FACTORS ASSOCIATED WITH INTRACYTOPLASMIC SPERM INJECTION OUTCOME IN MEN WITH AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA

[1]Donny Eka Putra, [2]Trisula Utomo.

[1]Department of Urology, Faculty of Medicine/Universitas Indonesia, Cipto Mangunkusumo General Hospital, Jakarta.
[2]Division of Urology/Department of Surgery, Faculty of Medicine/Gadjah Mada University, Sardjito General Hospital, Yogyakarta.

ABSTRACT

Objective: *This study is assigned to evaluate factors that associated with intracytoplasmic sperm injection (ICSI) outcome.* ***Material & methods:*** *A retrospective study design is used to evaluate couples who seeking help for infertility problems at Permata Hati Infertility Clinic, Sardjito General Hospital Yogyakarta. The outcome measures were fertilization and clinical pregnancies rates. All analyses were performed with SPSS statistical software, version 20.0.* ***Result:*** *Forty-five couples participated in this study with mean age 33.69 ± 5.38 years for men and 30.53 ± 4.79 years for women. Of 56 ICSI cycles, there were 43 successful fertilizations (95.6%) and 14 clinical pregnancies (31.1%). In multivariate analysis, factors associated with successful clinical pregnancies were women's age (p = 0.034, 95% CI 0.005-0.8), number of ICSI cycle (p = 0.045, 95% CI 0.001-0.93), and sperm morphology (p = 0.019, 95% CI 1.648- 253). In bivariate analysis, only men's age is significantly associated with successful fertilization (p = 0.006, 95% CI 7.22-16.43).* ***Conclusion:*** *Factors associated with outcome of ICSI are women's age, number of ICSI cycle, and sperm morphology.*

Keyword: *Intracytoplasmic sperm injection, azoospermia, severe oligozoospermia.*

ABSTRAK

Tujuan: *Penelitian ini bertujuan untuk mengevaluasi faktor yang berhubungan dengan hasil intracytoplasmic sperm injection (ICSI).* ***Bahan & cara:*** *Penelitian ini menggunakan desain retrospektif untuk mengevaluasi pasangan yang mencari bantuan untuk masalah infertilitas di Klinik Infertilitas Permata Hati, RSUD Sardjito Yogyakarta. Hasil penilaian adalah fertilisasi dan rate kehamilan klinis. Semua analisa dilakukan menggunakan software statistik SPSS, versi 20.0.* ***Hasil:*** *Sebanyak 45 pasangan berpartisipasi dalam penelitian ini dengan rerata usia 33.69 ± 5.38 tahun untuk laki-laki dan 30.53 ± 4.79 tahun untuk wanita. Dari 56 siklus, didapatkan 43 fertilisasi yang sukses (95.6%) dan 14 kehamilan klinis (31.1%). Pada analisa multivariat, faktor yang berhubungan dengan kehamilan klinis yang sukses adalah usia wanita (p = 0.034, 95% CI 0.005-0.8), jumlah siklus ICSI (p = 0.045, 95% CI 0.001-0.93), dan morfologi sperma (p = 0.019, 95% CI 1.648-253). Pada analisa bivariat, hanya usia laki-laki yang secara signifikan berhubungan dengan fertilisasi yang sukses (p = 0.006, 95% CI 7.22-16.43).* ***Simpulan:*** *Faktor yang berhubungan dengan hasil ICSI adalah usia wanita, jumlah siklus ICSI dan morfologi sperma.*

Kata kunci: *Intracytoplasmic sperm injection, azoospermia, oligozoospermia berat.*

Correspondence: Donny Eka Putra; c/o: Department of Urology, Faculty of Medicine/Universitas Indonesia, Cipto Mangunkusumo Hospital. Jl. Diponegoro No.71, Jakarta Pusat, DKI Jakarta 10430, Indonesia. Phone: +62 21 3152892, Fax: +62 21 3145592. Email: donnyputra8@gmail.com.

INTRODUCTION

Infertility is a major health problem of 10-15% couples who do not have children, and it is estimated that 8% men in reproductive age see a doctor for infertility problem.[1-4] One of male infertility problems is azoospermia which is defined as the absence of spermatozoon in ejaculation after centrifugation. Azoospermia occurs in approximately 1-3% male population and 10% infertile male population.[4]

In the last decade, the rapid development of intracytoplasmic sperm injection (ICSI) technique as a primary treatment in men who have severe

spermatogenesis disorder showed that spermatozoon taken from the epididymis or testis was proved to be able to produce fertilization and pregnancy. The success does not depend on semen specimen, whether the sperm used is fresh or after cryo preservation. In the case of men with abnormal anti sperm antibodies, ICSI has advantages compared to other IVF (in vitro fertilization) techniques. Since a high success rate, the use of ICSI becomes more frequent as a treatment for sperm disorders with or without abnormalities in female.[5]

In 2002, International Committee Monitoring Assisted Reproductive Technologies reported ICSI is widely used representing > 50% cycles in Australia and New Zealand (53.8%), Europe (53.9%), Latin America (76.1%), Middle East (92.5%), and North America (60.8%). Mean while in Asia, ICSI represented 47.5% of all IVF cycles.[5] However, the success rate of ICSI is lower in men with severe spermatogenesis disorder than men with normal spermatogenesis.

A multivariate research by Botelho et al. (2012) showed that the identification of spermatozoon in spermogram and no genetic abnormality were the independent factors of TESE success. In this study, maternal age is the predictor factor of pregnancy after ICSI. The same study stated that the follicle stimulating hormone (FSH) level which was 3 times higher than the upper limit of the reference range in azoospermia men, indicating the damage on testis and testicular biopsy in that patient was not necessary.[6]

A study showed that the pregnancy rate after ICSI was lower in azoospermia men than normal spermatogenesis men.[7] Therefore, the appropriate information to patients about the probability of TESE and ICSI success becomes important.

OBJECTIVE

This study aims to determine the factors associated with ICSI outcome in men with azoospermia and severe oligozoospermia.

MATERIAL & METHOD

A retrospective study was conducted from 2011-2012 in Permata Hati Infertility Clinic, Sardjito Hospital, Yogyakarta. The inclusion criteria were couples undergoing ICSI method with male factor abnormalities in the form of azoospermia in 2 times semen analysis or severe oligozoospermia (sperm count < 1 million/ml) at one time semen analysis in the same laboratory (Permata Hati Infertility Clinic). The exclusion criteria were patients with hypogonadotropic hypogonadism or androgen receptor failure as well as previous vasectomy and urinary tract surgery.

A medical record was obtained from couples in accordance with the inclusion and exclusion criteria. Each couple performed the examination procedures such as clinical and additional examination. Male clinical examination included history in the form of infertility history, previous surgery, the use of drugs that interfered fertility, testicular trauma, and cryptorchidism. Male physical examination included the sign of secondary sexual development, testicular size, the presence of vasdeferens, and varicocele. The additional examination was the sperm analysis and the level of FSH, lutenizing hormone (LH), and testosterone. The other additional examination such as antisperm antibody analysis and viability test was performed based on indication.

Sperm concentration, motility, and mor phology were evaluated based on the criteria of the World Health Organization (WHO) 2010.[8] All azoospermia men were performed TESE, and the sperm was used directly or after cryo preservation. Men with severe oligozoospermia using ejaculated sperm for ICSI procedure.

Female clinical examinations were performed in the form of history, physical examination and additional examination. All women with abnormal anatomy and physiology required surgery or drugs were treated before ICSI procedure. The minimum infertility duration used in this study was no pregnancy within 1 year with regular intercourse without contraception.

The dependent factors analyzed were the success rate of fertilization and clinical pregnancy. The independent factors hypothesized to affect the outcomes were couple age, the level of male FSH, LH, and testosterone, sperm motility, sperm morphology, sperm source (ejaculation or TESE). The statistical analysis used was bivariate analysis using independent T-test for numerical variables and Chi-square for nominal variables. The multivariate analysis was performed to evaluate factors associated with ICSI success. All statistical analyses used SPSS version 20 with $\alpha = 5\%$ and $p \leq 0.05$ was assessed significance.

RESULT

The participants were 45 couples with primary infertility complaint. The average age of men was 33.69 ± 5.38 years and women was 30.53 ± 4.79 years. The average infertility duration was 6.38 ± 4.39 years. Twenty women (44.4%) were diagnosed with abnormalities in the reproductive tract and thus requiring appropriate management of abnormalities before ICSI.

Based on the sperm analysis, most men were diagnosed with severe oligozoospermia (22/45; 48.9%). Twenty men (44.4%) were diagnosed with non-obstructive azoospermia and 3 men (6.7%) were diagnosed with obstructive azoospermia. The sperm motility showed that 18 men (40%) had normal motility and 27 men (60%) were abnormal. The result of sperm morphology indicated that 23 men (51.1%) had normal sperm morphology, meanwhile 22 men (48.9%) had abnormal sperm morphology. The average level of FSH, LH, and testosterone were 6.22 ± 2.78 mg/dl, 5.86 ± 3.34 mg/dl, and 5.13 ± 1.79 mg/dl respectively.

Table 1. General characteristis of study participants.

Variable	Mean	Std. deviation
Men age (year)	33.69	5.38
Women age (year)	30.53	4.79
Duration of infertility (year)	6.38	4.39
Men FSH level (IU)	6.22	2.78
Men LH level (IU)	5.86	3.34
Testosterone level (IU)	5.13	1.79

A total of 56 ICSI cycles were performed on 45 infertile couples according to the protocol in the Permata Hati (table 3). Twenty-three men (51.1%) underwent TESE and 20 men (86.9%) were found sperm on TESE. From 56 ICSI cycles, the fertilization success rate was 43 (95.6%) and the clinical pregnancy occurred in 14 couples (31.1%).

A multivariate logistic regression analysis was performed to evaluate the relationship between the characteristics of the subjects and the occurrence of fertilization and clinical pregnancy (table 4). The result of multivariate analysis showed there were statistically significant correlations between women's age, the number of ICSI cycles, sperm morphology and the success of clinical pregnancy with p-value and confidence interval were p = 0.034; 95% CI = 0.005-0.8, p = 0.045; 95% CI = 0.001-0.93, and p = 0.019; 95% CI = 1.648-253 respectively.

Table 2. Characteristics of sperm analysis and reproductive tract state in women.

Variable	N	%
Sperm count		
Severe oligozoospermia	22	48.9
Non-obstructive azoospermia	20	44.4
Obstructive azoospermia	3	6.7
Sperm motility		
Normal	18	40
Abnormal	27	60
Sperm morphology		
Normal	23	51.1
Abnormal	22	48.9
Women reproductive tract		
Normal	20	44.4
Abnormal	25	55.6

Table 3. Intracytoplasmic sperm injection (ICSI) outcomes.

Variable	N	%
ICSI cycle	56	100
Fertilization rate		
Successful	43	95.6
Unsuccessful	2	4.4
Pregnancy rate		
Successful	14	31.1
Unsuccessful	31	68.9

The multivariate analysis showed that there was a significant difference in women's age associated with clinical pregnancy outcome after ICSI. The average age of mother who succeeded to become pregnant was 28 ± 2.99 years compared to the failed one was 31.68 ± 5.04 years. Based on the receiver operating characteristic (ROC) curve, the correlation strength value of odds ratio (OR) was 0.061 with the area under curve (AUC) value of 80% (figure 1).

Based on the analysis of the association between sperm morphology and successful pregnancy, the frequency of pregnancy occurrence was higher in the normal sperm morphology group compared to the abnormal sperm morphology group (85.7% vs.14.3%). The correlation strength value of OR was 20.42 with the AUC value of 84.8% (figure 2).

Table 4. Multivariate regression analysis. There were statistically significant correlations between women's age, the number of ICSI cycles, sperm morphology and the success of clinical pregnancy.

Variable	B	S.E.	Sig	95% C.I. for EXP (B)	
				Lower	Upper
Women age	-2.794	1.315	0.034	0.005	0.805
ICSI cycle	-3.292	1.643	0.045	0.001	0.930
Sperm morphology	3.017	1.284	0.019	1.648	253.06

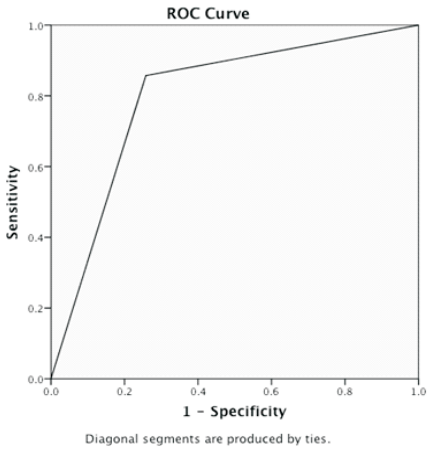

Figure 1. ROC curve between women's age and clinical pregnancy outcome. The correlation strength value of OR was 0.061 with the AUC value of 80%. The result showed strong discrimination capability.

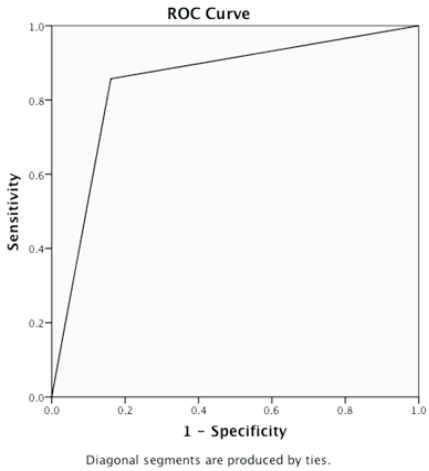

Figure 2. ROC curve between sperm morphology and clinical pregnancy outcome. The correlation strength value of OR was 20.42 with the AUC value of 84.8%. The result showed very strong discrimination capability.

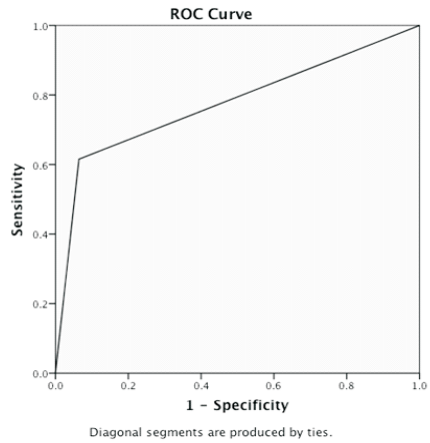

Figure 3. ROC curve between ICSI cycle and clinical pregnancy outcome. The OR value obtained at 0.037 and the AUC was 77.5%. This result described a weak correlated strength and medium discrimination value.

The number of ICSI cycles had a significant association with the pregnancy outcome. Couples who underwent more than one ICSI cycle had a higher clinical pregnancy than the ones who underwent one ICSI cycle. The OR value obtained at 0.037 and the AUC was 77.5% (figure 3).

The bivariate analysis indicated that there was a statistically significant association between male age and fertilization outcome, with p = 0.006; 95% CI = 7.22-16.43. This analysis showed that there was a statistically significant difference in the average male age to the fertilization success. The fertilization success occurred in 43 cycles with the average male age of 33.16 ± 4.88 years compared to the fertilization failure occurred in 2 cycles with the average male age of 45 ± 1.4 years

.

DISCUSSION

The result of this study indicated that as many as 56 ICSI cycles were performed on 45

infertile couples with 43 fertilization results (95.6%) and 14 clinical pregnancies (31.1%). There was relatively no difference in fertilization and pregnancy success rates compared to the other studies. A research by Botelho F, et al (2012) showed that the success rates of fertilization and pregnancy in couples with azoospermia after ICSI were 76.3% and 28.9% respectively.[6] Then, other research by Boitrelle F, et al (2012) indicated that the pregnancy rate with ICSI after TESE was 42.7%.[7]

In our study, the factors that associated with ICSI success were woman age, the number of ICSI cycles, and sperm morphology. According to Botelho F, et al. (2012), maternal age was the only factor that determines pregnancy after ICSI if TESE were successful. The other factors such as sperm quality, type of azoospermia, and the period between TESE and ICSI cycle had no significant difference in predicting pregnancy after ICSI.[6]

Other studies indicated that the older maternal age was associated with the lower probability of ICSI success.[9-11] A study by Rowe, et al (2006) showed that maternal age was the most important factor affecting the IVF success.[12] A different result was published by Boitrelle F, et al (2011) who concluded that maternal age, the number of ICSI cycles, and oocyte maturation factors were not significant in determining the ICSI success. In that study, pregnancy after ICSI was associated with the number of oocytes injected with sperms obtained from TESE procedure.[7]

Our result was largely in line with other studies that had been published. Maternal age was a variable that determined the ICSI success. In this study, however, maternal age was not the only factor associated with the ICSI success. Other factors associated with ICSI success were sperm morphology and the number of ICSI cycles.

A study by Zhu Y, et al (2013) concluded that ICSI increased the fertilization success rate, but it did not increase embryo quality, blastocyst formation, and pregnancy rates in couples with teratozoospermia.[13] Other studies showed a lower implantation rate in ICSI embryos using sperm with abnormal morphology.[14]

However, a journal published by De Vos A (2000) explained that the number of sperm, morphology (except globozoospermia), and high titer antisperm antibody did not affect the ICSI success. Sperm viability was one of the determining factors in the ICSI success.[15] Other study by French DB, et al (2010) explained that sperm morphology had little prognostic value to the ICSI success. In addition, sperm morphology can not affect the morphology or blastocyst development. A meta-analysis of teratozoospermia concluded that there was no decrease in the probability of conception success after ICSI using sperm from the teratozoospermia men.[16]

Our result was different from the previous study. Sperm morphology was associated with ICSI success. It might be because the ICSI success rate was higher (not statistically significant) in the severe oligozoospermia group compared to the azoospermia group. The severe oligozoospermia group had more normal sperm morphology than the azoospermia group.

Our study evaluated the ICSI success in couples with male factor abnormalities in the form of azoospermia and severe oligozoospermia. The statistical analysis showed that there was no significant difference between the azoospermia and severe oligozoospermia group in association with ICSI outcome ($p = 0.457$). In azoospermia, sperm was obtained by TESE, meanwhile sperm was obtained by ejaculation in severe oligozoospermia. The result of a literature review by Esteves SC, et al (2013) showed there was a contradiction between the ICSI success rate and sperm parameter. Some studies concluded that the pregnancy rate was lower with sperm obtained from non-obstructive azoospermia men than obstructive azoospermia and ejaculation. On the other hand, other studies showed no difference in the ICSI success rate from any type of azoospermia.[4]

In another study, it was reported that the patients with either obstructive or non-obstructive azoospermia, the success rate of ICSI was lower than the ones with normal sperma-togenesis. In men with obstructive azoospermia, the probability of clinical pregnancy after 3 ICSI cycles was 35%. In patients with non-obstructive azoospermia, the pregnancy rate after 3 ICSI cycles was lower at 17%. A relatively higher success rate in this study might be due to the difference in the patient selection for ICSI.[14]

This study had limitations in methodology and sample size. It is a retrospective study in which data were collected based on the medical records. The interpretation errors done by the researchers may lead to the result bias. Some indicators of female factors were not assessed in this study, such as abnormal anatomy, reproductive tract function, and the number of oocytes in insemination. These factors may affect the result. The sample size was small so

further research was needed with a larger sample size.

CONCLUSION

Factors associated with successful outcome of ICSI are women's age, number of ICSI cycle, and sperm morphology.

REFERENCES

1. Turek PJ. Male infertility. In Tanagho EA, McAninch JW, editor. Smith's General Urology. 17th ed. New York: McGraw-Hill; 2008. p. 684-718.
2. Taher A. Panduan penatalaksanaan medis infertilitas pria. JIBI. 2004; 32: 67-72.
3. De Kretser DM, Baker HWG. Infertility in men: recent advances and continuing controversies. J Clin Endocrinol Metab. 1999; 84: 3443-50.
4. Esteves SC, Agarwal A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: case series and systematic review. Clinics. 2013; 68: 141-9.
5. Palermo GD, Neri QV, Monahan D, Kocent J, Rosenwaks Z. Development and current applications of assisted fertilization. Fertil Steril. 2012; 97: 248-59.
6. Botelho F, Figueiredo L, Leite R, Carvalho A, Tomadal N, Vendeira P. Predictive daftors of a successful testicular biopsy and subsequent clinical pregnancy. Andrologia. 2012; 20: 1-6.
7. Boitrelle F, Robin G, Marcelli F, Albert M, Leroy-martin B, Dewailly D, et al. A predictive score for testicular sperm extraction quality and surgical ICSI outcome in non-obstructive azoospermia: a retrospective study. Hum Reprod. 2011; 0: 1-7.
8. Cooper TG, Aitken J, Auger J, Baker HWG, Barratt CLR, Behre HM, et al. WHO laboratory manual for the examination and processing of human semen [Internet]. Switzerland: WHO; 2010 [Cited 2015 Apr 20]. 271 p. Available from: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf?ua=1
9. Devroey P, Godoy H, Smitz J, Camus M, Tournaye H, Derde MP, et al. Female age predicts embryonic implantation after ICSI: a case-controlled study. Hum Reprod. 1996; 11: 1324–7.
10. Alrayyes S, Fakih H, Khan I. Effect of age and cycle responsiveness in patients undergoing intracyto-plasmic sperm injection. Fertil Steril. 1997; 68: 123–7.
11. Naru T, Sulaiman MN, Kidwai A, Ather MH, Waqar S, Virk S, et al. Intracytoplasmic sperm injection outcome using ejaculated sperm and retrieved sperm in azoospermic men. J Urol. 2008; 5: 106–10.
12. Rowe T. Fertility and a woman's age. J Reprod Med. 2006; 51: 157–63.
13. Zhu Y, Wu QF, Zhou XJ, Xin CL, Ling G, Lu GX. ICSI improves fertilization in isolated terato-zoospermic men: a study with strictly controlled external factors and WHO-5 standart. Syst Biol Reprod Med. 2013; 59: 21-26.
14. Tournaye H. Male factor infertility and ART. Asian J Androl. 2012; 14: 103-8.
15. De vos A. Intracytoplasmic sperm injection (ICSI). Hum reprod. 2000; 15: 59-64.
16. French DB, Sabanegh ES, Goldfarb J, Desai N. Does severe teratozoospermia affect blastocyst formation, live birth rate, and other clinical outcome parameters in ICSI cycles? Fertil Steril. 2010; 93: 1097-103.