Iranian Journal of Reproductive Medicine Vol.5. No.1. pp:23-27, Winter 2007

Role of embryo morphology in Intracytoplasmic Sperm Injection cycles for prediction of pregnancy

Mir Mehrdad Farsi, Ph.D., Ali Jorsaraei, Ph.D., Mahmood Hajiahmadi, Ph.D., Sedigheh Esmaelzadeh, M.D.

Babol University of Medical Sciences, Babol, Iran.

Received: 19 June 2006; accepted: 22 September 2006

Abstract

Background: Multiple factors have been suggested for prediction of pregnancy in Intracytoplasmic sperm injection (ICSI) cycles such as the number of injected oocytes, fertilization rate, embryo morphology and quality of transferred embryos. Predictive value of these factors is important in ICSI outcome.

Objectives: To evaluate the role of embryo morphology for prediction of pregnancy in ICSI cycles.

Materials and Methods: This retrospective study was done on 97 patients who were treated by ICSI in Fatemeh Zahra Fertility and Infertility Centre from April 2004 to March 2005. Number of retrieved oocytes, number of injected oocytes, fertilization rate, zygote morphology, rate of cytoplasmic fragmentation, number of four cell transferred embryos, and quality of embryo transfer, as predictors of pregnancy in ICSI cycles were evaluated. The results analysed by T-test, Mann-Whitney U test and Fisher's exact test. Logistic regression was used to estimate the significance of variables in the prediction of pregnancy probability.

Results: Out of 97 patients, 42 cases of pregnancy were detected (Pregnancy rate: 43.3%). The number of four cell transferred embryos was 112 (53.84%) in pregnant group. Pregnancy occurred in 33 (58.9%) patients with at least one good quality zygote. The mean number of four cell transferred embryos and the quality of zygotes had significant difference between pregnant and not pregnant groups ($p=0.006$ and $p=0.000$ respectively). In logistic regression analysis, the number of four-cell transferred embryos ($p=0.007$) and the quality of zygotes ($p=0.003$) were significant predictors of the pregnancy outcome.

Conclusions: Our results suggest that the number of four-cell transferred embryos with $\leq$ 15% cytoplasmic fragmentation and zygotes with centralized, apposed and polarized pronuclei in women <38 years old are significant predictors for pregnancy in ICSI cycles.

Key words: *Intracytoplasmic sperm injection, Pregnancy prediction, Zygote morphology, Four-cell transferred embryos*

Introduction

The predictive value of factors involving in intracytoplasmic sperm injection (ICSI) outcome is important in assisted conception treatment. Sperm parameters (1-3), parental age (4), skill of embryologist (5), and morphology of embryos are indicated as important predictors in ICSI outcome (6-11). The selection of embryos at early stages of development are proposed based on pronuclear orientation, polar body placement (6), number and distribution of nucleolar precursor bodies in each pronucleus in zygote stage (8) and number, fragmentation and equal size of blastomers for embryos in day 2 or 3 (10). These data suggest that developmental fate of human embryos is predictable by using these criteria. Tesarik and Greco (1999) found a strong correlation between pronucleus scoring system and implantation rate in a retrospective analysis (8). There is also clear correlation between embryo morphology and implantation rate. Faster cleaving embryos implant at a higher rate. However,

Correspondence Author:
Dr. Mir Mehrdad Farsi, Fatemeh Zahra Fertility and Infertility Centre, Babol, Iran.
E-mail: m_farsica@ yahoo.com

[Downloaded from ijrm.ir on 2024-10-12]

embryo morphology and cleavage rate alone do not always result high implantation of potential embryos (10). Furthermore, poor quality embryos may implant and result multiple pregnancy, whereas pregnancy might not occur despite the good quality transfer.

The clinical validation of these criteria is still questionable and more studies are needed. This study was focused on the role of embryo morphology in ICSI cycles for prediction of pregnancy. The embryo morphology was recorded in pregnant and not pregnant groups and the differences were analysed statistically. The number of retrieved oocytes, number of injected oocytes, fertilization rate, and quality of embryo transfer, were also evaluated as predictors.

Materials and methods

Patients

In total 97 couples, who were treated by ICSI procedure in Fatemeh Zahra Fertility and Infertility Centre from April 2004 to March 2005, were included in this study. The basal levels of FSH were ≤10 IU/L in all women. To exclude the influence of female age on analysis, women >38 years old were excluded.

Oocyte stimulation:

The standard long protocol of the pituitary suppression with gonadotropin releasing hormone (GnRH) was used in all patients. After 17 days of oral contraceptive, pre-treatment buserelin subcutaneous injection (Superfact; Hoechst, Frankfurt) was administrated at a daily dose of 0.5 cc until hMG (Pergonal; Serono,S.p.A., Rome) initiation on the second or third day of next cycle. On the day of initiation of hMG, the dose of buserelin was modified to 0.25 cc until hCG injection. The hMG was administrated at a daily dose of 150-300 IU for 6-7 days. The dose was modified according to the response. Transvaginal ultrasound was performed every 2-3 days to evaluate follicular size, number and quality. When the largest measured follicle(s) reached a maximum mean diameter of 18-19 mm, 10000 IU hCG (Pregnyle, Organon, Darupakhsh) was administrated intramuscularly. Oocytes were retrieved 36-38 hours after hCG injection through a transvaginal route.

Sperm preparation:

All semen samples were collected by aid of female partner at the clinical andrology laboratory, after an abstinence period of 3-5 days, on the morning of ovum pick-up. In general, routine sperm analysis was performed to measure sperm concentration, as well as percentage of motility and normal morphology. Swim-up washing was completed for all specimens.

Oocyte preparation, pre-embryo culture and transfer to uterine cavity

Immediately after the collection, oocyte cumulus complexes were put in ham's F_{10} medium containing 0.1 mg/ml hyaluronidase (Sigma, Aldrich Co., Germany) for maximum of 30 seconds. With hand-drawn glass pipette, oocytes were denuded completely. ICSI was performed in all M_{II} oocytes with normal appearance. Maximum of four injected oocytes were transferred to a drop of 20-30µl conventional G_{III} medium (Vitrolife, Sweden), and it was incubated at least 6 hours in 6% CO_2 air under mineral oil (Nidoil, Nidacone, Sweden). Injected oocytes were evaluated under an inverted light microscope (TE 300, Nikon, Japan) at × 200 magnifications for presence of two pronuclei (PN) 16-20 hours later. Zygotes with 1, 3 PN or more and 2PN with significant difference in size and vacuolated zygotes were eliminated. Zygotes were evaluated as good; if two pronuclei were centralized, apposed, and polarized. Zygotes let further culture for 24-30 hours. Embryos were assessed based on number of blastomeres and percentage of cytoplasmic fragments.

If there were four-cell embryos with <5% fragmentation, maximum of three embryos were transferred into the uterine cavity. Size of blastomeres and status of nucleus in blastomeres were not considered. Assisted hatching was performed mechanically in all pre-embryos, except for the cases that zona pellucida was hatched easily. Embryos were transferred in maximum of 30-40 µl media (G_{III}) by using ultrasound guided catheter (Frydman, CCD, France) and expelled at midpoint of uterine cavity. The quality of embryo transfer (bloody cervix only) was recorded in each case.

Luteal supplementation

The luteal phase was supplemented with a vaginal administration of 800 mg of natural progesterone (Cyclogest; Hoechst) daily beginning 24 hours after ovum pick-up, and continued until 8 weeks' gestation if pregnancy was occurred.

Outcome measures

Pregnancy test (β-hCG) was performed 15-16 days after hCG administration. A positive test of pregnancy was followed with an ultrasound to detect gestational sac at 5 weeks after menstrual age.

[Downloaded from ijrm.ir on 2024-10-12]

Statistical analysis

Statistical analysis including; T-test, Mann-Whitney U test and Fisher's Exact test were performed using SPSS (Version 10.05) software. Logistic regression was used to estimate the significance of variables in the prediction of pregnancy probability. p-value<0.05 was considered to be statistically significant.

Results

From the total of 97 couples treated by ICSI, 42 cases of pregnancy were detected (Pregnancy rate: 43.3%). In total 1140 Oocyte Cumulus Complex were collected, 91.66% of which were M_{II} (table I). Overall 259 out of 541 fertilized oocytes were in pregnant group. Pregnancy occurred in 33 (58.9%) patients with at least one good quality zygote (table II). The number of four-cell transferred embryos that resulted in pregnancy was 112 (53.84%). The mean and standard deviation of female age were 28.5 and 5.08 respectively in pregnant group. The mean number of four-cell transferred embryos and the quality of zygotes had significant difference between pregnant and not pregnant groups (p=0.006 and p=0.000 respectively; table I,II). In logistic regression analysis, quality of zygotes (p=0.003) and number of four-cell transferred embryos (p=0.007) were significant predictors (table III). Nagelkerke R^2 indicates that probability of analyzed factors for prediction of pregnancy was up to %32. The addition of female age, quality of embryo transfer and progressive motility of sperms as predictors to the L.R. model increased Nagelkerke R^2 from 0.32 to 0.34 (data not shown). These factors were not significant predictors.

Table I. Characteristics of patients in our study

	Pregnant group	Total	Significance of difference (P)
No. of patients (%)	42 (43.3)	97	
Female mean age (SD)	28.5 (5.08)		0.232[a]
No. of retrieved oocytes (%)	549 (48.16)	1140	
Mean (SD)	13.1(5.69)		0.068[a]
No. of injections (%)	498 (47.65)	1045 (91.66)	
Mean (SD)	11.9 (5.44)		0.168[a]
No. of fertilization (%)	259 (47.87)	541 (51.77)	
Mean (SD)	6.5 (2.94)		0.100[a]
No. of four-cell transferred embryos (%)	112 (53.84)	208 (38.44)	
Mean (SD)	2.7 (1.71)		0.006[b]

[a] *Statistical analysis with the T- test.*
[b] *Statistical analysis with the Mann-Whitney U test.*

Table II. Comparison of pregnancy between good and not good quality zygotes

	Pregnant group N (%)	Total N	Significance of difference (P)
Good (%)	33 (58.9)	56	
Not good (%)	9 (22.0)	41	0.000[a]
Total	42	97	

[a] *Statistical analysis with Fisher's Exact test.*

Table III. Comparison of pregnancy between <5% and <15% fragmented four-cell embryos

	Pregnant group N (%)	Total N	Significance of difference (P)
<5% fragmentation (%)	31 (49.2)	63	
<15% fragmentation (%)	11 (32.4)	34	0.135[a]
Total	42	97	

[a] *Statistical analysis with Fisher's Exact test.*

Discussion

Although ICSI is a well developed method in assisted conception treatment, the predictive value of the factors that affect its success rate is of great interest. Multiple factors have been suggested for prediction of pregnancy in ICSI cycles (4, 5, 7, 10). We focused on role of embryo morphology in ICSI cycles for prediction of pregnancy. We also analysed the number of retrieved oocytes, number of injected oocytes, fertilization rate, and quality of embryo transfer statistically. Our study differs from others because we used simple criteria in selection of zygotes based on their morphology and we analysed impact of

[Downloaded from ijrm.ir on 2024-10-12]

four-cell transferred embryos (≤ % 15 fragmentations) in pregnancy without considering other criteria in embryo morphology 48 hours after injection. We also put variables in a logistic regression model to analyse their predictable value in pregnancy. In our study zygote with big difference in size of pronuclei and large cytoplasmic vacuolization were eliminated out of the culture because strong cytoplasmic vacuolization and unequal pronuclei have been indicated as negative factors on blastocyst development (12). It has been shown that the number of retrieved oocytes and the number of mature oocytes decline significantly with advancing maternal age (4).To exclude the influence of female age on analysis, we evaluated couples with a female partner aged <38 years. The number and distribution of nucleolar precursor bodies (NPB) in pronuclei has been noted by some authors (8, 13). According to Tesarik and Greco (1999) classification of pronucleus morphology, good morphology embryos were significantly more frequent for zygotes with <3 difference in the number of NPB as compared with other patients. The distribution of NPBs was either polarized or non-polarized in this group. Out of 44 embryo transfer with at least one mentioned zygote pattern, 22(50%) clinical pregnancy resulted. Balaban *et al* (2001) also indicated that pronuclear morphology based on Tesarik and Greco scoring system is significantly related to cleavage rate and quality of blastocyst. In a group that had at least one blastocyst derived from zero PN pattern, clinical pregnancy/embryo transfer was 50% (13). Although these studies emphasize the effect of NPB number and distribution in embryo quality, proposed categories seems to be complex and using these criteria by embryologist encounters some pitfall in routine practice. Pronuclei morphology criteria used in our study was more simple and practical than the mentioned studies (8,13).

The present study shows that the probability of pregnancy elevates with the increasing of number of four-cell transferred embryos. This result is similar to Shen *et al* (2003) report. They analysed many factors in success of ICSI in a single population (5). In addition, transfer of embryos in four-cell stage 48 hours after injection emphasizes the importance of growth rate in pregnancy (14, 15). It is not unusual that high quality zygotes produce low quality embryos while low quality zygotes become top quality embryos, as indicated before (14). Placido *et al* (2002) indicated that selection of embryos based on morphology of zygote and embryo either independently or together has no predictive value (14). In spite of this study, our analysis shows that the presence of at least one high quality zygote, based on our criteria has predictive value in pregnancy.

The probability of factors for prediction of pregnancy in our study was more than Shen *et al* (2003). Ngelkerke R^2 was 0.32 versus 0.16 respectively (5).

Conclusion

In conclusion, the number of four-cell transferred embryos with ≤ 15% cytoplasmic fragmentation and zygotes with centralized, apposed and polarized pronuclei in women <38 years old have predictive value in pregnancy rate in ICSI cycles. The number of retrieved oocytes, number of injection, fertilization rate, rate of fragmentation (≤ 15%) and quality of embryo transfer (bloody cervix only) were not significant predictors of pregnancy.

Acknowledgments

We would like to thank our IVF lab technicians (F. Hashemi, F. N. Heidari and M. Fasihian) and all staff in Fatemeh Zahra Fertility and Infertility centre.

References

1. Gunalp S, Onculoglu C, Gurgan T, Kruger TF, Lombard CJ. A study of semen parameters with emphasis on sperm morphology in a fertile population: an attempt to develop clinical thresholds. *Hum Reprod* 2001; 16: 110-114.
2. Menkveld R, Wong WY, Lombard CJ, Wetzels AM, Thomas CM, Merkus HM, *et al.* Semen parameters, including WHO and strict criteria morphology, in a fertile and subfertile population: an effort towards standardization of in-vivo thresholds. *Hum Reprod* 2001; 16: 1165-1171.
3. Oehninger S, Chaturvedi S, Toner J, Morshedi M, Mayer J, Lanzendorf S, *et al*. Semen quality: Is there a paternal effect on pregnancy outcome in in-vitro fertilization/ intracytoplasmic sperm injection? *Hum Reprod* 1998; 13: 2161-2164.
4. Spandorfer SD, Avrech OM, Colombero LT, Palermo GD, Rosenwaks Z. Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. *Hum Reprod* 1998; 13: 334-338.
5. Shen S, Khabani A, Klein N, Battaglia D. Statistical analysis if factors affecting fertilization rates and clinical outcome associated with intracytoplasmic sperm injection. *Fertil steril* 2003; 79: 355-360.
6. Garello C, Baker H, Rai J, Montgomery S, Wilson P, Kennedy CR, Hartshorne GM. Pronuclear orientation, polar body placement, and embryo quality after intracytoplasmic sperm injection and in-vitro fertilization: further evidence for polarity in human oocytes? *Hum Reprod* 1999; 14: 2588-2595.

[Downloaded from ijrm.ir on 2024-10-12]

7. Kahraman S, Kumtepe Y, Sertyel S, Donmez E, Benkhalifa M, Findikli N, *et al*. Pronuclear morphology scoring and chromosomal status of embryos in severe male infertility. *Hum Reprod* 2002; 17: 3193-200.
8. Tesarik J, Greco E. The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. *Hum Reprod* 1999; 14: 1318-1323.
9. Sennl A, Urner F, Chanson A, Primi MP, Writhner D, Germond M. Morphological scoring of human pronuclear zygotes for prediction of pregnancy outcome. *Hum Reprod* 2006; 21: 234-239.
10. Fisch JD, Rodriguez H, Ross R, Overby G, Sher G. The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos. *Hum Reprod* 2001; 16: 1970-1975.
11. Saldeen P, Sundstrom P. Nuclear status of four-cell pre-embryos predicts implantation potential in invitro fertilization treatment cycles. *Fertil Steril* 2005; 84: 584-589.
12. Zollner U, Zollner KP, Hartl G, Dietl J, Steck T. The use of a detailed zygote score after IVF/ICSI to obtain good quality blastocysts: the German experience. *Hum reprod* 2002; 17: 1327-1333.
13. Balaban B, Urman B, Isiklar A, Alatas C, Aksoy S, Mercan R, *et al*. The effect of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome. *Hum Reprod* 2001; 16: 2357-2361.
14. Placido GD, Wilding M, Strina I, Alviggi E, Alviggi C, Mollo A, *et al*. High outcome predictability after IVF using a combined score for zygote and embryo morphology and growth rate. *Hum Reprod* 2002; 17: 2402-2409.
15. Balaban B, Urman B. Embryo culture as a diagnostic tool. *Reprod Biomed online* 2003; 7: 671-682.

[Downloaded from ijrm.ir on 2024-10-12]

[Downloaded from ijrm.ir on 2024-10-12]