The Impact of Maternal Age on Intracytoplasmic Sperm Injection (ICSI) Outcomes in Infertile Couples

Rabab Zahir[1], Al-yasiry[1], Haythem Ali Alsayigh[2], Muhjah Falah Hasan[3], Nawras Najah Alkafaji[1]

[1]Assistant Lecturer, College of Medicine, University of Babylon/Iraq, [2]Assistant Prof., College of Medicine, University of Babylon/Iraq, [3]Assistant Lecturer, College of Medicine, Karbala University/Iraq

Abstract

Objective: The aim of the study is to investigate the impact of maternal age on ICSI outcomes represented by the number of retrieved oocytes, number of fertilized oocytes, embryo quality and pregnancy rate in infertile couples undergoing ICSI.

Patients and Method: The study included sixty two infertile couples who underwent ICSI cycles. They divided in to two groups depending on females' age: group I with age less than 35 years and group II with age more than 35 years. Assessment of ICSI outcomes in form of retrieved oocytes number, fertilized oocytes number, quality of embryo and rate of pregnancy were done and the results were compared between both groups.

Results: The study showed that females whom their age more than 35 years produced significantly lower oocytes number with a lower fertilized oocytes when compared with females younger than 35 years. (the mean total number of retrieved oocytes 3.24±0.52 vs 10.84±0.81 and the mean total number of fertilized oocytes 2.29±0.26 vs 7.62±0.52, p-value =0.002 and 0.02 respectively. The total number of good quality embryos was significantly less in the females older than 35 years 1.12±0.18 vs 6.80±0.46, p-value=0.003 and the total number of bad quality embryos was significantly more 1.47±0.24 vs 0.60±0.1, p-value=0.02 when compared with females younger than 35 years. Pregnancy rate was significantly less in the older females p-value=0.0005.

Conclusion: The maternal age is one of the most important predictor of ICSI outcomes. An advanced females age has a negative impact on ICSI outcomes and females older than 35 years usually produced lower number of oocytes during oocytes' retrieval with a lower fertilization rate. Embryos produces from those females usually of bad quality and exhibited a lower implantation potential.

Keywords: *Sperm injection; Age; health; infertile couple.*

Introduction

Assisted reproduction; in vitro fertilization (IVF)/ ICSI is considered the final treatment modality for couples who fail to achieve pregnancy(1). ICSI is firstly used to treat infertile men and today, it becomes the most commonly applicable micromanipulator technique of all assisted cycles being used for most of infertility cases other than male factor infertility.

The international committee for assisted reproductive technologies monitoring was reported that ICSI was applied in 65% of cycles in Europe and this is highlighted in some areas of the world, ICSI is practiced in 100% of in vitro cycles(2).

It has been showed that older infertile women are at high risk of having poor ovarian reserve and producing

Corresponding Author:
Rabab Zahir Al-yasiry
Assistant Lecturer, College of Medicine, University of Babylon/Iraq
e-mail: Rababzahir7@gmail.com

bad quality oocytes following traditional medical infertility treatments, which makes ICSI of a great benefit in this group[3]. However, multiple factors tend to affect ICSI outcomes such as female age and reserve of ovaries[4]

The advance maternal age is defined 35 years or more at time of childbirth[5], it has been revealed that females' age is one of main important factors of reproductive outcome and fertility[6,7]. The chance of getting pregnancy after natural or assisted conception is highly dependent on maternal age.

The poor ovarian reserve and reduced the developmental competence of oocytes are the main reasons for age related infertility[8]. It has been reported that advanced female age was considered one of the factors that leads to oocyteaging, which may cause atypical fertilization and abnormal development, such as arrestdivision, implantation failure and abortion[9]. Inaddition, the aneuploidy rate hasbeen increased in the oocytes andembryos, which are produced fromold women[10]. Women with an age of 38 years have poor outcomes and this negative impact is increased with increasing age over 40 years[11].

Patients and Method

All included couples were taken from the IVF clinic in Fertility Center, AlSader Medical City, Al-Najaf Al-Ashraf in Iraq. All of them were assessed by history, physical examination, fertility investigations at the second day of the female cycles (Estrogen (E2), follicle stimulating hormone (FSH), lutenizing hormone (LH) prolactin, trans-vaginal ultrasound (TVUS) for assessment of ovarian reserve and endometrial thickness) and male partners' seminal fluid analysis. All couples were subjected to ICSI due to unexplained infertility. They divided into 2 groups according to female partners' age;GI: females whom their age between 20-34 years (n=45) and GII females whom their age between 35-45 years (n=17).Female partners were subjected to controlled ovarian stimulation from cycle day two by gonadotropin releasing hormone GnRH antagonist (Cetrotide 0.25 mg*1) followed by gonadotropins stimulation (Follitrope 75 iu*2) for 7-10 days to induce development of multiple follicles. When TVUS showed 6 or more follicles of size 18 mm, Human Chorionic Gonadotropin (hCG): Pregnyl 5000 iu *2 was given to induce final maturation of oocytes and all these were under the continuous monitoring by TVUS and serum E2 assay. Ovum pick up was performed under general anesthesia and TVUS 34-36hr after hCG injection. The retrieved follicles were denuded chemically by hyaluronidase enzyme and mechanically by frequent pipetting of cumulus-oocyte complex through different size pipettes. Only metaphase II (MII) oocytes are considered mature and used for injection. Preparation ofthe ejaculated semen to obtain viable sperms was done concomitantly by direct swim-up from pellet (according to WHO,2010). Injected oocytes were washed and incubated in culture media under 5% CO2 and 37Ċ. Normally the fertilized oocytes displayed two pronuclei and two polar bodies 16-18h after injection. Subsequent monitoring of the embryo quality was done based on blastomeres (number, shape and equality), nucleation and the percentage of fragmentations. The embryos were classified as good quality (grade I & II) when they have 4 cells at 48 hr or 6-8 cells 72 hr post injection,with even sized blastomeres and little or no fragmentation. Anything else were classified as bad quality embryos (grade III & IV)[12].

Up to 3 embryos(I,II) were delivered to the uterus at day 3 after injection under TVUS guidance followed by luteal phase support by vaginal progesterone; duphaston tablets 400 mg*2, aspirin tablets 100mg *1 and folic acid tablets 5 mg*1. The pregnancy was diagnosed chemically by measuring B-hcg titer in the serum of females whom underwent embryo transfer was performed 14 day after embryo transfer(ET). Pregnancy rate calculation was done by dividing the number of females who get on the total number of females whom embryos were transferred to the uterus *100%.

Study design & Statistical analysis: The study is cross sectional, observational done prospectively. Statistical analysis was done by SPSS version 20 by measuring mean and standard deviation for the continuous data using t-test. A p-value <0.05 was significant statistically.

Results

Table (1) reveals cycle day 2 hormonal profile, duration of infertility and endometrial thickness in the studied groups, no significant differences were presented in both groups except duration of infertility which was significantly longer in the older females, p-value=0.007.

Table(1): The hormonal levels, duration of infertility and endometrial thickness among study groups.

Parameters	Group I (age 20-35 years), (n=45) (mean ± SE)	Group II (age 3-45 years), (n=17) (mean ± SE)	P-value
Duration of infertility (day)	7.33±0.56	9.65±1.61	0.007
FSH (iu/l)	5.23±0.26	5.72±0.75	0.08
LH (iu/l)	4.36±0.41	3.06±0.41	0.11
E2 (pg/ml)	32.94±1.89	37.36±3.58	0.88
Endometrial thickness (mm)	4.17±0.11	3.88±0.18	0.56

Table (2) exhibits the response to controlled ovarian stimulation represented by duration of stimulated cycles, mean total number of retrieved oocytes, mean total number of fertilized oocytes and embryo quality in both groups. With the exception of cycle duration, there was a significant variation in both groups regarding these parameters being less in the females of age 35-45 years.

Table (2): A comparison of duration of stimulated cycles, number of retrieved oocytes, number of fertilized oocytes and embryos' quality between both groups.

Parameters	Group 1(age 20-34 years), (n=45) Mean±SD	Group II(age 35-45 years), (n=17) Mean±SD	P-value
Duration of cycles (days)	10.56±0.25	10.65±0.63	0.07
No of retrieved oocytes	10.84±0.81	3.24±0.52	0.002
No of fertilized oocytes	7.62±0.52	2.29±0.26	0.02
No. of embryos (I, II)	6.80±0.46	1.12±0.18	0.003
No. of embryos (III, IV)	0.60±0.1	1.47±0.24	0.02

Discussion

Females' age is the main important factor affecting her fertility and her chance of getting a baby. In most women,ovarian aging will have started before any changes in their menstrual cycle; therefore, they may be at high risk of reduced fertility[5]. Advanced women age have been associated with high rate of maternal and obstetrical complications such as maternal death, fetal and neonatal death, maternal hypertension, prematurity and operative delivery[13]. Many authors reported that poor IVF outcomes have been associated with increasing of maternal age[14]. Some centers of IVF restrict the maternal age for IVF as 43 years[10]. Older women have poor response to controlled ovarian hyper stimulation, decreased number of retrieved oocytes, decreased number of fertilized oocytes, poor quality embryos, low implantation rate, lower pregnancy rate, higher miscarriage rate and birth defects[9,11,15,16].

The present study showed that retrieved oocytes, fertilized oocytes, embryo quality and pregnancy chance depend primarily on maternal age. Some results were consistent with our findings[16]. Other authors reported that the outcome of IVF (retrieved oocytes number) was highest in women aged less than 30 years with poor quality embryo and lower percentage of pregnancy were observed in older women[11,15,17-19]. However, some reports, which exhibited, that there is no correlation between maternal age and pregnancy rate are still present[20]. This could be related to the facts that aging process will lead to ovulatory dysfunction, poor ovarian reserve which in turn lead to producing little number oocytes with poor developmental potential, reducing the chance of getting good quality embryos with a high implantation potential[21-26].

Conclusion

The maternal age is one of the most important predictor of ICSI outcomes. An advanced female's age has a negative impact on ICSI outcomes and females older than 35 years usually produced lower number of oocytes during oocytes' retrieval with a lower fertilization rate. Embryos produces from those females usually of bad quality and exhibited a lower implantation potential.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

Funding: Self-funding

References

1. TURHAN, Nilgün, et al. ICSI outcome in severely oligoasthenozoospermic patients and its relationship to prewash progressive sperm motility. Turkish Journal of Medical Sciences, 2011, 41.6: 995-999.
2. DYER, Silke, et al. International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology 2008, 2009 and 2010. Human reproduction, 2016, 31.7: 1588-1609.
3. TANNUS, Samer, et al. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. Human Reproduction, 2016, 32.1: 119-124.
4. SHRIDHARANI, And; SANDLOW, Jay I. Vasectomy reversal versus IVF with sperm retrieval: which is better?. Current opinion in urology, 2010, 20.6: 503-509.
5. Ishikawa T, Shiotani M, Izumi Y, Hashimoto H, Kokeguchi S, Goto S, et al. Fertilization and pregnancy using cryopreserved testicu–lar sperm for intracytoplasmic sperm injection with zoospermia. FertilSteril 2009;92:174-9.
6. KARIM, Shilan Hussein. The effect of maternal age on the outcomes of in vitro fertilization Sulaimani region. Mustansiriya Medical Journal, 2016, 15.1: 70-74.
7. De Mouzon J, Goossens V, Bhattacharya S, et al. Assisted reproductive technology in Europe,2006: results generated from European registers by ESHRE. Hum Reprod, 2010, 25: 1851–1862
8. Balasch J. Ageing and infertility: an overview. Gynecol Endocrinol,2010, 26: 855–860.
9. CIMADOMO, Danilo, et al. Impact of maternal age on oocyte and embryo competence. Frontiers in endocrinology, 2018, 9.
10. YAN, JunHao, et al. Effect of maternal age on the outcomes of in vitro fertilization and embryo transfer (IVF-ET). Science China Life Sciences, 2012, 55.8: 694-698.
11. Hassold T, Chen N, Funkhouser J, et al. A cytogenetic study of 100 spontaneous abortions. Ann Hum Genet, 1980, 44: 151–178
12. Denny S, David K.Evaluation of embryo quality, analysis of morphology and physiology. In : Textbook of assisted reproductive techniques. David K. and Colin M. (eds.).5th ed. CRC Press Taylor & Francis Group.,2018;225-242.
13. Thum M Y, Abdalla H I, Taylor D. Relationship between women's age and basal follicle stimulating hormone levels with aneuploidy risk in in vitro fertilization treatment. FertilSteril, 2008, 90: 315–321
14. LIU, Kimberly, et al. Advanced Reproductive Age and Fertility: No. 269, November 2011. International Journal of Gynecology & Obstetrics, 2012, 117.1: 95-102.
15. GNOTH, C., et al. Final ART success rates: a 10 years survey. Human reproduction, 2011, 26.8: 2239-2246.
16. HOURVITZ, Ariel, et al. Assisted reproduction in women over 40 years of age: how old is too old?. Reproductive biomedicine online, 2009, 19.4: 599-603.
17. GRIFFITHS, Alison, et al. A cost-effectiveness analysis of in-vitro fertilization by maternal age and number of treatment attempts. Human Reproduction, 2010, 25.4: 924-931.
18. AHMED, Mohamed, et al. Maternal age and intracytoplasmic sperm injection outcome in infertile couples at Khartoum, Sudan. F1000 Research, 2015, 4.
19. Tan TY, Lau SK, Loh SF, et al.: Female ageing and reproductive outcome in assisted reproduction cycles. Singapore Med J. 2014; 55(6): 305–9.
20. SANDALINAS, Mireia, et al. Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. Human Reproduction, 2001, 16.9: 1954-1958.

21. Nouri K, Tempfer CB, Walch K, et al.: Predictive value of the time interval between embryo loading and transfer for IVF/ICSI success: a prospective cohort study. Reprod Biol Endocrinol. 2015; 13: 51.
22. Simpson JL, Lobo RA, Kelsey J, et al.: Genetic programming in ovarian development and oogenesis. Menopause: biology and pathobiology. San Diego,Academic Press. 2000; 77–94.
23. Speroff L: The effect of aging on fertility. Curr Opin Obstet Gynecol. 1994; 6.2
24. Navot D, Bergh PA, Williams MA, et al.: Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet. 1991; 337(8754): 1375–7.
25. Hull MG, Fleming CF, Hughes AO, et al.: The age-related decline in female fecundity: a quantitative controlled study of implanting capacity and survival of individual embryos after in vitro fertilization. Fertil Steril. 1996; 65(4): 783–90.
26. Faddy MJ, Gosden RG, Gougeon A, et al.: Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod. 1992; 7(10): 1342–6.