RESEARCH ARTICLE

Embryo quality in intracytoplasmic sperm injection: A quasi experimental design in Pakistan

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Abstract

Objective: To assess the role of good-quality embryos in acquiring pregnancy after intracytoplasmic sperm injection and to predict factors required for development of embryos.

Methods: The quasi-experimental study was conducted from June 2010 to August 2012 at Islamabad Clinic Serving Infertile Couples and comprised infertile females who received long-term gonadotropin agonist protocol for intracytoplasmic sperm injection. Basal estradiol and antral follicle count was measured at baseline, while estradiol, progesterone and interleukin levels with pre-ovulatory follicle count were evaluated on the ovulation induction day. Follicular output rate was measured as ratio of pre-ovulatory follicle count to antral follicle count. The quality of embryos was graded as good, fair and poor on the third day after the injection. Linear regression was employed to determine unadjusted and adjusted estimate of effect of different factors on good-quality embryo.

Results: There were282 subjects with a mean age of 32.1±4.7 years (range: 23-41 years). There was an inverse correlation of body mass index with good-quality embryo (r=-0.131). In adjusted analyses, basal estradiol and progesterone on the ovulation induction day showed negative effect (95% confidence interval: -1.42 - -1.01), while estradiol and interleukin measured positive effect on quality of embryo ($p<0.05$). Follicular output rate was highly positively correlated with good quality embryo ($p<0.05$).

Conclusion: Females with low body mass index produced good-quality embryos. A high follicular output rate with production of estradiol and interleukin complemented good-quality embryos.

Keywords: Quality of embryo, Intracytoplasmic sperm injection, Antral follicle count, Pre-ovulatory follicle count, Ovulation induction. (JPMA 68: 1451; 2018)

Introduction

Development of medical proficiency has brought new horizons for effective treatment of infertility and giving them hope for conception.[1] Intracytoplasmic sperm injection (ICSI) is a new era in the treatment of untreatable cases of male infertility with poor sperm characteristics, especially due to oligo-astheno-teratozoospermia and azoospermia.[2,3] However, patients undergoing assisted reproductive techniques (ARTs) like in vitro fertilisation (IVF) and ICSI have risks of multiple pregnancies and poor outcome.[4] These risks can be avoided by selection of adequate number of good-quality embryos for transfer in uterus after ICSI.

The quality of embryo plays pivotal role in implantation, pregnancy and delivery rate in patients after ART.[5] Studies suggest that embryo fragmentation, cleavage stage, number of homogeneous transferred embryos have impact on pregnancy outcome after ARTs, including IVF and ICSI.[6] Lack of multinucleated blastomeres, 4-5 blastomeres on day 3, seven or more cells on day 3, $\leq$20% fragments without nuclei and great implantation potential are distinctive qualities of good embryos. Live birth rate after implantation highly depends upon the cleavage of embryo and the resultant development of blastocysts within the first two days. Rapid or slow cleavage of embryos during these days is associated with reduced implantation rates.[7]

It is still an ongoing debate as to whether the quality of embryo can influence further development of embryo and pregnancy outcome. However, a study has shown that the rate of fragmentation in an embryo has an impact on post-implantation development and loss of gestational sacs rate (LGSR).[8] Low delivery rate has been reported with fragmentation rate of more than 20% in comparison with no fragmentation on the 2nd day of development after IVF or ICSI.[8] Which of the embryos should be transferred for better pregnancy outcome is still a point of consideration by a number of researchers. Moreover, the factors which contribute to development of good quality embryos is also hypothesised.

The current study was planned to assess the impact of embryo quality on conception in patients undergoing

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ICSI, and to predict factors which may help in the development these embryos.

Patients and Methods

The quasi-experimental study was conducted from June 2010 to August 2012 at Islamabad Clinic Serving Infertile Couples and comprised infertile females who received long-term gonadotropin agonist protocol for ICSI. Approval was obtained from the Islamabad Clinic Serving Infertile Couples, Islamabad, Pakistan. The sample size was calculated on the basis of a recent study which stated that a minimum of two subjects per variable are required in linear regression model.[9] We used 15 variables in our model and had 18.8 subjects per variable which deemed appropriate for running multiple linear regression.

Those included were females aged 20-40 years, recommended for treatment of ICSI on the basis of infertility due to male factor (e.g. varicocele, prior surgeries and semen abnormalities), female factor (e.g. polycystic ovaries, uterine fibroids, endometriosis and tubal blockade etc.) or unexplained cause for a couple's failure to achieve pregnancy. Females with endocrine disorder, thyroid dysfunction, abnormal prolactin levels and with diagnosed cause of infertility in male as well as female were excluded. All subjects furnished written informed consent before enrolment.

Treatment protocol was followed as described in an earlier study.[10] Before initiation of stimulation, Follicle stimulating hormone (FSH) and basal estradiol were assessed and antral follicle count (AFC) was determined through a trans-vaginal ultrasound scan (TVS). The two ovaries were scanned and volume was measured in longitudinal (D1), antero-posterior (D2) and transverse (D3) dimensions. Basal ovarian volume was acquired by the sum of dimensions of each ovary derived by the formula ($D1 \times D2 \times D3 \times \pi/6$).[10] The pre-ovulatory follicle count (PFC) measured number of oocytes visible on TVS at the end of controlled ovarian stimulation (COH). Follicular output rate (FORT), which reflects follicular response to exogenous recombinant FSH (rFSH), was measured as ratio of PFC to AFC.[11] The steps of ICSI procedure were carried out as described in literature.[12]

Micromanipulation was performed and fertilisation and cleavage was assessed. The quality of embryos was graded as good (Grade I), fair (Grade II) and poor (Grade III) on the basis of cleavage rate and differentiation before embryo replacement. Good-quality embryo had blastomeres of same size, slight or no fragmentation, and a zonapellucida that was not enormously dense in appearance. Fair embryos had blastomeres of same size, little fragments in cytoplasm wrapping <10% of embryo. Poor-quality embryo had blastomeres of noticeably uneven size and moderate-to-significant fragments of cytoplasm, wrapping >10% of embryo external.[13] Results were categorised on the basis of β-subunit of human chorionic gonadotropin (β-hCG) concentrations performed 14 days after egg collection and ultrasound confirmation of an intrauterine gestational sac 14 days after results of β-hCG. Non-pregnant group had β-hCG<25 ml U/ml, and pregnant group had β-hCG>25 ml U/ml, an intrauterine gestational sac with cardiac activity confirmed by TVS.

Serum estradiol was determined by enzyme-linked immunosorbent assay (ELISA) of human estradiol (E2) (Kit Cat. No: 07BC-1111; MP Bio medicals, USA) before stimulation (basal) and on the day of ovulation induction (OI). Serum progesterone and serum interleukin (IL) were estimated by human progesterone enzyme immunoassay (Kit Cat No: KAP 1451 by Bio Source, Belgium and Human Interleukin Enzyme immunoassay Kit Cat. No: KAP 1211 by DIA source Immuno Assays, Belgium) on OI day.

To determine correlation of good-quality embryo (Grade I) with body mass index (BMI), FORT, AFC, PFC, number of oocyte per patient, in metaphase II, fertilised oocytes, estradiol, progesterone and IL levels on OI day, we used Spearman's correlation coefficient. Linear regression was employed to determine unadjusted and adjusted estimate of effect of different factors on good-quality embryo. Results were expressed with the coefficient of regression and 95% confidence interval (CI). If the interval contained zero, then the estimate was considered non-significant. $P<0.05$ indicated significant effect of the related factor. In addition, to check whether good-quality embryo yielded pregnancy, we measured the effect size using mean estimates of good, fair and poor embryos. A threshold of 0.5 was used to consider good effect on pregnancy.

Result

There were 282 females with mean age 32.1±4.7 years (range: 23-41 years), mean BMI 24.2±3.7kg/m2, mean AFC count 14.7±2.8, mean PFC count 7.8±1.87 and mean FORT 54.7±13.6 (range: 31.25-85.71). Mean oocyte number collected was 14.3±0.97 and the mean number of oocytes per patient was 7.7±1.7. Mean estradiol before treatment was 214.7±145.8pg/ml (range: 95-661pg/ml). On OI day,mean progesterone and interleukin were 1.5±0.7, 2323±298 and 116±63.5. Mean basal ovarian volume (BOV) by ultrasound was 11.6±3.1. The good quality embryos varied from 0 to 5 with a mean value of 2.1±1.6 (Table-1).

There was inverse relationship of good-quality embryo

Table-1: Descriptive characteristics of the study population.

	Min.-Max.	Mean ± SD	Median (IQR)
Body Mass Index (kg/m^2)	17-30	24.24±3.69	25 (7)
Female age (years)	23-41	32.11±4.66	32 (8)
Antral follicle count (number)	10-21	14.66±2.8hh	16 (5)
Preovulatory follicle count (number)	4-14	7.8±1.87	8 (3)
Rate of Follicular Output	31.25-85.71	54.69±13.6	58.82 (22.92)
Follicle Stimulating Hormone ((mIU/ mL)	4.5-9.8	6.69±1.09	6.5 (1.1)
Day of egg collection	13-16	14.34±0.97	14 (1)
No of oocytes/patient	4-13	7.69±1.67	8 (3)
No of oocytes Metaphase II	1-12	7.13±1.96	7 (2)
No of oocytes fertilized	1-10	5.95±1.56	6 (2)
Estradiol before treatment (pg/ml)	94.96-660.68	214.74±145.83	135.58 (177.19)
Progesterone at Day of Ovulation Inductionng/ml	0.23-3.8	1.47±0.74	1.38 (1.21)
Estradiol at Day of Ovulation Induction (pg/ml)	1402-3001	2322.97±298.22	2346.64 (185)
Interleukin at Day of Ovulation Inductionpg/ml.	26.2-272.5	115.95±63.51	116.65 (103.1)
Ovarian Volume by Ultrasound	5.28-23.52	11.55±3.07	11.25 (4.2)
Good Quality embryo (Grade I)	0-6	2.05±1.58	1 (2)

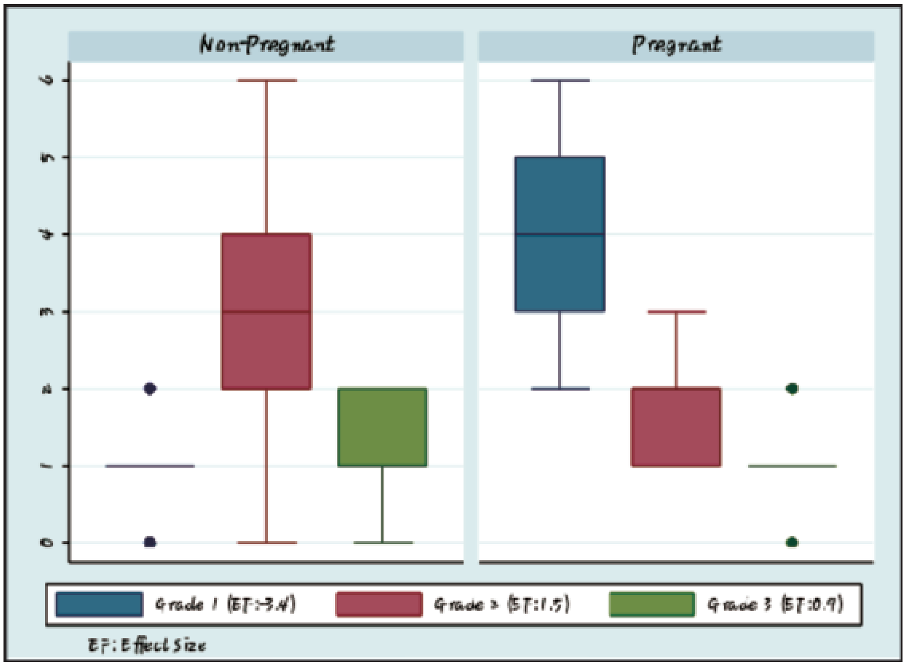
SD: Standar deviation
IQR: Interquartile range.

Table-2: Predictors of embryo quality.

	Correlation†	Unadjusted		Adjusted	
		Constant (95%CI)	Coefficient (95%CI)	Constant (95%CI)	Coefficient (95%CI)
Body Mass Index	-.131*	3.39 (2.16-4.62)	-0.06 (-0.11--0.005)	-2.77 (-10.22-4.67)	0.02 (-0.03-0.06)
Female age (years)	-0.004	2.34 (1.04-3.64)	-0.01 (-0.05-0.03)		-0.02 (-0.05-0.02)
Antral follicle count (number)	-.400**	4.85 (3.91-5.78)	-0.19 (-0.25--0.13)		0.09 (-0.31-0.49)
Preovulatory follicle count	.400**	-0.49 (-1.23-0.25)	0.33 (0.23-0.42)		-0.28 (-1.09-0.54)
Follicular Output Rate	.636**	-1.78 (-2.40--1.17)	0.07 (0.06-0.08)		0.05 (-0.06-0.16)
Follicle Stimulating Hormone	-.337**	5.50 (4.41-6.58)	-0.52 (-0.68--0.36)		-0.05 (-0.27-0.17)
Day of egg collection	-0.049	3.44 (0.69-6.19)	-0.10 (-0.29-0.09)		-0.02 (-0.20-0.16)
No of oocytes/patient	.413**	-0.97 (-1.77--0.17)	0.39 (0.29-0.49)		0.05 (-0.82-0.92)
No of oocytes Metaphase II	.563**	-1.19 (-1.77--0.61)	0.45 (0.38-0.53)		0.36 (-0.13-0.85)
No of oocytes fertilized	.563**	-1.32 (-1.93--0.71)	0.57 (0.47-0.67)		-0.01 (-0.56-0.54)
Estradiol before treatment	.414**	1.26 (0.95-1.57)	0.004 (0.002-0.005)		-0.001 (-0.002-0.000)
Progesterone at Day of Ovulation Induction	-.583**	3.84 (3.50-4.18)	-1.22 (-1.42--1.01)		-0.62 (-0.90--0.35)
Estradiol at Day of Ovulation Induction	.547**	-3.6 (-4.9--2.3)	0.002 (0.002-0.003)		0.001 (0.000-0.001)
Interleukin at Day of Ovulation Induction	.590**	0.8 (0.4-1.3)	0.015 (0.011-0.018)		0.004 (0.001-0.008)
Ovarian Volume via Ultrasound	-.187**	3.0 (2.3-3.7)	-0.08 (-0.14--0.02)		-0.03 (-0.09-0.02)

†: * P value <0.05, ** P value < 0.01.
CI: Confidence Interval.

with BMI (r=-0.131). With one kg/m^2 increase of BMI, the unadjusted significant decrease in good-quality embryo was ground up to 0.06 grade. The magnitude of correlation of AFC and PFC was highly significant, but both depicted opposite effect ($p<0.05$). With the increase of AFC, good quality embryo decreased to 0.19 (95% CI: -0.25- -0.13) unit while PFC elevated the grade up to 0.33 (95% CI: 0.23-0.42) unit. FORT yielded highly significant correlation with good quality embryo ($p<0.05$). With one unit increase of FSH, good-quality embryo significantly reduced to 0.52 (95% CI: -0.68 - -0.36) units. One oocyte increase at initial phase, metaphase and fertilisation phase surged good-quality embryo to 0.39, 0.45 and 0.57 unit respectively. Estradiol before treatment had significantly positive effect on good quality embryo ($p<0.05$). Increase in progesterone on OI day significantly reduced the grade up to 1.22 (95% CI: -1.42 - -1.01) units. The unadjusted effect of estradiol and IL on the OI day was significantly positive ($p<0.05$). Larger volume of ovary caused decrement in the quality of embryo. After



Grading based on quality of embryo:
Grade 1- Good
Grade 2- Fair
Grade 3- Poor

Figure: Effect of embryo grades on pregnancy status.

adjustment, multiple regression analyses suggested that, holding the effect of all other variables, estradiol before treatment, progesterone, estradiol and IL on OI day were independent predictors for good-quality embryo. In adjusted analyses, estradiol before treatment and progesterone on OI day showed negative effect, while estradiol and IL on OI day showed positive effect on quality of embryo (Table 2).

Good-quality embryos in subjects who did not get pregnant was 3.4 times less than those who did get pregnant (Figure). Fair and poor-quality embryos in non-pregnant females was 1.5 and 0.9 times more than in average pregnant females.

Discussion

The success in ART depends on the quality of transferred embryos.[14] Our study showed that good-quality embryos helped in acquiring pregnancy which is similar to a study in which pregnancy outcome and live birth after ICSI was based on the grading of embryo on the basis of cleavage and differentiation of blastomeres.[8]

Female's age is an analytical factor to estimate chances of success after ART procedures in infertile couples and an obvious failure in attainment of successful pregnancy; its outcome rate is witnessed as the age increases.[14] It is expected that embryo quality is better if females opt for ICSI at younger age. According to a research, irrespective of the quality, increase in quantity of shifted embryos (from one to two) improved pregnancy rate in patients above 30 years of age.[15] Another study recounted that older the patient becomes, the higher is the FSH level which makes the implantation rate lower.[16] A study documented that increasing age causes decrement in the number of follicles, retrieved oocytes and number of embryos.[14] However, we observed no such association in our patients. The current study showed that the female age was not a predictor of good-quality embryos.

Results also showed that BMI was a significant factor in the prediction of embryo quality. The finding is supported by studies which state that increase in BMI is correlated with decreased rate of pregnancy in normal and assisted methods of conceptions.[17,18] But one study reported that an increase in BMI did not affect IVF outcome though pre-conceptual counselling for reduction in BMI helped in pregnancy-related complications.[19] Another study also contradicts our study by stating that increased BMI does not affect oocyte quality or clinical pregnancy rates.[20]

The volume of estradiol produced by the follicles in a stimulated cycle is considered to be an important element of success in ART.[21] Our results show that OI estradiol (E2) levels was a significant predictor of good-quality embryo as well. One study supported our findings, stating that higher serum E2 level correlates with better oocycte and embryo quality.[22] A study also proposed that elevated E2 levels increase number of oocytes and embryos with provision of good-quality embryos for transfer and cryopreservation.[23]

In the field of ART, increased basal FSH and poor embryo quality remains a subject of excited debate. The current study shows that low FSH level is a significant predictor of good-quality embryo. The main problem of high FSH documented so far is decreased follicular response to oocyte stimulation. However, if these women complete stimulation and embryo transfer, chances of clinical pregnancy and viable delivery are related to women of their own age.[24] Another study suggests that females with raised FSH levels should attempt at least one cycle of IVF.[25]

FORT, considered to be an indicator of follicular response to FSH, was found to exert a positive impact on the quality of embryo and reproductive competence after ICSI. Similar results were observed by researchers who observed that increase in FORT improved quality of embryos, implantation and clinical pregnancy rates.[12,26]

The current study is limited since because of its uni-centre nature with a relatively small sample size. Moreover, we did not estimate concentration of anti-mullerian hormone (AMH) which can be a predictor of oocytes retrieved and embryo quality. However, this is the first study in Pakistan that highlights the factors which point towards the development of good-quality embryos required for implantation of embryo after ICSI.

Conclusion

Successful implantation after ICSI is facilitated by transfer of good quality of embryos. The factors which improve the quality are low BMI, high FORT and increase in production of E2 and IL during the development of embryos in ICSI cycles.

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