7-2019

Intracytoplasmic sperm injection and advanced maternal age: Success or treatment failure

Rehana Rehman

Hafsa Mahmood

Fatima Syed

Hareem Syed

Hina Gul

Intracytoplasmic sperm injection and advanced maternal age: Success or treatment failure?

Rehana Rehman[1]*, Hafsa Mahmood[2], Fatima Syed[3], Hareem Syed[2] and Hina Gul[5]
[1]Department of Biological & Biomedical sciences, Aga Khan University, Karachi, Pakistan
[2]Departmet of Medicne, Dow University of Health Sciences, Karachi, Pakistan
[3]Departmet of Medicine, Jinnah Sindh Medical University, Karachi, Pakistan
[4]Prontolinks Pvt. Ltd., Karachi, Pakistan

Abstract: Infertility rate documented in Pakistan is 21.9% with only 25% success rate even after procedures like intracytoplasmic sperm injection (ICSI). This rate is further on the decline with enhancement of female age. We aimed to observe the effect of female age on oocyte parameters and reproductive outcome after ICSI. It was done by retrospective analysis of a quasi- experimental design carried out after approval from "Ethical review board of Islamabad clinic serving infertile couples" from July 2010 to August 2011. The response to ovarian stimulation in (282) females was assessed on the basis of groups, A, B, C and D with age ranges up to 25years; 25.1 to 30years; 30.1 to 35years and >35years, respectively. The outcome was assessed as non-pregnant, preclinical abortion and clinical pregnancy groups on the basis of beta hCG and cardiac activity by trans-vaginal scan. We observed that maximum number of pregnancies 32 (38%) occurred in C group, and least 10 (10%) in group A. There was a statistical reduction in the number of mature and fertilized oocytes as the age advanced from group C to D ($p<0.05$).This shows that reduction in maturity and fertilization of oocytes with advancement of age recommends early referral of couples to assisted reproductive clinics.

Keywords: Female age, infertility, intracytoplasmic sperm injection, ovarian reserve.

INTRODUCTION

Infertility can be defined as incapability of a couple to enjoy parenthood after unprotected regular intercourse without any contraception over a period of at least 1-2 years (Rehman *et al.,* 2015). Intracytoplasmic sperm injection (ICSI) developed especially for treatment of sterile couples is an infertility treatment in which a sperm is directly injected in the oocyte (Spandorfer *et al.,* 1998, Rehman *et al.,* 2014). The procedure deals with in fecund males and also women of variable age groups and repeated failures after in-vitro fertilization (IVF) (Palermo GD *et al.,* 1995). ICSI has resulted in exponential rise in the number of children born as a result of infertility treatment plans (Wong and Ledger, 2013).

There is a decline in reproductive functions, ovarian reserve and its response to stimulation with the enhancement of maternal age (Marca *et al.,* 2011). The mean age of women carrying their first child has increased up to 5years due to a number of factors including delay in marriages, contraception, and reliance of assortment to assisted reproductive technology (ART). Female fertility progresses to a rapid decline after the age of 35 even after assisted reproductive techniques (ART) (Baird *et al.,* 2005). Advancing age deleteriously causes a diminution in ovarian reserve (OR) with a decrease in number and quality of oocyte, hormones of implantation, fertilization rate and success after ICSI (Rehman *et al.,* 2015 b). The effect of increasing maternal age on fertility is attributed to poor oocyte parameters that decrease pregnancy rate and multiplies risk of chromosomal abnormalities in the embryo (Kim *et al.,* 2013).

The association of maternal age and fertility documented by Hutterites declared that maximum infertility occurred after 35 years of age (Hassold *et al.,* 2009). In another investigation, there was a significant effect of female age on pregnancy rate and age group of 19-26 year old women had a higher number of pregnancies as compared to women belonging to 27-29year old group (Dunson and Baird, 2004). There was one-sixth the number of pregnancies in women above 40years of age as compared to women aged less than 34years (Oehniger *et al*., 1995). Since there is lack of available data on effect of female age on outcome after ICSI, we aimed to investigate the effects of maternal age on oocyte parameters and reproductive outcome after ICSI.

MATERIALS AND METHODS

It was a retrospective analysis of a quasi-experimental study conducted after approval from the Ethical Review Board of "Islamabad Clinic Serving Infertile Couples" from June 2010 till August 2011. Data of 282 females with age 18-41 years, infertility duration of more than 2 years, body mass index (BMI) of 18-27kg/m^{2}and both functional ovaries exhibiting cycle of 25-35 days with basal serum FSH level <10IU/mL was collected. Females with metabolic and endocrine abnormalities, uterine

**Corresponding author:* e-mail: drrehana7@gmail.com

fibroids and previous failed attempts of ICSI were excluded.

Treatment protocol

All subjects were given long desensitizing protocol for mid luteal pituitary down regulation of ovaries by subcutaneous administration of GnRHa Decapeptyl 3.75 mg depot or Decapeptyl 0.1mg daily from D-21 of the preceding cycle. Controlled Ovarian Stimulation was carried out by using subcutaneous administration of recombinant FSH (r FSH) 50IU preparation (Puregon registered; NV Organon, Oss, The Netherlands). The initiation dose was calculated on the basis of age of the subject, basal serum FSH concentrations, AFC and BMI. Dose adjustment was performed by follicular tracking with trans vaginal scan TVS (7.5MHz probe Aloka 500, Tokyo Japan) that commenced from the fifth day of COS on alternate days for assessment of follicular response and measurement of endometrial thickness. Ovulation Induction (OI) was decided on the basis of maximum number of follicles > 18±2mm in diameter on TVS (around 14±2 days of COS). It was made possible by administration of human chorionic gonadotrophin (hCG) by intra muscular injection of 10,000IU of hCG (Profasi®, Serono, Switzerland). On the day of OI (dhCG) venous sample was taken for initial estradiol (E2), and Progesterone (P) estimation (Endometrial thickness was measured by experienced sonographers in the midsagittal plane on the day of OI by two-dimensional ultrasound with a 7.5-MHz probe (Hitachi EUB 525; Hitachi, Tokyo). Oocytes were retrieved 36 hours after OI on 14th, 15th or 16th day of COS by vaginal ultrasound probe with 16G adapter and double lumen oocyte aspiration needle (Cook Australia; Queens land, Australia) under short general anesthesia. The follicle aspirate was immediately placed in to a 60mm labeled culture dish placed on a thermo plate at 37˚C (Falcon, Becton Dickinson and company, Franklin Lakes, USA) and examined under stero microscope (Leica MZ12; Wetzlar, Gernamy). They were then 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% CO2. All oocytes were collected in a similar manner. Soon after the oocyte was found it was transferred to mini incubator (K-MINC-1000, Australia). Sperm preparation for ICSI was made on the day of OPU after obtaining semen sample of husband in the clinic by masturbation following 3-5 days of abstinence. It was carried out using sperm Grad gradient (Vitro life) and preparations were kept in tri gas incubator (Sanyo MCO-18M- Germany).

Micromanipulation

ICSI was performed 3-6h after oocyte recovery on all morphologically intact eggs in Metaphase II stage. The procedure was carried out in labeled microinjection dish prepared by adding several 10ml droplets of G Gamete culture buffered media on the heated stage of an inverted phase contrast microscope under 200 x magnifications (Leica DMIRB, Leica Microsystems, Wetzlar, Germany) with the help of micro injection pipette.

Assessment of fertilization and cleavage

Fertilization was assessed 16-20 hours after ICSI. Only normally fertilized (with two pronuclei and two polar bodies) were considered for eventual embryo transfer. These were cultured for additional 24-30 hours at 37°C in fresh CO_2 -equilibrated G1 Plus medium till cleavage, growth and differentiation of embryo.

Assessment of embryo quality

Embryos were evaluated on alternate days and were graded {Grades 1-5 from best to worst}. The degree of fragmentation was recognized as the embryonic volume occupied by enucleated cytoplasmic fragment 322RBM Online.

Embryo transfer

ET was carried out using Sure View Wallace- Embryo Replacement Catheter (SIMS-Porter Limited, Hythe Kent, UK) on Day-five or six after OPU under abdominal ultrasound guidance. In the procedure cervix was visualized with the help of a vaginal speculum, which was then cleansed with culture media. The optimal target for embryo replacement known as the maximal implantation potential was identified by abdominal scan. In the first step of ET, catheter was flushed with Embryo Glue (Vitrolife), shaped to the degree of angulation of cervix, loaded with embryos on its tip sand transferred under ultrasound guidance.

Outcome measures

The outcome of procedure was categorized into three groups on the basis of serum beta hCG measurement and TVS performed 14 and 28 days after egg collection respectively. Non-pregnant females had a negative beta hCG, preclinical abortion group had positive beta hCG without any cardiac activity on TVS and clinical pregnancy group included females who had confirmed pregnancy by appearance of cardiac activity on TVS. The outcome was studied in groups A; less than 25years, B; 25. 1 till 30years, C; 30.1 till 35years and D; more than 35years of female age respectively. Distribution of patients on the basis of age showed that A and B groups both comprised of 30% females whereas 11% and 29% had age up to 25years (A) and above 35years (D) respectively.

STATISTICAL ANALYSIS

Interpretation and analysis of data was done via SPSS (Statistical Packages of Social Sciences) version 15.0. Results of continuous variables are represented as mean ± standard deviation. Comparison of female age groups was

done by one way analysis of variance (ANOVA) with results declared significant at p-value <0.05.

RESULTS

Table 1 shows outcome of ICSI in 282 females out of which 162 conceived, 61 (60%) were biochemical and 101 (36%) were clinical pregnancies. Maximum number of clinical pregnancies 42 (41%) was observed in 30.1-35years (C) whereas least number 10 (10%) were observed in less than 25years age group (A). Group D (35.1 to 40) had maximum preclinical abortions 20 (33%) in comparison to younger age group {(A);6(10%)}.The failure to conception was detected to be 12%, 31%, 30%and 27% in groups A, B, C and D respectively. No significant difference was observed in variables of body mass index, basal and peak hormones. AFC in age group D was significantly less (14.4±2.79) as compared to younger age groups ($p<0.001$). Table 2 shows ovarian responsiveness to stimulation in age groups. It was noticed that there was no statistical difference in number of oocytes in all age groups whereas mature and fertilized oocytes were less in group D as compared to group C ($p<0.05$). In addition to that, oocyte maturity and fertilization rate of females aged 35.1 to 40years (Group D) was lower than other groups; significantly low ($p<0.05$) as compared with group C. The association of oocyte maturity with female age is expressed in fig., which narrates a trend towards decline in oocyte maturity with increase in female age.

DISCUSSION

Female age, BMI, inheritance of OR, basal FSH and antral follicle count (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. The number of AFC present in the ovary depends on the number of primordial follicles present (Muttukrishna *et al.,* 2005). Although it represents number of remaining primordial pool in women and corresponds to the number of oocytes retrieved, there is still a debate on its influence on quality of oocyte and embryo quality and hence result of ICSI (Rehman *et al.,* 2015 b) According to our study, there was significant effect of age on AFC. Females up to 25years of age (A) had a higher AFC as compared to other age groups.

Literature reveals decline of AFC with age by a number of studies done in different decades. AFC declared to be a reliable marker for OR (Ferraretti *et al.,* 2011). A study by Gabrielle J.

Table 1: Comparison of outcome after intracytoplasmic Sperm Injection in females of different age groups

Group	Female age	Number (282)	Non- pregnant (120)	Preclinical abortion (61)	Clinical pregnancies (101)
A	Up to 25 years	30 (11%)	14 (12%)	6 (10%)	10 (10%)
B	25.1 to 30years	84 (30%)	37 (31%)	18 (29%)	29 (29%)
C	30.1 to 35years	85 (30%)	36 (30%)	17 (28%)	42* (41%)
D	35.1 to 40 years	83 (29%)	33 (27 %)	20 (33%)	20 (20%)

Results of age groups compared on the basis of outcome of ICSI in terms of clinical Pregnancy, Preclinical Abortions and no conceptions. (Percentages in columns)

Table 2: Comparison of ovarian responsiveness to stimulation in age groups

	A Upto 25 years	B 25.1 to 30years	C 30.1 to 35years	D 35.1 to 40 years
Total number of injections required for stimulation	56.71 ± 8.582	56.34 ± 8.193	58.1 ±11.206	57.35 ± 7.924
Number of injections in one day	4.02 ± 0.676	3.94 ±0.612	4.05 ± 0.79	4.02 ± 0.604
No of oocytes/patient	7.97 ±2.189	7.79 ±1.715	7.71 ± 1.419	7.48 ± 1.664
No of oocytes Metaphase II	7.4 ± 2.372	7.19 ± 1.917	7.08 ± 1.74	7.01 ±2.081*
No of oocytes fertilized	6.13 ± 1.833	5.98 ± 1.505	5.89 ± 1.397	4.90 ± 1.679*
Oocyte_retrieval_rate	98.37 ± 3.329	99.2 ±2.481	98.96 ± 4.883	99.05 ± 3.169
Oocyte maturity rate	92.64 ± 16.18	92.85 ± 16.617	94.52 ± 15.405	91.54 ± 21.202*
Fertilization Rate	77.11 ± 12.301	77.4 ±13.642	78.92 ± 13.179	77.33 ± 17.949*

Values expressed as Mean ± SD

Groups compared by one way analysis of variance

- p value <0.05 for comparison of group D with C
- Oocyte retrieval rate (%) = Total number of follicles at ultrasound/number of oocytes retrieved × 100 (
- Oocyte maturity rate(%) = Total number of metaphase II oocytes / Total number of oocytes retrieved × 100 (
- Fertilization rate (%) = Total number of 2 pronuclei/ Total number of oocytes microinjected × 100 (

Scheffer *et al*. showed that the AFC by the age of 37 reduces every year by 4.8% and with further increase in age AFC reduces by 11.7% (Scheffer *et al.,* 1999). A prospective study that was conducted by Ming-Yang Chang *et al*. portrayed that women with lower AFC had higher rate of miscarriages (Chang *et al.,* 1998). Our results are supported by a study done by Antonio La Marca *et al* which illustrated that there was linear decrease in AFC with increasing age (Marca *et al.,* 2011). In our study, a decrease in number of retrieved oocytes was observed with advancement in maternal age. This is in contrary to work conducted by Mille r JE and Smith TT 2001 in which no considerable differences in relation to number of recovered oocytes, maternal age and percentage of fertilized oocytes during ICSI was observed (Miller and Smith, 2001).

Advancing maternal age did show a great impact on the number of pregnancies by our group. Silber SJ *et al*. conducted an investigation which proved that age of female had no significant effect on the number of successful pregnancies (Silber *et al.,* 1997). The rate of preclinical abortions in group D was considerably higher which is in contrary to the results of a study in which females undergoing fertilization by ICSI had similar rates of abortions irrespective of age (Ulug U *et al.,* 2006). These results are supported by Staessen C. *et al*. which showed a higher number of pregnancy loss after assisted reproductive techniques (ART) with advancement of age (Staessen *et al.,* 2004).

This is a unicentric study with a very small sample size, however is the first study in Pakistan to draw evidence-based attention on awareness of age on reproductive outcome. The information obtained will help the women to know about the failure of treatment procedures, spontaneous pregnancy loss and likelihood of chromosomal abnormalities with progressive ageing and postponement of treatment.

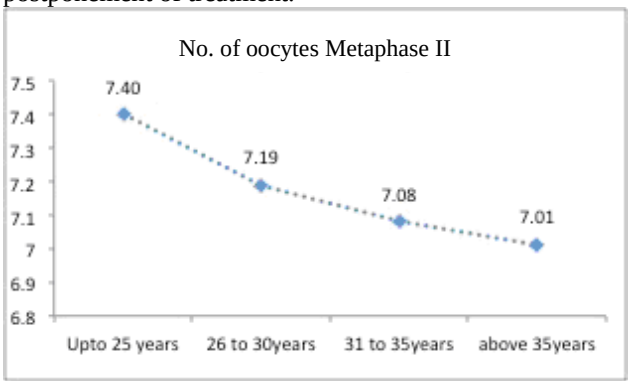

Fig. 1: Effect of age on maturity of oocytes.

CONCLUSION

Female age is the noteworthy variable that can be blamed for poor reproductive outcome as a result of changes that lead to failure of maturation of oocyte and fertilization after intracytoplasmic micro injection of spermatozoa.

REFERENCES

Chang MY, Chiang CH, Hseih TT, Soong YK and Hsu KH (1998). Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil. Steril.*, **69**(3): 505-510.

Dunson DB and Baird DD (2004). Increased infertility with age in men and women. *Obstet. Gynecol*., **103**(1): 51-56.

Baird DT, Collins J, Egozcue J, Evers LH, Gianaroli L, Leridon H, Sunde A, Templeton A, Van Steirteghem A, Cohen J, Crosignani PG, Devroey P, Diedrich K, Fauser BC, Fraser L, Glasier A, Liebaers I, Mautone G, Penney G, Tarlatzis B; ESHRE Capri Workshop Group (2005). Fertility and aging. *Hum. Reprod. Update*, **11**(3): 261-276.

Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE Working Group on Poor Ovarian Response Definition (2011). ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: The Bologna criteria. *Hum. Reprod.*, **26**(7): 1616-1624.

Hassold T and Hunt P (2009). Maternal age and chromosomally abnormal pregnancies: what we know and what we wish we knew. *Curr. Opin. Pediatr.*, **21**(6): 703-708.

Kim YJ, Lee JE, Kim SH, Shim SS and Cha DH (2013). Maternal age-specific rates of chromosomal abnormalities in Korean pregnant women of advanced maternal age. *Obstet. Gynecol. Sci*., **56**(3): 160-166.

Marca AL, Spada E, Sighinolfi G, Argento C, Tirelli A, Giulini S, Milani S, Volpe A. (2011). Age-specific nomogram for the decline in antral follicle count throughout the reproductive period. *Fertil. Steril.*, **95**(2): 684-688.

Miller JE and Smith TT(2001). The effect of intracytoplasmic sperm injection and semen parameters on blastocyst development *in vitro*. *Hum. Reprod.*, **16**(5): 918-924.

Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. (2005). Antral follicle count, anti-mullerian hormone and inhibin B: Predictors of ovarian response in assisted reproductive technology? *BJOG. Int. J. Obstet. Gy*., **112**(10): 1384-1390.

Oehninger S, Veeck L and Lanzendorf S Maloney M, Toner J, Muasher S (1995). Intracytoplasmic sperm injection: achievement of high pregnancy rates in couples with sever male factor infertility is dependant primarily upon female and not male factors. *Fertil. Steril*., **64**(5): 977-981.

Palermo GD, Cohen J and Alikani M, Adler A and Rosenwaks Z (1995). Intracytoplasmic sperm injection: A novel treatment for all forms of male factor infertility. *Fertil Steril*. **63**(6): 1231-1240.

Rehman R, Fatima K, Hussain M, Khan R and Fatima SS (2015). Predictors of number of oocytes in intra cytoplasmic sperm injection. *Pak. J. Med. Dent.,* **4**(1): 3-8.

Rehman R, Jawed S, Zaidi SF and Ahmad K (2015). Role of Interleukin-I β in conception after intracytoplasmic sperm injection. *J. Pak. Med. Assoc*., **65**: 49-53.

Rehman R, Mustafa R, Hoor T, Khan R and Gul H (2015). Importance of estimation of follicular output rate (FORT) in females assisted by intracytoplasmic sperm injection. *Int. J. Reprod. Contracept. Obstet. Gynecol*. **4**(1): 131-139.

Rehman R, Jawaid S, Gul H and Khan R (2014). Impact of peak estradiol levels on reproductive outcome of intracytoplasmic sperm injection. *Pak. J. Med. Sci*., **30**(5): 986-991.

Scheffer GJ, Broekmans FGM, Dorland M, Habbema JD, Looman CW and te Velde ERM (1999). Antral follicle counts by transvagnial ultrasonography are related to age in women with proven natural fertility. *Fertil. Steril*., **72**(5): 845-851.

Silber SJ, Nagy Z, Devroey P, Camus M and Van Steirteghem AC (1997). The effect of female age and ovarian reserve on pregnancy rate in male infertility: treatment of azospermia with sperm retrieval and intracytoplasmic sperm injection. *Hum. Reprod*., **12**(12): 2693-2700.

Spandorfer SD, Avrech OM and Colombero LT, Palermo GD and Rosenwaks Z (1998). Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. *Hum. Reprod*., **13**(2): 334-338.

Staessen C, Platteau P and Assche EV, Michiels A, Tournaye H, Camus M, Devroey P, Liebaers I and Van Steirteghem A (2004). Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: A prospective randomized controlled trial. *Hum. Reprod*., **19**(12): 2849-2858.

Ulug U, Tosun S and Jozwiak EA , Mesut A, Sismanoglu A and Bahceci M (2006). Subclincal pregnancy losses among women undergoing in-vitro fertilization with ICSI. *J. Assist. Reprod. Genet*., **23**(6): 261-267

Wong MYW and Ledger WL (2013). Is ICSI risky? *Obstet. Gynecol. Int*. 2013:473289. doi: 10.1155/2013/ 473289. Epub 2013 Feb 26.