

THE QUALITY OF SPERM AND FEMALE AGE AS FACTORS INFLUENCING PREGNANCY IN INTRACYTOPLASMIC SPERM INJECTION (ICSI)

¹Exsa Hadibrata, ²Trisula Utomo.

¹Division of Urology, Faculty of Medicine/Lampung University, Abdul Moeloek General Hospital, Lampung.

²Division of Urology, Faculty of Medicine/Gadjah Mada University, Sardjito General Hospital, Yogyakarta.

ABSTRACT

Objective: To evaluate the quality of sperm and the female age influence on fertilization and pregnancy rate after Intracytoplasmic Sperm Injection (ICSI) procedure. **Material & Methods:** This study was an observational analytic study with a retrospective cohort design. The data of infertile and subfertile male undergoing ICSI for five years were analyzed and evaluated to determine factors influencing pregnancy post-ICSI in Sardjito General Hospital. The Data were analyzed by using Chi-squared test, Kolmogorov-Smirnov test, and Mann-Whitney U test with the confidence level of $p < 0.05$ and $\alpha = 0.05$. **Results:** There was 52 male included in this study. A total of 52 males were infertile, with azoospermia, oligozoospermia, and oligoasthenoteratozoospermia. 2 patients (3.8%) were patients with oligozoospermia, 32 patients (61.5%) were patients with azoospermia and 18 patients (34.6%) were patients with oligoasthenoteratozoospermia. We evaluated 52 ICSI cycle, the percentage of fertilization and pregnancy are 84.6% and 36.5%, respectively. Bivariate analysis showed a significant relationship between wife under 37 years old variable and pregnancy post ICSI procedure with $p = 0.021$ and OR 9.0 (95% CI 1.05-76.4). There was no significant difference between azoospermia, oligozoospermia, and oligoasthenoteratozoospermia and the occurrence of pregnancy post ICSI procedure, $p = 0.986$. **Conclusion:** The percentage of fertilization and pregnancy post ICSI procedure were 84.6% and 36.5%. Azoospermia, oligozoospermia, and oligoasthenoteratozoospermia variables were not significantly associated with pregnancy post ICSI procedure. ICSI procedure must be carried out immediately before the age of the wife 37 years because of the high success rate.

Keywords: ICSI, sperm quality, female age.

ABSTRAK

Tujuan: Mengevaluasi pengaruh kualitas sperma dan usia istri pada fertilisasi dan kehamilan setelah tindakan Intracytoplasmic sperm injection (ICSI). **Bahan & Cara:** Penelitian ini adalah penelitian analitik observasional dengan design kohort retrospektif. Data pria infertil dan subfertil yang menjalani program ICSI selama 5 tahun kemudian dianalisis dan dievaluasi untuk mengetahui faktor-faktor yang mempengaruhi kehamilan pasca ICSI di RSUP Dr. Sardjito. Data dianalisis dengan menggunakan Chi-square test, Kolmogorov-Smirnov test dan Mann-Whitney U test dengan tingkat kepercayaan $p < 0.05$ dan $\alpha = 0.05$. **Hasil:** Terdapat 52 pria yang dilibatkan dalam penelitian ini. 52 sampel pria merupakan pria infertil dengan azoospermia, oligozoospermia, dan oligoasthenoteratozoospermia. Sebanyak 2 (3.8%) pasien merupakan pasien oligozoospermia, sebanyak 32 (61.5%) merupakan pasien azoospermia dan sebanyak 18 (34.6%) merupakan pasien dengan oligoasthenoteratozoospermia. Pasca prosedur ICSI, presentase fertilisasi dan kehamilan secara berturut-turut adalah 84.6% dan 36.5%. Pada analisis bivariat didapatkan hubungan yang bermakna antara variabel usia istri kurang dari 37 tahun dengan kehamilan pasca prosedur ICSI dengan $p = 0.021$ dan OR 9.0 (95% CI 1.05-76.4). Tidak didapatkan hubungan yang bermakna antara variabel azoospermia, oligozoospermia, dan oligoasthenoteratozoospermia dengan terjadinya kehamilan pasca prosedur ICSI dengan $p = 0.986$. **Simpulan:** Presentase fertilisasi dan kehamilan istri setelah prosedur ICSI adalah 84.6% dan 36.5%. Variabel azoospermia, oligozoospermia, dan oligoasthenoteratozoospermia tidak berhubungan secara bermakna terhadap kehamilan istri pasca prosedur ICSI. Prosedur ICSI harus dilakukan segera sebelum usia istri 37 tahun karena tingkat keberhasilan yang tinggi.

Kata Kunci: ICSI, kualitas sperma, usia istri.

Correspondence: Exsa Hadibrata; c/o: Division of urology, Faculty of Medicine/Lampung University, Abdul Moeloek General Hospital. Jl. Dr. Rivai No.6, Bandar Lampung, Lampung 35121, Indonesia. Phone: 0721 – 703312. Email: exsa.hadibrata@gmail.com.

INTRODUCTION

Infertility is a problem that has not yet been fully resolved. Infertility rates in the world are still quite high. The percentage of infertility in Indonesia is about 6%, with an age range of infertility between 25-49 year olds. The dominant factors that cause infertility on female are the tubal and ovulation factors. Male infertility in general is caused by the low count or poor quality of spermatozoa.¹

Assisted reproduction therapy (ART) consists of two methods, among others, artificial insemination and in vitro/Intracytoplasmic Sperm Injection (ICSI). In vitro fertilization consists of several stages: ovulation induction, oocyte retrieval, collection and preparation of spermatozoa, in vitro fertilization and embryo culture, and embryo implantation into the uterus. ART is particularly useful in the group of men with azoospermia, oligozoospermia, and oligoasthenoteratozoospermia. The chance of fertilization by using this method is about 70% to 80% with a pregnancy rate up to 45%.^{2,3}

The previous research states that poor semen quality would affect embryonic development after ICSI action, but research has no significant relationship to pregnancy or implantation of the embryo in the uterus.⁴ Another study states that the factors influence the success of ICSI were wife age and ovarian cycle. However, there was not a wife age limit in the study.^{5,6} Research in Sudan that the age of the wife above 35 years reduces the success rate of pregnancy to 29.2% compared to the age of the wife under 35 years by 57.7%.⁷

Based on this research, it has not clearly concluded how the relationship of sperm quality can determine the success of ICSI, and there was no research in Indonesia that state a wife's age limit on the success of ICSI actions. The purpose of this study was to determine the quality of sperm and the wife's age influence fertilization and pregnancy rate after ICSI procedure.

OBJECTIVE

This study aims to evaluate the quality of sperm and the female age influence on fertilization and pregnancy rate after Intracytoplasmic Sperm Injection (ICSI) procedure.

MATERIAL & METHODS

This study was an observational analytic research with a retrospective cohort design to

evaluate factors that influence pregnancy post-ICSI. Variables that influence pregnancy were assessed by using the medical record data of infertile men with azoospermia, oligozoospermia and oligoasthenoteratozoospermia and their partners who undergo ICSI program. Data of patients undergoing an ICSI program in Sardjito General Hospital during the period 2010-2014 were analyzed and evaluated to know the factors that influence the success of ICSI in Sardjito General Hospital.

The inclusion criteria for this research are patients diagnosed with azoospermia, oligozoospermia, and oligoasthenoteratozoospermia infertility that underwent the ICSI program and had a complete medical record data. ICSI program was performed in the Sardjito General Hospital period from January 2010 to December 2014. The exclusion criteria for this study, patients who did not have a complete medical record data and patients who are not undergoing ICSI procedure.

The data were analyzed by using SPSS 18. The analyzed data include the duration of infertility, age of husband and wife, male and female diagnosis, examination of testicular organs, hormonal analysis, spermatozoa analysis, male varicocele, Testicular sperm extraction (TESE), also fertilization and pregnancy post-ICSI. Then, the data are analyzed by using Chi-squared test, Kolmogorov-Smirnov test, and Mann-Whitney U test with the confidence level of $p < 0.05$ and $\alpha = 0.05$.

RESULTS

In this study, there were 52 couples, husband and wife. A total of 52 male samples were infertile with azoospermia, oligozoospermia, and oligoasthenoteratozoospermia. 2 patients (3.8%) were patients with oligozoospermia, 32 patients (61.5%) were patients with azoospermia, and 18 patients (34.6%) were patients with oligoasthenoteratozoospermia. The average husband's age was 36.3 ± 6.42 years, and wife's age was 32.5 ± 5.73 years. Before the ICSI procedure, 32 men with azoospermia and three men with oligoasthenoteratozoospermia performed Testicular Sperm Extraction (TESE) first. Patients with oligoasthenoteratozoospermia performed TESE because post-preparation of sperm and washing sperm no spermatozoa obtained with normal morphology and motility. Frozen sperm storage was performed in 35 patients (67.3%). We analyzed the wife diagnose, 31 (59.6%) patients were no abnormality, 7 (13.5%) patients were diagnosed endometriosis, 6 (11.5%) patients were diagnosed

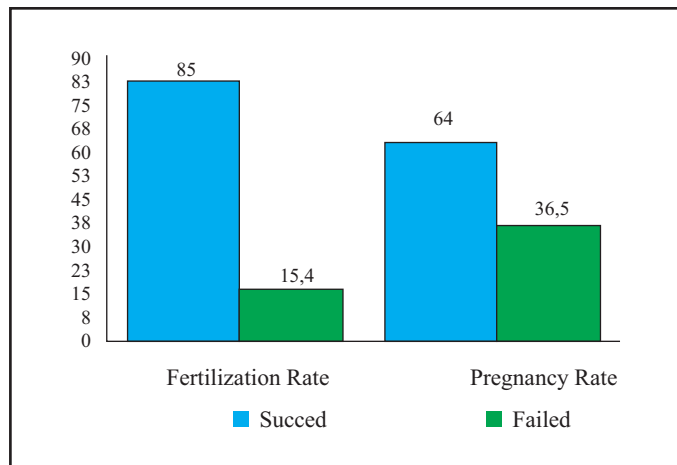
polyp endometrium, 4 (7.7%) patients were diagnosed no paten tuba, 2 (3.8%) patients were diagnosed leiomyoma, and 2 (3.8%) patients were diagnosed ovarian cyst.

We evaluated 52 ICSI cycle, the percentage of fertilization and pregnancy were 84.6% and 36.5%, respectively. Bivariate analysis showed a

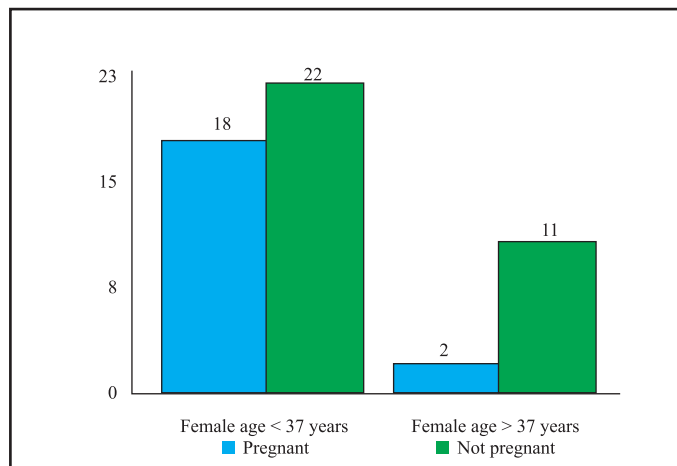
significant relationship between wife under 37 years old variable and pregnancy post ICSI procedure with $p=0.021$ and OR 9.0 (95% CI 1.05-76.4). There was no significant association between azoospermia, oligozoospermia, and oligoasthenoteratozoospermia and the occurrence of pregnancy post ICSI procedure, $p=0.986$.

Table 1. Clinical characteristic patient.

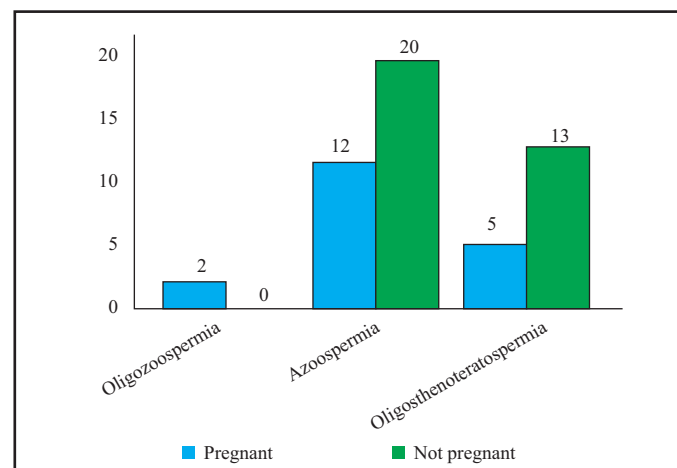
Variables	N (%)	Mean (SD)/Range
Male diagnose		
- Oligozoospermia	2 (3.8%)	
- Azoospermia	32 (61.5%)	
- Oligoasthenoteratospermia	18 (34.6%)	
Male age		36.3 ± 6.42 / (25-52)
Voricocele		
- Yes	17 (32.7%)	
- No	35 (67.3%)	
Sperm retrieval		
- Yes	35 (67.3%)	
- No	17 (32.7%)	
Male FSH Level		
- Normal (5-20 mIU/ml)	43 (82.7%)	
- Low (<5 mIU/ml)	6 (11.5%)	
- High (>20 mIU/ml)	3 (5.8%)	
Testicle size		
- Normal	42 (80.8%)	
- Hipogonad	10 (19.2%)	
Frozen sperm		
- Yes	35 (67.3%)	
- No	17 (32.7%)	
Female age		32.5 ± 5.73 / (22-47)
Female diagnose		
- Endometriosis	7 (13.5%)	
- Endometrial polyp	6 (11.5%)	
- Non paten tuba	4 (7.7%)	
- Leimyoma	2 (3.8%)	
- Ovarian cyst	2 (3.8%)	
- No Abnormality	31 (59.6%)	
Duration of Infertility (year)		6.2 (4.3) / (1-18)
FSH Level (mIU/ml)		7.3 (5.9) / (1.3-39.1)
Duration of frozen sperm (month)		4.1 (11.3) / (0-60)



Graphic 1. Fertilization and Pregnancy rate Post ICSI.



Graphic 2. Variable female age with pregnancy (p=0.021).



Graphic 3. Variable oligozoospermia, azoospermia and oligasthenoteratospermia with pregnancy (p=0.986).

Table 2. Clinical factors influencing pregnancy post ICSI.

Variables		Pregnant	P	OR
Female age <37 years	Yes	18	0.021 *	9.0 (95% CI 1.059-76.4)
	No	1		
Abnormality of spermatozoa	Oligozoospermia	2	0.986 #	
	Azoospermia	12		
	OAT	5		
Varicocele	Yes	4	0.989 *	0.9 (95% CI 0.24-3.94)
	No	15		
Female diagnosis	Endometriosis	2	1.00 #	
	Endometrial polyp	2		
	Nonpaten tube	2		
	Leimyoma	1		
	Ovarian cyst	1		
Male FSH level	Normal	17	0.99 #	
	Low	1		
	High	1		
Testicle size	Normal	16	0.633 #	1.46 (95% CI 0.32 – 6.36)
	Hipogonad	3		
Male age	25-30 years	5	0.88 #	
	31-35 years	4		
	36-40 years	8		
	>40 years	2		
Female age	20-25 years	4	0.359 #	
	26-30 years	6		
	31-35 years	6		
	36-40 years	3		
	>40 years	0		
Duration of infertility (years)		6.0 ± 3.74	0.907 **	
Male FSH level (mIU/ml)		7.1 ± 3.9	0.382 **	
Duration of cryopreservation sperm (months)		7.56 ± 18	0.420 **	

(*) we calculated using Chi-square test, (**) we calculated using Mann-Whitney U test, (#) we calculated using Kolmogorov-Smirnov test.

DISCUSSION

The chance of fertilization on people of all ages and spermatozoa of different types treated by the ICSI method was about 70% to 80% with a pregnancy rate of up to 45%.⁶ The obtained results were slightly different from this study. In this study, the number of pregnancy post ICSI procedure was 36.5%. It may be caused by small sample size. The samples taken in this study were limited only to men with azoospermia, oligozoospermia and oligoasthenoteratozoospermia.

Some journals mention the influence of wife's age on pregnancy. In this study, the average age of pregnant women is 30.1 ± 4.6 years, while the average age of non-pregnant women is 34.1 ± 5.9 years. The age of wife variable is significantly

associated with pregnancy post ICSI procedure with $p=0.033$. Women above 37 years old have a poor prognosis for pregnancy post ICSI procedures.⁷ Based on these data, this study also divide the samples into two groups. The group I consists of wives under 37 years old, group II consists of wives above 37 years old. In group I, the pregnancy rate was 45%. In group II, the pregnancy rate was 8.3%. In Chi-squared test, there was a significant relationship between wife under 37 years old and pregnancy post ICSI procedure. Other studies also mention that the age of wife influenced pregnancy post ICSI procedure. The pregnancy rate for wives between 41-43 years old was 12% and the delivery rate was 2-7%.^{7,8} Female age was the most important single variable influencing outcome in assisted reproduction. Compared to a female aged 25 years,

the fertility potential of a female aged 35 years is reduced to 50%, to 25% at 38 years, and less than 5% at over 40 years.⁹

Research in Sudan states that the wife's age after ICSI has significant value because the age below 30 years has a pregnancy success reaching 32.7% and the age above 40 years has a 4.5% pregnancy success. In addition to pregnancy, under 30 years have successful egg fertilization, the endometrial thickness is better than age over 40 years. This study also discusses that the age and thickness of the wife's endometrium will influence the process of conception, implantation, and pregnancy.^{5,6}

Another study based on the analysis of stepwise regression data for all azoospermia patients conducted by ICSI showed that the chance of achieving pregnancy after ICSI correlated significantly with the wife's age of a patient with an age limit below 38 years that had a highly significant value.⁷

Some studies report that abnormalities in both morphology and motility were associated with the decrease in fertilization and pregnancy rates and the increase in abortion rate. Morphological abnormalities may be in the form of head, neck, and tail defects. Spermatozoa with head abnormalities may have a large head (length >5.0 μm and a width >3.0 μm), small head (length <3.0 μm and width <2.0 μm) and tapered head (length <5.0 μm and width <3.0 μm). The neck of spermatozoa is abnormal if its width >2.0 μm , and the tail is abnormal if it is damaged or curled. Meanwhile, motility disorders may be in the form of low motility (5-25 $\mu\text{m/s}$), non-progressive motility (<5 $\mu\text{m/s}$) and immotility. The percentage of pregnancy post ICSI procedure with morphologically normal sperm was 36.7%, and morphologically abnormal sperm was 20.2%.¹⁰ In this study, the pregnancy rate with oligoasthenoteratozoospermic husbands was lower than those with azoospermic or oligozoospermic husbands. But, there was no statistically significant difference in the chance of pregnancy. Based on the literature, normal sperm morphology is a significant predictor of pregnancy, and sperm motility is an important predictor of spermatozoa functional aspects.² Therefore, there was a meaningful difference in the percentage of pregnancies with oligoasthenoteratospermia compared to pregnancies with normal sperm morphology and motility. The high pregnancy percentage with oligoasthenoteratospermic husband post ICSI procedure was

caused by the meticulous preparation and selection of spermatozoa. With high magnification microscopic motility and morphological selection, an embryologist will get the best spermatozoa possible for the ICSI process.¹⁰

It was reported that the sperm of 35 patients are stored frozen in this study. The duration of sperm storage varies from 1 month to 60 months. After analyzing by using Mann-Whitney U test, there was no significant difference between the duration of frozen sperm storage and pregnancy post ICSI procedure with $p=0.42$. Literature mentions that if the freezing process is maintained correctly, it will not damage the sperm quality. Several things need to be taken into account during the storage process, namely: the rate of temperature changes during the freezing and thawing process and the concentration of cryoprotectants used to obtain normal results. Freezing is executed slowly with a constant rate of temperature drop, 1°C per minute. Thus, spermatozoa will experience the process of exosmosis, which is the intracellular water passage until the chemical potential between intracellular and extracellular is balanced. The intracellular water passage causes an increase in the concentration of infra-cellular solutes and blocks the toxic effects due to the formation of ice in the cells.¹¹ Other studies report that stored frozen sperm will experience motility impairment, but, it is not related to the decrease of spermatozoa fertilization capacity.¹²

This study also analyzed male varicocele variable, wife diagnosis, level of FSH, testicular size, husband age, and duration of infertility and the relationship with pregnancy post ICSI procedure. In the Chi-squared test, Kolmogorov-Smirnov test and Mann-Whitney U test, p value >0.05 was obtained. The test showed that there was no significant relationship between male varicocele, wife diagnosis, level of FSH, testicular size, husband age, duration of infertility, and pregnancy of wives post ICSI procedure. What variables did not influence pregnancy post ICSI procedure because the ICSI procedure was a procedure that injects spermatozoa directly to the egg. The selection of a good spermatozoon in morphology and motility was one of the keys to the success of ICSI. The presence of varicocele abnormalities, testicular atrophy and hormonal disorders have no effect in fertilization as it took only one spermatozoon with good morphology and motility to fertilize the ovum. The limitation of this study was the small number of samples. We did only evaluate the first ICSI cycle

without learn the next ICSI cycle. This research was the first time conducted in the area of Jogjakarta.

CONCLUSION

The percentage of fertilization and pregnancy post ICSI procedure were 84.6% and 36.5%. The impact of this study was that ICSI action must be carried out immediately before the age of the wife 37 years because of the high success rate. Azoospermia, oligozoospermia, and oligoasthenoteratozoospermia variables were not significantly associated with pregnancy post ICSI procedure. Female under 37 years old were significantly associated with pregnancy. However, other variables such as varicocele, wife diagnosis, FSH level, testis size, husband age, duration of infertility and frozen sperm storage variables were not significantly associated with the occurrence of pregnancy post ICSI procedure.

REFERENCES

1. HIFERI, PERFETRI, IAUI, POGI. Konsensus Penanganan Infertilitas. Jakarta: HIFERI, PERFETRI, IAUI, POGI; 2013. p. 5-33.
2. Sabanegh E, Agarwal A. Male Infertility. Campbell Walsh Urology. 10th ed. Philadelphia: Elsevier; 2012. p. 616.
3. Hauser R, Yorgev L, Amit A, et al. Severe hypospermatogenesis in cases of nonobstructive azoospermia: should we use fresh or frozen testicular spermatozoa?. *J Androl*. 2005 Nov- Dec; 26(6): 772-8.
4. Loutradi KE, Tarlatzis BC, Goulis DG, Zepiridis L, Pagou T, Chatziioannou E, et al. The effects of sperm quality on embryo development after intracytoplasmic sperm injection. *J Assist Reprod Genet*. 2006; 23(2): 69–74.
5. Adam I, Ahmed M, Shareef O, Rayis D. Maternal age and intracytoplasmic sperm injection outcome in infertile couples at Khartoum, Sudan. *F1000Research*. 2015; 4(0): 1–11.
6. Friedler S. Factors influencing the outcome of ICSI in patients with obstructive and non-obstructive azoospermia: a comparative study. *Hum Reprod*. 2002; 17(12): 3114–21.
7. Palermo GD, et al. ICSI :Where we have been and where we are going. *Semin Reprod Med*. 2009; 27(2): 191-201.
8. Osmanagaoglu, Kaan, et al. Cumulative delivery rates after ICSI in women >37 tahun. *Human Reproduction Journal*. 2002; 17(4): 940-944.
9. Ron E R, et al. Outcome of assisted reproductive technology in women over the age of 41. *Fertil Steril Journal*. 2000 Sep; 74(3): 471-5.
10. Rowe T. Fertility and woman's age. *J Reprod Med*. 2006; 51(3): 157-63.
11. De Vos A, et al. Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. *Fertility and Sterility Journal*. 2003; 79(1).
12. Winarso H. Simpan beku sperma manusia. Post graduate course penatalaksanaan infertilitas pria dan analisis semen di Surabaya; 1999. p. 1-8.
13. Surg Lt Bhavni Oberoi A, et al. Study of human sperm motility post cryopreservation. *Medical Journal Armed Forces India*. 2000; 70: 349–353.