ORIGINAL ARTICLE

Predictors for Number of Retrieved Oocytes in Intracytoplasmic Sperm Injection

Oocyte Numbers and IC SI Success

Rehana Rehman[1], Kiran Fatima[2], Mehwish Hussain[3]. Rakhshaan Khan[4], Syeda Sadia Fatima[5]

ABSTRACT

Background: Successful ICSI depends on ovarian response to stimulation and maturation of oocytes retrieved.

Objective: To identify predictors of number of retrieved oocytes during intra cytoplasmic sperm injection (ICSI).

Methods: A cross sectional study carried out at Islamabad Clinic Serving Infertile Couples @ Saudi Pak Tower from January 2011 till December 2012. Female subjects (n= 282) aged 20 -40 years had base line estimation of follicle stimulating hormone, estradiol, antral follicle count and ovarian volume on the second day of cycle. Down regulation of ovaries was followed by controlled ovarian stimulation for 12 ± 2days. On the day of ovulation induction by intramuscular injection of human chorionic gonadotropin, pre-ovulatory follicle count (PFC) was measured by trans vaginal scan and serum estradiol, progesterone and interleukin I-β were estimated. Data was expressed as mean ± standard deviation and median with interquartile range. Correlation coefficient was measured to study degree of association of demographic and clinical factors with NRO. Univariate regression of NRO as dependent variable with predictors was confirmed by multivariate regression.

Results: Retrieved oocytes had strongest correlation with PFC (r = 0.98). Multivariable analysis stipulated that in the presence of other variables PFC had similar strongest effect on oocytes. AFC showed significant but negative effect on NRO by multiple regressions. Progesterone, E2, IL-I β, FSH and injections used for stimulation had insignificant effect in the presence of other variables.

[1] Rehana Rehman

Assistant Professor, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi.

[2] Kiran Fatima

Phil Scholar, Department of Physiology, DOW University of Health Sciences, Karachi.

[3] Mehwish Hussain

Senior Lecturer, Department of Biostatistics, DOW University of Health Sciences, Karachi.

[4] Rakhshaan Khan

Public Health Physician, Islamabad.

[5] Syeda Sadia Fatima

Senior Instructor, Department of Biological and Biomedical Sciences, Aga Khan University.

Corresponding Author

Syeda Sadia Fatima

Conclusion: PFC measured at the time of ovulation induction is strongest predictor of oocytes which are obtained during oocyte pick up procedure of ICSI.

KEY WORDS: *Intracytoplasmic Sperm Injection, Pre-ovulatory Follicle Count, Ovarian Reserve, Antral Follicle Count, Controlled Ovarian Stimulation.*

INTRODUCTION

Intra cytoplasm sperm injection (ICSI) is a highly advanced technique of "Assisted reproductive Clinics" (ARC) where conception does not occur by regular infertility treatment procedures.[1,2] The technique although employed predominantly for male factors like compromised; sperm concentration, motility or morphology yet successful conception depends on oocyte parameters like number of retrieved, mature and fertilized oocytes obtained as a result of treatment.[1]

Females have life time endowment of OR at 20 weeks of her gestation when approximately 6-7 million oogonia are there in her ovaries. This number decreases to approximately 2-3 million at birth and then drops off to 300,000 at the time of puberty. After that, during reproductive years, ovary starts to recruit at least 30-50 oocytes from ovarian reserve (OR).[3] They compete with each other to become the dominant follicle and ultimately ovulate to release an egg for fertilization. A good reserve generally points toward a large number of viable oocytes and low OR represents smaller number of their availability[1]. Diminished OR caused by increasing age is associated with decrease in number as well as quality of oocyte retrieved during assisted conception treatment.[3]

The likelihood of success depends on ovarian response to stimulation which is estimation of number of oocytes retrieved (NRO) from OR. Pre-treatment assessment of OR is made by ovarian reserve test (ORT) which gives an idea about probable response to ovarian stimulation.[4] This knowledge is important in order to calculate the appropriate dose of stimulation with some information about quantity and quality of oocytes, as well as risk of cycle cancellation.[5] Antral follicle count (AFC), a very common and noninvasive method for determining OR refers to the total number of primordial follicles presents in the both ovaries and suggests the responsiveness of antral follicles to FSH.[5] The objective of our study is to find out predictor of NRO in females who go through ICSI in our study population.

METHODOLOGY

A A cross sectional study was carried out in "Islamabad Clinic serving infertile couples" from January 2011 to December 2012. All females with age range 20 to 40 years, body mass index (BMI) 18-35 kg/m^2 with both functional ovaries were included. Women with polycystic ovaries (diagnosis of PCOS was made by existence of two of the three criteria: polycystic ovaries, oligo - and an ovulation, and clinical or biochemical evidence of hyper-androgenism) endometriosis and uterine fibroids were excluded.[6] The base line investigations done on the second day of female cycle were basal follicle stimulating hormone (FSH), basal estradiol (E2), AFC and basal ovarian volume (BOV). AFC was measured by Tran's vaginal scan (TVS), mean of two perpendicular measurements of all follicles in both ovaries were then added for the total AFC count.[7] The volume of each ovary was measured in three perpendicular directions by TVS and calculated by the formula (D1 × D2 × D3 × π/6). The volumes of both ovaries were added for the total BOV.[7]

Pituitary desensitization from mid luteal phase of preceding cycle was followed by COS with gonadotropins recombinant FSH (r FSH) 50 IU preparation (Puregon registered; NV Organon, Oss, The Netherlands) was administered by I/M or S/C route for 12 ± 2 days. Ovulation induction (OI) by intra muscular injection of hCG (Pregnyl 10,000 I.U) was planned with maximum follicles acquiring size of 20 mm on TVS. On this day, number of pre ovulatory follicles (PFC)

was noted and estimation of serum E2, Progesterone (P) and interleukin Iβ (IL- Iβ) was done. Oocyte pickup was performed 36 hours after OI by 16G adapter of vaginal ultrasound probe under short general anesthesia. As soon as the oocyte was found, it was picked up using 23 mm sterile glass Pasteur pipette (Hunter Scientific UK) and transferred into the culture dish containing culture medium GIVF Plus (Vitro life) overlaid with liquid paraffin oil (Ovoid virtolife, Tohai Hint Japan) preincubated at 37°C, 5% CO_2 and placed on a thermo plate at 37°C (Falcon, Becton Dickinson and company, Franklin Lakes, USA). Presence of oocyte was confirmed under phase contrast x10 magnification of stereomicroscope (Leica MZ12; Wetzlar, Gernamy), by the presence of corona radiate and thick cumulus mass around it. If the oocyte was not found in the first aspirate, the follicle was flushed 1–3 times, till was found or absence was confirmed. All oocytes were collected in a similar manner.[8] When all were collected, they were recounted and transferred to incubator (Sanyo MCO-18 M- Germany) for 1-2 hours.

The study was approved by the ethics committee of Islamabad Clinic serving infertile couples at Saudi Pak Tower No.11/R- ICSI. A written informed consent was obtained from all participants.

All data were entered in SPSS version 18 and analyzed in the same software. Descriptive measures for the variables were expressed in terms of mean with standard deviation and median with interquartile range. Correlation coefficient was measured to study degree of association of demographic and clinical factors with NRO. Crude regression coefficients were obtained by simple linear regression to identify potential significant predictors for NRO. At this univariable analysis stage, variables which produced P value less than 0.05 were selected for multivariable analysis. Thus, adjusted regression coefficient was estimated to measure the effect significant predictors on NRO counts. P value less than 5% level was considered to show significant effect.

RESULTS

Mean age of females was 32.1 ± 4.7 years with mean BMI was 24.2 ± 3.7 Kg /m^2. Pre ovulatory follicle was 7.8 ± 1.9; average retrieved oocytes of patients were 7.7 ± 1.7. At the day of OI, P was 1.5 ± 0.7 ng/ml, E2 2323 ± 298.2 and IL-Iβ was 116 ± 63.5 pg/ml. AFC was 14.7 ± 2.8 while BOV described by ultrasound was 11.6 ± 3.1. Basal E2 was 214.7 ± 145.8. Total count of r FSH used was 57.2 ± 9.2 (Table 1).

Table 1: Descriptive Measures of Study Variables

	Mean ± SD	Median	Inter-quartile Range	Minimum	Maximum
Female age	32.11±4.66	32	8	23	41
Body Mass Index (kg/m^2)	24.24 ± 3.69	25	7	17	30
Preovulatory follicle count	7.8 ± 1.9	8	3	4	14
Number of cleaved embryos	5.9 ± 1.5	6	2	1	9
Endometrial thickness	8.6 ± 3.4	9	6	3	14
Number of gestational sacs	0.5 ± 0.7	0	1	0	2
Progesterone at Day of Ovulation Induction	1.5 ± 0.7	1.4	1.21	0.23	3.8
Estradiol at Day of Ovulation Induction	233 ± 298	2346	185	1402	3001
Interleukin at Day of Ovulation Induction	116 ± 63.51	116	103.1	26.2	272.5
Antral follicle count	14.66 ± 2.8	16	5	10	21
Ovarian Volume by Ultrasound	11.55 ± 3.07	11.2	4.2	5.28	23.52

Follicle stimulating hormone	6.69 ± 1.09	6.5	1.1	4.5	9.8
Basal Estradiol	214.7 ± 145.8	135.6	177.1	95	660.7
Total number of gonadotrophin injections	57.2 ± 9.1	56	8.1	33	96
No of oocytes/patient	7.7 ± 1.6	8	3	4	13

Table 2 showed the effect of demographic and clinical factors on NRO. Age, BMI, BOV had no significant relationship with number of retrieved oocytes. Correlation analysis revealed that retrieved oocytes had strongest correlation of Preovulatory follicle count (r = 0.98). Univariable regression analysis studied crude effect of clinical factors on NRO. The regression coefficient of PFC revealed that with one amount increase of PFC, NRO increased up to 0.88 units. E2 and IL-I β at the OI day, AFC and basal E2 also showed positive significant effect on retrieved oocytes. On the other hand, Pat day of OI and FSH had negative significant effect. With the increase of one unit progesterone, NRO decreased up to 0.42 units at the day of ovulation induction. Also, one unit increase of FSH caused 0.29 units decreased in number of retrieved oocytes (Table 2).

Table 2: Relationship and Predictors for Oocytes Retrieval

	r	Crude Regression Coefficient	p-value	r Adjusted for Age and BMI	p-value
Female age	-0.06	-0.02	0.32	--	
BMI	-0.04	-0.02	0.54	--	
Pre ovulatory follicle count (PFC)	0.98	0.88	<0.0001	0.904	<0.0001
Progesterone at Day of Ovulation Induction	-0.19	-0.42	0.002	-0.01	0.62
Estradiol at Day of Ovulation Induction	0.17	0.001	0.005	-0.00003	0.56
Interleukin at Day of Ovulation Induction	0.18	0.01	0.02	0.00001	0.35
Antral follicle count (AFC)	0.21	0.13	<0.0001	-0.03	0.001
Ovarian Volume by Ultrasound (BOV)	0.003	0.002	0.96	--	
Follicle stimulating hormone (FSH)	-0.19	-0.29	0.001	0.034	0.46
Basal Estradiol	0.17	0.004	0.002	-0.00005	0.19
Total number of gonadotropin injections	-0.18	-0.03	0.002	-0.004	0.35

Multivariable analysis stipulated that in the presence of other variables PFC had still similar strongest effect on oocytes. AFC was another variable which showed significant but negative effect on NRO while keeping other clinical factors constant. Others predictors such as P, E2 (both at ovulation induction day and before treatment), IL-I β, FSH, total number of rFSH (Inj puregons) had insignificant effect at multivariable analysis stage (Table 2).

DISCUSSION

Success of treatment procedures after ICSI depends on retrieval of appropriate number of mature oocytes with minimum risks of ovarian hyper stimulation syndrome.[9] The ORT however define quantity rather than quality and ultrasound parameters like AFCs used alone or in combination with age and basal FSH, are not capable to predict the probability of

conception.[10,11]. Female age, BMI, inheritance of OR, basal FSH and AFC have an impact on dosage and duration of drugs required for favorable number of oocytes, quality of embryo and hence its implantation of after ICSI.[6,12]

When couples opt for infertility treatment procedures, specialists decisively look at the age of female partner. There is evidence that the chance of natural and assisted conception and on the top of that success of infertility treatment decreases with advancing age.[13] Ageing has been found to exert noteworthy deleterious effect on women's reproductive capability by decrease in NRO and has been established as a better predictive of live births as compared to rest of OR.[13,14] In our study female age did not turn out to be a predictor of NRO which is in contrast to Pinto etal, who have emphasized on female age as one of the predictors of ovarian response to stimulation.[15]

The responsiveness of antral follicles to gonadotropic drugs is an indicator of female health, reproductive competence, ultimate outcome of COS and success rates of ARC procedures. AFC has proved to be the single best predictor of ovarian aging[16] and type of response to COS[4,7,11]. Researchers found that patients with low AFC require high stimulation doses and predict low fertility rates.[6] In our study, AFC proved itself to be a better predictor of ovarian response to COS (measured by NRO) as compared to OV by Univariate regression which is comparable to other studies[17], however was not found to be a predictor by multivariate regression which is supported by Melo et al.[18]

Basal serum levels of FSH and E2 predicted number of immature oocytes and the pregnancy rate in women undergoing unstimulated IVM treatment.[19]. Basal FSH has been established as a reliable ORT during the pituitary suppression cycle of in vitro fertilization patients and turned out to be a negative predictor of number of oocytes in our study.[20] On the other hand, basal E2 turned out to be a sensitive positive predictor of NRO by Univariate regression which is comparable to other researchers.[21]

Increased requirement of injectable gonadotropin and increased cost of treatment was associated with an increase in NRO between cycles in women with normal ovarian reserve.[22] The number of injections however did not improve NRO in our study. A negative correlation observed with P levels is supported by work done by Ozcakir.[23] BMI was not found to be a predictor of NRO besides increase dose requirement of gonadotropins and a reduced amount of oocytes retrieved in females of obese (BMI ≥ 26) by Rehman et al.[5] The Univariate regression revealed IL-Iβ to be an indicator of NRO; positive correlation of IL-Iβ with NRO has been observed in literature.[24] Multiple regression analysis revealed a positive association of NRO with PFC which has recently been documented,[25] however results are not in association with Pinto etal in which treatment protocol and AFC showed a positive association.[15]

CONCLUSION

The success of ICSI from the aspect of NRO can be predicted by simple estimation of PFC. Instead of measurement of AFC, basal FSH, BOV and basal E2 before initiation of treatment, detection of PFC the time of OI can give an insight to probability of ovarian response to stimulation and likelihood of success after ICSI. In conclusion, instead of giving females hopes, expectations or reservations before initiation of treatment, it is good to start the treatment and then make out the decision.

The fertility specialists should not waste time, energy and money of infertile couples on basal E2, FSH and AFC estimation and should not exclude patients on the basis of advanced age, low basal E2, raised FSH and low AFC. The unfertile couples should be given trial with expectation of the best.

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