Factors Affecting Intra-Cytoplasm Sperm Injection (ICSI) and Pregnancy Outcome in the Fertility Center of Al – Najaf City

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ABSTRACT:

BACKGROUND:

Knowing predictors of pregnancy in IVF is helpful for clinicians to individualize the treatment plans and improve patient counseling and for patients to decide whether to undergo infertility treatment.

OBJECTIVE:

A prospective and retrospective study to determine the factors affecting results and outcome of Intra-Cytoplasm Sperm Injection (ICSI) ICSI in the treatment of sub-fertile patients and the pregnancy outcome.

PATIENTS AND METHODS:

140 couples underwent IVF cycle in the form of intra-cytoplasm sperm injection ICSI enrolled in the study, pregnancy rate was analyzed according to the age, type, cause and duration of sub-fertility, hormonal assay, endometrial thickness, type of treatment protocol, number of retrieved oocytes, number of embryos transferred and sperm parameter.

RESULTS:

From the total of 140 women, 23 (16.43%) were cancelled, among 117 cycle, the pregnancy rate was 25.27%, fertilization rate was 39.87%. Factors which significantly affect the outcome of ICSI included the age of the women as fertility decline with advanced age, type and causes of sub-fertility. Sperm quality was more important than sperm number, and the number of embryos transferred also affect pregnancy outcome.

CONCLUSION:

Multiple factors affecting ICSI outcome, the most important one is female age, The cause and type of sub-fertility also affect the outcome.

KEY WORDS: intracytoplasm-sperm injection (ICSI)

INTRODUCTION:

Subfertility is defined as failure to conceive within one year of unprotected regular sexual intercourse, for couples who have had no previous conception, this sub-fertility is defined as primary, while couples who have had a previous conception and have then not conceived again are defined as having secondary sub-fertility [(1)]. Sub-fertility causes great distress to many couples, and lead to increasing number of them who seeking specialist fertility care, about 15% of all couples are involuntarily sub-fertile and require fertility treatment [(2)]. In young healthy couples the probability of conception in one reproductive cycle is typically 20-25% and in one year it is approximately 90% [(3)].

Thirty four years after the birth of first infant of IVF, number of children born worldwide as a result of ICSI already exceeded three million, the increasing popularity of these techniques and the data generated has given a better understanding of the efficacy, consequence and cost of these procedure[(4)]. ICSI is an optional procedure used during IVF involves removing oocytes from the ovaries, fertilizing them in the laboratory by deposition of a single spermatozoon directly into the cytoplasm of the oocyte, then replacing a specific number of the embryos that develop into the uterus in an attempt to initiate a pregnancy[(5)]. As only one sperm required for each egg, this allows for treatment of couples that have very low sperm count [(6)].

Female age is a major detriment of success rate of sub-fertility treatment and was the first prognostic factor in ICSI, since fertility decreases rapidly with age, the number of women seeking fertility treatment because of advancing age increased, the lower pregnancy rates seen in older women seem to be due to

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partly to a worsening response in spite of increased dosages of gonadotropins, older women yield fewer oocytes and have higher cancelation rates [7]. The pregnancy rate per embryo transfer declined from 26% in patients younger than 30 years to 9% in those aged 37 years, whereas the miscarriage rate increased from 29% in women under the age of 40 to 50% in those ≥ 40 years old [8]. Low response is also affect the ICSI outcome, it observed when the outcome of ovarian stimulation is suboptimal (low peak estradiol level <300-500pg⁄ml and/or low number of growing follicles < 3-5 on day of HCG) leading to a low chance of pregnancy after ICSI; low response is one of the significant problems of IVF because it occurs in up to 24% of cases [9].

The chance of pregnancy increases along with the number and good quality of embryos transferred, so that the implantation probability of a given embryo is increased by 22% for each additionally implanted embryo [10]. Regarding the obesity, obese women require higher gonadotropin doses, fewer oocytes are collected and decreased cumulative pregnancy rate as only 17% of obese patients have a live birth compared with 21% of normal weight women. Male subfertility and endometriosis have also been associated with a low chance of live birth. Other factors with an effect on the outcome of ICSI are smoking, primary infertility, a long duration of infertility and a high number of previous ICSI treatments carried poor prognostic effects [11].

PATIENTS AND METHODS:

This is retrospective and prospective study, conducted at fertility center in Al-Najaf city from October 2011 to April 2012. 140 women underwent ICSI program in these 6 months, complete history from each women include: name, age, address, type and duration of infertility. The patients were divided into 3 age groups <25years, 25-35years, and >35years old. In cycle day two, baseline vaginal ultrasound for the uterus, endometrium and ovarian size and texture, as well as hormonal study which include FSH, LH, E2, prolactin, and TSH were done. Seminal fluid analysis was done and the results were classified into: 1-Azoospermia, when no sperms in the ejaculate so we did to them MESA or TESE under local or general anesthesia by urologist.2-Oligospermia, when sperms number less than 20milion per ml.3-Asthenospermia, less than 50% activity.4- normospermia, normal ejaculate as defined by reference value(3). According to the history, ultrasound and hormonal results the type of protocol was decided either long or short protocol. In long protocol, long acting depot GnRH agonist triptorelin (Decapeptyle 3.57mg, Ferring, Germany) used on cycle day 21and gonadotropins started on cycle day two of the next cycle, while in short protocol short acting GnRH agonist (Decapeptyle 0.1mg, Ipsen, Ireland) used from cycle day two followed by gonadotropins in the same cycle. The starting dose of gonadotropins was determined according to the patient's age, BMI, antral follicular count in the baseline ultrasound and the outcome of previous treatments, we used recombinant FSH (Gonal-f®, Serono International SA, Geneva, Switzerland). Follow up was done by serial vaginal ultrasound and serial estradiol (E2) levels to evaluate the patient response and predict hyper-stimulation. The cycle was cancelled if less than three growing follicles are observed on day 10 of stimulation. Then patient prepared for oocyte retrieval procedure under general anesthesia and the oocytes received by embryologist for further preparation for ICSI procedure to be done then embryos transferred back to the mother uterus after 2-3 days and a high dose of progestogen (800mg) vaginally or rectally was given for luteal phase support. β-hCG blood test was done fourteen days after embryo transfer to diagnose pregnancy which later on followed up by ultrasound two weeks later after positive test to diagnose a clinical pregnancy and the number of gestational sacs.

Statistical analysis: SPSS . ver.18 statistical software for window was used to analysed data. Independent-sample t-test was used to detect the significant differences between each two groups of continuous variables. Non parametric data tested by Chi squared test (χ^2). $P<0.05$, $p<0.01$ were considered to be significant at 5% and 1% respectively.

RESULTS:

The total number of our patients were 140, from them (117) 83.6% reached ovum pick-up and (23) 16.4% cancelled of multiple causes as below, from those 117 women (91) 77.8% reached the embryo transfer, and from them (23) 25.3% got pregnancy.

Table 1:Classification of total No. of patients.

Total No of patients	140	
No. of patients reach step of ovum pickup	117 (83.57%)	
No. of patients didn't reach ovum pickup(cancelling)	23(16.43%)	
Causes	No.	%from 140
Poor responder	17	12.14
Anaesthesia fitness	2	1.43
No sperm	4	2.85
No. of patients reach step of embryo transfer	91	77.78
No. of patients didn't reach step of embryo transfer	26	22.22
No. of pregnancy	23	25.27

In table-2 out of 86 patients of 25 – 35 years, 14 (60.87%) showed positive pregnancy outcome, and differ significantly (P≤0.01) from other two groups.

Table 2: Age groups of total sample and their pregnancy outcome.

Age Groups	No.	Mean±SD	Pregnancy outcome	P
<25 years	18	22.55±1.688	0 (0%)	0.000
25 – 35 years	86	29.44±3.135	14(60.87%)	
>35 years	36	39.33±2.541	9(39.13%)	

In table 3, the number of primary infertility was 118(84.29%) which is higher than secondary type (22, 15.7%), but the pregnancy outcome in secondary infertility was higher 27.27% compared with 14.41% in primary infertility group. Women with less than 10 years duration of infertility were more liable to become pregnant (17.35%) than those with more than 10 years infertility (14.29%) although this was not significant. About the causes of infertility male factor was predominant than other causes (56.43%), and tubal cause carried good prognostic outcome than other causes (+ve outcome 32%) which was significant

Table 3: Type, duration and causes of infertility in the studied groups.

Parameters	+ve outcome		-ve outcome		Total No.(%)	*P*
Type of Infertility	No.	%	No.	%		
Primary	17	14.41	101	85.59	118(84.29)	0.035
Secondary	6	27.27	16	72.73	22(15.71)	
Duration of Infertility						
<10	17	17.35	81	82.65	98(70.00)	0.696
>10	6	14.29	36	85.71	42(30.00)	
of infertility						
Male cause	10	12.66	69	87.34	79(56.43)	0.000
Tubal	8	32	17	68	25(17.86)	
Ovarian	0	00.00	03	100	03(02.14)	
Unexplained	5	15.15	28	84.85	33(23.57)	

In table 4.The hormonal criteria for each woman underwent ICSI in this study was of normal cycle day2 hormonal assay to start with, we found that E2 level at day of hCG injection was more in women that have got pregnancy than in women that haven't got pregnancy which reflects good ovarian response , but there were no significant differences.

Table 4: FSH, LH and E2 levels of studied women.

Parameters	+ outcome	- outcome	*P*
	Mean±SD	Mean±SD	
FSH(CD2)	4.73±2.776	5.03±2.111	0.573NS
LH(CD2)	2.82±2.254	2.77±1.768	0.915 NS
E2(CD2)	43.46±15.559	38.27±15.538	0.161 NS
E2(hCG day)	2427.57±1307.593	2346.0±1497.616	0.815 NS

NS = non significant differences at ($P \leq 0.05$) -Independent-Sample t-test

In table 5, normal sperm represented 44.3% of all studied sample and significantly ($P \leq 0.01$) differ from other types of sperm analysis; however cases with normal sperm were higher in positive outcome than negative outcome group, and those with abnormal sperm activity had poor prognostic value than the abnormal number of sperms.

For the azoospermia we did testicular biopsy or aspiration and sometimes with freezing, Total azoospermia underwent testicular biopsies in this study whether MESA or TESE were 23, from them only 10 (43.48%) yielded a +ve result and from them only 2 (20%) got pregnancy.

Table 5: Sperm analysis of studied sample.

SFA	+ outcome		- outcome		Total	P
	No	%	No	%	No(%)	
Oligo + Asthenospermia	5	21.74	21	17.95	26(18.57)	0.000
Asthenospermia	1	4.35	14	11.97	15(10.71)	
Oligospermia	3	13.04	11	9.40	14(10)	
Azoospermia	2	8.70	21	17.95	23(16.43)	
Normospermia	12	52.17	50	42.74	62(44.29)	

About the relation between the treatment protocol type and success rate (table 6) we found that long protocol gives good results than short protocol which was significant.

Table 6: Types of protocol.

Type of Protocol	+ outcome		- outcome		Total No. (%)	P value
	No	%	No	%		
Short	14	12.39	99	87.61	113(80.71)	0.001**
Long	9	33.33	18	66.67	27(19.29)	

We studied the oocytes in metaphase two (MII) because it is the mature one and liable for injection with sperm and become embryo, the mean No. of metaphase II was higher ($P \leq 0.01$) in women that got pregnancy than women that didn't get pregnancy.

Both groups of women showed no differences in their endometrial thickness, however women with positive pregnancy outcome have slightly more thickness. The mean No. of embryos was higher in pregnant women than those who didn't get pregnancy, which was significant

Table 7: No. of MII oocytes, endometrial thickness and No. of embryos.

Parameters	+ outcome	- outcome	P
	Mean±SD	Mean±SD	
No. of Oocytes in Metaphase II	7.09±4.048	4.54±3.959	0.008**
Endometrium thickness(mm)	10.14±2.056	9.68±2.239	0.379 NS
No. of Embryos	3.90±2.773	1.59±1.913	0.000**

In this study the total No. of oocytes in metaphaseII were 690 from them 275 fertilized and became embryo, so the fertilization rate was 39.87%.

We studied the relation of embryo types with pregnancy outcome, we divided the embryo as grade 1, 2, 3 and 4, we found most of pregnancy occurred with high grade embryos.

Table 8: Type of embryo in the studied groups.

Type of embryo (Grades) (No. of Patients)	+ outcome		- outcome	
	Mean±SD	Range	Mean±SD	Range
G1(44)	2.43±2.15	0-10	0.76±1.219	0-6
G2(36)	1.33±1.527	0-5	0.56±0.988	0-5
G3(20)	0.09±0.300	0-1	0.22±0.602	0-4
G4(5)	0.05±0.218	0-1	0.06±0.311	0-2

DISCUSSION:

With a growing experience in performing ICSI a better understanding of factors necessary for ART success. The majority of these factors are characteristic of female rather than male patient. The most pertinent concern to the infertile couple has always been the success rate of specific ART treatments. Therefore, numerous studies have been dedicated to identify reliable markers that may help in predicting outcomes in IVF cycles, as well as the feasibility of applying these markers in patient counseling prior to treatment initiation

Our study shows that the chance of conceiving after a completed treatment cycle decreases as age increases especially in women >35 years. This finding suggests that the age-related decline on the success in IVF most likely lies in the progressively diminished ovarian reserve, with decreases in both quantity and quality of oocytes, this in agreement with Wang JG et.al. findings(12, 2010).We also found the pregnancy rate low in young age group > 25 years this may be because most of these patients are in teenagers and their infertility cause is sever male factor or azoospermia which gives poor results.

Our study showed that the secondary infertility had better prognosis than primary infertility which goes with Johnson J study in 2004(13), and low duration of infertility associated with better results than long duration of infertility as Van Loendersloot et.al. study in 2010 (14). Tubal cause carried good prognostic outcome than other causes this indicate the quality of sperms and oocytes are very important in success of ICSI, although Templeton et.al. 2009 found the cause of infertility does not affect IVF outcome(15).

About the semen analysis, sperm activity had poor prognostic value than the number of the sperms, because in ICSI we need only one sperm to each oocyte retrieved.

The long protocol had good pregnancy outcome than short protocol in our study, in meta-analysis of 26 prospective and randomized studies there is 4.6% increase in the clinical pregnancy rate with long protocol(16). The better results may be due to the better control of LH secretion and complete its suppression with long protocol.

About the oocytes and embryos, the mean number of metaphaseII oocytes was higher in the pregnant group than not pregnant, this agree with Sunkara SK et.al. in 2011(17). And the mean number of embryos was higher in pregnant women which was significant as Hyden-Granskog C study who found that the chance of pregnancy increases along with number of embryo transferred in 22% for each embryo this explained by the increased placental mass in early multiple implantation which produce more HCG and progesterone than single placenta (10) However, the benefits of multiple implantation are lost in later pregnancy, as multiple pregnancies often result in the premature births of infants with low birth weight. These results suggest that the total number of embryos contributes additional information to the predictive model that the total number of good-quality embryos may be a surrogate marker for hormone factors and may act through the uterine receptivity (18, Cai QF et al., 2011). However, El-Mazny(8, 2011) showed the embryo grading cannot give an accurate prediction of pregnancy.

CONCLUSION AND RECOMMENDATION:

Multiple factors affecting ICSI outcome, the most important one is female age. So these factors may be used as markers of fertility for counseling IVF/ICSI candidates to increase success rate and decrease the cancellation cycles.

REFERENCES:

1. Edward Arnold, David M. Luesley, Philip N. Baker, Hany A. Lashen. Female infertility in: Obstetrics & gynaecology an evidence-based text for MRCOG. 2004;52:566-87.
2. Keith Edmond. Infertility in: Dewhurst's text book of obstetrics & gynaecology, 7th edition.2007: 440.
3. John Studd. Factors affecting the outcome of in vitro fertilization in Progress in obstetrics & gynaecology 2005;16;259.
4. Peter R. Brinsden. Textbook of in vitro fertilization and assested reproduction, 3rd edition. 2007.
5. Ash Monga. Gynaecology by ten teachers. 18th edition;2006:81.
6. David K., Ariel Weissman, Colin M. Howles, Zeev Shohman. Tetbook of reproductive technologies, 3rd edition 2009.
7. Bhattacharya S, Templeton A. What is the most relevant standard of success in assisted reproduction? Redefining success in the context of elective single embryo transfer. 2004;19:1939-42.
8. Akmal El-Mazny, Waleed El-Khayat, Mustafa Mahmood. Factors affecting IVF\ICSI outcome: A review of literature. Kasr Al-Aini J. of obs.& gyne. 2011;2.
9. Fisch JD, Keskintepe L & Sher G (2008) Gonadotropin-releasing hormone agonist/antagonist conversion with estrogen priming in low responders with prior in vitro fertilization failure. Fertil Steril 2007; 89: 342–47.
10. Hyden-Granskog C, Unkila Kallio L, Halttunen M. Number of embryo transfer in an option in frozen embryo transfer. Hum Reprod. 2005;20:2934-38.
11. Olli Vuolteenaho, Kirsti Nukkala. Factors affecting outcome of IVF\ICSI. University of Oulu, Finland; 2008.
12. Wang JG, Douglas NC, Nakhuda GS, Choi JM, Park SJ, Thornton MH, Guarnaccia MM, Sauer MV: The association between anti-Müllerian hormone and IVF pregnancy outcomes is influenced by age. Reprod Biomed Online 2010;21**:**757-61.
13. Johnson J, Canning J, Kaneko T, et al. Germ stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428:145-50.
14. Van Loendersloot LL, Van Wely M, Limpens J, Bossuyt PM, Repping S, vander Veen F. Predictive factors in in vitrofertilization (IVF): a systematic review and meta-analysis. Hum Reprod Update 2010;16:577–89.
15. Templeton A, Morris JK, Parslow W.Factors that affect outcome of IVF treatment. Lancet 2009; 348: 234-40.
16. Basil C. Tarlatzis, Grigoris G. Ovulation induction2002;12:155-69.
17. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J. Association between the number of eggs and live birth in IVF treatment: an analysis of 400135 treatment cycles. Hum Reprod. 2011;26:1768-74.
18. Cai QF, Wan F, Hang R, Zhang HW. Factors predicting the cumulative outcome of IVF\ICSI treatment; amulti-variable analysis of 2450 patients. Hum Reprod. 2011;26:2532-40.