after ICSI [1] the procedure has also been used in couples with unexplained infertility, borderline semen quality, immunologic infertility, previous failure of fertilization in conventional in vitro fertilization (IVF) and tubal factor infertility.

However, ICSI costs an extra $600 per fresh cycle completed [2] and if ICSI would have been advocated for all patients requiring IVF $60,000 would be needed to gain one additional live birth [3]. Ovarian response and uterine receptivity are also important determinants of success in assisted reproductive technology, as a significant association between the number of aspirated oocytes [4], the proportion of fertilized oocytes [5], the number and quality of embryos, semen quality [6] and clinical pregnancy were demonstrated.

The aim of this study was to analyze the predictors of intracytoplasmic sperm injection outcome in couples with and without male factor infertility.

Materials and Method

A total of 100 consecutively performed ICSI cycles (one cycle/couple) performed between 1/1/2001 and 1/1/2003 were included in the study. All women had regular menstrual cycles every 22-35 days. All the patients had cycle day 3 serum FSH, LH, E2, prolactin and TSH levels within normal limits. The presence of a normal uterine cavity was confirmed at hysterosalpingography and/or hysteroscopy performed ≤ 1 year before ICSI. Male patients that need testicular sperm extraction were excluded from the study. Eighteen couples with tubal factor infertility plus normal semen and 82 couples with varying degrees of oligoasthenoteratozoospermia as the sole cause of infertility were eligible for analysis.

Semen analysis and sperm processing

Semen samples were obtained after three to four days of sexual abstinence. Samples were allowed to liquefy for at least 20 min at 37°C before analysis. Sperm concentration and motility, and oocyte number were evaluated according to the recommendations of the World Health Organization [7] and sperm morphology was evaluated by strict criteria [8].

Ovarian stimulation, oocyte retrieval and handling

Ovarian stimulation was carried out with long protocol GnRHa (Leuprolide, Lucrin®, Abbott, Turkey) plus HMG (Pergonal®, Serono, Turkey) combinations. Follicular development was then stimulated with an injection of FSH ± HMG and the dose of gonadotropin hormone was individualized according to the patients' age and previous stimulation history or response to stimulation. Ovulation was induced by injection of 10,000 IU hCG (Pregnyl®, Organon, Turkey) when at least two follicles reached a diameter > 18 mm. Oocytes were retrieved 36 hours after hCG administration by the guidance of transvaginal sonography (Logic-α200, General Electric, USA). Two hours after ovum pick-up hyaluronidase 80 IU/ml (Hyase®, Vitrolife, Sweden) was added to gamete-20 medium to denude the cumulus cells surrounding the oocytes under phase contrast dissection microscope (Nikon Smz 800, Japan) for 10-15 sec. Then

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the oocytes were rinsed with gamete-20 and incubated in bicarbonate buffered medium (G1.2®, Vitrolife, Sweden).

ICSI procedure

The microinjection procedure was performed on a heated stage of an inverted microscope and micromanupilator (Nikon®, Eclipse TE300, Japan). The injecting micropipettes (Hunter scientific pipette, 2005-6, UK) and the holding pipettes (Hunter scientific pipette, 2005-2, UK) were ready made. The oocyte was held with a holding pipette with the polar body at the 6 or 12 o'clock position. The injecting pipette containing the spermatozoa was introduced at the 3 o'clock position through the zona pellucida, oolemma and deeply injected into the cytoplasm. A small part of the cytoplasm was aspirated and the spermatozoa was injected with the cytoplasm. After injection, oocytes were incubated in IVF medium (G1 medium, Vitrolife, Sweden). Fertilization was assessed 15-18 hours after microinjection. Oocytes were classed as fertilized if two pronuclei (2PN) were present and the second polar body had been extruded.

Embryo quality grading, transfer and luteal support

The morphological condition (grading) of cleaving embryos was assessed immediately before transfer on day 2 or 3. The grading was carried out according to the criteria by Veeck [9]: Grade 1: the best morphological condition, embryo with blastomeres of equal size; no cytoplasmic fragments. Grade 2: embryo with blastomeres of equal size; minor cytoplasmic fragments or blebs. Grade 3: embryo with blastomeres of distinctly unequal size; few cytoplasmic fragments or none. Grade 4: embryo with blastomeres of equal or unequal size; significant cytoplasmic fragmentation. Grade 5: embryo with few blastomeres of any size, severe or complete fragmentation. The cleaving status represented the number of blastomeres in the embryos judged immediately before transfer. Cleaved embryos were transferred using the Wallace catheter (cat. no: 1816N, H.G. Wallace limited, UK). Luteal phase support was provided by 8% progesterone vaginal gel (Crinone®, Serono, Turkey) for 14 days, beginning two days after oocyte retrieval. Serum hCG levels were detected 12 days or later after embryo transfer.

Definitions and statistical analysis

The measures evaluated were fertilization rate (number of embryos/number of good quality oocytes), implantation rate (fetal heart beat/embryo replaced), percentage of 4-cell embryo cleavage (number of 4-cell embryos/all embryos), clinical pregnancy rate (fetal/embryo heart beat detected via ultrasound) and live birth rate. All statistical analyses were performed via SPSS version 10.0 (SPSS Inc, Chicago, IL). For the initial statistical evaluation the chi-square and independent samples t-test were used. Forward stepwise logistic regression analysis was performed to determine the predictors of clinical pregnancy and live birth. The association between the continuous data was analyzed using correlation analysis and coefficient of determination (r^2); $p < 0.05$ was considered statistically significant.

Results

The female age of the 100 couples included in the study ranged between 20 to 42 years and male age ranged between 20 to 50 years. The demographic variables of the study group according to the presence of male factor infertility are presented in Table 1. Eighteen patients without male factor infertility were found to have a significantly higher mean male and female age when compared with 82 patients with male factor infertility. Tubal factor infertility was also present in 23% of the couples with male factor infertility. The mean infertility period, male and female tobacco use, nulligravida, previous attempts of ovulation induction, intrauterine insemination and IVF were comparable in cases with or without male factor infertility.

Table 1. — *Demographic variables of the study group.*

Variable	Male factor infertility		p
	Present (n = 82)	Absent (n = 18)	
Male age (years)	35 ± 5.3	39 ± 4.5	0.01*
Female age (years)	32.6 ± 4.8	35.8 ± 4.4	0.004*
Infertility period (months)	89.5 ± 52.1	115.2 ± 49.1	0.06
Female tobacco use (%)	2 (2.4)	–	0.5
Male tobacco use (%)	9 (11)	3 (16.7)	0.5
Nulligravida (%)	70 (85.4)	12 (66.7)	0.06
Tubal factor (%)	19 (23.8)	18 (100)	< 0.001*
Previous ovulation induction (%)	40 (48.8)	12 (66.7)	0.1
Previous intrauterine insemination (%)	37 (45.1)	12 (66.7)	0.09
Previous in vitro fertilization failure (%)	7 (8.5)	2 (11.1)	0.7

*Statistically significant ($p < 0.05$, Students' t-test).

A summary of the semen indices of the study group is presented in Table 2. Moderate and severe oligospermia was found in 26% of the cases, asthenospermia in 26% of the cases, teratospermia in 76% of the cases and leucocytospermia in 33% of the cases. The mean endometrial thickness on the day of hCG administration was 10 ± 1.6 mm (6.9-14) while the mean estradiol level was 2,113 ± 1,232 pg/mg (215-7000).

Table 2. — *Summary of the semen indices.*

Variable	
Sperm volume (ml)	3.2 ± 1.4
Sperm number (10^6)	52.5 ± 41.8
Total motile sperm (10^6)	22.9 ± 21.3
Total progressive motile sperm (10^6)	22.4 ± 22
Leucocyte count (10^6/ml)	1.15 ± 0.65
Oligospermia	
Moderate (5-20 x 10^6) (%)	15 (15)
Severe (< 5 x 10^6) (%)	11 (11)
Asthenospermia (WHO a+b < 50%)	26 (26)
Teratospermia (%) (≤ 4% normal morphology)	76 (76)
Leucocytospermia (> 1 x 10^6/ml) (%)	33 (33)

The comparison of ICSI outcome according to male factor infertility is presented in Table 3. The mean estradiol level on the day of hCG, leucocyte count in sperm and number of oocytes retrieved and injected were similar in the two groups. The fertilization rate of the patients with male factor infertility was significantly lower than in cases without male factor infertility (respectively 39.9 ± 28.5 and 59.7 ± 30.7, $p = 0.01$). Despite this difference in fertilization rate, percent of 4-cell embryo cleavage, number of embryos transferred and implantation rate were similar in the patients with or

Table 3. — *Comparison of ICSI outcome according to male factor infertility.*

Variable	Male factor infertility		p
	Present (n = 82)	Absent (n = 18)	
E_2 on hCG day (pg/ml)	2,145 ± 1,228	1,968 ± 1,273	0.5
Leukocyte count (10^3/ml)	1.1 ± 0.6	1.1 ± 0.4	0.9
No. of oocytes retrieved	10.5 ± 5.1	8.1 ± 5.2	0.09
No. of oocytes injected	8.4 ± 4.9	6.4 ± 4.2	0.1
Fertilization rate (%)	39.9 ± 28.5	59.7 ± 30.7	0.01*
% 4-cell embryo cleavage	77.4 ± 32.4	84.4 ± 21.1	0.3
No. of embryo transferred	2.6 ± 0.7	2.5 ± 0.7	0.5
Implantation rate	15.6 ± 23.4	9.2 ± 15.3	0.2
Biochemical pregnancies	6 (7.3)	–	0.2
Miscarriages	7 (8.5)	2 (11.1)	0.3
Deliveries	26 (31.7)	2 (11.1)	0.07

*Statistically significant ($p < 0.05$, Students' t-test).

without male factor infertility. A total of 43 pregnancies occurred out of 100 cycles. Biochemical pregnancy occurred in six cases and miscarriage occurred in nine cases. Twenty-eight patients (28%) delivered live fetuses.

Logistic regression analysis of the predictors of clinical pregnancy revealed that every 1,000 pg/ml increase in hCG-day E2 level caused a 54% decrease in clinical pregnancy rate (OR = 0.46, CI: 0.25-0.83, p = 0.01), 1% decrease in the rate of normal morphology caused a 19% decrease in clinical pregnancy rate (OR = 0.81, CI: 0.67-0.98, p = 0.03) and every increase in the number of good quality embryo transferred caused a two-time increase in the clinical pregnancy rate (OR = 2.1, CI: 1.2-4, p = 0.01). None of the motility parameters were found to affect clinical pregnancy.

When predictors of live birth were analyzed via logistic regression analysis every 1,000 pg/ml increase in hCG day E_2 level caused a 44% decrease (OR = 0.5, CI: 0.32-0.96, p = 0.03), 1% decrease in the rate of normal morphology caused a 34% decrease (OR = 0.66, CI: 0.5-0.86, p = 0.002) in live birth rate. On the other hand, every increase in the number of four-cell cleavage embryos (OR = 1.02, CI: 1.002-1.04), p = 0.03), hCG-day endometrial thickness (OR = 1.6, CI: 1.15-2.24, p = 0.005) were found to increase the live birth rate. Surprisingly, male tobacco use was found to be an independent factor to increase the live birth rate (OR = 5.6, CI: 1.1-27.9, p = 0.03).

Linear regression analysis of the effect of hCG day E_2 level on fertilization rate revealed a negative linear regression equation; fertilization rate = 60.83 + (–0.01 x hCG-day E2), R-square = 0.11. The equation is shown in Figure 1.

The leucocyte count was not significantly different in patients with > 50% fertilization rate (n = 55, m = 1.09 ± 0.4 x 10^6/ml) compared with poor fertilization cases (n = 45, m = 1.2 ± 0.8 x 10^6/ml, p = 0.3). The mean leucocyte number was similar in cases with live births (n = 28, m = 1.18 ± 1 x 10^6/ml) and without live births (n = 72, m = 1.13 ± 0.4 x 10^6/ml, p = 0.7). On the other hand, implantation rate (m = 8.3 x 14.6) was significantly lower in cases with leucocytospermia (> 1 x 10^6/ml leucocyte, n = 33) compared to cases without leucocytospermia (n = 67, m = 17.4 ± 24.6, p = 0.02).

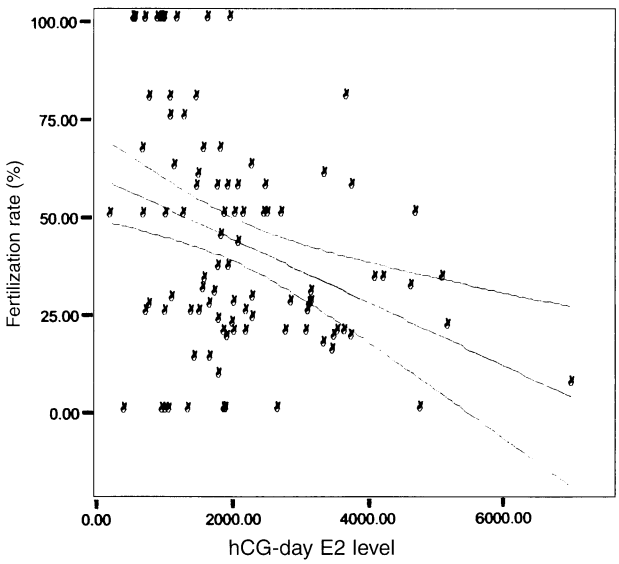

Figure 1. — Linear regression equation of fertilization rate = 60.83 + (-0.01 x hCG-day E2). R-square = 0.11, with 95% mean prediction interval.

Discussion

Despite considerable debate against its use for all cases of in-vitro conception, the use of ICSI is rising throughout the world [3]. Until today, most of the theoretical safety issues and concerns related to the ICSI technique remained unproven [9]. Besides being more expensive, the ICSI procedure itself has been proposed to have a detrimental effect on blastocyst development and quality, possibly due to physical disruption of nuclear or cytoplasmic components [10]. A more evidence-based explanation is the transmission of paternal genetic problems. Sperm from male infertility cases was shown to have aneuploidy, microdeletions of the Y chromosome, abnormal chromatic structure and DNA strand breaks [11, 12].

Several studies compared IVF and ICSI outcome in non-male factor causes. ICSI was found to have a similar implantation rate compared to IVF in tubal factor infertility and unexplained infertility in the presence of normal semen parameters [13, 14]. Whenever the cases with male factor infertility were studied, ICSI was found to have a superior fertilization rate than standard IVF, but similar to high insemination concentration IVF [15, 16]. On the other hand, Kihaile *et al.* found a similar development from ≥ 6 cells up to blastocyst stage in sibling oocytes from severe teratozoospermic patients with IVF and ICSI, although higher initial fertilization rates were obtained with ICSI [17].

We performed a logistic regression analysis of the many factors that might have an impact on ICSI outcome. We also included cases with non-male factor infertility to perform an analysis of the effect of semen indices on fertilization, implantation, clinical pregnancy and live birth rate. The group with normal semen parameters had a higher fertilization rate but similar four-cell embryo cleavage, implantation and delivery rate, although they

had known poor prognostic variables like advanced female age and longer infertility period.

When analyzed all together, the cleavage status, sperm morphology and the number of good quality embryos transferred were found to affect the clinical pregnancy rate and live birth rate after ICSI, parallel with previous published data [18, 19]. Several studies argued that outcome of ICSI is not related to strict morphology of the sperm used for microinjection [20, 21]. This proposal seems logical at a glance as "most normal looking" spermatozoa observed under the inverted microscope are selected and introduced into the oocyte thus bypassing the two barriers; the zona pellucida and oolemma. Besides the above-mentioned genetic problems, the ability of genetically damaged spermatoza to achieve normal fertilization after ICSI has been documented [22]. However, the fate of the zygote after several divisions and its ability to implant is less well understood.

In their study, de Vos *et al.* [19] found that normal looking sperm cells can be injected in 82% of completely teratozoospermic samples but a high biochemical pregnancy rate was observed. Our finding that sperm morphology is an important factor for ICSI outcome indicate that to produce more morphologically normal looking sperm may interfere with embryo development and implantation. This proposal is further supported by the findings that speculated possible paternal genetic and epigenetic problems may affect embryo cleavage speed, morphology, blastocyst formation and implantation rate [23, 24].

Other factors in the seminal fluid may also be a part of the paternal effect on ICSI outcome. We found that implantation rate is lower in men with leucocytospermia. There is growing evidence that reactive oxygen species (ROS) concentration in the seminal plasma may mediate this effect. The ROS production in sperm preparation is mediated mainly by leucocytes but spermatozoa may also produce a small fraction of ROS in semen [25]. The ROS production was found not to effect fertility in men with normal concentration and motility of spermatozoa [26]. On the other hand, ROS may further hamper the unstable DNA configuration in spermatozoa of oligoasthenoteratozoospermic samples. Not suprisingly, in our multivariate analysis sperm morphology was found to be a more important predictor of clinical pregnancy whereas leucocytospermia remained outside the model.

The ability to implant also depends on the hormonal milieu and endometrial thickness. High serum estradiol concentrations on the day of human chorionic gonadotropin administration has been a source of controversy. High serum estradiol concentrations were found not to affect oocyte and embryo quality in oocyte donation cycles [27], neither do they impair implantation and pregnancy rates in the subsequent frozen thawed embryo transfer cycles [28]. We found that, increasing hCG-day E2 level caused a significantly lower clinical pregnancy and live birth rate while thicker endometrium increased these outcomes. Few studies have found a favorable outcome with high serum E2 concentration on the day of hCG administration [29] or no relationship at all [30].

Endometrial thickness is a reflection of endometrial proliferation due to estradiol stimulation. Higher pregnancy rates were achieved when endometrium was at least 10 mm thick [31]. On the other hand, lower implantation and pregnancy rates were reported with an endometrial thickness ≥ 14 mm [32]. The abnormal estradiol-progesterone ratios around the time of implantation in high responders may be the explanation for these findings [33]. Also, supraphysiological concentrations of steroid hormones cause dyssynchrony between endometrial glands and stroma [34], impaired development of the endometrial glands [35], and advanced stromal development [36]. These data suggest that the essential estradiol concentration is overshot in some stimulated patients and this may have an adverse effect on uterine receptivity.

In conclusion, these results indicate that leucocytospermia, hCG-day E2 level and endometrial thickness, normal sperm morphology, and number of good quality of embryo are factors affecting implantation, clinical pregnancy and live birth rate following ICSI. Also, our study demonstrates that high fertilization rates achieved via ICSI do not guarantee a successful outcome. A good genetic background, high quality embryos and appropriate uterine receptivity are still the main predictors of clinical pregnancy and live birth rate.

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