The Outcomes of Invitro Fertilization and Embryo Transfer in Endometriosis-Associated Infertility: A Case Control Study

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Abstract --- The pathophysiology of infertility in endometriosis is still controversial and widely investigated. Several mechanisms are put forward. Whether In Vitro Fertilization-Embryo Transfer (IVF-ET) program in endometriosis is a solution to improve the fertility still needs further studied.
Objective : To compare the output of IVF-ET between endometriosis infertility and that of non-endometriosis infertility. **Methods :** This research is non experimental research with case control design. A retrospective approach with secondary data was conducted in 2017. The samples are fifty endometrial infertility patients and fifty infertility tubal factor and or sperm undergoing IVF-ET program at Permata Hati Clinic, Dr. Sardjito Hospital Yogyakarta.
Results : The research output is fertilization rate, i.e. a number of good quality embryo and the success of pregnancy based on pregnancy test. Data analysis was done by statistical test of T test and Chi Square. Multivariate analysis was done by logistic regression. Characteristics of both study groups are similar: in terms of age, BMI, duration of infertility, basal FSH, basal E2, stimulation type and gonadotropin needs. A number of good quality embryos in endometriosis group is lower (2.34 ± 2.33 vs 3.44 ± 2.97; $p < 0.039$), but the fertilization rate is the same (48.58 ± 27.16 vs 55.19 ± 30.9; $p > 0.259$). Compared to non-endometriosis, endometriosis group has bigger risk of pregnancy failure (OR 3.758; CI95% 1.11-12.68; $p < 0.03$).
Conclusion : There is no difference in fertilization rates between endometriosis and non-endometriosis. The number of good quality embryo on endometriosis group is lower than non-endometriosis. Pregnancy failure on endometriosis group is higher than non-endometriosis group.

Keywords: endometriosis, embryo transfer, infertility, in vitro fertilization, pregnancy rate.

I. INTRODUCTION

Endometriosis is the growth of gland and stroma of endometrial tissue outside the uterine cavity or myometrium. Endometriosis is a gynecological disorder associated with infertility. Manifestation of endometriosis lesions greatly varies from minimal lesions until endometriotic cyst on the ovary that is damaging the anatomy tubes and ovaries and cause severe adhesions with the surrounding tissue. The prevalence of endometriosis in the general population is unknown. Its estimation is based on visualization of gynecological organs during the laparoscopic examination. Approximately 12-32% of reproductive age women who underwent laparoscopic due to pelvic pain and about 9-50% of infertile women suffer from endometriosis s. In general, growth of endometriotic implants are dependent on the ovarian steroids, so that endometriosis is more prevalent in women at reproductive age and rarely in pre-menarche or post-menopause [1].

Immune system factors have been known involved in the development and growth of endometriosis. Some researchers reported that women with endometriosis have an increased amount of peritoneal fluid, increased activated macrophages, cytokines, and peritoneal prostaglandins. The peritoneal fluid that diffuses into the tubes and endometrial environments may affect sperm-egg interactions, affect the early stages of embryonic development and reduce endometrial receptibility [2].

The hypothesis why endometriosis causes infertility is still controversial and much debated despite there are many researchers that are trying to answer that question. Some literatures explained some of the mechanisms suspected to be associated with infertility in women with endometriosis. It is suspected that fertility disorder caused by anatomical distortion due to pelvic adhesion and due to the influence of endometriotic implant products. Endometriotic lesions cause disorder of the development of oocytes, tubal transport, folliculogenesis, follicular steroidogenesis and embryonic development. It is also affects cytokines and prostaglandins involved on implantation process thereby it decreases implantation rates and pregnancy rates. The mechanism of implantation impairment is still unclear and involves many factors

including the quality of oocytes and embryos. Allegedly poor oocyte quality occurs since the process of folliculogenesis before it is released to the abdominal cavity and mixed with the peritoneal fluid [3].

The aim of this study is to compare the outcome of IVF-ET between endometriosis and non-endometriosis. The outcomes that will be observed are the fertilization rate, the number of good quality embryos and the success of pregnancy.

II. METHODS

This study used a retrospective case control design using secondary data. The research had been conduct at February until December 2017. The subjects of this research were infertility patients who participated in IVF-ET program at Infertility Clinic Permata Hati RS dr. Sardjito Jogjakarta Indonesia that met the criteria of inclusion and exclusion. The inclusion criteria were patients who have been diagnosed as infertile due to endometriosis and infertility due to tubal factor and or sperm factor. The exclusion criteria were patients who did not receive ovulation stimulation with gonadotropin and patients who stopped the cycle. The sample size was determined statistically by taking the results of previous studies [4]. The sample size based on the proportion of pregnancy (with significance level was 95% and 80% of the research strength) was 36.08 cycles for each group. This study used 50 cycles for each group. Samples of this study are taken randomly.

The variables in this study include 3 kinds of independent variables, dependent variables (fertilization rate, number of good quality embryo, and the success of pregnancy) and external variables (age, duration of infertility, BMI, basal E2 levels, Basal FSH and type of stimulation protocol). The diagnosis of endometriosis was determine using laparoscopy and classified according to the American Society of Reproductive Medicine. This study used endometriosis patients at all stages. The fertilization rate is the total percentage of 2 cell embryos and the number of good embryos is the number of embryos that have scores I and II when embryo transfer is carried out. [5].

Two types of analysis have been done, and those are bivariate analysis and multivariate analysis. Independent sample T-test was used to compare the fertilization rate and the number of embryos, whereas the Chi-square test was used to compare the success of pregnancy. Multivariate analysis was conducted to see the relationship between independent variables and dependent with controlling outside variables. The statistical test used in this study was logistic regression analysis.

III. RESULTS & DISCUSSION

1. Research Group Comparability

Table 1. Endometriosis and non endometriosis Group Comparability

Variable	Endometriosis (n = 50) Mean ±SD	Non endometriosis (n = 50) Mean ± SD
Age (Y)	32.92 ± 4.69	34.11 ± 4.85
<35	37 (69.8%)	32 (60.4%)
≥35	16 (3.2%)	21 (39.6%)
BMI	22.46 ± 2.74	22.21 ± 2.68
Infertility length (y)	5.6 ± 3.89	4.85 ± 2.89
E_2 basal (pg/mL)	42.05 ± 22.56	51.56 ± 32.39
FSH basal (mIU/mL)	7.65 ± 6.11	5,99 ± 3,11
Stimulation types		
Long protocol	31 (58.5%)	26 (49.1%)
Short protocol	22 (41.5%)	27 (50.9%)
Amount of gonadotropin in total (IU)	2266.98 ± 1080.8	2471.89 ± 978.61
Amount of Daily gonadotropin (IU)	261.16±118.3	281.15 ± 103.15
Total number of follicles	13.04 ± 9.280	13.81 ± 5.96
Total number of mature follicel	1.42 ± 0.908	2.7 ± 2.04
E_2 peak (pg/mL)	1394.58 ± 832.4	1841.76 ± 906.9

The subject was someone who met the inclusion and exclusion criteria obtained 76 cycles from the endometriosis group and 105 cycles from the non-endometriosis group. Furthermore, it was taken randomly to obtain 100 cycles consisting of 50 cycles for each group. Characteristics of research subjects in both endometriosis and non-endometriosis groups were same. The age, prolonged infertility, BMI, basal estradiol levels and basal FSH levels showed no significant difference. Similarly, the type of ovarian stimulation protocol used and the amount of gonadotropin required for 1 cycle and the average number of gonadotropins per day also did not show significant differences between the 2 groups ($p > 0.05$). Hence, it can be said that the subjects were homogeneous in both groups. Such comparability will help to minimize research results disruption.

2. Fertilization Rate, Amount of Good Quality Embryo and Pregnancy Rate on Endometriosis and Non Endometriosis Group

Table 2 shows the research output data in the form of fertilization rates and the number of good embryos. The number of good quality embryos in the endometriosis group is lower compared to non-endometriosis (p= 0.039), although the fertilization rate is not significantly different (p= 0,259)

Table2. Fertilization rate and amount of good quality embryo on Endometriosis and Non Endometriosis Group

Variable	Endometriosis (n = 50) Mean ±SD	Non endometriosis (n = 50) Mean ± SD	△ mean	p
Fertilization rate (%)	48.58±27.16	55.19±30.91	9.61	0.259
Amount of good quality embryo	2.34± 2.33	3.44± 2.91	1.1	0.039 *

Note: *: means (p<0.05)

The association between the causes of infertility and pregnancy rates is shown in Table 3. It appears that the endometriosis group has a risk of 4,041 times for the failure of pregnancy compared to the non-endometriosis group (p = 0,).

Table3. The correlation between infertility causes with the success of pregnancy

Groups	Pregnancy		RR (CI 95%)	Value p
	failed	succes		
Infertility causes: Endometriosis (n=50)	46 (92%)	4 (8%)	4.041	0.031*
Non endometriosis (n=50)	37 (74%)	13 (36%)	(1.21-13.43)	

Note: * : Means (p<0.05)
RR: relative risk
CI: *confidence interval*

3. Relationship between independent variable, outside variable and dependent variable

Table 4. The correlation between variables and pregnancy

Variable	Pregnancy		RR (CI 95%)	Value p
	Failed	succes		
Infertility causes				
Endometriosis	46 (92%)	4 (8%)	4.041	0.031*
Non endometriosis	37 (74%)	13 (26%)	(1.21-13.43)	
Age (year)				
>35	32 (88.9%)	4 (11.1%)	2.039	0.281
≤35	51 (79.7%)	13(20.3%)	(0.61-6.8)	
Infertil Length (year)				
>5	37 (90.2%)	4 (9.8%)	2.641	0.175
≤5	46 (78%)	13 (22%)	(0.78-8.69)	
BMI (kg/m2)				
≥25	14(87.5%)	2(12.5%)	1.522	0.459
<25	69 (82.1%)	15(17.9%)	(0.31-7.41)	
FSH basal (mIU/ml)				
≥ 10	14 (100%)	0 (0%)	0.831	0.119
< 10	69 (80.2%)	17 (19.8%)	(0.75-0.92)	
E_2 basal (pg/ml)				
≥ 80	10 (83.3%)	2 (16.7%)	1.027	0.668
< 80	73 (83%)	15 (17%)	(0.2-5.17)	
Kinds of stimulation				
Short protocol	40 (85.1%)	7 (14.9%)	1.329	0.597
Long protocol	43 (81.1%)	10 (18.9%)	(0.46-3.83)	
E2 peak				
<=500	3(100%)	0 (0%)	0.964	0.568
>500	80 (82.5%)	17(17.5%)	(0.93-1.01)	

Note: * Means (p<0.05)

To see the relationship between independent variables, external variables, and dependent variables, the overall data were multivariate analysis, however, a preliminary bivariate analysis was done to determine what variables were significantly associated with the success of pregnancy (Table 4). Multivariate analysis used in this study was logistic regression analysis (Table 5).

Table 4 shows the results of bivariate analysis between independent variables and outside variables with the success of pregnancy. It seems that the success of pregnancy was significantly different in the variables due to infertility (p= 0,031). Furthermore, from a multivariate analysis with logistic regression, it is known that only infertile causes are associated with pregnancy failure (Table 5). The endometriosis group had a risk of pregnancy failure of 3.75 times greater than the non-endometriosis group (p = 0,033).

Table 5. Analysis of some logystic regresion variable towards pregnancy

Variabel	OR	CI 95%	Nilai p
Infertilitas causes			
Endometriosis	3.758	1.11-12.68	0.033*
Non endometriosis	1		
Age (year)			
>35	2.055	0.58-7.349	0.268
≤35	1		
Infertil length(tahun)			
>5	1.69	0.475-6.01	0.418
≤5	1		
BMI (kg/m2)			
≥25	1.054	0.174-6.373	0.955
<25	1		
E_2 basal (pg/ml)			
≥ 80	1.238	0.22-6.9	0.807
< 80	1		
Kinds of stimulation			
Short protocol	1.113	0.346-3.58	0.857
Long protocol	1		
Need of gonadotropin/day			
>300 IU	0.525	0.058-4.75	0.566
≤300 IU	1		

Note : * means ($p<0.05$)

The link between endometriosis and infertility is still debated to date. The rate of normal partner fecundity is 0.15 to 0.20 per month and decreases with age. In women with endometriosis the level of fecundity decreased to 0.02-0.1 per month. In addition, endometriosis is also widely associated with lower live birth rates. Even though large-scale research has been conducted, there is no agreement how endometriosis cause infertility. Several mechanisms have been proposed to explain the relationship between endometriosis and infertility. These mechanisms include anatomical distortion of the pelvis, endocrine and ovulatory abnormalities, changes in peritoneal function, and changes in hormone function and cell-mediated function in the endometrium, ectopic endometrial implants and also disrupts embryo implantation [1].

Peritoneal fluid in endometriosis contains various macrophages include prostaglandin, IL-1, TNF, and proteases. This change in macrophages is thought to be related to disturbances in oocyte, sperm, embryonic, or fallopian tube motility. In addition, an ovum capture inhibitor (OCI) is associated with a failure in taking oocytes by fimbria. In endometriosis,the level of IgG and IgA antibodies to endometrial antigen and lymphocytes are increase. This abnormality can affect endocrine receptivity and embryo implantation [1,6].

Several studies have shown that women with endometriosis have experience of increasing peritoneal fluid, increasing macrophage activity, increasing prostaglandin, IL-1, TNF and protease concentrations. Abnormalities of this fluid affect gametes, embryos and tubal functions. The occurrence of pelvic adhesion plays an important role in infertility through the mechanism of disruption of ovum interruption, transport of sperm to cavity peritonei and inhibit tubal pickup oocyst, tubal motility, and tubal patency [1,6,7].

In endometriosis there is a disruption in the process of folliculogenesis. There is a decrease in the number of preantral follicles and the number of mature follicles and reduced dominant follicular diameters. In addition, the dominant level of estradiol follicles decreases and there are changes in hormone profiles including reduced estrogen, androgens and progesterone and increased activin. Follicular fluid of patients with endometriosis contains pro-inflammatory factors such as cytokines and growth factors (IL-1β, IL-8, IL-6, TNF α dan VEGF) suspected of being involved in the growth of endometriosis lesions and cause follicular less optimum environment for oocyte maturation [8]. Impaired follicular growth in endometriosis patients is associated with the increased apoptosis of granulose follicle cells that cause granulose cell damage and impaired synthesis of estrogen and progesterone hormones. The high apoptosis of granulose cell bodies of endometriosis patients performed by ovulation induction is associated with low oocyte quantity and quality. This level of apoptosis bodies increases with the degree of endometriosis and the presence of endometriomas increases in number [6,9]. Seino et al. compares the occurrence of oxidative stress as indicated by high 8-hydroxydeoxyguanosine to infertility endometriosis and non-endometriosis patients attending IVF program. Endometriosis patients have high levels of 8-OhdG. This increase in 8OHdG shows a negative correlation with preovulatory estradiol levels, embryo quality, and fertilization rates [10]. Other studies have shown that due to the disfunction NK cells there is a decrease in cytotoxicity in endometriosis cells. In the peritoneal fluid of women with endometriosis and around endometriosis lesions there is an increase in the number of leukocytes and macrophages. The result is the release of cytokines and growth factors (IL-1.6 and IL-8, TNF, RANTES, VEGF) and subsequently spur angiogenesis around implants. With the formation of new capillaries around the lesion, endometriosis cells can proliferate well [11].

The increase in proinflammatory mediator due to endometrioma has been shown to cause disruption in oocyte and ovulation maturation and cause disruption of the luteal phase in endometriosis. This luteal phase defect is thought to arise as a result of progesterone receptor dysregulation and the effects on the progesterone target

gene. Furthermore, it can cause a decrease in endometrial receptivity. Increased number of inflammatory cells in the peritoneal fluid but has also been shown to have toxic effects on the embryo. The increase in free radicals in the enometrium as shown by the increased expression of glutathione peroxidase and catalase also has a negative impact on embryonic development [9,11].

Implantation failure in endometriosis involves resistance to progesterone and progesterone receptor dysregulation. The role of the progesteron in the implantation process is very large, progesterone will induce desidualization of the endometrium in the luteal phase. In the normal endometrium, before implantation low progesterone receptor regulation occurs but this process is delayed in endometriosis. In addition, eutopic and ectopic endometrium have been shown to be resistant to progesterone, causing a state of estrogen that is not contradictory which may not be suitable for implantation. During the process of implantation the progesterone receptor decreases while the expression of α-integrin epithelium increases. In patients with endometriosis integrin expression decreases so that it can cause interference with embryo implantation.There is a correlation between the effects of embryonic fluid peritoneal and serum of endometriosis patients. This suggested that embryotoxic factors enter and circulate in the systemic circulation and affect early embryogenesis in the reproductive organs [11,12]. In endometriosis,the level of IgG and IgA antibodies to endometrial antigen and lymphocytes are increase. This abnormality can affect endocrine receptivity and embryo implantation [1,6]. Autoantibodies to endometrial antigens increase in women with endometriosis. Endometriosis is associated with recurrent abortion and possible infertility due to abnormal autoantibodies [11].

IV. CONCLUSION & RECOMMENDATION

There is no difference in fertilization rates between endometriosis and non-endometriosis. The number of good quality embryo on endometriosis group is lower than non-endometriosis. Pregnancy failure on endometriosis group is higher than non-endometriosis group.

REFERENCES

[1] Bulletti C, Coccia MA, Battistoni S, Borini A. Endometriosis and Infertility. *J Assist Reprod Genet* 2010; 27:441–447

[2] Lebovic D, Mueller M, Robert . Immunobiology of endometriosis. *Fertil Steril 2010 ;75: 1-10*

[3] Julie A. W. Stilley & Julie A. Birt & Kathy L. Sharpe-Timms , 2012, Cellular and molecular basis for endometriosis-associated infertility, Cell Tissue Res (2012) 349:849–862

[4] Aboulghar MA, Ragaa TM, Gamal IS, Hesham GA, Mona MA. The outcome of in vitro fertilization in advanced endometriosis with previous surgery: a case-controlled study. *AJOG, February, 2003 ;188(2): 371-375*

[5] Shahine LK, Burney RO, Behr B, Milki AA, Westphal LM, Lathi RB. Embryo quality before and after surgical treatment of endometriosis in infertile patients. *Journal of Assisted Reproduction and Genetics*. 2009;26(2-3):69-73.

[6] Fadhlaoui, A., Bouquet de la Jolinière, J., & Feki, A. 2014. Endometriosis and infertility: how and when to treat?. Frontiers in surgery, 1, 24.

[7] Pahlajani G, Falcone T. Laparoscopic surgery for endometriosis-associated infertility: a pathophysiologic approach. *Gynecol Surg* 2010; 7:319–328

[8] Stilley, J. A., Birt, J. A., & Sharpe-Timms, K. L. 2012. Cellular and molecular basis for endometriosis-associated infertility. Cell and tissue research, 349(3), 849–862.

[9] Králíčková M, Vetvicka V. Immunological aspects of endometriosis: a review. Annals of Translational Medicine. 2015;3(11):153.

[10] Seino T, Saito H, Kaneko T, Takahashi T, Kawachiya S, Kurachi H. Eight-hydroxy-2-deoxyguanosinase in granulosa cells is corelated with the quality of oosites and embryo in an in vitro fertilization-embryo transfer program. *Fertil Steril* 2001;77(6):1184-90

[11] Máté G, Bernstein LR and Török AL (2018) Endometriosis Is a Cause of Infertility. Does Reactive Oxygen Damage to Gametes and Embryos Play a Key Role in the Pathogenesis of Infertility Caused by Endometriosis? Front. Endocrinol. 9:725.

[12] Shu J, Xing L, Ding G, Luo Q, Liu X, Yan Q, et al. (2013) The Effect of Peritoneal Fluid from Patients with Endometriosis on Mitochondrial Function and Development of Early Mouse Embryos. *PLoS ONE* 2013; 8(12): e82334.